

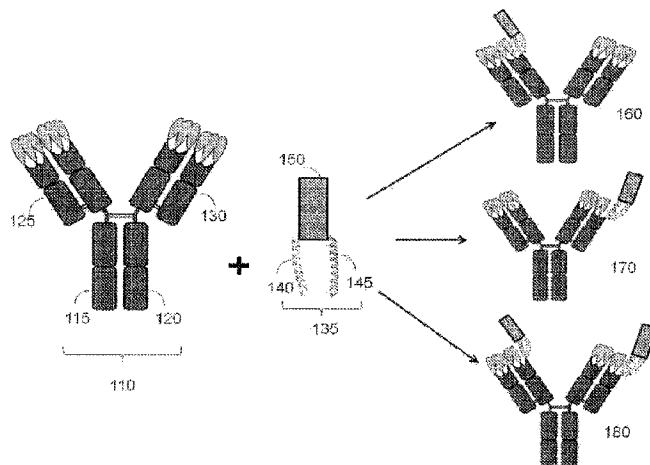


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(54) Title: COILED COIL IMMUNOGLOBULIN FUSION PROTEINS AND COMPOSITIONS THEREOF



(57) **Abrégé/Abstract:**

Disclosed herein are immunoglobulin fusion proteins comprising a first antibody region, a first therapeutic agent, and a first connecting peptide; wherein the first therapeutic agent is attached to the first antibody region by the connecting peptide; and wherein the connecting peptide does not comprise a region having beta strand secondary structure. The connecting peptide may comprise an extender peptide. The extender peptide may have an alpha helical secondary structure. The connecting peptide may comprise a linker peptide. The linker peptide may not comprise any secondary structure. Also disclosed herein are compositions comprising the immunoglobulin fusion proteins and methods for using the immunoglobulin fusion proteins for the treatment or prevention of a disease or condition in a subject.

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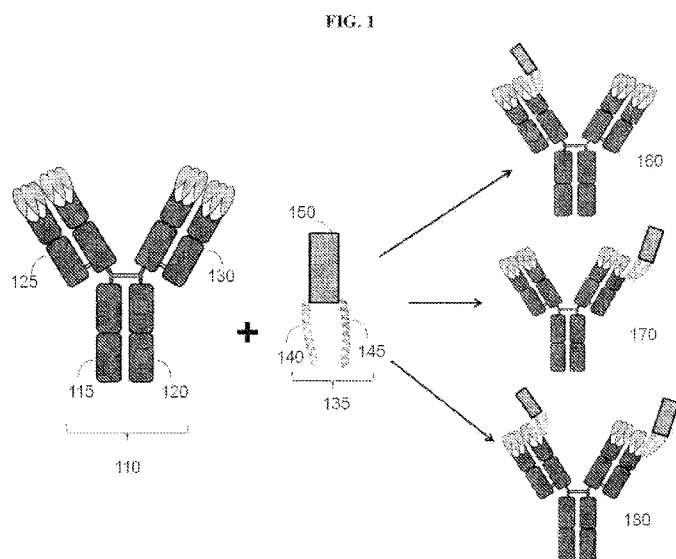
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(54) Title: COILED COIL IMMUNOGLOBULIN FUSION PROTEINS AND COMPOSITIONS THEREOF

(57) Abstract: Disclosed herein are immuno-
globulin fusion proteins comprising a first anti-
body region, a first therapeutic agent, and a first
connecting peptide; wherein the first therapeu-
tic agent is attached to the first antibody region
by the connecting peptide; and wherein the con-
necting peptide does not comprise a region hav-
ing beta strand secondary structure. The con-
necting peptide may comprise an extender pep-
tide. The extender peptide may have an alpha
helical secondary structure. The connecting
peptide may comprise a linker peptide. The
linker peptide may not comprise any secondary
structure. Also disclosed herein are composi-
tions comprising the immunoglobulin fusion
proteins and methods for using the immuno-
globulin fusion proteins for the treatment or
prevention of a disease or condition in a sub-
ject.

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COILED COIL IMMUNOGLOBULIN FUSION PROTEINS AND COMPOSITIONS THEREOF

[0001]

BACKGROUND OF THE INVENTION

[0002] Antibodies are natural proteins that the vertebrate immune system forms in response to foreign substances (antigens), primarily for defense against infection. For over a century, antibodies have been induced in animals under artificial conditions and harvested for use in therapy or diagnosis of disease conditions, or for biological research. Each individual antibody producing cell produces a single type of antibody with a chemically defined composition, however, antibodies obtained directly from animal serum in response to antigen inoculation actually comprise an ensemble of non-identical molecules (*e.g.*, polyclonal antibodies) made from an ensemble of individual antibody producing cells.

[0003] Antibody fusion constructs can be used to improve the delivery of drugs or other agents to target cells, tissues and tumors. Antibody fusion constructs may comprise a chemical linker to attach a drug or other agent to antibody. Exemplary antibody fusion constructs and methods of producing antibody fusion constructs are disclosed in US patent application numbers 2006/0182751, 2007/0160617 and US patent number 7,736,652.

[0004] Disclosed herein are novel immunoglobulin fusion proteins and methods of producing such immunoglobulin fusion proteins. Further disclosed herein are uses of the immunoglobulin fusion proteins for the treatment of various diseases and conditions. Methods of extending the half-life of a therapeutic agent are also disclosed herein.

SUMMARY OF THE INVENTION

[0005] In one aspect of the disclosure, provided herein is an immunoglobulin fusion protein comprising a first antibody region, a first therapeutic agent, and a first connecting peptide; wherein the first therapeutic agent is attached to the first antibody region by the connecting peptide; and wherein the connecting peptide does not comprise a region having beta strand secondary structure.

[0006] In one embodiment, the connecting peptide comprises a first extender peptide. The first extender peptide may comprise one or more regions having alpha helical secondary structure. In one instance, the first extender peptide does not comprise more than 7 consecutive amino acids that are based on or derived from a bovine ultralong CDR3 amino acid sequence.

[0007] In one embodiment, the connecting peptide comprises a first linking peptide. The first linking peptide may not comprise alpha helical or beta strand secondary structure. The first linking peptide may comprise from about 0 to about 50 amino acids.

[0008] In one embodiment, the connecting peptide comprises from about 0 to about 50 amino acids. The connecting peptide may comprise from about 4 to about 100 amino acids.

[0009] In one embodiment, the first connecting peptide is attached to a CDR of the first antibody region. In one embodiment, the first therapeutic peptide replaces one or more regions of the first antibody region. In another embodiment, the first connecting peptide replaces one or more regions of the first antibody region.

[0010] In one embodiment, the immunoglobulin fusion protein further comprises a second connecting peptide. In one embodiment, the second connecting peptide does not comprise a region having beta strand secondary structure. The second connecting peptide may comprise a second extender peptide. The second extender peptide may comprise one or more regions having alpha helical secondary structure. The second peptide may comprise a second linking peptide. The second linking peptide may not comprise alpha helical or beta strand secondary structure.

[0011] In one embodiment, the immunoglobulin fusion protein further comprises a second therapeutic agent.

[0012] In one embodiment, the first antibody region comprises an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 19-36 and 271-273. In one embodiment, the first antibody region comprises an amino acid sequence that is at least about 50% identical and/or homologous to an amino acid sequence of any one of SEQ ID NOs: 19-36 and 271-273. In one embodiment, the first antibody region comprises an amino acid sequence that is at least about 80% identical and/or homologous to an amino acid sequence of any one of SEQ ID NOs: 19-36 and 271-273.

[0013] In one embodiment, the first antibody region comprises an amino acid sequence that is based on or derived from a trastuzumab immunoglobulin. In one embodiment, the first antibody region comprises an amino acid sequence that is at least about 50% identical and/or homologous to an amino acid sequence of trastuzumab immunoglobulin. In one embodiment, the first antibody region comprises an amino acid sequence that is at least about 80% identical and/or homologous to an amino acid sequence of trastuzumab immunoglobulin.

[0014] In one embodiment, the first antibody region comprises an amino acid sequence that is based on or derived from a palivizumab immunoglobulin. In one embodiment, the first antibody region comprises an amino acid sequence that is at least about 50% identical and/or homologous to an amino acid sequence of palivizumab immunoglobulin. In one embodiment, the first antibody region comprises an amino acid sequence that is at least about 80% identical and/or homologous to an amino acid sequence of palivizumab immunoglobulin.

[0015] In one embodiment, the immunoglobulin fusion protein further comprises a second antibody region. In one instance, the first antibody region comprises a region of an antibody light chain and the second antibody region comprises a region of an antibody heavy chain. In one instance, the first antibody region comprises a region of an antibody heavy chain and the second antibody region comprises a region of an antibody light chain. In one instance, the first antibody region comprises a first region of an antibody light chain and the second antibody region comprises a second region of an antibody light chain.

In one instance, the first antibody region comprises a first region of an antibody heavy chain and the second antibody region comprises a second region of an antibody heavy chain. In one embodiment, the second antibody region comprises an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 19-36 and 271-273. In one embodiment, the second antibody region comprises an amino acid sequence that is at least about 50% identical and/or homologous to an amino acid sequence of any one of SEQ ID NOs: 19-36 and 271-273. In one embodiment, the second antibody region comprises an amino acid sequence that is at least about 80% identical and/or homologous to an amino acid sequence of any one of SEQ ID NOs: 19-36 and 271-273.

[0016] In one embodiment, the second antibody region comprises an amino acid sequence that is based on or derived from a trastuzumab immunoglobulin. In one embodiment, the second antibody region comprises an amino acid sequence that is at least about 50% identical and/or homologous to an amino acid sequence of trastuzumab immunoglobulin. In one embodiment, the second antibody region comprises an amino acid sequence that is at least about 80% identical and/or homologous to an amino acid sequence of trastuzumab immunoglobulin. In one embodiment, the second antibody region comprises an amino acid sequence that is based on or derived from a palivizumab immunoglobulin. In one embodiment, the second antibody region comprises an amino acid sequence that is at least about 50% identical and/or homologous to an amino acid sequence of palivizumab immunoglobulin. In one embodiment, the second antibody region comprises an amino acid sequence that is at least about 80% identical and/or homologous to an amino acid sequence of palivizumab immunoglobulin.

[0017] In one embodiment, the first connecting peptide comprises an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 144-185. In one embodiment, the first connecting peptide comprises an amino acid sequence that is at least about 50% identical and/or homologous to an amino acid sequence of any one of SEQ ID NOs: 144-185. In one embodiment, the first connecting peptide comprises an amino acid sequence that is at least about 80% identical and/or homologous to an amino acid sequence of any one of SEQ ID NOs: 144-185.

[0018] In one embodiment, the second connecting peptide comprises an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 144-185. In one embodiment, the second connecting peptide comprises an amino acid sequence that is at least about 50% identical and/or homologous to an amino acid sequence of any one of SEQ ID NOs: 144-185. In one embodiment, the second connecting peptide comprises an amino acid sequence that is at least about 80% identical and/or homologous to an amino acid sequence of any one of SEQ ID NOs: 144-185.

[0019] In one embodiment, the first connecting peptide comprises a protease cleavage site. In one embodiment, the second connecting peptide comprises a protease cleavage site. In one instance, the first therapeutic agent comprises an amino acid sequence configured for recognition by a protease. In one instance, the second therapeutic agent comprises an amino acid sequence configured for recognition by a protease.

[0020] In one embodiment, the first connecting peptide comprises one or more extender peptides and one or more linker peptides. In one embodiment, the first connecting peptide comprises one or more extender

peptides, one or more linker peptides, and one or more protease cleavage sites. In one embodiment, the first connecting peptide comprises one or more extender peptides and one or more protease cleavage sites. In one embodiment, the first connecting peptide comprises one or more linker peptides and one or more protease cleavage sites.

[0021] In one embodiment, the second connecting peptide comprises one or more extender peptides and one or more linker peptides. In one embodiment, the second connecting peptide comprises one or more extender peptides, one or more linker peptides, and one or more protease cleavage sites. In one embodiment, the second connecting peptide comprises one or more extender peptides and one or more protease cleavage sites. In one embodiment, the second connecting peptide comprises one or more linker peptides and one or more protease cleavage sites.

[0022] In one embodiment, the first therapeutic agent comprises an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 227-267. In one embodiment, the first therapeutic agent comprises an amino acid sequence that is at least about 50% identical and/or homologous to an amino acid sequence of any one of SEQ ID NOs: 227-267. In one embodiment, the first therapeutic agent comprises an amino acid sequence that is at least about 80% identical and/or homologous to an amino acid sequence of any one of SEQ ID NOs: 227-267. In one embodiment, the first therapeutic agent comprises from about 5 to about 1,000 amino acids comprising from about 5 to about 350 amino acids identical and/or homologous to any one of SEQ ID NOs: 227-267.

[0023] In one embodiment, the immunoglobulin fusion protein comprises an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 68-99. In one embodiment, the immunoglobulin fusion protein comprises an amino acid sequence that is at least about 50% identical and/or homologous to an amino acid sequence of any one of SEQ ID NOs: 68-99. In one embodiment, the immunoglobulin fusion protein comprises an amino acid sequence that is at least about 80% identical and/or homologous to an amino acid sequence of any one of SEQ ID NOs: 68-99. In one embodiment, the immunoglobulin fusion protein comprises from about 5 to about 3,000 amino acids comprising from about 50 to about 700 amino acids identical and/or homologous to any one of SEQ ID NOs: 68-99.

[0024] In another aspect, provided herein is a first genetic construct comprising nucleic acids encoding the immunoglobulin fusion protein of any of SEQ ID NOs: 68-99. In one embodiment, a first genetic construct comprises nucleic acids derived from any one of SEQ ID NOs: 37-67. In one embodiment, a first genetic construct comprises a nucleic acid sequence that is at least about 50% identical and/or homologous to a nucleic acid sequence of any one of SEQ ID NOs: 37-67. In one embodiment, a first genetic construct comprises a nucleic acid sequence that is at least about 80% identical and/or homologous to a nucleic acid sequence of any one of SEQ ID NOs: 37-67.

[0025] In another aspect, provided herein is a first expression vector comprising a first genetic construct comprising nucleic acids encoding the immunoglobulin fusion protein of any of SEQ ID NOs: 68-99. In one embodiment, provided herein is a first expression vector comprising a first genetic construct comprising nucleic acids derived from any one of SEQ ID NOs: 37-67. In one embodiment, provided herein is a first expression vector comprising a first genetic construct comprising a nucleic acid sequence

that is at least about 50% identical and/or homologous to a nucleic acid sequence of any one of SEQ ID NOs: 37-67. In one embodiment, provided herein is a first expression vector comprising a first genetic construct comprising a nucleic acid sequence that is at least about 80% identical and/or homologous to a nucleic acid sequence of any one of SEQ ID NOs: 37-67. In one instance, provided herein is a mammalian expression host comprising a first expression vector. In one embodiment, provided herein is a method of producing an immunoglobulin fusion protein comprising (a) transfecting a first expression vector transiently in a mammalian cell culture, (b) growing the cell culture in an expression medium at a controlled temperature and percentage CO₂, (c) and harvesting the secreted immunoglobulin fusion protein. In one embodiment, the method of producing an immunoglobulin fusion protein further comprises purifying the immunoglobulin fusion protein.

[0026] In another aspect, provided herein is a method of treating a disease or condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount an immunoglobulin fusion protein comprising an amino acid sequence derived from any one of SEQ ID NOs 68-99. In another aspect, provided herein is a method of treating a disease or condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount an immunoglobulin fusion protein comprising an amino acid sequence that is at least about 50% identical and/or homologous to any one of SEQ ID NOs 68-99. In another aspect, provided herein is a method of treating a disease or condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount an immunoglobulin fusion protein comprising an amino acid sequence that is at least about 80% identical and/or homologous to any one of SEQ ID NOs 68-99.

[0027] In another aspect, provided herein is a pharmaceutical composition comprising an immunoglobulin fusion protein derived from any one of SEQ ID NOs: 68-99. In another aspect, provided herein is a pharmaceutical composition comprising an immunoglobulin fusion protein comprising an amino acid sequence that is at least about 50% identical and/or homologous to any one of SEQ ID NOs: 68-99. In another aspect, provided herein is a pharmaceutical composition comprising an immunoglobulin fusion protein comprising an amino acid sequence that is at least about 80% identical and/or homologous to any one of SEQ ID NOs: 68-99. In one embodiment, the pharmaceutical composition further comprises a pharmaceutically acceptable excipient.

[0028] In one embodiment, the immunoglobulin fusion protein comprises an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 122-143. In one embodiment, the immunoglobulin fusion protein comprises an amino acid sequence that is at least about 50% identical and/or homologous to an amino acid sequence of any one of SEQ ID NOs: 122-143. In one embodiment, the immunoglobulin fusion protein comprises an amino acid sequence that is at least about 80% identical and/or homologous to an amino acid sequence of any one of SEQ ID NOs: 122-143. In one embodiment, the first immunoglobulin fusion protein comprises from about 5 to about 3,000 amino acids comprising from about 50 to about 700 amino acids identical and/or homologous to any one of SEQ ID NOs: 122-143.

[0029] In another aspect, provided herein is a first genetic construct comprising nucleic acids encoding the immunoglobulin fusion protein of any of SEQ ID NOs: 122-143. In one embodiment, a first genetic construct comprises nucleic acids derived from any one of SEQ ID NOs: 100-121. In one embodiment, a first genetic construct comprises a nucleic acid sequence that is at least about 50% identical and/or homologous to a nucleic acid sequence of any one of SEQ ID NOs: 100-121. In one embodiment, a first genetic construct comprises a nucleic acid sequence that is at least about 80% identical and/or homologous to a nucleic acid sequence of any one of SEQ ID NOs: 100-121.

[0030] In another aspect, provided herein is a first expression vector comprising a first genetic construct comprising nucleic acids encoding the immunoglobulin fusion protein of any of SEQ ID NOs: 122-143. In one embodiment, provided herein is a first expression vector comprising a first genetic construct comprising nucleic acids derived from any one of SEQ ID NOs: 100-121. In one embodiment, provided herein is a first expression vector comprising a first genetic construct comprising a nucleic acid sequence that is at least about 50% identical and/or homologous to a nucleic acid sequence of any one of SEQ ID NOs: 100-121. In one embodiment, provided herein is a first expression vector comprising a first genetic construct comprising a nucleic acid sequence that is at least about 80% identical and/or homologous to a nucleic acid sequence of any one of SEQ ID NOs: 100-121. In one instance, provided herein is a mammalian expression host comprising a first expression vector. In one embodiment, provided herein is a method of producing an immunoglobulin fusion protein comprising (a) transfecting a first expression vector transiently in a mammalian cell culture, (b) growing the cell culture in an expression medium at a controlled temperature and percentage CO₂, (c) and harvesting the secreted immunoglobulin fusion protein. In one embodiment, the method of producing an immunoglobulin fusion protein further comprises purifying the immunoglobulin fusion protein.

[0031] In another aspect, provided herein is a method of treating a disease or condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount an immunoglobulin fusion protein comprising an amino acid sequence derived from any one of SEQ ID NOs: 122-143. In another aspect, provided herein is a method of treating a disease or condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount an immunoglobulin fusion protein comprising an amino acid sequence that is at least about 50% identical and/or homologous to any one of SEQ ID NOs: 122-143. In another aspect, provided herein is a method of treating a disease or condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount an immunoglobulin fusion protein comprising an amino acid sequence that is at least about 80% identical and/or homologous to any one of SEQ ID NOs: 122-143.

[0032] In another aspect, provided herein is a pharmaceutical composition comprising an immunoglobulin fusion protein derived from any one of SEQ ID NOs: 122-143. In another aspect, provided herein is a pharmaceutical composition comprising an immunoglobulin fusion protein comprising an amino acid sequence that is at least about 50% identical and/or homologous to any one of SEQ ID NOs: 122-143. In another aspect, provided herein is a pharmaceutical composition comprising an

immunoglobulin fusion protein comprising an amino acid sequence that is at least about 80% identical and/or homologous to any one of SEQ ID NOs: 122-143. In one embodiment, the pharmaceutical composition further comprises a pharmaceutically acceptable excipient.

[0033] In one embodiment, the first therapeutic peptide is configured to treat neutropenia and/or a neutropenia related disease. In one embodiment, the first therapeutic peptide is configured to treat diabetes and/or a diabetes related disease. In one embodiment, the first therapeutic peptide is configured to treat obesity and/or an obesity related disease. In one embodiment, the first therapeutic peptide is configured to treat an autoimmune disease and/or an autoimmune related disease. In one embodiment, the first therapeutic peptide is configured to treat anemia and/or an anemia related disease. In one embodiment, the first therapeutic peptide is configured to treat growth hormone deficiency and/or a growth hormone related disease. In one embodiment, the first therapeutic peptide is configured to treat chronic obstructive pulmonary disease (COPD) and/or a COPD related disease. In one embodiment, the first therapeutic peptide is configured to treat pain. In one embodiment, the first therapeutic peptide is configured to treat irritable bowel syndrome (IBS) and/or an IBS related disease. In one embodiment, the first therapeutic peptide is configured to treat Crohn's disease and/or a Crohn's disease related illness. In one embodiment, the first therapeutic peptide is configured to treat neutropenia and/or a neutropenia related disease. In one embodiment, the first therapeutic peptide is configured to treat a metabolic disorder and/or a disease resulting from said metabolic disorder. In one embodiment, the metabolic disorder includes lipodystrophy, diabetes, and hypertriglyceridemia. In one embodiment, the first therapeutic peptide is configured to treat short bowel syndrome and/or a short bowel syndrome related disease. In one embodiment, the first therapeutic peptide is configured to treat a patient with heart failure. In one embodiment, the first therapeutic peptide is configured to treat fibrosis and/or a fibrosis related disease.

[0034] In one embodiment, the immunoglobulin fusion protein is configured to treat neutropenia and/or a neutropenia related disease. In one embodiment, the immunoglobulin fusion protein is configured to treat diabetes and/or a diabetes related disease. In one embodiment, the immunoglobulin fusion protein is configured to treat obesity and/or an obesity related disease. In one embodiment, the immunoglobulin fusion protein is configured to treat an autoimmune disease and/or an autoimmune related disease. In one embodiment, the immunoglobulin fusion protein is configured to treat anemia and/or an anemia related disease. In one embodiment, the immunoglobulin fusion protein is configured to treat growth hormone deficiency and/or a growth hormone related disease. In one embodiment, the immunoglobulin fusion protein is configured to treat chronic obstructive pulmonary disease (COPD) and/or a COPD related disease. In one embodiment, the immunoglobulin fusion protein is configured to treat pain. In one embodiment, the immunoglobulin fusion protein is configured to treat irritable bowel syndrome (IBS) and/or an IBS related disease. In one embodiment, the immunoglobulin fusion protein is configured to treat Crohn's disease and/or a Crohn's disease related illness. In one embodiment, the immunoglobulin fusion protein is configured to treat neutropenia and/or a neutropenia related disease. In one embodiment, the immunoglobulin fusion protein is configured to treat a metabolic disorder and/or a disease resulting

from said metabolic disorder. In one embodiment, the metabolic disorder includes lipodystrophy, diabetes, and hypertriglyceridemia. In one embodiment, the immunoglobulin fusion protein is configured to treat short bowel syndrome and/or a short bowel syndrome related disease. In one embodiment, the immunoglobulin fusion protein is configured to treat a patient with heart failure. In one embodiment, the immunoglobulin fusion protein is configured to treat fibrosis and/or a fibrosis related disease. In one embodiment, the first therapeutic peptide is configured to treat pulmonary arterial hypertension, ventilator-induced injury of the immature lung and/or lung transplant rejection.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] The foregoing summary, as well as the following detailed description of the disclosure, will be better understood when read in conjunction with the appended figures. For the purpose of illustrating the disclosure, shown in the figures are embodiments which are presently preferred. It should be understood, however, that the disclosure is not limited to the precise arrangements, examples and instrumentalities shown. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity.

[0036] In some figures, trastuzumab is referred to as Herceptin. It is to be understood that trastuzumab and Herceptin may be used interchangeably throughout this disclosure. In some figures, an immunoglobulin fusion protein is described in the following order: antibody, coil or direct, therapeutic agent, and antibody region to which the therapeutic agent is attached; for example, trastuzumab-coil hEPO (CDRH3). The immunoglobulin fusion protein may be described in any other manner, for example, trastuzumab-CDRH3-coil-hEPO is the same fusion as trastuzumab-coil hEPO (CDRH3). In some instances, an antibody is abbreviated in the figures, for example, bAb and BLVH12 are abbreviations for a bovine antibody. PBS is an abbreviation of phosphate buffered saline. In some instances, hAb is an abbreviation for Herceptin or trastuzumab antibody. In some instances, H2 is an abbreviation of CDRH2, H3 is an abbreviation of CDRH3, and L3 is an abbreviation of CDRL3. In some instances, CDRH3 and CDR3H indicate a complementary determining region 3 of a heavy chain, CDRH2 and CDR2H indicate a complementary determining region 2 of a heavy chain, and CDRL3 and CDR3L indicate a complementary determining region 3 of a light chain.

[0037] Provided herein are immunoglobulin fusion proteins comprising the term coil, wherein in some instances, these immunoglobulin fusion proteins comprising at least one extender peptide comprising amino acids having an alpha helical secondary structure. Provided herein are immunoglobulin fusion proteins comprising the term direct, wherein in some instances, these immunoglobulin fusion proteins do not comprise an extender peptide.

[0038] Included in the drawings are the following figures.

[0039] **FIG. 1** depicts a schematic of various immunoglobulin fusion proteins with an extender peptide comprising an alpha helix (e.g., coil) structure.

[0040] **FIG. 2A-2G** depict schematics of various non-antibody regions.

- [0041] FIG. 3 depicts a schematic of various immunoglobulin fusion proteins with extender peptides.
- [0042] FIG. 4 depicts an SDS-PAGE gel of bovine-coil IgG, bovine-coil bGCSF IgG, trastuzumab IgG, and trastuzumab-coil bGCSF IgG.
- [0043] FIG. 5 depicts a schematic of various immunoglobulin fusion proteins with a therapeutic peptide directly inserted into an antibody region.
- [0044] FIG. 6 depicts a graph of the in vitro activity of bovine-coil IgG, bovine-coil bGCSF IgG, trastuzumab IgG, and trastuzumab-coil bGCSF IgG in mouse NFS-60 cells.
- [0045] FIG. 7 depicts a graph of the binding affinity of a trastuzumab-coil bGCSF IgG to a Her2 receptor.
- [0046] FIG. 8 depicts a Western blot of BLV1H12-coil betatrophin IgG, with and without DTT.
- [0047] FIG. 9 depicts a schematic of various immunoglobulin fusion proteins without extender peptides.
- [0048] FIG. 10 depicts an SDS-PAGE of trastuzumab-direct bGCSF fusion proteins, with and without DTT.
- [0049] FIG. 11 depicts a graph of the in vitro activity of trastuzumab-direct bGCSF fusion protein and bGCSF in proliferating mouse NFS-60 cells.
- [0050] FIG. 12 depicts an SDS-PAGE gel of trastuzumab-coil exendin-4 (CDRH3) fusion protein and trastuzumab-coil exendin-4 fusion protein cleaved with Factor Xa to generate trastuzumab-coil exendin-4 RN.
- [0051] FIG. 13 depicts a chromatograph of an electrospray ionization mass spectrometry (ESI-MS) of trastuzumab-coil exendin-4 (CDRH3) fusion protein treated with peptide N-glycosidase and DTT.
- [0052] FIG. 14A-14B depicts a chromatograph of an ESI-MS of trastuzumab-coil exendin-4 RN (CDRH3) fusion protein treated with peptide N-glycosidase and DTT, (A) N-terminal fragment, (B) C-terminal fragment.
- [0053] FIG. 15 depicts a graph of the in vitro activities of exendin-4 (Ex-4), trastuzumab, trastuzumab-coil exendin-4 (CDRH3), and trastuzumab-coil exendin-4 (CDRH3) RN in HEK 293 cells overexpressing GLP-1R receptor and cAMP responsive element (CRE)-luciferase (Luc) reporter.
- [0054] FIG. 16 depicts a graph of the in vitro activities of glucagon, Ex-4, trastuzumab, trastuzumab-coil exendin-4 (CDRH3), and trastuzumab-coil exendin-4 (CDRH3) RN in HEK 293 cells overexpressing glucagon receptor (GCGR) and CRE-Luc reporter.
- [0055] FIG. 17A-17B depicts a graph of the pharmacokinetics of trastuzumab-coil exendin-4 (CDRH3) IgG with (A) intravenous injection and (B) subcutaneous injection in mice.
- [0056] FIG. 18A-18R depicts the pharmacodynamics of trastuzumab-coil exendin-4 (CDRH3) IgG in mice at different time points.
- [0057] FIG. 19A-19N depicts the pharmacodynamics of various concentrations of trastuzumab-coil exendin-4 (CDRH3) IgG in mice at different time points.
- [0058] FIG. 20 depicts an SDS-PAGE gel of trastuzumab-coil Moka IgG and trastuzumab-coil Vm24 IgG, with and without DTT.

- [0059] FIG. 21 depicts a graph of the in vitro activities of trastuzumab-coil Moka IgG and trastuzumab-coil Vm24 IgG on T-cell activation in human peripheral blood mononucleated cells (PBMCs).
- [0060] FIG. 22 depicts an SDS-PAGE of trastuzumab-coil hGCSF (CDRH2) and trastuzumab-coil hGCSF (CDRL3), with and without DTT.
- [0061] FIG. 23A-23B depicts an ESI-MS of (A) trastuzumab-coil hGCSF (CDRH2) treated with peptide N-glycosidase and DTT, and (B) trastuzumab-coil hGCSF (CDRL3) treated with peptide N-glycosidase and DTT.
- [0062] FIG. 24 depicts a histogram of trastuzumab-coil hGCSF (CDRH2) and trastuzumab-coil hGCSF (CDRL3) binding to HER2 receptor.
- [0063] FIG. 25 depicts a graph of the in vitro activity of trastuzumab-coil hGCSF (CDRH2) and trastuzumab-coil hGCSF (CDRL3) in proliferating mouse NFS-60 cells.
- [0064] FIG. 26 depicts an SDS-PAGE gel of trastuzumab-coil hEPO (CDRH3), with and without DTT.
- [0065] FIG. 27 depicts an ESI-MS of trastuzumab-coil hEPO (CDRH3) IgG treated with peptide N-glycosidase and DTT.
- [0066] FIG. 28 depicts a histogram of trastuzumab-coil hEPO (CDRH3) binding to HER2 receptor.
- [0067] FIG. 29 depicts a graph of the in vitro activity of trastuzumab-coil hEPO (CDRH3) IgG in proliferating human TF-1 cells.
- [0068] FIG. 30 depicts an SDS-PAGE gel of dual fusion trastuzumab-coil hEPO (CDRH3)-trastuzumab-coil hGCSF (CDRL3) IgG, with and without DTT.
- [0069] FIG. 31A-31B depicts an ESI-MS of the light (A) and heavy (B) chains of dual fusion trastuzumab-coil hEPO (CDRH3)-trastuzumab-coil hGCSF (CDRL3) IgG treated with peptide N-glycosidase and DTT.
- [0070] FIG. 32 depicts a histogram of dual fusion trastuzumab-coil hEPO (CDRH3)-trastuzumab-coil hGCSF (CDRL3) IgG binding to HER2 receptor.
- [0071] FIG. 33 depicts a graph of the in vitro activity of dual fusion trastuzumab-coil hEPO (CDRH3)-trastuzumab-coil hGCSF (CDRL3) IgG in proliferating human TF-1 cells.
- [0072] FIG. 34 depicts a graph of the in vitro activity of dual fusion trastuzumab-coil hEPO (CDRH3)-trastuzumab-coil hGCSF (CDRL3) IgG in proliferating mouse NFS-60 cells.
- [0073] FIG. 35 depicts an SDS-PAGE gel of trastuzumab-coil hGH (CDRH3) IgG after purification, trastuzumab-direct fusion hGH (CDRH2) and trastuzumab-coil hGH (CDRH2) IgGs, with and without DTT.
- [0074] FIG. 36A-36B depicts the pharmacokinetics of trastuzumab-coil hGH (CDRH3) IgG in rat by (A) intravenous injection and (B) subcutaneous injection.
- [0075] FIG. 37 depicts a graph indicating the percentage of body weight change in rats injected with a vehicle, genotropin, trastuzumab, and trastuzumab-coil hGH (CDRH3) IgG (denoted in the figure as Ab-GH).

[0076] FIG. 38 depicts an SDS-PAGE gel of trastuzumab-coil hLeptin (CDRH2), trastuzumab-coil hLeptin (CDRH3), and trastuzumab-coil hLeptin (CDRL3), with and without DTT.

[0077] FIG. 39A-39A depicts graphs of the in vitro activity of (A) hLeptin, trastuzumab-coil hLeptin (CDRH2), trastuzumab-coil hLeptin (CDRH3), and (B) hLeptin, trastuzumab-coil hLeptin (CDRL3) in activating human leptin receptor (LepR).

[0078] FIG. 40A-40C depicts histograms of SKBR3 cell binding with wild-type (wt) trastuzumab, trastuzumab-coil hLeptin (CDRH2), and trastuzumab-coil hLeptin (CDRH3).

[0079] FIG. 41 depicts an SDS-PAGE gel of trastuzumab-coil elafin (CDRH3), with and without DTT.

[0080] FIG. 42A-42B depicts graphs of the in vitro inhibition activity of trastuzumab-coil elafin (CDRH3).

[0081] FIG. 43 depicts an SDS-PAGE gel of trastuzumab-coil GLP2 (CDRH3), with and without DTT.

[0082] FIG. 44 depicts an SDS-PAGE gel of trastuzumab-coil relaxin (insulin c-peptide) (CDRH3), with and without DTT.

[0083] FIG. 45 depicts an SDS-PAGE gel of trastuzumab-coil relaxin (CDRH3) co-transfected with the cleavage enzyme prohormone convertase 2 (PC2), with and without DTT.

[0084] FIG. 46 depicts an SDS-PAGE gel of trastuzumab-coil relaxin (XTEN35) with 6x HIS (CDRH3) IgG co-transfected with PC2, with and without DTT.

DETAILED DESCRIPTION OF THE INVENTION

[0085] Disclosed herein are immunoglobulin fusion proteins and methods of producing such immunoglobulin fusion proteins. Disclosed herein are immunoglobulin fusion proteins comprising an antibody region and a non-antibody region, wherein the non-antibody region comprises: (a) a first extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The secondary structure may be an alpha helix. In some embodiments, the alpha helix is configured to form a coiled coil. The therapeutic agent may be a therapeutic peptide. The non-antibody region may further comprise a linker. The linker may be a peptide linker. The peptide linker may have no regular secondary structure. The non-antibody region may further comprise a proteolytic cleavage site. In some embodiments, the non-antibody region replaces at least a portion of the antibody region. In some embodiments, the extender peptide connects the therapeutic agent to the antibody region. In some instances, the non-antibody region is connected to a CDR of an antibody. The CDR may be CDR1, CDR2, or CDR3. The CDR may be part of a light chain or a heavy chain. In an exemplary embodiment, the first extender peptide is a connecting peptide or a portion of a connecting peptide. In some embodiments, the peptide linker is a connecting peptide or a portion of a connecting peptide. In some embodiments, the protease cleavage site is a connecting peptide or a portion of a connecting peptide.

[0086] Further disclosed herein are immunoglobulin fusion proteins comprising an antibody region and an extender fusion region, wherein the extender fusion region comprises: (a) a first extender peptide,

wherein the first extender peptide comprises an amino acid sequence comprising an alpha helix or coiled coil secondary structure, and (b) a therapeutic agent. The therapeutic agent may be a therapeutic peptide. The extender fusion region may further comprise a linker. The linker may be a peptide linker. The peptide linker may have no regular secondary structure. The extender fusion region may further comprise a proteolytic cleavage site. In some embodiments, the extender fusion region replaces at least a portion of the antibody region. In some embodiments, the extender peptide connects the therapeutic agent to the antibody region. In some instances, the extender fusion region is connected to a CDR of an antibody. The CDR may be CDR1, CDR2, or CDR3. The CDR may be part of a light chain or a heavy chain. In an exemplary embodiment, the first extender peptide is a connecting peptide or a portion of a connecting peptide. In some embodiments, the peptide linker is a connecting peptide or a portion of a connecting peptide. In some embodiments, the protease cleavage site is a connecting peptide or a portion of a connecting peptide.

[0087] Further disclosed herein are immunoglobulin fusion proteins comprising an antibody region directly attached to a non-antibody region, wherein the non-antibody region comprises a therapeutic agent. These immunoglobulin fusion proteins, in some instances, may be referred to as direct immunoglobulin fusion proteins. In some instances, the therapeutic agent is a therapeutic peptide. In some embodiments, the therapeutic agent is attached to the antibody region without the use of a peptide comprising a secondary structure. In some embodiments, the therapeutic agent replaces at least a portion of the antibody region to which the therapeutic agent is attached. In some embodiments, the therapeutic peptide is attached to the antibody region by one or more linkers comprising no regular secondary structure (e.g., no alpha helices or beta strands). In some embodiments, the linker is a peptide linker. In some embodiments, the immunoglobulin fusion protein further comprises one or more protease cleavage sites. In some embodiments, the therapeutic agent is attached to a CDR of an antibody. The CDR may be CDR1, CDR2, or CDR3. The CDR may be part of a light chain or a heavy chain. In an exemplary embodiment, the peptide linker is a connecting peptide or a portion of a connecting peptide. In some embodiments, the protease cleavage site is a connecting peptide or a portion of a connecting peptide.

[0088] Further disclosed herein are immunoglobulin fusion proteins comprising an antibody region directly attached to an extender fusion region, wherein the extender fusion region comprises a therapeutic agent. These immunoglobulin fusion proteins, in some instances, may be referred to as direct immunoglobulin fusion proteins. In some instances, the therapeutic agent is a therapeutic peptide. In some embodiments, the therapeutic agent is attached to the antibody region without the use of a peptide comprising a secondary structure. In some embodiments, the therapeutic agent replaces at least a portion of the antibody region to which the therapeutic agent is attached. In some embodiments, the therapeutic agent is attached to the antibody region by one or more linkers comprising no regular secondary structure (e.g., no alpha helices or beta strands). In some embodiments, the linker is a peptide linker. In some embodiments, the immunoglobulin fusion protein further comprises one or more protease cleavage sites. In some embodiments, the therapeutic agent is attached to a CDR of an antibody. The CDR may be CDR1, CDR2, or CDR3. The CDR may be part of a light chain or a heavy chain. In an exemplary

embodiment, the peptide linker is a connecting peptide or a portion of a connecting peptide. In some embodiments, the protease cleavage site is a connecting peptide or a portion of a connecting peptide.

[0089] Further disclosed herein are immunoglobulin fusion proteins comprising a first antibody region, a first therapeutic agent, and a first connecting peptide; wherein the first therapeutic agent is attached to the first antibody region by the connecting peptide; and wherein the connecting peptide does not comprise a region having beta strand secondary structure. In an exemplary embodiment, the first therapeutic agent and the first connecting peptide are components of a non-antibody region. In an exemplary embodiment, the first therapeutic agent and the first connecting peptide are components of an extender fusion region. In some embodiments, the connecting peptide comprises one or more extender peptides. In some embodiments, the connecting peptide comprises one or more linking peptides. In some embodiments, the connecting peptide comprises one or more protease cleavage sites. In some embodiments, the first connecting peptide comprises one or more extender peptides and one or more linker peptides. In some embodiments, the first connecting peptide comprises one or more extender peptides, one or more linker peptides, and one or more protease cleavage sites. In some embodiments, the first connecting peptide comprises one or more extender peptides and one or more protease cleavage sites. In some embodiments, the first connecting peptide comprises one or more linker peptides and one or more protease cleavage sites.

[0090] Further disclosed herein are immunoglobulin fusion proteins comprising (a) a non-antibody region; and (b) an antibody region, wherein the non-antibody region replaces at least a portion of an antibody from which the antibody region is based on or derived from. The non-antibody region may replace at least a portion of a complementarity determining region. The non-antibody region may replace at least a portion of a variable domain. The non-antibody region may replace at least a portion of a constant domain. The non-antibody region may replace at least a portion of a heavy chain. The non-antibody region may replace at least a portion of a light chain. The non-antibody region may comprise a therapeutic peptide. The non-antibody region may comprise a connecting peptide.

[0091] Further disclosed herein are immunoglobulin fusion proteins comprising (a) an extender fusion region; and (b) an antibody region, wherein the extender fusion region replaces at least a portion of an antibody from which the antibody region is based on or derived from. The extender fusion region may replace at least a portion of a complementarity determining region. The extender fusion region may replace at least a portion of a variable domain. The extender fusion region may replace at least a portion of a constant domain. The extender fusion region may replace at least a portion of a heavy chain. The extender fusion region may replace at least a portion of a light chain. The extender fusion may comprise a therapeutic peptide. The extender fusion region may comprise a connecting peptide.

[0092] Further disclosed herein are dual fusion proteins comprising two or more therapeutic agents attached to one or more antibody regions or fragments thereof. At least one therapeutic agent may be inserted into or attached to the antibody or fragment thereof. Two or more therapeutic agents may be inserted into or attached to the antibody or fragment thereof. The therapeutic agents may replace at least a portion of the antibody or fragment thereof. In some instances, a dual fusion protein comprises two

therapeutic agents attached to a heavy chain. In some instances, a dual fusion protein comprises two therapeutic agents attached to a light chain. In some instances, a dual fusion protein comprises one therapeutic agent attached to a heavy chain and another therapeutic agent attached to a light chain.

[0093] In some embodiments, the non-antibody region is an extender fusion region. In some instances, the extender fusion region comprises (a) a first extender peptide comprising at least one secondary structure, and (b) a therapeutic agent. The secondary structure may be an alpha helix. The secondary structure may be configured to form a coiled coil. The therapeutic agent may be a therapeutic peptide. In some embodiments, the non-antibody region comprises a linker. The linker may have no regular secondary structure. In some embodiments, the non-antibody region comprises a protease cleavage site.

[0094] In some embodiments, an immunoglobulin fusion protein comprising an extender peptide, wherein the extender peptide forms an alpha helix and may be configured to form a coiled coil, is referred to as a coiled coil immunoglobulin fusion protein. In some embodiments, an immunoglobulin fusion protein which does not comprise an extender peptide having secondary structure is referred to as a direct immunoglobulin fusion protein.

[0095] The extender peptide may be based on or derived from an ultralong CDR3. The extender peptide may comprise 7 or fewer amino acids from an ultralong CDR3 sequence. Alternatively, or additionally, the extender peptide does not comprise an amino acid sequence based on or derived from an ultralong CDR3. The extender peptide may comprise one or more secondary structures. The one or more secondary structures may be an alpha helix.

[0096] Exemplary immunoglobulin fusion proteins comprising two extender peptides comprising a coiled coil structure (e.g., each extender peptide has an alpha helix secondary structure) are depicted in FIG. 1. As shown in FIG. 1, an antibody region (110) comprising two immunoglobulin heavy chains (115, 120) and two immunoglobulin light chains (125, 130) is attached to a non-antibody region (135) comprising two extender peptides (140, 145) and a therapeutic agent (150) to produce immunoglobulin fusion proteins (160, 170, 180). As shown in FIG. 1, the immunoglobulin fusion protein (160) comprises a non-antibody region attached to one of the immunoglobulin heavy chains of the antibody region. As shown in FIG. 1, the immunoglobulin fusion protein (170) comprises a non-antibody region attached to one of the immunoglobulin light chains of the antibody region. Also shown in FIG. 1, the immunoglobulin fusion protein (180) comprises two non-antibody regions attached two immunoglobulin chains of the antibody region. The two extender peptides may form a coiled coil. The two extender peptides may form anti-parallel coiled coil.

[0097] Exemplary direct immunoglobulin fusion proteins in which the non-antibody region/extender fusion region (e.g., therapeutic agent) is directly inserted into the antibody without the aid of an extender peptide having secondary structure are depicted in FIG. 5. As shown in FIG. 5, an antibody region (1010) comprising two immunoglobulin heavy chains (1015, 1020) and two immunoglobulin light chains (1025, 1030) is attached to a non-antibody region (1050) to produce immunoglobulin fusion proteins (1060, 1070, 1080). As shown in FIG. 5, the immunoglobulin fusion protein (1060) comprises a non-antibody

region attached to one of the immunoglobulin heavy chains of the antibody region. As shown in FIG. 5, the immunoglobulin fusion protein (1070) comprises a non-antibody region attached to one of the immunoglobulin light chains of the antibody region. Also shown in FIG. 5, the immunoglobulin fusion protein (1080) comprises two non-antibody regions attached two immunoglobulin chains of the antibody region.

[0098] Additional exemplary coiled coil immunoglobulin fusion proteins are depicted in FIG. 3. Formula IA of FIG. 3 depicts an immunoglobulin fusion protein comprising an antibody region (A^1) attached to an extender fusion region comprising an extender peptide (E^1) attached to a therapeutic agent (T^1).

[0099] Formula IIA of FIG. 3 depicts an immunoglobulin fusion protein comprising an antibody region (A^1) attached to an extender fusion region comprising two extender peptides (E^1 and E^2) attached to a therapeutic agent (T^1).

[00100] Formula IIIA of FIG. 3 depicts an immunoglobulin dual fusion protein comprising two antibody regions (A^1 and A^2) attached to each other. The immunoglobulin dual fusion protein may comprise (a) a first antibody region (A^1) attached to a first extender fusion region comprising two extender peptides (E^1 and E^2) attached to a first therapeutic agent (T^1); and (b) a second antibody region (A^2) attached to a second extender fusion region comprising two extender peptides (E^3 and E^4) attached to a second therapeutic agent (T^2).

[00101] Formula IVA of FIG. 3 depicts an immunoglobulin fusion protein comprising an antibody region (A^1) attached to an extender fusion region comprising a linker (L^1) attached to a therapeutic agent (T^1), with the linker and therapeutic agent located between two extender peptides (E^1 and E^2).

[00102] Formula VA of FIG. 3 depicts an immunoglobulin fusion protein comprising an antibody region (A^1) attached to an extender fusion region comprising a proteolytic cleavage site (P^1) attached to a therapeutic agent (T^1), with the proteolytic cleavage site and therapeutic agent located between two extender peptides (E^1 and E^2). Formula VB of FIG. 3 depicts the clipped version of Formula VA, wherein the proteolytic cleavage site is cleaved by a protease, which results in release of one end of the therapeutic agent. An immunoglobulin fusion protein which may be cleaved to release the amino-terminus of a therapeutic agent is referred to as RN, for released N-terminus. For example, trastuzumab-coil hGH RN indicates that upon proteolytic cleavage, the N-terminus of hGH is released.

[00103] Formula VIA of FIG. 3 depicts an immunoglobulin fusion protein comprising an antibody region (A^1) attached to an extender fusion region comprising a therapeutic agent (T^1) attached to a linker (L^1) and a proteolytic cleavage site (P^1), which the therapeutic agent, linker and proteolytic cleavage site located between two extender peptides (E^1 and E^2). Formula VIB of FIG. 3 depicts the clipped version of Formula VIA, wherein the proteolytic cleavage site is cleaved by a protease, which results in release of one end of the therapeutic agent. An immunoglobulin fusion protein which may be cleaved to release the carboxyl-terminus of a therapeutic agent is referred to as RC, for released C-terminus. For example, trastuzumab-coil hGH RC indicates that upon proteolytic cleavage, the C-terminus of hGH is released.

[00104] Formula VIIA of FIG. 3 depicts an immunoglobulin dual fusion protein comprising two antibody regions (A^1 and A^2). The first antibody region (A^1) is attached to a first extender fusion region comprising a therapeutic agent (T^1) with two linkers (L^1 and L^2) on each end, with the therapeutic agent and linkers located between two extender peptides (E^1 and E^2). The second antibody region (A^2) is attached to a second extender fusion region comprising a therapeutic agent (T^2) attached to a proteolytic cleavage site (P^1). The therapeutic agent and proteolytic cleavage site in the second extender fusion region are flanked by two linkers (L^3 and L^4). The therapeutic agent, proteolytic cleavage site and the two linkers of the second extender region are flanked by two extender peptides (E^1 and E^2).

[00105] Formula VIIIA of FIG. 3 depicts an immunoglobulin fusion protein comprising an antibody region (A^1) attached to an extender fusion region comprising two extender peptides (E^1 and E^2), two linkers (L^1 and L^2), two proteolytic cleavage sites (P^1 and P^2) and a therapeutic agent (T^1). Formula VIIIB of FIG. 3 depicts the clipped version of Formula VIIIA, wherein the proteolytic cleavage sites located on the N- and C-termini of the therapeutic agent are cleaved by a protease, which results in release of the therapeutic agent from the immunoglobulin fusion protein.

[00106] Additional exemplary immunoglobulin fusion proteins without extender peptides (direct immunoglobulin fusion proteins) are depicted in FIG. 9. Formula IXA of FIG. 9 depicts an immunoglobulin fusion protein comprising an antibody region (A^1) attached to a non-antibody region comprising a therapeutic agent (T^1).

[00107] Formula XA of FIG. 9 depicts an immunoglobulin fusion protein comprising an antibody region (A^1) attached to a non-antibody region comprising a linker (L^1) attached to a therapeutic agent (T^1). Formula XIA of FIG. 9 depicts an immunoglobulin dual fusion protein comprising two antibody regions (A^1 and A^2) attached to each other. The immunoglobulin dual fusion protein may comprise (a) a first antibody region (A^1) attached to a first non-antibody region comprising a first therapeutic agent (T^1); and (b) a second antibody region (A^2) attached to a second non-antibody region comprising a second therapeutic agent (T^2).

[00108] Formula XIIA of FIG. 9 depicts an immunoglobulin fusion protein comprising an antibody region (A^1) attached to a non-antibody region comprising a linker (L^1), a proteolytic cleavage site (P^1) and a therapeutic agent (T^1), wherein the proteolytic cleavage site is located between the linker and the therapeutic agent. The proteolytic cleavage site in the second non-antibody region has been cleaved by a protease, resulting in release of one end of the second therapeutic agent.

[00109] Formula XIII A of FIG. 9 depicts an immunoglobulin fusion protein comprising an antibody region (A^1) attached to a non-antibody region comprising a proteolytic cleavage site (P^1) attached to a therapeutic agent (T^1). Formula XIII B of FIG. 9 depicts the clipped version of Formula XIII A, wherein the proteolytic cleavage site is cleaved by a protease, which results in release of one end of the therapeutic agent.

[00110] Formula XIVA of FIG. 9 depicts an immunoglobulin fusion protein comprising an antibody region (A^1) attached to a non-antibody region comprising a linker (L^1), a therapeutic agent (T^1), and a proteolytic cleavage site (P^1), wherein the therapeutic agent is located between the linker and the

proteolytic cleavage site. Formula XIVB of FIG. 9 depicts the clipped version of Formula XIVA, wherein the proteolytic cleavage site is cleaved by a protease, which results in release of one end of the therapeutic agent.

[00111] Formula XVA of FIG. 9 depicts an immunoglobulin dual fusion protein comprising two antibody regions (A^1 and A^2). The first antibody region (A^1) is attached to a first non-antibody region comprising a therapeutic agent (T^1) with two linkers (L^1 and L^2) on each end. The second antibody region (A^2) is attached to a second non-antibody region comprising a second therapeutic agent (T^2) attached to a proteolytic cleavage site (P^1). The therapeutic agent and proteolytic cleavage site in the second non-antibody region are flanked by two linkers (L^3 and L^4). The proteolytic cleavage site in the second non-antibody region has been cleaved by a protease, resulting in release of one end of the second therapeutic agent.

[00112] Formula XVIA of FIG. 9 depicts an immunoglobulin fusion protein comprising an antibody region (A^1) attached to a non-antibody region comprising two linkers (L^1 and L^2), two proteolytic cleavage sites (P^1 and P^2) and a therapeutic agent (T^1). Formula XVIB of FIG. 9 depicts the clipped version of Formula XVIA, wherein the proteolytic cleavage sites located on the N- and C-termini of the therapeutic agent are cleaved by a protease, which results in release of the therapeutic agent from the immunoglobulin fusion protein

[00113] Further disclosed herein are methods of treating a disease or condition in a subject in need thereof. The method may comprise administering to the subject an immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide and (b) a therapeutic agent, and wherein the extender peptide does not have secondary structure comprising a beta strand. The method may comprise administering to the subject an immunoglobulin fusion protein comprising an antibody region attached to non-immunoglobulin region, wherein the non-immunoglobulin region comprises (a) an extender peptide and (b) a therapeutic agent, and wherein the extender peptide does not have secondary structure comprising a beta strand. The method may comprise administering to the subject a direct immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises a therapeutic agent, and wherein the therapeutic agent is attached to the antibody region without using an extender peptide or linking peptide having secondary structure. The method may comprise administering to the subject an immunoglobulin fusion protein comprising an antibody region attached to non-immunoglobulin region, wherein the non-immunoglobulin region comprises a therapeutic agent, and wherein the therapeutic agent is attached to the antibody region without using an extender peptide or linking peptide having secondary structure. The method may comprise administering to the subject an immunoglobulin fusion protein comprising an antibody region attached to a therapeutic peptide via a connecting peptide.

[00114] Further disclosed herein are methods of extending the half-life of a therapeutic agent. The method may comprise attaching a therapeutic agent to an antibody region. The method may comprise attaching a therapeutic agent to an antibody region using one or more linker peptides having no regular

secondary structure. The method may comprise attaching a therapeutic agent to an antibody region using one or more protease cleavage sites. The method may comprise attaching a therapeutic agent to an extender fusion peptide. The method may comprise attaching a therapeutic agent to an antibody region using an extender fusion peptide. The method may comprise attaching a therapeutic agent to a connecting peptide. The method may comprise attaching a therapeutic agent to an antibody region using a connecting peptide.

[00115] Further disclosed herein are methods of extending the half-life of a therapeutic agent. The method may comprise attaching an antibody region to the therapeutic agent to produce an immunoglobulin fusion protein. The method may further comprise attaching one or more linkers or proteolytic cleavage sites to the immunoglobulin fusion protein. The one or more linkers may be attached to an N- and/or C-terminus of the therapeutic agent. The one or more proteolytic cleavage sites may be attached to an N- and/or C-terminus of the therapeutic agent. The one or more proteolytic cleavage sites may be inserted into the therapeutic agent.

[00116] Further disclosed herein are methods of improving the delivery of a therapeutic agent. The method may comprise attaching an extender peptide to a therapeutic agent. The method may further comprise attaching an antibody region to the extender peptide, therapeutic agent, or extender fusion peptide. The method may comprise attaching a therapeutic peptide directly to an antibody region. The method may comprise attaching a connecting peptide to a therapeutic agent. The method may further comprise attaching an antibody region to the connecting peptide and therapeutic agent.

[00117] Further disclosed herein are methods of improving the delivery of a therapeutic agent. The method may comprise attaching an antibody region to a therapeutic agent to produce an immunoglobulin fusion protein. The method may further comprise attaching one or more linkers or proteolytic cleavage sites to the immunoglobulin fusion protein. The one or more linkers may be attached to an N- and/or C-terminus of the therapeutic agent. The one or more proteolytic cleavage sites may be attached to an N- and/or C-terminus of the therapeutic agent. The one or more proteolytic cleavage sites may be inserted into the therapeutic agent.

[00118] Before the present methods and compositions are described, it is to be understood that this invention is not limited to a particular method or composition described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims. Examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

[00119] The terms "homologous," "homology," or "percent homology" when used herein to describe to an amino acid sequence or a nucleic acid sequence, relative to a reference sequence, can be determined using the formula described by Karlin and Altschul (Proc. Natl. Acad. Sci. USA 87: 2264-2268, 1990, modified as in Proc. Natl. Acad. Sci. USA 90:5873-5877, 1993). Such a formula is incorporated into the basic local alignment search tool (BLAST) programs of Altschul et al. (J. Mol. Biol. 215: 403-410, 1990). Percent homology of sequences can be determined using the most recent version of BLAST, as of the filing date of this application.

[00120] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[00121] 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 invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, some potential and preferred methods and materials are now described. All publications mentioned herein are referenced to disclose and describe the methods and/or materials in connection with which the publications are cited. It is understood that the present disclosure supersedes any disclosure of a referenced publication to the extent there is a contradiction.

[00122] As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present invention. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

[00123] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the peptide" includes reference to one or more peptides and equivalents thereof, e.g. polypeptides, known to those skilled in the art, and so forth.

[00124] As used herein, an amino acid sequence that is based on another amino acid sequence comprises one or more consecutive amino acid portions of the another amino acid sequence. Consecutive amino acid portions include any number of amino acids in the another amino acid sequence. Consecutive amino acids may be 1-10%, 1-20%, 10-20%, 10-30%, 20-30%, 20-40%, 30-40%, 40-50%, 50-60%, 50-

100%, 60-70%, 60-100%, 70-100%, 80-100%, 80-90%, 90-95%, 90-100%, or 1-100% identical to any consecutive amino acid region in the another amino acid sequence.

[00125] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

[00126] Immunoglobulin fusion proteins

[00127] The immunoglobulin fusion proteins disclosed herein may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. The immunoglobulin domain may be from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. The mammalian antibody may be a murine antibody. The mammalian antibody may be a non-human primate antibody.

[00128] The immunoglobulin fusion proteins disclosed herein may comprise a therapeutic agent, wherein the therapeutic agent is a functional peptide. The immunoglobulin fusion protein may comprise a functional peptide grafted into an antibody scaffold. The functional peptide may be a linear peptide. The functional peptide may be a modified cyclic peptide. The functional peptide may comprise a peptide modified to comprise a β -hairpin structure. The β -hairpin structure may be locked into a β -hairpin conformation by one or more bonds between two or more amino acid residues of the β -hairpin structure. The N terminus and/or the C terminus of the functional peptide may be grafted to the extender fusion region of the immunoglobulin fusion protein. The N terminus of the functional peptide may be grafted to a first extender peptide of the extender fusion region and the C terminus of the functional peptide may be grafted to a second extender peptide of the extender fusion region. The functional peptide may comprise a peptide modified to comprise a conformationally constrained peptide. A conformationally constrained peptide may have a greatly improved binding affinity and/or specificity to a target relative to an endogenous or naturally-occurring binding partner of the target. An endogenous or naturally-occurring binding partner of the target may be a ligand or substrate of the target. By non-limiting example, the conformationally constrained peptide may be a peptide comprising a β -hairpin structure. The conformationally constrained peptide may comprise a region that binds to a binding site of a target. The target may be a receptor. The target may be an enzyme. The binding site of the target may be a deep pocket of a ligand binding domain or substrate binding domain. The functional peptide or portion thereof may bind the deep pocket of a ligand binding domain or substrate binding domain such that it blocks a target ligand and/or substrate from binding. The functional peptide or portion thereof may bind the deep

pocket of a ligand binding domain or substrate binding domain such that it partially blocks the target ligand and/or substrate from binding. The functional peptide or portion thereof may bind the deep pocket of a ligand binding domain or substrate binding domain such that it completely blocks the target ligand or substrate from binding. The functional peptide or portion thereof may bind the surface of the ligand binding domain or substrate binding domain. The functional peptide may be an agonist. The functional peptide may be an antagonist. The functional peptide may be an inhibitor. The functional peptide may be a ligand. The functional peptide may be a substrate.

[00129] The immunoglobulin fusion protein may comprise an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 68-99, and 122-143. The immunoglobulin fusion protein may comprise an amino acid sequence that is at least about 50% homologous to any one of SEQ ID NOs: 68-99, and 122-143. The immunoglobulin fusion protein may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% homologous to any one of SEQ ID NOs: 68-99, and 122-143. The immunoglobulin fusion protein may comprise an amino acid sequence that is at least about 70% homologous to any one of SEQ ID NOs: 68-99, and 122-143. The immunoglobulin fusion protein may comprise an amino acid sequence that is at least about 80% homologous to any one of SEQ ID NOs: 68-99, and 122-143.

[00130] The immunoglobulin fusion protein may comprise an amino acid sequence comprising 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids based on or derived from any one of SEQ ID NOs: 68-99, and 122-143. The immunoglobulin fusion protein may comprise an amino acid sequence comprising 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 450, 500 or more amino acids based on or derived from any one of SEQ ID NOs: 68-99, and 122-143. The immunoglobulin fusion protein may comprise an amino acid sequence comprising 10 or more amino acids based on or derived from any one of SEQ ID NOs: 68-99, and 122-143. The immunoglobulin fusion protein may comprise an amino acid sequence comprising 50 or more amino acids based on or derived from any one of SEQ ID NOs: 68-99, and 122-143. The immunoglobulin fusion protein may comprise an amino acid sequence comprising 100 or more amino acids based on or derived from any one of SEQ ID NOs: 68-99, and 122-143. The immunoglobulin fusion protein may comprise an amino acid sequence comprising 200 or more amino acids based on or derived from any one of SEQ ID NOs: 68-99, and 122-143. The amino acids may be consecutive. Alternatively, or additionally, the amino acids are nonconsecutive.

[00131] The immunoglobulin fusion protein may be encoded by a nucleotide sequence that is based on or derived from any one of SEQ ID NOs: 37-67, and 100-121. The immunoglobulin fusion protein may be encoded by a nucleotide sequence that is at least about 50% homologous to any one of SEQ ID NOs: 37-67, and 100-121. The immunoglobulin fusion protein may be encoded by a nucleotide sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% homologous to any one of SEQ ID NOs: 37-67, and 100-121. The immunoglobulin fusion protein may be encoded by a nucleotide sequence that is at least about 70% homologous to any one of SEQ ID NOs: 37-67, and 100-121. The immunoglobulin fusion protein may be encoded by a nucleotide sequence that is at least about 80% homologous to any one of SEQ ID NOs: 37-67, and 100-121.

[00132] The immunoglobulin fusion protein may be encoded by a nucleotide sequence comprising 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more nucleotides based on or derived from any one of SEQ ID NOs: 37-67, and 100-121. The immunoglobulin fusion protein may be encoded by a nucleotide sequence comprising 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 450, 500 or more nucleotides based on or derived from any one of SEQ ID NOs: 37-67, and 100-121. The immunoglobulin fusion protein may be encoded by a nucleotide sequence comprising 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more nucleotides based on or derived from any one of SEQ ID NOs: 37-67, and 100-121. The immunoglobulin fusion protein may be encoded by a nucleotide sequence comprising 1100, 1200, 1300, 1400, 1500 or more nucleotides based on or derived from any one of SEQ ID NOs: 37-67, and 100-121. The immunoglobulin fusion protein may be encoded by a nucleotide sequence comprising 100 or more nucleotides based on or derived from any one of SEQ ID NOs: 37-67, and 100-121. The immunoglobulin fusion protein may be encoded by a nucleotide sequence comprising 500 or more nucleotides based on or derived from any one of SEQ ID NOs: 37-67, and 100-121. The immunoglobulin fusion protein may be encoded by a nucleotide sequence comprising 1000 or more nucleotides based on or derived from any one of SEQ ID NOs: 37-67, and 100-121. The immunoglobulin fusion protein may be encoded by a nucleotide sequence comprising 1300 or more nucleotides based on or derived from any one of SEQ ID NOs: 37-67, and 100-121. The nucleotides may be consecutive. Alternatively, or additionally, the nucleotides are nonconsecutive.

[00133] The immunoglobulin fusion protein may further comprise one or more immunoglobulin light chains. The immunoglobulin fusion protein may comprise at least two immunoglobulin light chains. The immunoglobulin light chain may comprise one or more portions of an immunoglobulin light chain. The immunoglobulin light chain may be an immunoglobulin fusion light chain. The immunoglobulin fusion light chain comprises an antibody region derived from an immunoglobulin light chain and a therapeutic agent. The therapeutic agent may be attached to the antibody region by one or more connecting peptides. The immunoglobulin light chain may comprise an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 19-21, 28, 36, 68, 80, 94, 98, and 122. The immunoglobulin light chain may comprise an amino acid sequence that is at least about 50% homologous to any one of SEQ ID NOs: 19-21, 28, 36, 68, 80, 94, 98, and 122. The immunoglobulin light chain may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% homologous to any one of SEQ ID NOs: 19-21, 28, 36, 68, 80, 94, 98, and 122. The immunoglobulin light chain may comprise an amino acid sequence that is at least about 70% homologous to any one of SEQ ID NOs: 19-21, 28, 36, 68, 80, 94, 98, and 122. The immunoglobulin light chain may comprise an amino acid sequence that is at least about 80% homologous to any one of SEQ ID NOs: 19-21, 28, 36, 68, 80, 94, 98, and 122.

[00134] The immunoglobulin light chain may comprise an amino acid sequence comprising 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids based on or derived from any one of SEQ ID NOs: 19-21, 28, 36, 68, 80, 94, 98, and 122. The immunoglobulin light chain may comprise an amino acid sequence comprising 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 450, 500 or more

amino acids based on or derived from any one of SEQ ID NOs: 19-21, 28, 36, 68, 80, 94, 98, and 122. The immunoglobulin light chain may comprise an amino acid sequence comprising 10 or more amino acids based on or derived from any one of SEQ ID NOs: 19-21, 28, 36, 68, 80, 94, 98, and 122. The immunoglobulin light chain may comprise an amino acid sequence comprising 50 or more amino acids based on or derived from any one of SEQ ID NOs: 19-21, 28, 36, 68, 80, 94, 98, and 122. The immunoglobulin light chain may comprise an amino acid sequence comprising 100 or more amino acids based on or derived from any one of SEQ ID NOs: 19-21, 28, 36, 68, 80, 94, 98, and 122. The immunoglobulin light chain may comprise an amino acid sequence comprising 200 or more amino acids based on or derived from any one of SEQ ID NOs: 19-21, 28, 36, 68, 80, 94, 98, and 122. The amino acids may be consecutive. Alternatively, or additionally, the amino acids are nonconsecutive.

[00135] The immunoglobulin light chain may be encoded by a nucleotide sequence that is based on or derived from any one of SEQ ID NOs: 1-3, 10, 18, 37, 49, 63, 67, and 100. The immunoglobulin light chain may be encoded by a nucleotide sequence that is at least about 50% homologous to any one of SEQ ID NOs: 1-3, 10, 18, 37, 49, 63, 67, and 100. The immunoglobulin light chain may be encoded by a nucleotide sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% homologous to any one of SEQ ID NOs: 1-3, 10, 18, 37, 49, 63, 67, and 100. The immunoglobulin light chain may be encoded by a nucleotide sequence that is at least about 70% homologous to any one of SEQ ID NOs: 1-3, 10, 18, 37, 49, 63, 67, and 100. The immunoglobulin light chain may be encoded by a nucleotide sequence that is at least about 80% homologous to any one of SEQ ID NOs: 1-3, 10, 18, 37, 49, 63, 67, and 100.

[00136] The immunoglobulin light chain may be encoded by a nucleotide sequence comprising 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-3, 10, 18, 37, 49, 63, 67, and 100. The immunoglobulin light chain may be encoded by a nucleotide sequence comprising 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-3, 10, 18, 37, 49, 63, 67, and 100. The immunoglobulin light chain may be encoded by a nucleotide sequence comprising 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-3, 10, 18, 37, 49, 63, 67, and 100. The immunoglobulin light chain may be encoded by a nucleotide sequence comprising 1100, 1200, 1300, 1400, 1500 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-3, 10, 18, 37, 49, 63, 67, and 100. The immunoglobulin light chain may be encoded by a nucleotide sequence comprising 100 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-3, 10, 18, 37, 49, 63, 67, and 100. The immunoglobulin light chain may be encoded by a nucleotide sequence comprising 500 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-3, 10, 18, 37, 49, 63, 67, and 100. The immunoglobulin light chain may be encoded by a nucleotide sequence comprising 1000 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-3, 10, 18, 37, 49, 63, 67, and 100. The immunoglobulin light chain may be encoded by a nucleotide sequence comprising 1300 or more nucleotides based on or derived from any one of SEQ ID

NOs: 1-3, 10, 18, 37, 49, 63, 67, and 100. The nucleotides may be consecutive. Alternatively, or additionally, the nucleotides are nonconsecutive.

[00137] The immunoglobulin fusion protein may further comprise one or more immunoglobulin heavy chains. The immunoglobulin fusion protein may comprise at least two immunoglobulin heavy chains. The immunoglobulin heavy chain may comprise one or more portions of an immunoglobulin heavy chain. The immunoglobulin heavy chain may be an immunoglobulin fusion heavy chain. The immunoglobulin fusion heavy chain comprises an antibody region derived from an immunoglobulin heavy chain and a therapeutic agent. The therapeutic agent may be attached to the antibody region by one or more connecting peptides. The immunoglobulin heavy chain may comprise an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 22-27, 29-35, 69-79, 81-93, 95-97, 99, and 123-143. The immunoglobulin heavy chain may comprise an amino acid sequence that is at least about 50% homologous to any one of SEQ ID NOs: 22-27, 29-35, 69-79, 81-93, 95-97, 99, and 123-143. The immunoglobulin heavy chain may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% homologous to any one of SEQ ID NOs: 22-27, 29-35, 69-79, 81-93, 95-97, 99, and 123-143. The immunoglobulin heavy chain may comprise an amino acid sequence that is at least about 70% homologous to any one of SEQ ID NOs: 22-27, 29-35, 69-79, 81-93, 95-97, 99, and 123-143. The immunoglobulin heavy chain may comprise an amino acid sequence that is at least about 80% homologous to any one of SEQ ID NOs: 22-27, 29-35, 69-79, 81-93, 95-97, 99, and 123-143.

[00138] The immunoglobulin heavy chain may comprise an amino acid sequence comprising 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids based on or derived from any one of SEQ ID NOs: 22-27, 29-35, 69-79, 81-93, 95-97, 99, and 123-143. The immunoglobulin heavy chain may comprise an amino acid sequence comprising 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 450, 500 or more amino acids based on or derived from any one of SEQ ID NOs: 22-27, 29-35, 69-79, 81-93, 95-97, 99, and 123-143. The immunoglobulin heavy chain may comprise an amino acid sequence comprising 10 or more amino acids based on or derived from any one of SEQ ID NOs: 22-27, 29-35, 69-79, 81-93, 95-97, 99, and 123-143. The immunoglobulin heavy chain may comprise an amino acid sequence comprising 50 or more amino acids based on or derived from any one of SEQ ID NOs: 22-27, 29-35, 69-79, 81-93, 95-97, 99, and 123-143. The immunoglobulin heavy chain may comprise an amino acid sequence comprising 100 or more amino acids based on or derived from any one of SEQ ID NOs: 22-27, 29-35, 69-79, 81-93, 95-97, 99, and 123-143. The immunoglobulin heavy chain may comprise an amino acid sequence comprising 200 or more amino acids based on or derived from any one of SEQ ID NOs: 22-27, 29-35, 69-79, 81-93, 95-97, 99, and 123-143. The amino acids may be consecutive. Alternatively, or additionally, the amino acids are nonconsecutive.

[00139] The immunoglobulin heavy chain may be encoded by a nucleotide sequence that is based on or derived from any one of SEQ ID NOs: 4-9, 11-17, 38-48, 50-62, 64-66, and 101-121. The immunoglobulin heavy chain may be encoded by a nucleotide sequence that is at least about 50% homologous to any one of SEQ ID NOs: 4-9, 11-17, 38-48, 50-62, 64-66, and 101-121. The immunoglobulin heavy chain may be encoded by a nucleotide sequence that is at least about 60%, 65%,

70%, 75%, 80%, 85%, 90%, 95%, or 97% homologous to any one of SEQ ID NOs: 4-9, 11-17, 38-48, 50-62, 64-66, and 101-121. The immunoglobulin heavy chain may be encoded by a nucleotide sequence that is at least about 70% homologous to any one of SEQ ID NOs: 4-9, 11-17, 38-48, 50-62, 64-66, and 101-121. The immunoglobulin heavy chain may be encoded by a nucleotide sequence that is at least about 80% homologous to any one of SEQ ID NOs: 4-9, 11-17, 38-48, 50-62, 64-66, and 101-121.

[00140] The immunoglobulin heavy chain may be encoded by a nucleotide sequence comprising 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more nucleotides based on or derived from any one of SEQ ID NOs: 4-9, 11-17, 38-48, 50-62, 64-66, and 101-121. The immunoglobulin heavy chain may be encoded by a nucleotide sequence comprising 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 450, 500 or more nucleotides based on or derived from any one of SEQ ID NOs: 4-9, 11-17, 38-48, 50-62, 64-66, and 101-121. The immunoglobulin heavy chain may be encoded by a nucleotide sequence comprising 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more nucleotides based on or derived from any one of SEQ ID NOs: 4-9, 11-17, 38-48, 50-62, 64-66, and 101-121. The immunoglobulin heavy chain may be encoded by a nucleotide sequence comprising 1100, 1200, 1300, 1400, 1500 or more nucleotides based on or derived from any one of SEQ ID NOs: 4-9, 11-17, 38-48, 50-62, 64-66, and 101-121. The immunoglobulin heavy chain may be encoded by a nucleotide sequence comprising 100 or more nucleotides based on or derived from any one of SEQ ID NOs: 4-9, 11-17, 38-48, 50-62, 64-66, and 101-121. The immunoglobulin heavy chain may be encoded by a nucleotide sequence comprising 500 or more nucleotides based on or derived from any one of SEQ ID NOs: 4-9, 11-17, 38-48, 50-62, 64-66, and 101-121. The immunoglobulin heavy chain may be encoded by a nucleotide sequence comprising 1000 or more nucleotides based on or derived from any one of SEQ ID NOs: 4-9, 11-17, 38-48, 50-62, 64-66, and 101-121. The immunoglobulin heavy chain may be encoded by a nucleotide sequence comprising 1300 or more nucleotides based on or derived from any one of SEQ ID NOs: 4-9, 11-17, 38-48, 50-62, 64-66, and 101-121. The nucleotides may be consecutive. Alternatively, or additionally, the nucleotides are nonconsecutive.

[00141] The immunoglobulin fusion protein may comprise (a) a first immunoglobulin fusion heavy chain comprising an amino acid sequence that is based on or derived from SEQ ID NOs: 69-79, 81-93, 95-97, 99, and 123-143; and (b) a first immunoglobulin light chain comprising an amino acid sequence that is based on or derived from SEQ ID NOs: 19-21, 28, and 36. The immunoglobulin fusion protein may comprise (a) a first immunoglobulin fusion heavy chain comprising an amino acid sequence that is at least about 50% identical to SEQ ID NOs: 69-79, 81-93, 95-97, 99, and 123-143; and (b) a first immunoglobulin light chain comprising an amino acid sequence that is at least about 50% identical to SEQ ID NOs: 19-21, 28, and 36. The first immunoglobulin fusion heavy chain may comprise an amino acid sequence that is at least about 60%, 70%, 75%, 80%, 90%, 95%, or 97% identical to SEQ ID NOs: 69-79, 81-93, 95-97, 99, and 123-143. The first immunoglobulin light chain comprising an amino acid sequence that is at least about 60%, 70%, 75%, 80%, 90%, 95%, or 97% identical to SEQ ID NOs: 19-21, 28, and 36.

[00142] The immunoglobulin fusion protein may comprise (a) a first immunoglobulin fusion heavy chain encoded by a nucleotide sequence of SEQ ID NOs: 38-48, 50-62, 64-66, and 101-121; and (b) a first immunoglobulin light chain encoded by a nucleotide sequence of SEQ ID NOs: 1-3, 10 and 18. The immunoglobulin fusion protein may comprise (a) a first immunoglobulin fusion heavy chain encoded by a nucleotide sequence that is at least 50% or more homologous to a nucleotide sequence of SEQ ID NOs: 38-48, 50-62, 64-66, and 101-121; and (b) a first immunoglobulin light chain encoded by a nucleotide sequence that is at least 50% or more homologous to a nucleotide sequence of SEQ ID NOs: 1-3, 10 and 18. The first immunoglobulin fusion heavy chain encoded by a nucleotide sequence that is at least 60%, 70%, 75%, 80%, 90%, 95%, or 97% or more homologous to a nucleotide sequence of SEQ ID NOs: 38-48, 50-62, 64-66, and 101-121. The first immunoglobulin light chain encoded by a nucleotide sequence that is at least 60%, 70%, 75%, 80%, 90%, 95%, or 97% or more homologous to a nucleotide sequence of SEQ ID NOs: 1-3, 10 and 18.

[00143] The immunoglobulin fusion protein may comprise (a) a first immunoglobulin heavy chain comprising an amino acid sequence that is based on or derived from SEQ ID NOs: 22-27, and 29-35; and (b) a first immunoglobulin fusion light chain comprising an amino acid sequence that is based on or derived from SEQ ID NOs: 68, 80, 94, 98, and 122. The immunoglobulin fusion protein may comprise (a) a first immunoglobulin heavy chain comprising an amino acid sequence that is at least about 50% identical to SEQ ID NOs: 22-27, and 29-35; and (b) a first immunoglobulin fusion light chain comprising an amino acid sequence that is at least about 50% identical to SEQ ID NOs: 68, 80, 94, 98, and 122. The first immunoglobulin heavy chain may comprise an amino acid sequence that is at least about 60%, 70%, 75%, 80%, 90%, 95%, or 97% identical to SEQ ID NOs: 22-27, and 29-35. The first immunoglobulin fusion light chain comprising an amino acid sequence that is at least about 60%, 70%, 75%, 80%, 90%, 95%, or 97% identical to SEQ ID NOs: 68, 80, 94, 98, and 122.

[00144] The immunoglobulin fusion protein may comprise (a) a first immunoglobulin heavy chain encoded by a nucleotide sequence of SEQ ID NOs: 4-9 and 11-17; and (b) a first immunoglobulin fusion light chain encoded by a nucleotide sequence of SEQ ID NOs: 37, 49, 63, 67, and 100. The immunoglobulin fusion protein may comprise (a) a first immunoglobulin heavy chain encoded by a nucleotide sequence that is at least 50% or more homologous to a nucleotide sequence of SEQ ID NOs: 4-9 and 11-17; and (b) a first immunoglobulin fusion light chain encoded by a nucleotide sequence that is at least 50% or more homologous to a nucleotide sequence of SEQ ID NOs: 37, 49, 63, 67, and 100. The first immunoglobulin heavy chain encoded by a nucleotide sequence that is at least 60%, 70%, 75%, 80%, 90%, 95%, or 97% or more homologous to a nucleotide sequence of SEQ ID NOs: 4-9 and 11-17. The first immunoglobulin fusion light chain encoded by a nucleotide sequence that is at least 60%, 70%, 75%, 80%, 90%, 95%, or 97% or more homologous to a nucleotide sequence of SEQ ID NOs: 37, 49, 63, 67, and 100.

[00145] The immunoglobulin fusion protein may comprise (a) a first immunoglobulin fusion heavy chain comprising an amino acid sequence that is based on or derived from SEQ ID NOs: 69-79, 81-93, 95-97, 99, and 123-143; and (b) a first immunoglobulin fusion light chain comprising an amino acid

sequence that is based on or derived from SEQ ID NOs: 68, 80, 94, 98, and 122. The immunoglobulin fusion protein may comprise (a) a first immunoglobulin fusion heavy chain comprising an amino acid sequence that is at least about 50% identical to SEQ ID NOs: 69-79, 81-93, 95-97, 99, and 123-143; and (b) a first immunoglobulin fusion light chain comprising an amino acid sequence that is at least about 50% identical to SEQ ID NOs: 68, 80, 94, 98, and 122. The first immunoglobulin fusion heavy chain may comprise an amino acid sequence that is at least about 60%, 70%, 75%, 80%, 90%, 95%, or 97% identical to SEQ ID NOs: 69-79, 81-93, 95-97, 99, and 123-143. The first immunoglobulin fusion light chain comprising an amino acid sequence that is at least about 60%, 70%, 75%, 80%, 90%, 95%, or 97% identical to SEQ ID NOs: 68, 80, 94, 98, and 122.

[00146] The immunoglobulin fusion protein may comprise (a) a first immunoglobulin fusion heavy chain encoded by a nucleotide sequence of SEQ ID NOs: 38-48, 50-62, 64-66, and 101-121; and (b) a first immunoglobulin fusion light chain encoded by a nucleotide sequence of SEQ ID NOs: 37, 49, 63, 67, and 100. The immunoglobulin fusion protein may comprise (a) a first immunoglobulin fusion heavy chain encoded by a nucleotide sequence that is at least 50% or more homologous to a nucleotide sequence of SEQ ID NOs: 38-48, 50-62, 64-66, and 101-121; and (b) a first immunoglobulin fusion light chain encoded by a nucleotide sequence that is at least 50% or more homologous to a nucleotide sequence of SEQ ID NOs: 37, 49, 63, 67, and 100. The first immunoglobulin fusion heavy chain encoded by a nucleotide sequence that is at least 60%, 70%, 75%, 80%, 90%, 95%, or 97% or more homologous to a nucleotide sequence of SEQ ID NOs: 38-48, 50-62, 64-66, and 101-121. The first immunoglobulin fusion light chain encoded by a nucleotide sequence that is at least 60%, 70%, 75%, 80%, 90%, 95%, or 97% or more homologous to a nucleotide sequence of SEQ ID NOs: 37, 49, 63, 67, and 100.

[00147] Further disclosed herein are immunoglobulin dual fusion proteins comprising (a) an antibody region attached to a non-antibody region, wherein the non-antibody region comprises (i) a first extender peptide, wherein the first extender peptide comprises an amino acid sequence comprising an alpha helix secondary structure and wherein the first extender peptide does not comprise an ultralong CDR3, and (ii) a first therapeutic agent; and (b) a second therapeutic agent. Attachment of the antibody region to the non-antibody region may comprise insertion of the non-antibody region into the antibody region. The first therapeutic agent and the second therapeutic agent may be the same. The first therapeutic agent and the second therapeutic agent may be different. The dual fusion protein may further comprise a second antibody region. The first and second therapeutic agent may be attached to a first antibody region. The first and second therapeutic agent may be each attached to a first antibody region and a second antibody region. The first and second antibody regions may be connected. The first and second antibody regions may be connected by one or more disulfide bonds. The first and second antibody regions may be part of one immunoglobulin light or heavy chain. The immunoglobulin dual fusion protein may further comprise one or more additional extender peptides. The immunoglobulin dual fusion protein may further comprise one or more linker peptides. The immunoglobulin dual fusion protein may further comprise one or more protease cleavage sites.

[00148] Alternatively, the immunoglobulin dual fusion protein comprises (a) an antibody region attached to a non-antibody region, wherein the non-antibody region comprises (i) a first extender peptide, wherein the first extender peptide comprises an amino acid sequence comprising an alpha helix secondary structure and wherein the extender peptide comprises 7 or fewer amino acids based on or derived from an ultralong CDR3; and (ii) a first therapeutic agent; and (b) a second therapeutic agent. Attachment of the antibody region to the non-antibody region may comprise insertion of the non-antibody region into the antibody region. The first therapeutic agent and the second therapeutic agent may be the same. The first therapeutic agent and the second therapeutic agent may be different. The dual fusion protein may further comprise a second antibody region. The first and second therapeutic agent may be attached to a first antibody region. The first and second therapeutic agent may be each attached to a first antibody region and a second antibody region. The first and second antibody regions may be connected. The first and second antibody regions may be connected by one or more disulfide bonds. The first and second antibody regions may be part of one immunoglobulin light or heavy chain. The immunoglobulin dual fusion protein may further comprise one or more additional extender peptides. The immunoglobulin dual fusion protein may further comprise one or more linker peptides. The immunoglobulin dual fusion protein may further comprise one or more protease cleavage sites.

[00149] Alternatively, the immunoglobulin dual fusion protein comprises (a) an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (i) a first extender peptide, wherein the first extender peptide comprises an amino acid sequence comprising an alpha helix secondary structure and wherein the first extender peptide does not comprise an ultralong CDR3, and (ii) a first therapeutic agent; and (b) a second therapeutic agent. Attachment of the antibody region to the extender fusion region may comprise insertion of the extender fusion region into the antibody region. The first therapeutic agent and the second therapeutic agent may be the same. The first therapeutic agent and the second therapeutic agent may be different. The dual fusion protein may further comprise a second antibody region. The first and second therapeutic agent may be attached to a first antibody region. The first and second therapeutic agent may be each attached to a first antibody region and a second antibody region. The first and second antibody regions may be connected. The first and second antibody regions may be connected by one or more disulfide bonds. The first and second antibody regions may be part of one immunoglobulin light or heavy chain. The immunoglobulin dual fusion protein may further comprise one or more additional extender peptides. The immunoglobulin dual fusion protein may further comprise one or more linker peptides. The immunoglobulin dual fusion protein may further comprise one or more protease cleavage sites.

[00150] Alternatively, the immunoglobulin dual fusion protein comprises (a) an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (i) a first extender peptide, wherein the first extender peptide comprises an amino acid sequence comprising an alpha helix secondary structure and wherein the extender peptide comprises 7 or fewer amino acids based on or derived from an ultralong CDR3; and (ii) a first therapeutic agent; and (b) a second therapeutic agent. Attachment of the antibody region to the extender fusion region may comprise insertion of the extender

fusion region into the antibody region. The first therapeutic agent and the second therapeutic agent may be the same. The first therapeutic agent and the second therapeutic agent may be different. The dual fusion protein may further comprise a second antibody region. The first and second therapeutic agent may be attached to a first antibody region. The first and second therapeutic agent may be each attached to a first antibody region and a second antibody region. The first and second antibody regions may be connected. The first and second antibody regions may be connected by one or more disulfide bonds. The first and second antibody regions may be part of one immunoglobulin light or heavy chain. The immunoglobulin dual fusion protein may further comprise one or more additional extender peptides. The immunoglobulin dual fusion protein may further comprise one or more linker peptides. The immunoglobulin dual fusion protein may further comprise one or more protease cleavage sites.

[00151] Alternatively, the immunoglobulin dual fusion protein comprises (a) an antibody region attached to a non-antibody region, wherein the non-antibody region comprises (i) a first linking peptide, wherein the first linking peptide does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure, and (ii) a first therapeutic agent; and (b) a second therapeutic agent. Attachment of the antibody region to the non-antibody region may comprise insertion of the non-antibody region into the antibody region. The first therapeutic agent and the second therapeutic agent may be the same. The first therapeutic agent and the second therapeutic agent may be different. The dual fusion protein may further comprise a second antibody region. The first and second therapeutic agent may be attached to a first antibody region. The first and second therapeutic agent may be each attached to a first antibody region and a second antibody region. The first and second antibody regions may be connected. The first and second antibody regions may be connected by one or more disulfide bonds. The first and second antibody regions may be part of one immunoglobulin light or heavy chain. The immunoglobulin dual fusion protein may further comprise one or more additional linker peptides. The immunoglobulin dual fusion protein may further comprise one or more protease cleavage sites.

[00152] Alternatively, the immunoglobulin dual fusion protein comprises (a) an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (i) a first linking peptide, wherein the first linking peptide does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure; and (ii) a first therapeutic agent; and (b) a second therapeutic agent. Attachment of the antibody region to the extender fusion region may comprise insertion of the extender fusion region into the antibody region. The first therapeutic agent and the second therapeutic agent may be the same. The first therapeutic agent and the second therapeutic agent may be different. The dual fusion protein may further comprise a second antibody region. The first and second therapeutic agent may be attached to a first antibody region. The first and second therapeutic agent may be each attached to a first antibody region and a second antibody region. The first and second antibody regions may be connected. The first and second antibody regions may be connected by one or more disulfide bonds. The first and second antibody regions may be part of one immunoglobulin light or heavy chain. The immunoglobulin dual fusion protein may further comprise one or more additional linker peptides. The immunoglobulin dual fusion protein may further comprise one or more protease cleavage sites.

[00153] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first non-antibody region, wherein the first non-antibody region comprises (i) a first extender peptide, wherein the first extender peptide comprises an amino acid sequence comprising an alpha helix secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to a second non-antibody region, wherein the second non-antibody region comprises (i) a second extender peptide comprising at least one secondary structure and (ii) a second therapeutic agent. In some embodiments, the first extender peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the second extender peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the first extender peptide does not comprise amino acids having a beta strand secondary structure. In some embodiments, the second extender peptide does not comprise amino acids having a beta strand secondary structure. The dual fusion antibody may further comprise one or more peptide linkers. The dual fusion antibody may further comprise one or more protease cleavage sites.

[00154] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first non-antibody region, wherein the first non-antibody region comprises (i) a first extender peptide, wherein the first extender peptide comprises an amino acid sequence comprising an alpha helix secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to an extender fusion region, wherein the extender fusion region comprises (i) a second extender peptide comprising at least one secondary structure and (ii) a second therapeutic agent. In some embodiments, the first extender peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the second extender peptide is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more peptide linkers. The dual fusion antibody may further comprise one or more protease cleavage sites. In some embodiments, the first extender peptide does not comprise amino acids having a beta strand secondary structure. In some embodiments, the second extender peptide does not comprise amino acids having a beta strand secondary structure.

[00155] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first extender fusion region, wherein the first extender fusion region comprises (i) a first extender peptide, wherein the first extender peptide comprises an amino acid sequence comprising an alpha helix secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to a non-antibody region, wherein the non-antibody region comprises (i) a second extender peptide comprising at least one secondary structure and (ii) a second therapeutic agent. In some embodiments, the first extender peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the second extender peptide is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more peptide linkers. The dual fusion antibody may further comprise one or more protease cleavage sites. In some embodiments, the first extender peptide does not comprise amino acids

having a beta strand secondary structure. In some embodiments, the second extender peptide does not comprise amino acids having a beta strand secondary structure.

[00156] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first extender fusion region, wherein the first extender fusion region comprises (i) a first extender peptide, wherein the first extender peptide comprises an amino acid sequence comprising an alpha helix secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to a second extender fusion region, wherein the extender fusion region comprises (i) a second extender peptide comprising at least one secondary structure and (ii) a second therapeutic agent. In some embodiments, the first extender peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the second extender peptide is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more peptide linkers. The dual fusion antibody may further comprise one or more protease cleavage sites. In some embodiments, the first extender peptide does not comprise amino acids having a beta strand secondary structure. In some embodiments, the second extender peptide does not comprise amino acids having a beta strand secondary structure.

[00157] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first non-antibody region, wherein the first non-antibody region comprises (i) a first peptide linker, wherein the first peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to a second non-antibody region, wherein the second non-antibody region comprises (i) a second peptide linker, wherein the second peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a second therapeutic agent. In some embodiments, the first linker peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the second linker peptide is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more protease cleavage sites.

[00158] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first non-antibody region, wherein the first non-antibody region comprises (i) a first peptide linker, wherein the first peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to an extender fusion region, wherein the extender fusion region comprises (i) a second peptide linker, wherein the second peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a second therapeutic agent. In some embodiments, the first linker peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the second linker peptide is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more protease cleavage sites.

[00159] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first extender fusion region, wherein the first extender fusion region comprises (i) a first peptide linker, wherein the first peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to a non-antibody region, wherein the non-antibody region comprises (i) a second peptide linker, wherein the second peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a second therapeutic agent. In some embodiments, the first linker peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the second linker peptide is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more protease cleavage sites.

[00160] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first extender fusion region, wherein the first extender fusion region comprises (i) a first peptide linker, wherein the first peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to a second extender fusion region, wherein the extender fusion region comprises (i) a second peptide linker, wherein the second peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a second therapeutic agent. In some embodiments, the first linker peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the second linker peptide is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more protease cleavage sites.

[00161] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first non-antibody region, wherein the first non-antibody region comprises (i) a first peptide linker, wherein the first peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to a second non-antibody region, wherein the second non-antibody region comprises (i) an extender peptide comprising at least one secondary structure and (ii) a second therapeutic agent. In some embodiments, the extender peptide comprises amino acids having an alpha helix secondary structure. In some embodiments, the extender peptide does not comprise amino acids having a beta strand secondary structure. In some embodiments, the extender peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the peptide linker is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more protease cleavage sites. The dual fusion antibody may further comprise one or more additional linkers. The second non-antibody region of the second immunoglobulin fusion protein may further comprise one or more additional extender peptides.

[00162] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first non-antibody region, wherein the first non-antibody region comprises (i) a first peptide linker, wherein the first peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to an extender fusion region, wherein the extender fusion region comprises (i) an extender peptide comprising at least one secondary structure and (ii) a second therapeutic agent. In some embodiments, the extender peptide comprises amino acids having an alpha helix secondary structure. In some embodiments, the extender peptide does not comprise amino acids having a beta strand secondary structure. In some embodiments, the extender peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the peptide linker is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more protease cleavage sites. The dual fusion antibody may further comprise one or more additional linkers. The extender fusion region of the second immunoglobulin fusion protein may further comprise one or more additional extender peptides.

[00163] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first extender fusion region, wherein the first extender fusion region comprises (i) a first peptide linker, wherein the first peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to a non-antibody region, wherein the non-antibody region comprises (i) an extender peptide comprising at least one secondary structure and (ii) a second therapeutic agent. In some embodiments, the extender peptide comprises amino acids having an alpha helix secondary structure. In some embodiments, the extender peptide does not comprise amino acids having a beta strand secondary structure. In some embodiments, the extender peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the peptide linker is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more protease cleavage sites. The dual fusion antibody may further comprise one or more additional linkers. The non-antibody region of the second immunoglobulin fusion protein may further comprise one or more additional extender peptides.

[00164] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first extender fusion region, wherein the first extender fusion region comprises (i) a first peptide linker, wherein the first peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to a second extender fusion region, wherein the extender fusion region comprises (i) a second extender peptide comprising at least one secondary structure and (ii) a second therapeutic agent. In some embodiments, the extender peptide comprises amino acids having an alpha helix secondary structure. In some embodiments, the extender peptide does not comprise amino acids having a beta strand secondary structure. In some embodiments, the extender peptide is a connecting peptide or is a part of a connecting

peptide. In some embodiments, the peptide linker is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more protease cleavage sites. The dual fusion antibody may further comprise one or more additional linkers. The second extender fusion region of the second immunoglobulin fusion protein may further comprise one or more additional extender peptides.

[00165] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first non-antibody region, wherein the first non-antibody region comprises (i) a first extender peptide, wherein the first extender peptide comprises an amino acid sequence comprising an alpha helix secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to a second non-antibody region, wherein the second non-antibody region comprises (i) a peptide linker, wherein the peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a second therapeutic agent. In some embodiments, the extender peptide does not comprise amino acids having a beta strand secondary structure. In some embodiments, the extender peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the peptide linker is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more protease cleavage sites. The dual fusion antibody may further comprise one or more additional linkers. The first non-antibody region of the first immunoglobulin fusion protein may further comprise one or more additional extender peptides.

[00166] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first non-antibody region, wherein the first non-antibody region comprises (i) a first extender peptide, wherein the first extender peptide comprises an amino acid sequence comprising an alpha helix secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to an extender fusion region, wherein the extender fusion region comprises (i) a peptide linker, wherein the second peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a second therapeutic agent. In some embodiments, the extender peptide does not comprise amino acids having a beta strand secondary structure. In some embodiments, the extender peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the peptide linker is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more protease cleavage sites. The dual fusion antibody may further comprise one or more additional linkers. The first non-antibody region of the first immunoglobulin fusion protein may further comprise one or more additional extender peptides.

[00167] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first extender fusion region, wherein the first extender fusion region comprises (i) a first extender peptide, wherein the first extender peptide comprises an amino acid sequence comprising an alpha helix secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to a non-antibody region,

wherein the non-antibody region comprises (i) a peptide linker, wherein the peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a second therapeutic agent. In some embodiments, the extender peptide does not comprise amino acids having a beta strand secondary structure. In some embodiments, the extender peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the peptide linker is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more protease cleavage sites. The dual fusion antibody may further comprise one or more additional linkers. The first extender fusion region of the first immunoglobulin fusion protein may further comprise one or more additional extender peptides.

[00168] The dual fusion antibody may comprise (a) a first immunoglobulin fusion protein comprising a first antibody region attached to a first extender fusion region, wherein the first extender fusion region comprises (i) a first extender peptide, wherein the first extender peptide comprises an amino acid sequence comprising an alpha helix secondary structure and (ii) a first therapeutic agent; and (b) a second immunoglobulin fusion protein comprising a second antibody region attached to a second extender fusion region, wherein the extender fusion region comprises (i) a peptide linker, wherein the peptide linker does not comprise an amino acid sequence comprising an alpha helix or beta strand secondary structure and (ii) a second therapeutic agent. In some embodiments, the extender peptide does not comprise amino acids having a beta strand secondary structure. In some embodiments, the extender peptide is a connecting peptide or is a part of a connecting peptide. In some embodiments, the peptide linker is a connecting peptide or is a part of a connecting peptide. The dual fusion antibody may further comprise one or more protease cleavage sites. The dual fusion antibody may further comprise one or more additional linkers. The first extender fusion region of the first immunoglobulin fusion protein may further comprise one or more additional extender peptides.

[00169] The first therapeutic agent and the second therapeutic agent may be the same. The first therapeutic agent and the second therapeutic agent may be different. The immunoglobulin dual fusion protein may further comprise one or more additional therapeutic agents. The two or more therapeutic agents may be the same. Alternatively, or additionally, the two or more therapeutic agents may be different. The first therapeutic agent may be a therapeutic peptide. The second therapeutic agent may be a therapeutic peptide. One or more of the additional therapeutic agents may be a therapeutic peptide. The first therapeutic agent may comprise a therapeutic peptide. The second therapeutic agent may comprise a therapeutic peptide. One or more of the additional therapeutic agents may comprise one or more therapeutic peptides. A therapeutic agent may comprise one or more therapeutic peptides or regions of therapeutic peptides. A therapeutic agent may comprise, for example, a first therapeutic peptide or portion thereof, an internal peptide, and a second therapeutic peptide or portion thereof. The internal peptide may include, for example, a protease cleavage site or an affinity tag, such as a histidine tag (6X HIS). The internal peptide may include, for example, another therapeutic peptide or portion thereof. For example, a therapeutic agent may comprise, a first portion of a first therapeutic peptide, a first portion of a second therapeutic peptide, and a second portion of a first therapeutic peptide.

[00170] The first antibody region and the second antibody region may be the same. For example, the first antibody region and the second antibody region comprise an immunoglobulin heavy chain. Alternatively, the first antibody region and the second antibody region may comprise an immunoglobulin light chain. The first antibody region and the second antibody region may be different. For example, the first antibody region comprises an immunoglobulin heavy chain and the second antibody region comprises an immunoglobulin light chain or vice versa. The immunoglobulin dual fusion protein may further comprise one or more additional antibody regions. The two or more antibody regions may be the same. Alternatively, or additionally, the two or more antibody regions may be different.

[00171] The immunoglobulin dual fusion protein may further comprise one or more extender peptides. The one or more extender peptides may be the same. Alternatively, or additionally, the one or more extender peptides are different. In some embodiments, the extender peptide comprises 7 or fewer amino acids based on or derived from an ultralong CDR3.

[00172] The immunoglobulin dual fusion protein may further comprise one or more additional antibody regions. The two or more antibody regions may be the same. Alternatively, or additionally, the two or more antibody regions are different.

[00173] The immunoglobulin dual fusion protein may further comprise one or more linkers. The immunoglobulin dual fusion protein may further comprise two or more linkers. The two or more linkers may be the same. Alternatively, or additionally, the two or more linkers are different.

[00174] The immunoglobulin dual fusion protein may further comprise one or more proteolytic cleavage sites. The immunoglobulin dual fusion protein may further comprise two or more proteolytic cleavage sites. The two or more proteolytic cleavage sites may be the same. Alternatively, or additionally, the two or more proteolytic cleavage sites are different.

[00175] The immunoglobulin dual fusion protein may further comprise one or more therapeutic agents comprising internal peptides. An internal peptide may comprise an affinity tag or label, such as a HHHHHH (6X) Histidine tag. An internal peptide may comprise a portion of a therapeutic peptide.

[00176] Exemplary immunoglobulin dual fusion proteins are depicted in FIG. 3, Formula IIIA and Formula VIIA. As shown in Formula IIIA of FIG. 8, the immunoglobulin dual fusion protein may comprise (a) a first antibody region (A^1) attached to a first extender fusion region comprising a first therapeutic agent (T^1) attached to two extender peptides (E^1 , E^2); and (b) a second antibody region (A^2) attached to a second extender fusion region comprising a second therapeutic agent (T^2) attached to two extender peptides (E^3 , E^4). The immunoglobulin dual fusion proteins may further comprise one or more linkers and one or more proteolytic cleavage sites. The one or more proteolytic cleavage sites may be attached to the N- and/or C-terminus of a therapeutic agent. Proteolytic cleavage of the proteolytic cleavage site may release the N- and/or C-terminus of the therapeutic agent from the immunoglobulin fusion protein. Formula VIIA of FIG. 3 depicts an exemplary immunoglobulin dual fusion protein in which the N-terminus of the second therapeutic agent (T^2) has been released.

[00177] Antibody Region

[00178] The immunoglobulin fusion proteins disclosed herein comprise one or more antibody regions. The antibody region may comprise an immunoglobulin or a fragment thereof. The antibody region may comprise at least a portion of an immunoglobulin heavy chain, immunoglobulin light chain, or a combination thereof. The antibody region may comprise two or more immunoglobulin chains or portions thereof. The antibody region may comprise three or more immunoglobulin chains or portions thereof. The antibody region may comprise four or more immunoglobulin chains or portions thereof. The antibody region may comprise five or more immunoglobulin chains or portions thereof. The antibody region may comprise two immunoglobulin heavy chains and two immunoglobulin light chains.

[00179] The antibody region may comprise an entire immunoglobulin molecule or any polypeptide comprising fragment of an immunoglobulin including, but not limited to, heavy chain, light chain, variable domain, constant domain, complementarity determining region (CDR), framework region, fragment antigen binding (Fab) region, Fab', F(ab')₂, F(ab')₃, Fab', fragment crystallizable (Fc) region, single chain variable fragment (scFV), di-scFv, single domain immunoglobulin, trifunctional immunoglobulin, chemically linked F(ab')₂, and any combination thereof. The immunoglobulin region may comprise one or more mutations. The Fc region may be a mutated Fc region. The mutated Fc region may comprise one or more mutations that eliminate an antibody-dependent cellular cytotoxicity (ADCC) effect of an Fc region. The mutated Fc region may comprise about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 1 to about 10, about 1 to about 20, or about 1 to about 30 mutations.

[00180] In some embodiments, an immunoglobulin heavy chain may comprise an entire heavy chain or a portion of a heavy chain. For example, a variable domain or region thereof derived from a heavy chain may be referred to as a heavy chain or a region of a heavy chain. In some embodiments, an immunoglobulin light chain may comprise an entire light chain or a portion of a light chain. For example, a variable domain or region thereof derived from a light chain may be referred to as a light chain or a region of a light chain. A single domain immunoglobulin includes, but is not limited to, a single monomeric variable immunoglobulin domain, for example, a shark variable new antigen receptor immunoglobulin fragment (VNAR).

[00181] The immunoglobulin may be derived from any type known to one of skill in the art including, but not limited to, IgA, IgD, IgE, IgG, IgM, IgY, IgW. The antibody region may comprise one or more units, including but not limited to, 1, 2, 3, 4, and 5 units. Functional units may include, but are not limited to, non-antibody regions, heavy chain, light chain, variable domain, constant domain, complementarity determining region (CDR), framework region, fragment antigen binding (Fab) region, Fab', F(ab')₂, F(ab')₃, Fab', fragment crystallizable (Fc) region, single chain variable fragment (scFV), di-scFv, single domain immunoglobulin, trifunctional immunoglobulin, chemically linked F(ab')₂, and any combination or fragments thereof. Non-antibody regions include, but are not limited to, carbohydrates, lipids, small molecules and therapeutic peptides. The antibody region may comprise one or more units connected by one or more disulfide bonds. The antibody region may comprise one or more units connected by a

peptide linker, for example, a scFv immunoglobulin. The immunoglobulin may be a recombinant immunoglobulin including immunoglobulins with amino acid mutations, substitutions, and/or deletions. The immunoglobulin may be a recombinant immunoglobulin comprising chemical modifications. The immunoglobulin may comprise a whole or part of an immunoglobulin-drug conjugate.

[00182] The antibody region may comprise at least a portion of an immunoglobulin heavy chain. The antibody region may comprise one or more immunoglobulin heavy chains or a portion thereof. The antibody region may comprise two or more immunoglobulin heavy chains or a portion thereof. The antibody region may comprise an amino acid sequence that is at least about 50% homologous to an immunoglobulin heavy chain. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to an immunoglobulin heavy chain. The antibody region may comprise an amino acid sequence that is at least about 70% homologous to an immunoglobulin heavy chain. The antibody region may comprise an amino acid sequence that is at least about 80% homologous to an immunoglobulin heavy chain. The antibody region may comprise an amino acid sequence that is at least about 90% homologous to an immunoglobulin heavy chain. The immunoglobulin heavy chain may comprise amino acids based on or derived from any one of SEQ ID NOs: 22-27, and 29-35. In some embodiments, the antibody region comprises an amino acid sequence that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% homologous to an amino acid sequence of any one of SEQ ID NOs: 22-27, and 29-35. In some embodiments, the antibody region comprises an amino acid sequence that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% identical to an amino acid sequence of any one of SEQ ID NOs: 22-27, and 29-35.

[00183] The antibody region may comprise an amino acid sequence comprising 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more amino acids of an immunoglobulin heavy chain. The antibody region may comprise an amino acid sequence comprising 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900 or more amino acids of an immunoglobulin heavy chain. The amino acids may be consecutive. Alternatively, or additionally, the amino acids are non-consecutive.

[00184] The immunoglobulin heavy chain may be encoded by a nucleotide sequence based on or derived from SEQ ID NOs: 4-9, and 11-17. The immunoglobulin heavy chain may be encoded by a nucleotide sequence that is at least about 50% homologous to SEQ ID NOs: 4-9, and 11-17. The immunoglobulin heavy chain may be encoded by a nucleotide sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to SEQ ID NOs: 4-9, and 11-17. The immunoglobulin heavy chain may be encoded by a nucleotide sequence that is at least about 75% homologous to SEQ ID NOs: 4-9, and 11-17. The immunoglobulin heavy chain may be encoded by a nucleotide sequence that is at least about 85% homologous to SEQ ID NOs: 4-9, and 11-17. In some embodiments, the antibody region is encoded by a nucleotide sequence that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% homologous to a nucleotide sequence of any one of SEQ ID NOs: 4-9, and 11-17. In some embodiments, the antibody region is encoded by a nucleotide

sequence that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% identical to a nucleotide sequence of any one of SEQ ID NOs: 4-9, and 11-17.

[00185] The antibody region may comprise at least a portion of an immunoglobulin light chain. The antibody region may comprise one or more immunoglobulin light chains or a portion thereof. The antibody region may comprise two or more immunoglobulin light chains or a portion thereof. The antibody region may comprise an amino acid sequence that is at least about 50% homologous to an immunoglobulin light chain. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to an immunoglobulin light chain. The antibody region may comprise an amino acid sequence that is at least about 70% homologous to an immunoglobulin light chain. The antibody region may comprise an amino acid sequence that is at least about 80% homologous to an immunoglobulin light chain. The antibody region may comprise an amino acid sequence that is at least about 90% homologous to an immunoglobulin light chain. The immunoglobulin light chain may comprise amino acids based on or derived from any one of SEQ ID NOs: 19-21, 28, and 36. In some embodiments, the antibody region comprises an amino acid sequence that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% homologous to an amino acid sequence of any one of SEQ ID NOs: 19-21, 28, and 36. In some embodiments, the antibody region comprises an amino acid sequence that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% identical to an amino acid sequence of any one of SEQ ID NOs: 19-21, 28, and 36.

[00186] The antibody region may comprise an amino acid sequence comprising 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90 or more amino acids of an immunoglobulin light chain. The antibody region may comprise an amino acid sequence comprising 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900 or more amino acids of an immunoglobulin light chain. The amino acids may be consecutive. Alternatively, or additionally, the amino acids are non-consecutive.

[00187] The immunoglobulin light chain may be encoded by a nucleotide sequence based on or derived from SEQ ID NOs: 1-3, 10, and 18. The immunoglobulin light chain may be encoded by a nucleotide sequence that is at least about 50% homologous to SEQ ID NOs: 1-3, 10, and 18. The immunoglobulin light chain may be encoded by a nucleotide sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to SEQ ID NOs: 1-3, 10, and 18. The immunoglobulin light chain may be encoded by a nucleotide sequence that is at least about 75% homologous to SEQ ID NOs: 1-3, 10, and 18. The immunoglobulin light chain may be encoded by a nucleotide sequence that is at least about 85% homologous to SEQ ID NOs: 1-3, 10, and 18. In some embodiments, the antibody region is encoded by a nucleotide sequence that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% homologous to a nucleotide sequence of any one of SEQ ID NOs: 1-3, 10, and 18. In some embodiments, the antibody region is encoded by a nucleotide sequence that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% identical to a nucleotide sequence of any one of SEQ ID NOs: 1-3, 10, and 18.

[00188] The antibody region may comprise at least a portion of a variable domain. The antibody region may comprise one or more variable domains or portions thereof. The antibody region may comprise 2, 3, 4, 5 or more variable domains or portions thereof. The antibody region may comprise an amino acid sequence comprising 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 350, 400, 500 or more amino acids based on or derived from an amino acid sequence of one or more variable domains. The amino acids may be consecutive. The amino acids may be non-consecutive.

[00189] The antibody region may comprise at least a portion of a constant domain. The antibody region may comprise one or more constant domains or portions thereof. The antibody region may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10 or more constant domains or portions thereof. The antibody region may comprise an amino acid sequence comprising 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 350, 400, 500, 600, 700, 800, 900, 1000, 1200, 1400 or more amino acids based on or derived from an amino acid sequence of one or more constant domains. The amino acids may be consecutive. The amino acids may be non-consecutive.

[00190] The antibody region may comprise at least a portion of a complementarity-determining region (CDR). The antibody region may comprise one or more complementarity-determining regions (CDRs) or portions thereof. The antibody region may comprise 2, 3, 4, 5 or more complementarity-determining regions (CDRs) or portions thereof. The antibody region may comprise 6, 7, 8 or more complementarity-determining regions (CDRs) or portions thereof. The antibody region may comprise four or more complementarity-determining regions (CDRs) or portions thereof. The antibody region may comprise 9, 10, 11 or more complementarity-determining regions (CDRs) or portions thereof. The one or more CDRs may be CDR1, CDR2, CDR3 or a combination thereof. The one or more CDRs may be CDR1. The one or more CDRs may be CDR2. The one or more CDRs may be CDR3. The CDR may be a heavy chain CDR. The one or more CDRs may be a light chain CDR.

[00191] The antibody region may comprise an amino acid sequence comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids based on or derived from an amino acid sequence of a CDR. The antibody region may comprise an amino acid sequence comprising 3 or more amino acids based on or derived from an amino acid sequence of a CDR. The antibody region may comprise an amino acid sequence comprising 5 or more amino acids based on or derived from an amino acid sequence of a CDR. The antibody region may comprise an amino acid sequence comprising 10 or more amino acids based on or derived from an amino acid sequence of a CDR. The amino acids may be consecutive. The amino acids may be non-consecutive.

[00192] The antibody region may be based on or derived from at least a portion of an anti-T cell receptor immunoglobulin. The antibody region may be based on or derived from at least a portion of an anti-B cell receptor immunoglobulin.

[00193] The antibody region may be based on or derived from at least a portion of an anti-T cell co-receptor immunoglobulin. The antibody region may be based on or derived from at least a portion of an anti-CD3 immunoglobulin. The antibody region may be based on or derived from an anti-CD3 immunoglobulin. The anti-CD3 immunoglobulin may be UCHT1. The antibody region may be based on

or derived from at least a portion of a Fab fragment of an anti-CD3 immunoglobulin. The antibody region may be based on or derived from an immunoglobulin fragment of an anti-CD3 immunoglobulin.

[00194] The antibody region may be based on or derived from an immunoglobulin or immunoglobulin fragment that binds to at least a portion of a receptor on a cell. The antibody region may be based on or derived from an immunoglobulin or immunoglobulin fragment that binds to at least a portion of a co-receptor on a cell. The antibody region may be based on or derived from an immunoglobulin or immunoglobulin fragment that binds to at least a portion of an antigen or cell surface marker on a cell. The cell may be a hematopoietic cell. The hematopoietic cell may be a myeloid cell. The myeloid cell may be an erythrocyte, thrombocyte, neutrophil, monocyte, macrophage, eosinophil, basophil, or mast cell. The hematopoietic cell may be a lymphoid cell. The lymphoid cell may be a B-cell, T-cell, or NK-cell. The hematopoietic cell may be a leukocyte. The hematopoietic cell may be a lymphocyte.

[00195] The antibody region may be based on or derived from an immunoglobulin or immunoglobulin fragment that binds to at least a portion of a receptor on a T-cell. The receptor may be a T-cell receptor (TCR). The TCR may comprise TCR alpha, TCR beta, TCR gamma and/or TCR delta. The receptor may be a T-cell receptor zeta.

[00196] The antibody region may be based on or derived from an immunoglobulin or immunoglobulin fragment that binds to at least a portion of a receptor on a lymphocyte, B-cell, macrophage, monocytes, neutrophils and/or NK cells. The receptor may be an Fc receptor. The Fc receptor may be an Fc-gamma receptor, Fc-alpha receptor and/or Fc-epsilon receptor. Fc-gamma receptors include, but are not limited to, FcγRI (CD64), FcγRIIA (CD32), FcγRIIB (CD32), FcγRIIIA (CD16a) and FcγRIIIB (CD16b). Fc-alpha receptors include, but are not limited to, FcαRI. Fc-epsilon receptors include, but are not limited to, FcεRI and FcεRII. The receptor may be CD89 (Fc fragment of IgA receptor or FCAR).

[00197] The antibody region may be based on or derived from an immunoglobulin or immunoglobulin fragment that binds at least a portion of a co-receptor on a T-cell. The co-receptor may be a CD3, CD4, and/or CD8. The antibody region may be based on or derived from an immunoglobulin fragment that binds to a CD3 co-receptor. The CD3 co-receptor may comprise CD3-gamma, CD3-delta and/or CD3-epsilon. CD8 may comprise CD8-alpha and/or CD8-beta chains.

[00198] In some embodiments, the antibody region is not specific for a mammalian target. In some embodiments, the immunoglobulin is an anti-viral immunoglobulin. In some embodiments, the immunoglobulin is an anti-bacterial immunoglobulin. In some embodiments, the immunoglobulin is an anti-parasitic immunoglobulin. In some embodiments, the immunoglobulin is an anti-fungal immunoglobulin. In some embodiments, the antibody region is derived from an immunoglobulin vaccine.

[00199] In some embodiments, the antibody region is based on or derived from immunoglobulins including, but not limited to, actoxumab, bezlotoxumab, CR6261, edobacomab, efungumab, exbivirumab, felvizumab, foravirumab, ibalizumab (TMB-355, TNX-355), libivirumab, motavizumab, nebacumab, pagibaximab, palivizumab, panobacumab, rafivirumab, raxibacumab, regavirumab, sevirumab (MSL-109), suvizumab, tefibazumab, tuvirumab, and urtoxazumab.

[00200] In some embodiments, the antibody region is based on or derived from immunoglobulins targeting *Clostridium difficile*, Orthomyxoviruses (Influenzavirus A, Influenzavirus B, Influenzavirus C, Isavirus, Thogotovirus), *Escherichia coli*, *Candida*, Rabies, Human Immunodeficiency Virus, Hepatitis, *Staphylococcus*, Respiratory Syncytial Virus, *Pseudomonas aeruginosa*, *Bacillus anthracis*, Cytomegalovirus, or *Staphylococcus aureus*.

[00201] The antibody region may be based on or derived from an anti-viral immunoglobulin. The anti-viral immunoglobulin may be directed against an epitope of a viral protein. The anti-bacterial immunoglobulin may target one or more viruses including, but not limited to, Adenoviruses, Herpesviruses, Poxviruses, Parvoviruses, Reoviruses, Picornaviruses, Togaviruses, Orthomyxoviruses, Rhabdoviruses, Retroviruses and Hepadnaviruses. The viral protein may be from a respiratory syncytial virus. The viral protein may be an F protein of the respiratory syncytial virus. The epitope may be in the A antigenic site of the F protein. The anti-viral immunoglobulin may be based on or derived from palivizumab. The immunoglobulin may be based on or derived from an anti-viral vaccine. The anti-viral immunoglobulin may be based on or derived from exbivirumab, foravirumab, libivirumab, raffivirumab, regavirumab, sevirumab, tuvirumab, felvizumab, motavizumab, palivizumab, and/or suvizumab.

[00202] The antibody region may be based on or derived from an anti-viral immunoglobulin G. The antibody region may comprise at least a portion of an anti-viral immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 50% homologous to at least a portion of an anti-viral immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to at least a portion of an anti-viral immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 70% homologous to at least a portion of an anti-viral immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 80% homologous to at least a portion of an anti-viral immunoglobulin G. In some embodiments the antibody region comprises an amino acid sequence based on or derived from an anti-viral immunoglobulin M.

[00203] The antibody region may comprise an amino acid sequence that comprises 10, 20, 30, 40, 50, 60, 70, 80, 90 or more amino acids of an anti-viral immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 100, 200, 300, 400, 500, 600, 700, 800, 900 or more amino acids of an anti-viral immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 50 or more amino acids of an anti-viral immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 100 or more amino acids of an anti-viral immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 200 or more amino acids of an anti-viral immunoglobulin G sequence.

[00204] The antibody region may be based on or derived from a palivizumab immunoglobulin. The antibody region may comprise at least a portion of a palivizumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 50% homologous to at least a portion of a palivizumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to at least a

portion of a palivizumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 70% homologous to at least a portion of a palivizumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 80% homologous to at least a portion of a palivizumab immunoglobulin.

[00205] The antibody region may comprise an amino acid sequence that comprises 10, 20, 30, 40, 50, 60, 70, 80, 90 or more amino acids of a palivizumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 100, 200, 300, 400, 500, 600, 700, 800, 900 or more amino acids of a palivizumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 50 or more amino acids of a palivizumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 100 or more amino acids of a palivizumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 200 or more amino acids of a palivizumab immunoglobulin sequence.

[00206] The antibody region may be based on or derived from an exbivirumab, foravirumab, libivirumab, rafivirumab, regavirumab, sevirumab, tuvirumab, felvizumab, motavizumab, palivizumab, and/or suvizumab immunoglobulin. The antibody region may comprise at least a portion of an exbivirumab, foravirumab, libivirumab, rafivirumab, regavirumab, sevirumab, tuvirumab, felvizumab, motavizumab, palivizumab, and/or suvizumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 50% homologous to at least a portion of an exbivirumab, foravirumab, libivirumab, rafivirumab, regavirumab, sevirumab, tuvirumab, felvizumab, motavizumab, palivizumab, and/or suvizumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to at least a portion of an exbivirumab, foravirumab, libivirumab, rafivirumab, regavirumab, sevirumab, tuvirumab, felvizumab, motavizumab, palivizumab, and/or suvizumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 70% homologous to at least a portion of an exbivirumab, foravirumab, libivirumab, rafivirumab, regavirumab, sevirumab, tuvirumab, felvizumab, motavizumab, palivizumab, and/or suvizumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 80% homologous to at least a portion of an exbivirumab, foravirumab, libivirumab, rafivirumab, regavirumab, sevirumab, tuvirumab, felvizumab, motavizumab, palivizumab, and/or suvizumab immunoglobulin.

[00207] The antibody region may comprise an amino acid sequence that comprises 10, 20, 30, 40, 50, 60, 70, 80, 90 or more amino acids of an exbivirumab, foravirumab, libivirumab, rafivirumab, regavirumab, sevirumab, tuvirumab, felvizumab, motavizumab, palivizumab, and/or suvizumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 100, 200, 300, 400, 500, 600, 700, 800, 900 or more amino acids of an exbivirumab, foravirumab, libivirumab, rafivirumab, regavirumab, sevirumab, tuvirumab, felvizumab, motavizumab, palivizumab, and/or suvizumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 50 or more amino acids of an exbivirumab, foravirumab, libivirumab, rafivirumab, regavirumab, sevirumab, tuvirumab, felvizumab, motavizumab, palivizumab, and/or suvizumab

immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 100 or more amino acids of an exbivirumab, foravirumab, libivirumab, rafivirumab, regavirumab, sevirumab, tuvirumab, felvizumab, motavizumab, palivizumab, and/or suvizumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 200 or more amino acids of an exbivirumab, foravirumab, libivirumab, rafivirumab, regavirumab, sevirumab, tuvirumab, felvizumab, motavizumab, palivizumab, and/or suvizumab immunoglobulin sequence.

[00208] The antibody region may be based on or derived from an anti-bacterial immunoglobulin. The anti-bacterial immunoglobulin may be directed against an epitope of a bacterial protein. The anti-bacterial immunoglobulin may target bacteria including, but not limited to, *Acetobacter aurantius*, *Agrobacterium radiobacter*, *Anaplasma phagocytophilum*, *Azorhizobium caulinodans*, *Bacillus anthracis*, *Bacillus brevis*, *Bacillus cereus*, *Bacillus subtilis*, *Bacteroides fragilis*, *Bacteroides gingivalis*, *Bacteroides melaninogenicus*, *Bartonella quintana*, *Bordetella bronchiseptica*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Burkholderia cepacia*, *Calymmatobacterium granulomatis*, *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter jejuni*, *Campylobacter pylori*, *Chlamydia trachomatis*, *Chlamydophila pneumoniae*, *Chlamydophila psittaci*, *Clostridium botulinum*, *Clostridium difficile*, *Corynebacterium diphtheriae*, *Corynebacterium fusiforme*, *Coxiella burnetii*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus gallinarum*, *Enterococcus maloratus*, *Escherichia coli*, *Francisella tularensis*, *Fusobacterium nucleatum*, *Gardnerella vaginalis*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Haemophilus pertussis*, *Haemophilus vaginalis*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Lactobacillus acidophilus*, *Lactococcus lactis*, *Legionella pneumophila*, *Listeria monocytogenes*, *Methanobacterium extroquens*, *Microbacterium multifforme*, *Micrococcus luteus*, *Moraxella catarrhalis*, *Mycobacterium phlei*, *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pasteurella multocida*, *Pasteurella tularensis*, *Peptostreptococcus*, *Porphyromonas gingivalis*, *Prevotella melaninogenica*, *Pseudomonas aeruginosa*, *Rhizobium radiobacter*, *Rickettsia rickettsii*, *Rothia dentocariosa*, *Salmonella enteritidis*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Stenotrophomonas maltophilia*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Treponema pallidum*, *Treponema denticola*, *Vibrio cholerae*, *Vibrio comma*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. The immunoglobulin may be based on or derived from a bacterial vaccine. The anti-viral immunoglobulin may be based on or derived from nebacumab, panobacumab, raxibacumab, edobacomab, pagibaximab, and/or tefibazumab.

[00209] The antibody region may be based on or derived from an anti-bacterial immunoglobulin G. The antibody region may comprise at least a portion of an anti-bacterial immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 50% homologous to at least a portion of an anti-bacterial immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to at least

a portion of an anti-bacterial immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 70% homologous to at least a portion of an anti-bacterial immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 80% homologous to at least a portion of an anti-bacterial immunoglobulin G. In some embodiments the antibody region comprises an amino acid sequence based on or derived from an anti-viral immunoglobulin M.

[00210] The antibody region may comprise an amino acid sequence that comprises 10, 20, 30, 40, 50, 60, 70, 80, 90 or more amino acids of an anti-bacterial immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 100, 200, 300, 400, 500, 600, 700, 800, 900 or more amino acids of an anti-bacterial immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 50 or more amino acids of an anti-bacterial immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 100 or more amino acids of an anti-bacterial immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 200 or more amino acids of an anti-bacterial immunoglobulin G sequence.

[00211] The antibody region may be based on or derived from a Nebacumab, Panobacumab, Raxibacumab, Edobacomab, Pagibaximab, and/or Tefibazumab immunoglobulin. The antibody region may comprise at least a portion of a nebacumab, panobacumab, raxibacumab, edobacomab, pagibaximab, and/or tefibazumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 50% homologous to at least a portion of a nebacumab, panobacumab, raxibacumab, edobacomab, pagibaximab, and/or tefibazumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to at least a portion of a nebacumab, panobacumab, raxibacumab, edobacomab, pagibaximab, and/or tefibazumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 70% homologous to at least a portion of a nebacumab, panobacumab, raxibacumab, edobacomab, pagibaximab, and/or tefibazumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 80% homologous to at least a portion of a nebacumab, panobacumab, raxibacumab, edobacomab, pagibaximab, and/or tefibazumab immunoglobulin.

[00212] The antibody region may comprise an amino acid sequence that comprises 10, 20, 30, 40, 50, 60, 70, 80, 90 or more amino acids of a nebacumab, panobacumab, raxibacumab, edobacomab, pagibaximab, and/or tefibazumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 100, 200, 300, 400, 500, 600, 700, 800, 900 or more amino acids of a nebacumab, panobacumab, raxibacumab, edobacomab, pagibaximab, and/or tefibazumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 50 or more amino acids of a nebacumab, panobacumab, raxibacumab, edobacomab, pagibaximab, and/or tefibazumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 100 or more amino acids of a nebacumab, panobacumab, raxibacumab, edobacomab, pagibaximab, and/or tefibazumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that

comprises 200 or more amino acids of a nebacumab, panobacumab, raxibacumab, edobacomab, pagibaximab, and/or tefibazumab immunoglobulin sequence.

[00213] The antibody region may be based on or derived from an anti-parasitic immunoglobulin. The anti-parasitic immunoglobulin may be directed against an epitope of a parasite protein. The anti-parasitic immunoglobulin may target parasites or parasite proteins including, but not limited to parasites *Acanthamoeba*, *Balamuthia mandrillaris*, *Babesia* (*B. divergens*, *B. bigemina*, *B. equi*, *B. microfti*, *B. duncani*), *Balantidium coli*, *Blastocystis*, *Cryptosporidium*, *Dientamoeba fragilis*, *Entamoeba histolytica*, *Giardia lamblia*, *Isospora belli*, *Leishmania*, *Naegleria fowleri*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale curtisi*, *Plasmodium ovale wallikeri*, *Plasmodium malariae*, *Plasmodium knowlesi*, *Rhinosporidium seeberi*, *Sarcocystis bovihominis*, *Sarcocystis suihominis*, *Toxoplasma gondii*, *Trichomonas vaginalis*, *Trypanosoma brucei*, *Trypanosoma cruzi*, *Cestoda*, *Taenia multiceps*, *Diphyllobothrium latum*, *Echinococcus granulosus*, *Echinococcus multilocularis*, *Echinococcus vogeli*, *Echinococcus oligarthrus*, *Hymenolepis nana*, *Hymenolepis diminuta*, *Taenia saginata*, *Taenia solium*, *Bertiella mucronata*, *Bertiella studeri*, *Spirometra erinaceieuropaei*, *Clonorchis sinensis*; *Clonorchis viverrini*, *Dicrocoelium dendriticum*, *Fasciola hepatica*, *Fasciola gigantica*, *Fasciolopsis buski*, *Gnathostoma spinigerum*, *Gnathostoma hispidum*, *Metagonimus yokogawai*, *Opisthorchis viverrini*, *Opisthorchis felinus*, *Clonorchis sinensis*, *Paragonimus westermani*; *Paragonimus africanus*; *Paragonimus caliensis*; *Paragonimus kellicotti*; *Paragonimus skrjabini*; *Paragonimus uterobilateralis*, *Schistosoma* sp., *Schistosoma mansoni*, *Schistosoma haematobium*, *Schistosoma japonicum*, *Schistosoma mekongi*, *Echinostoma echinatum*, *Trichobilharzia regenti*, *Schistosomatidae*, *Ancylostoma duodenale*, *Necator americanus*, *Angiostrongylus costaricensis*, *Anisakis*, *Ascaris* sp. *Ascaris lumbricoides*, *Baylisascaris procyonis*, *Brugia malayi*, *Brugia timori*, *Dioctophyme renale*, *Dracunculus medinensis*, *Enterobius vermicularis*, *Enterobius gregorii*, *Halicephalobus gingivalis*, *Loa filaria*, *Mansonella streptocerca*, *Onchocerca volvulus*, *Strongyloides stercoralis*, *Thelazia californiensis*, *Thelazia callipaeda*, *Toxocara canis*, *Toxocara cati*, *Trichinella spiralis*, *Trichinella britovi*, *Trichinella nelsoni*, *Trichinella nativa*, *Trichuris trichiura*, *Trichuris vulpis*, *Wuchereria bancrofti*, *Archiacanthocephala*, *Moniliformis moniliformis*, *Linguatula serrata*, *Oestroidea*, *Calliphoridae*, *Sarcophagidae*, *Tunga penetrans*, *Dermatobia hominis*, *Ixodidae*, *Argasidae*, *Cimex lectularius*, *Pediculus humanus*, *Pediculus humanus corporis*, *Pthirus pubis*, *Demodex folliculorum/brevis/canis*, *Sarcoptes scabiei*, *Cochliomyia hominivorax*, and *Pulex irritans*.

[00214] The antibody region may be based on or derived from an anti-parasitic immunoglobulin G. The antibody region may comprise at least a portion of an anti-parasitic immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 50% homologous to at least a portion of an anti-parasitic immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to at least a portion of an anti-parasitic immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 70% homologous to at least a portion of an anti-parasitic immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 80% homologous to at

least a portion of an anti-parasitic immunoglobulin G. In some embodiments the antibody region comprises an amino acid sequence based on or derived from an anti-parasitic immunoglobulin M.

[00215] The antibody region may comprise an amino acid sequence that comprises 10, 20, 30, 40, 50, 60, 70, 80, 90 or more amino acids of an anti-parasitic immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 100, 200, 300, 400, 500, 600, 700, 800, 900 or more amino acids of an anti-parasitic immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 50 or more amino acids of an anti-parasitic immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 100 or more amino acids of an anti-parasitic immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 200 or more amino acids of an anti-parasitic immunoglobulin G sequence.

[00216] The antibody region may be based on or derived from an anti-fungal immunoglobulin. The anti-bacterial immunoglobulin may be directed against an epitope of a fungal protein. The anti-fungal immunoglobulin may target fungi or fungal proteins including, but not limited to *Cryptococcus neoformans*, *Cryptococcus gattii*, *Candida albicans*, *Candida tropicalis*, *Candida stellatoidea*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida viswanathii*, *Candida lusitanae*, *Rhodotorula mucilaginosa*, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, *Brettanomyces bruxellensis*, *Candida stellata*, *Schizosaccharomyces pombe*, *Torulaspora delbrueckii*, *Zygosaccharomyces bailii*, *Yarrowia lipolytica*, *Saccharomyces exiguus* and *Pichia pastoris*. The anti-fungal immunoglobulin may be based on or derived from efungumab.

[00217] The antibody region may be based on or derived from an anti-fungal immunoglobulin G. The antibody region may comprise at least a portion of an anti-fungal immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 50% homologous to at least a portion of an anti-fungal immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to at least a portion of an anti-fungal immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 70% homologous to at least a portion of an anti-fungal immunoglobulin G. The antibody region may comprise an amino acid sequence that is at least about 80% homologous to at least a portion of an anti-fungal immunoglobulin G. In some embodiments the antibody region comprises an amino acid sequence based on or derived from an anti-fungal immunoglobulin M.

[00218] The antibody region may comprise an amino acid sequence that comprises 10, 20, 30, 40, 50, 60, 70, 80, 90 or more amino acids of an anti-fungal immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 100, 200, 300, 400, 500, 600, 700, 800, 900 or more amino acids of an anti-fungal immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 50 or more amino acids of an anti-fungal immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 100 or more amino acids of an anti-fungal immunoglobulin G sequence. The antibody region may comprise an amino acid sequence that comprises 200 or more amino acids of an anti-fungal immunoglobulin G sequence.

[00219] The antibody region may be based on or derived from an efungumab immunoglobulin. The antibody region may comprise at least a portion of an efungumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 50% homologous to at least a portion of an efungumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to at least a portion of an efungumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 70% homologous to at least a portion of an efungumab immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 80% homologous to at least a portion of an efungumab immunoglobulin.

[00220] The antibody region may comprise an amino acid sequence that comprises 10, 20, 30, 40, 50, 60, 70, 80, 90 or more amino acids of an efungumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 100, 200, 300, 400, 500, 600, 700, 800, 900 or more amino acids of an efungumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 50 or more amino acids of an efungumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 100 or more amino acids of an efungumab immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 200 or more amino acids of an efungumab immunoglobulin sequence.

[00221] The antibody region may be based on or derived from a trastuzumab immunoglobulin G immunoglobulin. The antibody region may comprise at least a portion of a trastuzumab immunoglobulin G immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 50% homologous to at least a portion of a trastuzumab immunoglobulin G immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to at least a portion of a trastuzumab immunoglobulin G immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 70% homologous to at least a portion of a trastuzumab immunoglobulin G immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 80% homologous to at least a portion of a trastuzumab immunoglobulin G immunoglobulin. The antibody region may comprise a mutated trastuzumab antibody. The antibody region may comprise a trastuzumab antibody that comprises a heptad mutation in the IgG1 heavy chain. The antibody region may comprise a trastuzumab antibody that comprises a triple mutation in the IgG4 heavy chain.

[00222] The antibody region may comprise an amino acid sequence that comprises 10, 20, 30, 40, 50, 60, 70, 80, 90 or more amino acids of a trastuzumab immunoglobulin G immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 100, 200, 300, 400, 500, 600, 700, 800, 900 or more amino acids of a trastuzumab immunoglobulin G immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 50 or more amino acids of a trastuzumab immunoglobulin G immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 100 or more amino acids of a trastuzumab immunoglobulin G

immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 200 or more amino acids of a trastuzumab immunoglobulin G immunoglobulin sequence.

[00223] The antibody region may be based on or derived from an anti-Her2 immunoglobulin. The antibody region may comprise at least a portion of an anti-Her2 immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 50% homologous to at least a portion of an anti-Her2 immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to at least a portion of an anti-Her2 immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 70% homologous to at least a portion of an anti-Her2 immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 80% homologous to at least a portion of an anti-Her2 immunoglobulin.

[00224] The antibody region may comprise an amino acid sequence that comprises 10, 20, 30, 40, 50, 60, 70, 80, 90 or more amino acids of an anti-Her2 immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 100, 200, 300, 400, 500, 600, 700, 800, 900 or more amino acids of an anti-Her2 immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 50 or more amino acids of an anti-Her2 immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 100 or more amino acids of an anti-Her2 immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 200 or more amino acids of an anti-Her2 immunoglobulin sequence.

[00225] The antibody region may be based on or derived from an anti-CD47 immunoglobulin. The antibody region may comprise at least a portion of an anti-CD47 immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 50% homologous to at least a portion of an anti-CD47 immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, or 97% or more homologous to at least a portion of an anti-CD47 immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 70% homologous to at least a portion of an anti-CD47 immunoglobulin. The antibody region may comprise an amino acid sequence that is at least about 80% homologous to at least a portion of an anti-CD47 immunoglobulin.

[00226] The antibody region may comprise an amino acid sequence that comprises 10, 20, 30, 40, 50, 60, 70, 80, 90 or more amino acids of an anti-CD47 immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 100, 200, 300, 400, 500, 600, 700, 800, 900 or more amino acids of an anti-CD47 immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 50 or more amino acids of an anti-CD47 immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 100 or more amino acids of an anti-CD47 immunoglobulin sequence. The antibody region may comprise an amino acid sequence that comprises 200 or more amino acids of an anti-CD47 immunoglobulin sequence.

[00227] The antibody region may be based on or derived from an anti-cancer immunoglobulin. Examples of anti-cancer immunoglobulin include, but are not limited to, abciximab, adalimumab,

alemtuzumab, basiliximab, belimumab, bevacizumab, brentuximab, canakinumab, certolizumab, cetuximab, daclizumab, denosumab, eculizumab, efalizumab, gemtuzumab, golimumab, ibritumomab, infliximab, ipilimumab, muromonab-cd3, natalizumab, ofatumumab, omalizumab, palivizumab, panitumumab, ranibizumab, rituximab, tocilizumab, tositumomab, trastuzumab.

[00228] The antibody region may comprise at least a portion of a human immunoglobulin. The antibody region may comprise at least a portion of a humanized immunoglobulin. The antibody region may comprise at least a portion of a chimeric immunoglobulin. The antibody region may be based on or derived from a human immunoglobulin. The antibody region may be based on or derived from a humanized immunoglobulin. The antibody region may be based on or derived from a chimeric immunoglobulin. The antibody region may be based on or derived from a monoclonal immunoglobulin. The antibody region may be based on or derived from a polyclonal immunoglobulin. The antibody region may comprise at least a portion of an immunoglobulin from a mammal, avian, reptile, amphibian, or a combination thereof. The mammal may be a human. The mammal may be a non-human primate. The mammal may be a dog, cat, sheep, goat, cow, rabbit, or mouse.

[00229] The antibody region may comprise a sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragment sequences. The antibody region may comprise a sequence that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or more homologous to a sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments. The antibody region may comprise a sequence that is at least about 70% homologous to a sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments. The antibody region may comprise a sequence that is at least about 80% homologous to a sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments. The antibody region may comprise a sequence that is at least about 90% homologous to a sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments. The antibody region may comprise a sequence that is at least about 95% homologous to a sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments. The sequence may be a peptide sequence. The sequence may be a nucleotide sequence.

[00230] The antibody region may comprise a peptide sequence that differs from a peptide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than or equal to about 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, 17, 15, 12, 10, 8, 6, 5, 4 or fewer amino acids. The antibody region may comprise a peptide sequence that differs from a peptide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than or equal to about 4 or fewer amino acids. The antibody region may comprise a peptide sequence that differs from a peptide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than or equal to about 3 or fewer amino acids. The antibody region may comprise a peptide sequence that differs from a peptide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than or equal to about 2 or fewer amino acids. The antibody region may comprise a peptide sequence that differs from a peptide sequence based on or

derived from one or more immunoglobulin and/or immunoglobulin fragments by less than or equal to about 1 or fewer amino acids. The amino acids may be consecutive, nonconsecutive, or a combination thereof. For example, the antibody region may comprise a peptide sequence that differs from a peptide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than about 3 consecutive amino acids. Alternatively, or additionally, the antibody region may comprise a peptide sequence that differs from a peptide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than about 2 non-consecutive amino acids. In another example, the antibody region may comprise a peptide sequence that differs from a peptide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than about 5 amino acids, wherein 2 of the amino acids are consecutive and 2 of the amino acids are non-consecutive.

[00231] The antibody region may comprise a nucleotide sequence that differs from a nucleotide sequence based on or derived from one or more antibodies and/or immunoglobulin fragments by less than or equal to about 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4 or fewer nucleotides or base pairs. The antibody region may comprise a nucleotide sequence that differs from a nucleotide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than or equal to about 15 or fewer nucleotides or base pairs. The antibody region may comprise a nucleotide sequence that differs from a nucleotide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than or equal to about 12 or fewer nucleotides or base pairs. The antibody region may comprise a nucleotide sequence that differs from a nucleotide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than or equal to about 9 or fewer nucleotides or base pairs. The antibody region may comprise a nucleotide sequence that differs from a nucleotide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than or equal to about 6 or fewer nucleotides or base pairs. The antibody region may comprise a nucleotide sequence that differs from a nucleotide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than or equal to about 4 or fewer nucleotides or base pairs. The antibody region may comprise a nucleotide sequence that differs from a nucleotide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than or equal to about 3 or fewer nucleotides or base pairs. The antibody region may comprise a nucleotide sequence that differs from a nucleotide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than or equal to about 2 or fewer nucleotides or base pairs. The antibody region may comprise a nucleotide sequence that differs from a nucleotide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than or equal to about 1 or fewer nucleotides or base pairs. The nucleotides or base pairs may be consecutive, nonconsecutive, or a combination thereof. For example, the antibody region may comprise a nucleotide sequence that differs from a nucleotide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than about 3 consecutive nucleotides or base pairs.

Alternatively, or additionally, the antibody region may comprise a nucleotide sequence that differs from a nucleotide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than about 2 non-consecutive nucleotides or base pairs. In another example, the antibody region may comprise a nucleotide sequence that differs from a nucleotide sequence based on or derived from one or more immunoglobulin and/or immunoglobulin fragments by less than about 5 nucleotides or base pairs, wherein 2 of the nucleotides or base pairs are consecutive and 2 of the nucleotides or base pairs are non-consecutive.

[00232] The peptide sequence of the antibody region may differ from the peptide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by one or more amino acid substitutions. The peptide sequence of the antibody region may differ from the peptide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by two or more amino acid substitutions. The peptide sequence of the antibody region may differ from the peptide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by three or more amino acid substitutions. The peptide sequence of the antibody region may differ from the peptide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by four or more amino acid substitutions. The peptide sequence of the antibody region may differ from the peptide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by five or more amino acid substitutions. The peptide sequence of the antibody region may differ from the peptide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by six or more amino acid substitutions. The peptide sequence of the antibody region may differ from the peptide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 15, 17, 20, 25 or more amino acid substitutions. The peptide sequence of the antibody region may differ from the peptide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by about 20-30, 30-40, 40-50, 50-60, 60-70, 80-90, 90-100, 100-150, 150-200, 200-300 or more amino acid substitutions.

[00233] The nucleotide sequence of the antibody region may differ from the nucleotide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by one or more nucleotide and/or base pair substitutions. The nucleotide sequence of the antibody region may differ from the nucleotide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by two or more nucleotide and/or base pair substitutions. The nucleotide sequence of the antibody region may differ from the nucleotide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by three or more nucleotide and/or base pair substitutions. The nucleotide sequence of the antibody region may differ from the nucleotide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by four or more nucleotide and/or base pair substitutions. The nucleotide sequence of the antibody region may differ from the nucleotide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by five or more nucleotide and/or base pair substitutions. The nucleotide sequence of the

antibody region may differ from the nucleotide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by six or more nucleotide and/or base pair substitutions. The nucleotide sequence of the antibody region may differ from the nucleotide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by nine or more nucleotide and/or base pair substitutions. The nucleotide sequence of the antibody region may differ from the nucleotide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by twelve or more nucleotide and/or base pair substitutions. The nucleotide sequence of the antibody region may differ from the nucleotide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by fifteen or more nucleotide and/or base pair substitutions. The nucleotide sequence of the antibody region may differ from the nucleotide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by eighteen or more nucleotide and/or base pair substitutions. The nucleotide sequence of the antibody region may differ from the nucleotide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by 20, 22, 24, 25, 27, 30 or more nucleotide and/or base pair substitutions. The nucleotide sequence of the antibody region may differ from the nucleotide sequence of the immunoglobulin or immunoglobulin fragment that it is based on and/or derived from by about 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-200, 200-300, 300-400 or more nucleotide and/or base pair substitutions.

[00234] The antibody region may comprise at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids. The antibody region may comprise at least about 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700 or more amino acids. The antibody region may comprise at least about 100 amino acids. The antibody region may comprise at least about 200 amino acids. The antibody region may comprise at least about 400 amino acids. The antibody region may comprise at least about 500 amino acids. The antibody region may comprise at least about 600 amino acids.

[00235] The antibody region may comprise less than about 2000, 1900, 1800, 1700, 1600, 1500, 1400, 1300, 1200 or 1100 amino acids. The antibody region may comprise less than about 1000, 950, 900, 850, 800, 750, or 700 amino acids. The antibody region may comprise less than about 1500 amino acids. The antibody region may comprise less than about 1000 amino acids. The antibody region may comprise less than about 800 amino acids. The antibody region may comprise less than about 700 amino acids.

[00236] The immunoglobulin fusion protein may further comprise an antibody region comprising 30 or fewer consecutive amino acids of a complementarity determining region 3 (CDR3). The antibody region may comprise 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or fewer consecutive amino acids of a CDR3. The antibody region may comprise 15 or fewer consecutive amino acids of a CDR3. The antibody region may comprise 14 or fewer consecutive amino acids of a CDR3. The antibody region may comprise 13 or fewer consecutive amino acids of a CDR3. The antibody region may comprise 12 or fewer consecutive amino acids of a CDR3. The antibody region may comprise 11 or fewer consecutive amino acids of a CDR3. The antibody region may comprise 10 or fewer

consecutive amino acids of a CDR3. The antibody region may comprise 9 or fewer consecutive amino acids of a CDR3. The antibody region may comprise 8 or fewer consecutive amino acids of a CDR3. The antibody region may comprise 7 or fewer consecutive amino acids of a CDR3. The antibody region may comprise 6 or fewer consecutive amino acids of a CDR3. The antibody region may comprise 5 or fewer consecutive amino acids of a CDR3. The antibody region may comprise 4 or fewer consecutive amino acids of a CDR3. The antibody region may comprise 3 or fewer consecutive amino acids of a CDR3. The antibody region may comprise 2 or fewer consecutive amino acids of a CDR3. The antibody region may comprise 1 or fewer consecutive amino acids of a CDR3. In some instances, the antibody region does not contain a CDR3.

[00237] The immunoglobulin fusion protein may comprise a first antibody region comprising 6 or fewer consecutive amino acids of a complementarity determining region 3 (CDR3). The first antibody region may comprise 5 or fewer consecutive amino acids of a CDR3. The first antibody region may comprise 4 or fewer consecutive amino acids of a CDR3. The first antibody region may comprise 3 or fewer consecutive amino acids of a CDR3. The first antibody region may comprise 2 or fewer consecutive amino acids of a CDR3. The first antibody region may comprise 1 or fewer consecutive amino acids of a CDR3. In some instances, the first antibody region does not contain a CDR3.

[00238] The immunoglobulin fusion protein may further comprise a second antibody region comprising 30 or fewer consecutive amino acids of a complementarity determining region 3 (CDR3). The second antibody region may comprise 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 15 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 14 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 13 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 12 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 11 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 10 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 9 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 8 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 7 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 6 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 5 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 4 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 3 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 2 or fewer consecutive amino acids of a CDR3. The second antibody region may comprise 1 or fewer consecutive amino acids of a CDR3. In some instances, the second antibody region does not contain a CDR3.

[00239] The antibody region may comprise an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 19-36 and 271-273. The antibody region may comprise an amino acid sequence that is at least about 50% homologous to any one of SEQ ID NOs: 19-36 and 271-273. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%,

90%, 95%, or 97% homologous to any one of SEQ ID NOs 19-36 and 271-273. The antibody region may comprise an amino acid sequence that is at least about 70% homologous to any one of SEQ ID NOs: 19-36 and 271-273. The antibody region may comprise an amino acid sequence that is at least about 80% homologous to any one of SEQ ID NOs: 19-36 and 271-273. The antibody region may comprise an amino acid sequence that is at least about 50% identical to any one of SEQ ID NOs: 19-36 and 271-273. The antibody region may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% identical to any one of SEQ ID NOs 19-36 and 271-273. The antibody region may comprise an amino acid sequence that is at least about 70% identical to any one of SEQ ID NOs: 19-36 and 271-273. The antibody region may comprise an amino acid sequence that is at least about 80% identical to any one of SEQ ID NOs: 19-36 and 271-273. The antibody region may comprise an amino acid sequence that is 100% identical to any one of SEQ ID NOs: 19-36 and 271-273. In some embodiments, the antibody region comprises an amino acid sequence that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% homologous to an amino acid sequence of any one of SEQ ID NOs: 19-36 and 271-273. In some embodiments, the antibody region comprises an amino acid sequence that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% identical to an amino acid sequence of any one of SEQ ID NOs: 19-36 and 271-273.

[00240] The antibody region may comprise an amino acid sequence comprising 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids based on or derived from any one of SEQ ID NOs: 19-36 and 271-273. The antibody region may comprise an amino acid sequence comprising 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 450, 500 or more amino acids based on or derived from any one of SEQ ID NOs: 19-36 and 271-273. The antibody region may comprise an amino acid sequence comprising 10 or more amino acids based on or derived from any one of SEQ ID NOs: 19-36 and 271-273. The antibody region may comprise an amino acid sequence comprising 50 or more amino acids based on or derived from any one of SEQ ID NOs: 19-36 and 271-273. The antibody region may comprise an amino acid sequence comprising 100 or more amino acids based on or derived from any one of SEQ ID NOs: 19-36 and 271-273. The antibody region may comprise an amino acid sequence comprising 200 or more amino acids based on or derived from any one of SEQ ID NOs: 19-36 and 271-273. The amino acids may be consecutive. Alternatively, or additionally, the amino acids are nonconsecutive. In some embodiments, the antibody region may comprise amino acids derived from any one of SEQ ID NOs: 19-36 and 271-273 and amino acids not derived from any one of SEQ ID NOs: 19-36 and 271-273. In some embodiments, the antibody region may comprise amino acids derived from one or more of SEQ ID NOs: 19-36 and 271-273 and amino acids not derived from any one of SEQ ID NOs: 19-36 and 271-273. In some embodiments, the antibody region comprises amino acids derived from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more of SEQ ID NOs: 19-36 and 271-273.

[00241] The antibody region may be encoded by a nucleotide sequence that is based on or derived from any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence that is at least about 50% homologous to any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence that is at least about 60%, 65%, 70%, 75%,

80%, 85%, 90%, 95%, or 97% homologous to any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence that is at least about 70% homologous to any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence that is at least about 80% homologous to any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence that is at least about 50% identical to any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% identical to any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence that is at least about 70% identical to any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence that is at least about 80% identical to any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence that is 100% identical to any one of SEQ ID NOs: 1-18 and 268-270.

[00242] The antibody region may be encoded by a nucleotide sequence comprising 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence comprising 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence comprising 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence comprising 1100, 1200, 1300, 1400, 1500 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence comprising 100 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence comprising 500 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence comprising 1000 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-18 and 268-270. The antibody region may be encoded by a nucleotide sequence comprising 1300 or more nucleotides based on or derived from any one of SEQ ID NOs: 1-18 and 268-270. The nucleotides may be consecutive. In some embodiments, the antibody region is encoded by a nucleotide sequence comprising nucleotides derived from any one of SEQ ID NOs: 1-18 and 268-270 and nucleotides not derived from any one of SEQ ID NOs: 1-18 and 268-270. In some embodiments, the antibody region is encoded by a nucleotide sequence comprising nucleotides derived from one or more of SEQ ID NOs: 1-18 and 268-270 and nucleotides not derived from any one of SEQ ID NOs: 1-18 and 268-270. In some embodiments, the antibody region is encoded by a nucleotide sequence derived from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more of SEQ ID NOs: 1-18 and 268-270.

[00243] Non-antibody region

[00244] The immunoglobulin fusion proteins disclosed herein may comprise one or more non-antibody regions. The immunoglobulin fusion proteins disclosed herein may comprise two or more non-antibody regions. The immunoglobulin fusion proteins disclosed herein may comprise 3, 4, 5, 6, 7, 8, 9, 10 or more non-antibody regions.

[00245] The two or more non-antibody regions may be attached to one or more antibody regions. The two or more non-antibody regions may be attached to two or more antibody regions. The two or more non-antibody regions may be attached to one or more immunoglobulin chains. The two or more non-antibody regions may be attached to two or more immunoglobulin chains. The two or more non-antibody regions may be attached to one or more subunits within the one or more antibody regions. The two or more non-antibody regions may be attached to two or more subunits within the one or more antibody regions.

[00246] The non-antibody regions may comprise one or more therapeutic agents. The non-antibody regions may comprise two or more therapeutic agents. The non-antibody regions may comprise 3, 4, 5, 6, 7 or more therapeutic agents. The therapeutic agents may be different. The therapeutic agents may be the same.

[00247] The non-antibody regions may comprise one or more extender peptides. The non-antibody regions may comprise two or more extender peptides. The non-antibody regions may comprise 3, 4, 5, 6, 7 or more extender peptides. The extender peptides may be different. The extender peptides may be the same. In some embodiments, the extender peptide comprises an amino acid sequence having an alpha helical secondary structure. In some embodiments, the extender peptide does not comprise amino acids having a beta strand secondary structure. In some embodiments, the extender fusion region comprises two extender peptides, wherein the two extender peptides are configured to form a coiled coil. In some instances, the non-antibody region does not comprise an extender peptide. The extender peptide may directly connect a therapeutic peptide to an antibody region.

[00248] The non-antibody regions may comprise one or more linkers. The non-antibody regions may comprise two or more linkers. The non-antibody regions may comprise 3, 4, 5, 6, 7 or more linkers. The linkers may be different. The linkers may be the same. The linker may directly connect the therapeutic agent to the antibody region. The linker may connect the therapeutic peptide to an extender peptide. In some instances, the non-antibody region does not comprise a linker. In some embodiments, the linker peptide does not comprise amino acids having alpha helical or beta strand secondary structure.

[00249] The non-antibody regions may comprise one or more protease cleavage sites. The non-antibody regions may comprise two or more protease cleavage sites. The cleavage sites may be different. The cleavage sites may be the same. The cleavage site may be directly connect the therapeutic agent to the antibody region. The cleavage site may connect the therapeutic agent to a linker peptide. The cleavage site may connect the therapeutic agent to an extender peptide. In some embodiments, the therapeutic agent comprises a protease cleavage site. In some instances, the non-antibody region does not comprise a protease cleavage site.

[00250] The extender fusion regions may comprise one or more connecting peptides. A connecting peptide may comprise an extender peptide. A connecting peptide may comprise a linker peptide. A connecting peptide may comprise a protease cleavage site. A connecting peptide may comprise any sequence of amino acids which are configured for connecting a therapeutic agent to an antibody region.

[00251] The non-antibody region may be inserted into the antibody region. Insertion of the non-antibody region into the antibody region may comprise removal or deletion of a portion of the antibody from which the antibody region is based on or derived from. The non-antibody region may replace at least a portion of a heavy chain. The non-antibody region may replace at least a portion of a light chain. The non-antibody region may replace at least a portion of a V region. The non-antibody region may replace at least a portion of a D region. The non-antibody region may replace at least a portion of a J region. The non-antibody region may replace at least a portion of a variable region. The non-antibody region may replace at least a portion of a constant region. The non-antibody region may replace at least a portion of a complementarity determining region (CDR). The non-antibody region may replace at least a portion of a CDR1. The non-antibody region may replace at least a portion of a CDR2. The non-antibody region may replace at least a portion of a CDR3. The non-antibody region may replace at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more of the antibody or portion thereof. For example, the non-antibody region may replace at least about 50% of a CDR. The non-antibody region may replace at least about 70% of a CDR. The non-antibody region may replace at least about 80% of a CDR. The non-antibody region may replace at least about 90% of a CDR. The non-antibody region may replace at least about 95% of a CDR.

[00252] Non-antibody regions may comprise (a) one or more extender peptides; (b) one or more therapeutic agents; (c) optionally, one or more linkers; and (d) optionally, one or more proteolytic cleavage sites. In some embodiments, the one or more extender peptides comprise amino acid sequences having alpha helical secondary structures. In some instances, an immunoglobulin fusion protein comprising an antibody region and a non-antibody region, wherein the non-antibody region comprises one or more extender peptides comprising amino acids having alpha helical secondary structures, is referred to as a coil immunoglobulin fusion protein.

[00253] Non-antibody regions may comprise (a) one or more linker peptides; (b) one or more therapeutic agents; and (c) optionally, one or more proteolytic cleavage sites. In some embodiments, the one or more linker peptides do not comprise amino acid sequences having alpha helical or beta strand secondary structures. In some instances, an immunoglobulin fusion protein comprising an antibody region and a non-antibody region, wherein the one or more linker peptides do not comprise amino acid sequences having alpha helical or beta strand secondary structure, is referred to as a direct immunoglobulin fusion protein.

[00254] In some embodiments, a non-antibody region is an extender fusion region.

[00255] Extender fusion region

[00256] The immunoglobulin fusion proteins disclosed herein may comprise one or more extender fusion regions. The immunoglobulin fusion proteins may comprise two or more extender fusion regions. The immunoglobulin fusion proteins may comprise 3, 4, 5, 6, 7, 8, 9, 10 or more extender fusion regions.

[00257] The two or more extender fusion regions may be attached to one or more antibody regions. The two or more extender fusion regions may be attached to two or more antibody regions. The two or more extender fusion regions may be attached to one or more immunoglobulin chains. The two or more

extender fusion regions may be attached to two or more immunoglobulin chains. The two or more extender fusion regions may be attached to one or more subunits within the one or more antibody regions. The two or more extender fusion regions may be attached to two or more subunits within the one or more antibody regions.

[00258] The extender fusion regions may comprise one or more extender peptides. The extender fusion regions may comprise two or more extender peptides. The extender fusion regions may comprise 3, 4, 5, 6 or more extender peptides. The extender peptides may be different. The extender peptides may be the same. In some embodiments, the extender peptide comprises an amino acid sequence having an alpha helical secondary structure. In some embodiments, the extender peptide does not comprise amino acids having a beta strand secondary structure. In some embodiments, the extender fusion region comprises two extender peptides, wherein the two extender peptides are configured to form a coiled coil. In some instances, the extender fusion region does not comprise an extender peptide. In some embodiments, the extender peptide directly connects a therapeutic agent to an antibody region.

[00259] The extender fusion regions may comprise one or more therapeutic agents. The extender fusion regions may comprise two or more therapeutic agents. The extender fusion regions may comprise 3, 4, 5, 6, 7 or more therapeutic agents. The therapeutic agents may be different. The therapeutic agents may be the same.

[00260] The extender fusion regions may comprise one or more linkers. The extender fusion regions may comprise two or more linkers. The extender fusion regions may comprise 3, 4, 5, 6, 7 or more linkers. The linkers may be different. The linkers may be the same. The linker may connect a therapeutic agent to an extender peptide. The linker may connect a therapeutic agent directly to an antibody region. In some instances, the extender fusion region does not comprise a linker. In some embodiments, the linker peptide does not comprise amino acids having alpha helical or beta strand secondary structure.

[00261] The extender fusion regions may comprise one or more protease cleavage sites. The extender fusion regions may comprise two or more protease cleavage sites. The cleavage sites may be different. The cleavage sites may be the same. The cleavage site may be directly connect the therapeutic agent to the antibody region. The cleavage site may connect a therapeutic agent to an extender peptide. The cleavage site may connect a therapeutic agent to a linker peptide. In some instances, the extender fusion region does not comprise a protease cleavage site.

[00262] The extender fusion regions may comprise one or more connecting peptides. A connecting peptide may comprise an extender peptide. A connecting peptide may comprise a linker peptide. A connecting peptide may comprise a protease cleavage site. A connecting peptide may comprise any sequence of amino acids which are configured for connecting a therapeutic agent to an antibody region.

[00263] The immunoglobulin fusion proteins disclosed herein may comprise an antibody region attached to an extender fusion region. The extender fusion region may be attached to the N-terminus, C-terminus, or N- and C-terminus of the antibody region. The antibody region may be directly attached to the extender fusion region. Alternatively, or additionally, the antibody region may be indirectly attached to the non-antibody sequence. Attachment of the extender fusion region to the antibody region may

comprise covalent attachment. Attachment may comprise fusion of the extender fusion region to the antibody region. Attachment may comprise chemical conjugation.

[00264] Alternatively, or additionally, attachment comprises insertion of the extender fusion region into the antibody region. The extender fusion region may be inserted into a heavy chain of the antibody region. The extender fusion region may be inserted into a light chain of the antibody region. The extender fusion region may be inserted into a variable domain of the antibody region. The extender fusion region may be inserted into a constant domain of the antibody region. The extender fusion region may be inserted into a complementarity-determining region (CDR) of the antibody region.

[00265] The extender fusion region may replace at least a portion of an antibody from which the antibody region is based on or derived. The extender fusion region may replace at least a portion of a heavy chain of an antibody from which the antibody region may be based on or derived. The extender fusion region may replace at least a portion a light chain of an antibody from which the antibody region may be based on or derived. The extender fusion region may replace at least a portion of a variable domain of an antibody from which the antibody region may be based on or derived. The extender fusion region may replace at least a portion of a variable domain of an antibody from which the antibody region may be based on or derived. The extender fusion region may replace at least a portion of a complementarity-determining region (CDR) of an antibody from which the antibody region may be based on or derived. The extender fusion region may replace at least a portion of a CDR1, CDR2, CDR3, or a combination thereof of an antibody from which the antibody or fragment thereof may be based on or derived. The extender fusion region may replace at least a portion of a CDR3 of an antibody from which the antibody region may be based on or derived.

[00266] The extender fusion region may replace at least about 1, 2, 3, 4, 5, 6, 7, 8, 9 or more amino acids of an antibody from which the antibody region is based on or derived. The extender fusion region may replace at least about 1 or more amino acids of an antibody from which the antibody region is based on or derived. The extender fusion region may replace at least about 3 or more amino acids of an antibody from which the antibody region is based on or derived. The extender fusion region may replace at least about 5 or more amino acids of an antibody from which the antibody region is based on or derived.

[00267] The extender fusion region may comprise at least about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acids. The extender fusion region may comprise at least about 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1500, 2000 or more amino acids. The extender fusion region may comprise at least about 10 or more amino acids. The extender fusion region may comprise at least about 25 or more amino acids. The extender fusion region may comprise at least about 50 or more amino acids. The extender fusion region may comprise at least about 75 or more amino acids. The extender fusion region may comprise at least about 100 or more amino acids.

[00268] The extender fusion region may comprise less than about 2000, 1500, 1000, 900, 800, 700, 600, or 500 amino acids. The extender fusion region may comprise less than about 450, 400, 350, 300, 275, 250, 225, 200, 175, 150, 125, 100, 90, 80, 70, 60, 50 amino acids. The extender fusion region may

comprise less than about 400 amino acids. The extender fusion region may comprise less than about 300 amino acids. The extender fusion region may comprise less than about 250 amino acids.

[00269] The extender fusion region may comprise between about 10 to about 1000 amino acids. The extender fusion region may comprise between about 10 to about 500 amino acids. The extender fusion region may comprise between about 10 to about 400 amino acids. The extender fusion region may comprise between about 10 to about 300 amino acids. The extender fusion region may comprise between about 10 to about 250 amino acids. The extender fusion region may comprise between about 20 to about 500 amino acids. The extender fusion region may comprise between about 20 to about 400 amino acids. The extender fusion region may comprise between about 20 to about 300 amino acids.

[00270] Extender fusion regions may comprise (a) one or more extender peptides; (b) one or more therapeutic agents; (c) optionally, one or more linkers; and (d) optionally, one or more proteolytic cleavage sites. Exemplary extender fusion regions are depicted in FIG. 2A-G. For example, as shown in FIG. 2A, an extender fusion region comprises an extender peptide (210) and a therapeutic agent (220). As shown in FIG. 2B, an extender fusion region comprises two extender peptides (210, 230) and a therapeutic agent (220). As shown in FIG. 2C, an extender fusion region comprises an extender peptide (210) and a therapeutic agent (220) connected by a linker (240). As shown in FIG. 2D, an extender fusion region comprises an extender peptide (210), and therapeutic agent (220) flanked by two linkers (240, 250). As shown in FIG. 2E, an extender fusion region comprises an extender peptide (210), a therapeutic agent (220) and a proteolytic cleavage site (260), wherein the proteolytic cleavage site (260) is inserted between the extender peptide and therapeutic agent. As shown on FIG. 2F, an extender fusion region comprises two extender peptides (210, 230), two linkers (240, 250) and a therapeutic agent (220). As shown on FIG. 2G, an extender fusion region comprises two extender peptides (210, 230), two linkers (240, 250), a proteolytic cleavage site (260) and a therapeutic agent (220).

[00271] The extender fusion regions may comprise (a) a first extender peptide, wherein the first extender peptide comprises (i) an amino acid sequence comprising an alpha helix secondary structure; and (ii) 7 or fewer amino acids based on or derived from an ultralong CDR3; and (b) a therapeutic agent. The extender fusion regions may further comprise one or more additional extender peptides comprising at least one secondary structure. The extender fusion regions may further comprise one or more linkers. The extender fusion regions may further comprise one or more proteolytic cleavage sites.

[00272] The extender fusion regions may comprise (a) a first extender peptide, wherein the first extender peptide comprises (i) an amino acid sequence comprising an alpha helix secondary structure; and (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a first therapeutic agent. The extender fusion regions may further comprise one or more additional extender peptides comprising at least one secondary structure. The extender fusion regions may further comprise one or more linkers. The extender fusion regions may further comprise one or more proteolytic cleavage sites.

[00273] Extender fusion regions may comprise (a) one or more extender peptides; (b) one or more therapeutic agents; (c) optionally, one or more linkers; and (d) optionally, one or more proteolytic cleavage sites. In some embodiments, the one or more extender peptides comprise amino acid sequences

having alpha helical secondary structures. In some instances, an immunoglobulin fusion protein comprising an antibody region and an extender fusion region, wherein the extender fusion region comprises one or more extender peptides comprising amino acids having alpha helical secondary structures, is referred to as a coil immunoglobulin fusion protein.

[00274] Extender fusion regions may comprise (a) one or more linker peptides; (b) one or more therapeutic agents; and (c) optionally, one or more proteolytic cleavage sites. In some embodiments, the one or more linker peptides do not comprise amino acid sequences having alpha helical or beta strand secondary structures. In some instances, an immunoglobulin fusion protein comprising an antibody region and an extender fusion region, wherein the one or more linker peptides do not comprise amino acid sequences having alpha helical or beta strand secondary structure, is referred to as a direct immunoglobulin fusion protein.

[00275] In some embodiments, an extender fusion region does not comprise amino acids based on or derived from an antibody. In some instances, an extender fusion region is a non-antibody region.

[00276] Extender peptide

[00277] The immunoglobulin fusion proteins disclosed herein may comprise one or more extender peptides. The immunoglobulin fusion proteins disclosed herein may comprise two or more extender peptides. The one or more extender peptides may be attached to the N-terminus, C-terminus, or N- and C-terminus of a therapeutic agent. The one or more extender peptides may be attached to each end of a therapeutic agent. The one or more extender peptides may be attached to different ends of a therapeutic agent.

[00278] The extender fusion region of the immunoglobulin fusion proteins disclosed herein may comprise one or more extender peptides. The extender fusion region may comprise 2 or more extender peptides. The extender fusion region may comprise 3 or more extender peptides. The extender fusion region may comprise 4 or more extender peptides. The extender fusion region may comprise 5 or more extender peptides. The extender fusion region may comprise a first extender peptide and a second extender peptide.

[00279] The extender peptide may comprise one or more secondary structures. The extender peptide may comprise two or more secondary structures. The extender peptide may comprise 3, 4, 5, 6, 7 or more secondary structures. The two or more extender peptide may comprise one or more secondary structures. The two or more extender peptides may comprise two or more secondary structures. The two or more extender peptides may comprise 3, 4, 5, 6, 7 or more secondary structures. Each extender peptide may comprise at least one secondary structure. The secondary structures of the two or more extender peptides may be the same. Alternatively, the secondary structures of the two or more extender peptides may be different.

[00280] Alternatively, or additionally, the one or more secondary structures may comprise one or more alpha helices. The extender peptides may comprise two or more alpha helices. For example, the first extender peptide comprises a first alpha helix and the second extender peptide comprises a second alpha helix. The extender peptides may comprise 3, 4, 5, 6, 7 or more alpha helices. The two or more alpha

helices may be anti-parallel. The two or more alpha helices may be parallel. The two or more alpha helices may form one or more coiled coil domains.

[00281] The one or more extender peptides may comprise at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids. The one or more extender peptides may comprise at least about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or more amino acids. The one or more extender peptides may comprise at least about 35, 40, 45, 50, 60, 70, 80, 90, 100 or more amino acids.

[00282] The one or more extender peptides may comprise less than about 100 amino acids. The one or more extender peptides may comprise less than about 95, 90, 85, 80, 75, 70, 65, 60, 55, or 50 amino acids. The one or more extender peptides may comprise less than about 90 amino acids. The one or more extender peptides may comprise less than about 80 amino acids. The one or more extender peptides may comprise less than about 70 amino acids.

[00283] The two or more extender peptides may be the same length. For example, the first extender peptide and the second extender peptide are the same length. Alternatively, the two or more extender peptides are different lengths. In another example, the first extender peptide and the second extender peptide are different lengths. The two or more extender peptides may differ in length by at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acids. The two or more extender peptides may differ in length by at least about 1 or more amino acids. The two or more extender peptides may differ in length by at least about 3 or more amino acids. The two or more extender peptides may differ in length by at least about 5 or more amino acids.

[00284] The extender peptide may be adjacent to an antibody region. The extender peptide may be attached to the N- terminus, C-terminus, or N- and C-terminus of the antibody region. The extender peptide may be adjacent to a non-antibody region. The extender peptide may be attached to the N-terminus, C-terminus, or N- and C-terminus of the non-antibody region. The extender peptide may be adjacent to a therapeutic agent. The extender peptide may be attached to the N-terminus, C-terminus, or N- and C-terminus of the therapeutic agent. The extender peptide may be adjacent to a linker. The extender peptide may be attached to the N-terminus, C-terminus, or N- and C-terminus of the linker. The extender peptide may be adjacent to a proteolytic cleavage site. The extender peptide may be attached to the N-terminus, C-terminus, or N- and C-terminus of the proteolytic cleavage site.

[00285] The extender peptide may connect the therapeutic agent to the antibody region. The extender peptide may be between the antibody region and the therapeutic agent, linker, and/or proteolytic cleavage site. The extender peptide may be between two or more antibody regions, therapeutic agents, linkers, proteolytic cleavage sites or a combination thereof. The extender peptide may be N-terminal to the antibody region, therapeutic agent, the linker, the proteolytic cleavage site, or a combination thereof. The extender peptide may be C-terminal to the antibody region, therapeutic agent, the linker, the proteolytic cleavage site, or a combination thereof.

[00286] The extender peptide may comprise an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 144-175. The extender peptide may comprise an amino acid sequence that is at least about 50% homologous to an amino acid sequence based on or derived from any one of SEQ ID

NOs: 144-175. The extender peptide may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or more homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-175. The extender peptide may comprise an amino acid sequence that is at least about 70% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-175. The extender peptide may comprise an amino acid sequence that is at least about 80% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-175. The extender peptide may comprise an amino acid sequence that is at least about 85% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-175.

[00287] The first extender peptide may comprise an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 144-175. The first extender peptide may comprise an amino acid sequence that is at least about 50% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-175. The first extender peptide may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or more homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-175. The first extender peptide may comprise an amino acid sequence that is at least about 75% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-175. The first extender peptide may comprise an amino acid sequence that is at least about 80% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-175.

[00288] The first extender peptide may comprise an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 144-153. The first extender peptide may comprise an amino acid sequence that is at least about 50% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-153. The first extender peptide may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or more homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-153. The first extender peptide may comprise an amino acid sequence that is at least about 75% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-153. The first extender peptide may comprise an amino acid sequence that is at least about 80% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-153.

[00289] The second extender peptide may comprise an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 144-175. The second extender peptide may comprise an amino acid sequence that is at least about 50% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-175. The second extender peptide may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or more homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-175. The second extender peptide may comprise an amino acid sequence that is at least about 70% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-175. The second extender peptide may comprise an amino acid sequence that is at least about 80% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 144-175.

[00290] The second extender peptide may comprise an amino acid sequence that is based on or derived from any one of SEQ ID NOs: 154-163. The second extender peptide may comprise an amino acid sequence that is at least about 50% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 154-163. The second extender peptide may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or more homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 154-163. The second extender peptide may comprise an amino acid sequence that is at least about 70% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 154-163. The second extender peptide may comprise an amino acid sequence that is at least about 80% homologous to an amino acid sequence based on or derived from any one of SEQ ID NOs: 154-163.

[00291] The extender peptides disclosed herein may be based on or derived from a CDR3. The CDR3 may be an ultralong CDR3. An “ultralong CDR3” or an “ultralong CDR3 sequence”, used interchangeably herein, may comprise a CDR3 that is not derived from a human antibody sequence. An ultralong CDR3 may be 35 amino acids in length or longer, for example, 40 amino acids in length or longer, 45 amino acids in length or longer, 50 amino acids in length or longer, 55 amino acids in length or longer, or 60 amino acids in length or longer. The ultralong CDR3 may be a heavy chain CDR3 (CDR-H3 or CDRH3). The ultralong CDR3 may comprise a sequence derived from or based on a ruminant (*e.g.*, bovine) sequence. An ultralong CDR3 may comprise one or more cysteine motifs. An ultralong CDR3 may comprise at least 3 or more cysteine residues, for example, 4 or more cysteine residues, 6 or more cysteine residues, or 8 or more cysteine residues. Additional details on ultralong CDR3 sequences can be found in Saini SS, et al. (Exceptionally long CDR3H region with multiple cysteine residues in functional bovine IgM antibodies, *European Journal of Immunology*, 1999), Zhang Y, et al. (Functional antibody CDR3 fusion proteins with enhanced pharmacological properties, *Angew Chem Int Ed Engl*, 2013), Wang F, et al. (Reshaping antibody diversity, *Cell*, 2013) and United States Patent Number 6,740,747.

[00292] The extender peptides may comprise 7 or fewer amino acids based on or derived from a CDR. The extender peptides may comprise 6, 5, 4, 3, 2, 1 or fewer amino acids based on or derived from a CDR. The amino acids may be consecutive. The amino acids may be non-consecutive. The CDR may be CDR1. The CDR may be CDR2. The CDR may be CDR3. The CDR may be an ultralong CDR.

[00293] The extender peptides may be based on or derived from a CDR, wherein the CDR is not an ultralong CDR3. The extender peptides may comprise 10 or fewer amino acids based on or derived from a CDR3. The extender peptides may comprise 9, 8, 7, 6, 5, 4, 3, 2, 1 or fewer amino acids based on or derived from a CDR3. The extender peptides may comprise 8 or fewer amino acids based on or derived from a CDR3. The extender peptides may comprise 7 or fewer amino acids based on or derived from a CDR3. The extender peptides may comprise 5 or fewer amino acids based on or derived from a CDR3.

[00294] The extender peptides may comprise an amino acid sequence that is less than about 50% identical to an amino acid sequence comprising an ultralong CDR3. The extender peptides may comprise an amino acid sequence that is less than about 45%, 40%, 35%, 30%, 25%, 20%, 25%, or 10% identical to an amino acid sequence comprising an ultralong CDR3. The extender peptides may comprise an amino

acid sequence that is less than about 30% identical to an amino acid sequence comprising an ultralong CDR3. The extender peptides may comprise an amino acid sequence that is less than about 25% identical to an amino acid sequence comprising an ultralong CDR3. The extender peptides may comprise an amino acid sequence that is less than about 20% identical to an amino acid sequence comprising an ultralong CDR3.

[00295] The extender peptide may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more amino acids attached to or inserted into an ultralong CDR3-based portion of the extender peptide. The extender peptide may comprise 1 or more amino acids attached to or inserted into an ultralong CDR3-based portion of the extender peptide. The extender peptide may comprise 3 or more amino acids attached to or inserted into an ultralong CDR3-based portion of the extender peptide. The extender peptide may comprise 5 or more amino acids attached to or inserted into an ultralong CDR3-based portion of the extender peptide. The two or more amino acids attached to or inserted into the ultralong CDR3 may be contiguous. Alternatively, or additionally, the two or more amino acids attached to or inserted into the ultralong CDR3 are not contiguous.

[00296] The extender peptide may comprise 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10 or fewer amino acids attached to or inserted into an ultralong CDR3-based portion of the extender peptide. The extender peptide may comprise 20 or fewer amino acids attached to or inserted into an ultralong CDR3-based portion of the extender peptide. The extender peptide may comprise 15 or fewer amino acids attached to or inserted into an ultralong CDR3-based portion of the extender peptide. The extender peptide may comprise 10 or fewer amino acids attached to or inserted into an ultralong CDR3-based portion of the extender peptide. The amino acids attached to or inserted into the ultralong CDR3 may be contiguous. Alternatively, or additionally, the amino acids attached to or inserted into the ultralong CDR3 are not contiguous.

[00297] The extender peptide may comprise the sequence $X^1X^2X^3X^4X^5X^6X^7X^8X^9X^{10}X^{11}X^{12}X^{13}X^{14}$ (SEQ ID NO: 144). In some embodiments, a first extender peptide comprises the sequence $X^1X^2X^3X^4X^5X^6X^7X^8X^9X^{10}X^{11}X^{12}X^{13}X^{14}$ (SEQ ID NO: 144). A first extender peptide, in some instances, is located between the amino terminus of a therapeutic agent and an antibody region. X^1 - X^{14} may be independently selected from a positively charged amino acid or a hydrophobic amino acid. X^1 - X^{14} may be independently selected from the group comprising alanine (A), asparagine (N), isoleucine (I) leucine (L), valine (V), glutamine (Q), glutamic acid (E) and lysine (K). X^1 - X^{14} may be independently selected from the group comprising alanine (A), leucine (L) and lysine (K). Alanine may comprise at least about 30% of the total amino acid composition. Alanine may comprise less than about 70% of the total amino acid composition. Leucine may comprise at least about 20% of the total amino acid composition. Leucine may comprise less than about 50% of the total amino acid composition. Lysine may comprise at least about 20% of the total amino acid composition. Lysine may comprise less than about 50% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 50% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 60% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 70% of the total

amino acid composition. The hydrophobic amino acids may comprise less than about 90% of the total amino acid composition.

[00298] The extender peptide may comprises the sequence $(X^1X^2X^3X^4X^5X^6X^7)_n$ (SEQ ID NO. 145). In some embodiments, a first extender peptide comprises the sequence $(X^1X^2X^3X^4X^5X^6X^7)_n$ (SEQ ID NO. 145). A first extender peptide, in some instances, is located between the amino terminus of a therapeutic agent and an antibody region. In some embodiments, n is between about 1 and about 10. In some embodiments, n is between about 1 and about 5. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. N may be from about 1 to about 3. X^1 - X^7 may be independently selected from a positively charged amino acid or a hydrophobic amino acid. X^1 - X^7 may be independently selected from the group comprising alanine (A), asparagine (N), isoleucine, (I), leucine (L), valine (V), glutamine (Q), glutamic acid (E) and lysine (K). Alanine (A) may comprise at least about 30% of the total amino acid composition. Alanine (A) may comprise less than about 70% of the total amino acid composition. Leucine may comprise at least about 20% of the total amino acid composition. Leucine may comprise less than about 50% of the total amino acid composition. Lysine may comprise at least about 20% of the total amino acid composition. Lysine may comprise less than about 50% of the total amino acid composition. Asparagine may comprise about 50% of the total amino acid composition. Isoleucine may comprise about 50% of the total amino acid composition. Valine may comprise about 50% of the total amino acid composition. Glutamine may comprise about 50% of the total amino acid composition. Glutamic acid may comprise about 50% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 50% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 60% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 70% of the total amino acid composition. The hydrophobic amino acids may comprise less than about 90% of the total amino acid composition.

[00299] The extender peptide may comprise the sequence $X^aX^bX^cX^d(X^1X^2X^3X^4X^5X^6X^7)_n$ (SEQ ID NO: 148). In some embodiments, a first extender peptide comprises the sequence $X^aX^bX^cX^d(X^1X^2X^3X^4X^5X^6X^7)_n$ (SEQ ID NO: 146). A first extender peptide, in some instances, is located between the amino terminus of a therapeutic agent and an antibody region. In some embodiments, n is between about 1 and about 10. In some embodiments, n is between about 1 and about 5. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. N may be from about 1 to about 3. X^a , X^b and X^d may be independently selected from a hydrophobic amino acid. X^c may be a polar, uncharged amino acid. X^a , X^b and X^d may be the same amino acid. X^a , X^b and X^d may be different amino acids. X^1 - X^7 may be independently selected from a positively charged amino acid or a hydrophobic amino acid. X^1 - X^7 may be independently selected from the group comprising alanine (A), asparagine (N), isoleucine, (I), leucine (L), valine (V), glutamine (Q), glutamic acid (E) and lysine (K). X^1 - X^7 may be independently selected from the group comprising A, L and K. A may comprise at least about 30% of the total amino acid composition. A may comprise less than about 70% of the total amino acid composition. L may comprise at least about 20% of the total amino acid composition. L may comprise less than about 50% of the total amino acid composition. K may comprise at least about 20% of the total amino acid composition. K may

comprise less than about 50% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 50% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 60% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 70% of the total amino acid composition. The hydrophobic amino acids may comprise less than about 90% of the total amino acid composition. In some embodiments, X^a is glycine (G). In some embodiments, X^b is G. In some embodiments, X^d is glycine. X^a , X^b and X^d may be glycine (G). X^c may be serine (S).

[00300] The extender peptide may comprise the sequence $X^aX^bX^cX^d(AKLAALK)_n$ (SEQ ID NO. 147). In some embodiments, a first extender peptide comprises the sequence $X^aX^bX^cX^d(AKLAALK)_n$ (SEQ ID NO. 147). A first extender peptide, in some instances, is located between the amino terminus of a therapeutic agent and an antibody region. In some embodiments, n is between about 1 and about 10. In some embodiments, n is between about 1 and about 5. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. N may be from about 1 to about 3. X^a , X^b and X^d may be independently selected from a hydrophobic amino acid. X^c may be a polar, uncharged amino acid. X^a , X^b and X^d may be the same amino acid. X^a , X^b and X^d may be different amino acids. In some embodiments, X^a is glycine (G). In some embodiments, X^b is glycine. In some embodiments, X^d is glycine. X^a , X^b and X^d may be glycine (G). X^c may be serine (S).

[00301] The extender peptide may comprise the sequence $(AKLAALK)_n$ (SEQ ID NO. 148). In some embodiments, a first extender peptide comprises the sequence $(AKLAALK)_n$ (SEQ ID NO. 148). A first extender peptide, in some instances, is located between the amino terminus of a therapeutic agent and an antibody region. In some embodiments, n is between about 1 and about 10. In some embodiments, n is between about 1 and about 5. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. N may be from about 1 to about 3.

[00302] The extender peptide may comprise the sequence $GGSG(AKLAALK)_n$ (SEQ ID NO: 149). In some embodiments, a first extender peptide comprises the sequence $GGSG(AKLAALK)_n$ (SEQ ID NO: 149). A first extender peptide, in some instances, is located between the amino terminus of a therapeutic agent and an antibody region. In some embodiments, n is between about 1 and about 10. In some embodiments, n is between about 1 and about 5. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. N may be from about 1 to about 3.

[00303] The extender peptide may comprise the sequence $X^1X^2X^3X^4X^5X^6X^7X^8X^9X^{10}X^{11}X^{12}X^{13}X^{14}$ (SEQ ID NO: 154); wherein in X^1 - X^{14} are independently selected from a negatively charged amino acid or a hydrophobic amino acid. In some embodiments, a second extender peptide comprises the sequence $X^1X^2X^3X^4X^5X^6X^7X^8X^9X^{10}X^{11}X^{12}X^{13}X^{14}$ (SEQ ID NO: 154); wherein in X^1 - X^{14} are independently selected from a negatively charged amino acid or a hydrophobic amino acid. A second extender peptide, in some instances, is located between the carboxyl terminus of a therapeutic agent and an antibody region. In some embodiments, n is between about 1 and about 10. In some embodiments, n is between about 1 and about 5. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. N may be from about 1 to about 3. In some embodiments, X^1 - X^{14} are independently selected from the group comprising alanine (A),

leucine (L) and glutamic acid (E). In one embodiment, A comprises at least about 30% of the total amino acid composition. In one embodiment, A comprises less than about 70% of the total amino acid composition. In one embodiment, L comprises at least about 20% of the total amino acid composition. In one embodiment, L comprises less than about 50% of the total amino acid composition. In one embodiment, E comprises at least about 20% of the total amino acid composition. In one embodiment, E comprises less than about 50% of the total amino acid composition. In one embodiment, the hydrophobic amino acids comprises at least about 50% of the total amino acid composition. In one embodiment, the hydrophobic amino acids comprises at least about 60% of the total amino acid composition. In one embodiment, the hydrophobic amino acids comprises at least about 70% of the total amino acid composition. In one embodiment, the hydrophobic amino acids comprises less than about 90% of the total amino acid composition.

[00304] The second extender peptide may comprise the sequence $(X^1X^2X^3X^4X^5X^6X^7)_n$ (SEQ ID NO: 155). In some embodiments, a second extender peptide comprises the sequence $(X^1X^2X^3X^4X^5X^6X^7)_n$ (SEQ ID NO: 155). A second extender peptide, in some instances, is located between the carboxyl terminus of a therapeutic agent and an antibody region. In some embodiments, n is between about 1 and about 10. In some embodiments, n is between about 1 and about 5. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. N may be from about 1 to about 3. X^1 - X^7 may be independently selected from a positively charged amino acid or a hydrophobic amino acid. X^1 - X^7 may be independently selected from the group comprising alanine (A), asparagine (N), isoleucine, (I), leucine (L), valine (V), glutamine (Q), glutamic acid (E) and lysine (K). Alanine (A) may comprise at least about 30% of the total amino acid composition. Alanine (A) may comprise less than about 70% of the total amino acid composition. Leucine may comprise at least about 20% of the total amino acid composition. Leucine may comprise less than about 50% of the total amino acid composition. Lysine may comprise at least about 20% of the total amino acid composition. Lysine may comprise less than about 50% of the total amino acid composition. Asparagine may comprise about 50% of the total amino acid composition. Isoleucine may comprise about 50% of the total amino acid composition. Valine may comprise about 50% of the total amino acid composition. Glutamine may comprise about 50% of the total amino acid composition. Glutamic acid may comprise about 50% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 50% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 60% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 70% of the total amino acid composition. The hydrophobic amino acids may comprise less than about 90% of the total amino acid composition.

[00305] The extender peptide may comprise the sequence $(X^1X^2X^3X^4X^5X^6X^7)_n X^aX^bX^cX^d$ (SEQ ID NO: 158). In some embodiments, a second extender peptide comprises the sequence $(X^1X^2X^3X^4X^5X^6X^7)_n X^aX^bX^cX^d$ (SEQ ID NO: 156). A second extender peptide, in some instances, is located between the carboxyl terminus of a therapeutic agent and an antibody region. In some embodiments, n is between about 1 and about 10. In some embodiments, n is between about 1 and about 5. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. N may be from about 1 to about 3. X^1 - X^7 may be independently

selected from a positively charged amino acid or a hydrophobic amino acid. X^1 - X^7 may be independently selected from the group comprising alanine (A), leucine (L) and lysine (K). A may comprise at least about 30% of the total amino acid composition. A may comprise less than about 70% of the total amino acid composition. L may comprise at least about 20% of the total amino acid composition. L may comprise less than about 50% of the total amino acid composition. K may comprise at least about 20% of the total amino acid composition. K may comprise less than about 50% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 50% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 60% of the total amino acid composition. The hydrophobic amino acids may comprise at least about 70% of the total amino acid composition. The hydrophobic amino acids may comprise less than about 90% of the total amino acid composition. X^a , X^b and X^d may be independently selected from a hydrophobic amino acid. X^c may be a polar, uncharged amino acid. X^a , X^b and X^d may be the same amino acid. X^a , X^b and X^d may be different amino acids. In some embodiments, X^a is glycine (G). In some embodiments, X^b is glycine. In some embodiments, X^d is glycine. X^a , X^b and X^d may be glycine (G). X^c may be serine (S).

[00306] The extender peptide may comprise the sequence (ELAALEA)_n $X^aX^bX^cX^d$ (SEQ ID NO: 157). In some embodiments, a second extender peptide comprises the sequence (ELAALEA)_n $X^aX^bX^cX^d$ (SEQ ID NO: 157). A second extender peptide, in some instances, is located between the carboxyl terminus of a therapeutic agent and an antibody region. In some embodiments, n is between about 1 and about 10. In some embodiments, n is between about 1 and about 5. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. N may be from about 1 to about 3. X^a , X^b and X^d may be independently selected from a hydrophobic amino acid. X^c may be a polar, uncharged amino acid. X^a , X^b and X^d may be the same amino acid. X^a , X^b and X^d may be different amino acids. In some embodiments, X^a is glycine (G). In some embodiments, X^b is glycine. In some embodiments, X^d is glycine. X^a , X^b and X^d may be glycine (G). X^c may be serine (S).

[00307] The extender peptide may comprise the sequence (ELAALEA)_n (SEQ ID NO: 158). In some embodiments, a second extender peptide comprises the sequence (ELAALEA)_n (SEQ ID NO: 158). A second extender peptide, in some instances, is located between the carboxyl terminus of a therapeutic agent and an antibody region. In some embodiments, n is between about 1 and about 10. In some embodiments, n is between about 1 and about 5. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. N may be from about 1 to about 3.

[00308] The extender peptide may comprise the sequence (ELAALEA)_nGGSG (SEQ ID NO: 159). In some embodiments, a second extender peptide comprises the sequence (ELAALEA)_nGGSG (SEQ ID NO: 159). A second extender peptide, in some instances, is located between the carboxyl terminus of a therapeutic agent and an antibody region. In some embodiments, n is between about 1 and about 10. In some embodiments, n is between about 1 and about 5. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. N may be from about 1 to about 3.

[00309] The immunoglobulin fusion protein may comprise (a) a first extender peptide comprising an amino acid sequence based on or derived from SEQ ID NO: 151; and (b) a second extender peptide

comprising an amino acid sequence based on or derived from SEQ ID NO: 161. The immunoglobulin fusion protein may comprise (a) a first extender peptide comprising an amino acid sequence that is at least about 50% homologous to an amino acid sequence of SEQ ID NO: 151; and (b) a second extender peptide comprising an amino acid sequence that is at least about 50% homologous to an amino acid sequence of SEQ ID NO: 161. The first extender peptide may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more homologous to an amino acid sequence of SEQ ID NO: 151. The second extender peptide may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more homologous to an amino acid sequence of SEQ ID NO: 161. The first extender peptide may comprise an amino acid sequencing comprising 3, 4, 5, 6, 7 or more amino acids based on or derived from an amino acid sequence of SEQ ID NO: 151. The first extender peptide may comprise an amino acid sequencing comprising 5 or more amino acids based on or derived from an amino acid sequence of SEQ ID NO: 151. The second extender peptide may comprise an amino acid sequencing comprising 3, 4, 5, 6, 7 or more amino acids based on or derived from an amino acid sequence of SEQ ID NO: 161. The second extender peptide may comprise an amino acid sequencing comprising 5 or more amino acids based on or derived from an amino acid sequence of SEQ ID NO: 161.

[00310] The aliphatic amino acids may comprise at least about 20% of the total amino acids of the extender peptides. The aliphatic amino acids may comprise at least about 22%, 25%, 27%, 30%, 32%, 35%, 37%, 40%, 42%, 45% or more of the total amino acids of the extender peptides. The aliphatic amino acids may comprise at least about 22% of the total amino acids of the extender peptides. The aliphatic amino acids may comprise at least about 27% of the total amino acids of the extender peptides.

[00311] The aliphatic amino acids may comprise less than about 50% of the total amino acids of the extender peptides. The aliphatic amino acids may comprise less than about 47%, 45%, 43%, 40%, 38%, 35%, 33% or 30% of the total amino acids of the extender peptides.

[00312] The aliphatic amino acids may comprise between about 20% to about 45% of the total amino acids of the extender peptides. The aliphatic amino acids may comprise between about 23% to about 45% of the total amino acids of the extender peptides. The aliphatic amino acids may comprise between about 23% to about 40% of the total amino acids of the extender peptides.

[00313] The aromatic amino acids may comprise less than about 20% of the total amino acids of the extender peptides. The aromatic amino acids may comprise less than about 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11% or 10% of the total amino acids of the extender peptides. The aromatic amino acids may comprise between 0% to about 20% of the total amino acids of the extender peptides.

[00314] The non-polar amino acids may comprise at least about 30% of the total amino acids of the extender peptides. The non-polar amino acids may comprise at least about 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, or 40% of the total amino acids of the extender peptides. The non-polar amino acids may comprise at least about 32% of the total amino acids of the extender peptides.

[00315] The non-polar amino acids may comprise less than about 80% of the total amino acids of the extender peptides. The non-polar amino acids may comprise less than about 77%, 75%, 72%, 70%, 69%, or 68% of the total amino acids of the extender peptides.

[00316] The non-polar amino acids may comprise between about 35% to about 80% of the total amino acids of the extender peptides. The non-polar amino acids may comprise between about 38% to about 80% of the total amino acids of the extender peptides. The non-polar amino acids may comprise between about 38% to about 75% of the total amino acids of the extender peptides. The non-polar amino acids may comprise between about 35% to about 70% of the total amino acids of the extender peptides.

[00317] The polar amino acids may comprise at least about 20% of the total amino acids of the extender peptides. The polar amino acids may comprise at least about 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 35% or more of the total amino acids of the extender peptides. The polar amino acids may comprise at least about 23% of the total amino acids of the extender peptides.

[00318] The polar amino acids may comprise less than about 80% of the total amino acids of the extender peptides. The polar amino acids may comprise less than about 77%, 75%, 72%, 70%, 69%, or 68% of the total amino acids of the extender peptides. The polar amino acids may comprise less than about 77% of the total amino acids of the extender peptides. The polar amino acids may comprise less than about 75% of the total amino acids of the extender peptides. The polar amino acids may comprise less than about 72% of the total amino acids of the extender peptides.

[00319] The polar amino acids may comprise between about 25% to about 70% of the total amino acids of the extender peptides. The polar amino acids may comprise between about 27% to about 70% of the total amino acids of the extender peptides. The polar amino acids may comprise between about 30% to about 70% of the total amino acids of the extender peptides.

[00320] Alternatively, the immunoglobulin fusion proteins disclosed herein do not comprise an extender peptide.

[00321] Therapeutic agent

[00322] The immunoglobulin fusion proteins disclosed herein may comprise one or more therapeutic agents. The therapeutic agent may be a peptide. The therapeutic agent may be a small molecule. The immunoglobulin fusion proteins disclosed herein may comprise two or more therapeutic agents. The immunoglobulin fusion proteins disclosed herein may comprise 3, 4, 5, 6 or more therapeutic agents. The two or more therapeutic agents may be the same. The two or more therapeutic agents may be different.

[00323] The therapeutic agent may comprise any secondary structure, for example alpha helix or beta strand or comprise no regular secondary structure. The therapeutic agent may comprise amino acids with one or more modifications including, but not limited to, myristoylation, palmitoylation, isoprenylation, glypiation, lipoylation, acylation, acetylation, alkylolation, methylation, glycosylation, malonylation, hydroxylation, iodination, nucleotide addition, oxidation, phosphorylation, adenylation, propionylation, succinylation, sulfation, selenoylation, biotinylation, pegylation, deimination, deamidation, eliminolysis, and carbamylation. The therapeutic agent may comprise one or more amino acids conjugated to one or more small molecules, for example a drug. In some embodiments, the therapeutic agent comprises one or more non-natural amino acids. In some embodiments, the therapeutic agent comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50 or more non-natural amino acids. In some embodiments, the therapeutic agent comprises one or more amino acids substitutions. In some

embodiments, the therapeutic agent comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50 or more amino acid substitutions.

[00324] The therapeutic agent may be inserted into the immunoglobulin region. Insertion of the therapeutic agent into the immunoglobulin region may comprise removal or deletion of a portion of the immunoglobulin from which the immunoglobulin region is based on or derived from. The therapeutic agent may replace at least a portion of a heavy chain. The therapeutic agent may replace at least a portion of a light chain. The therapeutic agent may replace at least a portion of a variable domain. The therapeutic agent may replace at least a portion of a constant domain. The therapeutic agent may replace at least a portion of a complementarity determining region (CDR). The therapeutic agent may replace at least a portion of a CDR1. The therapeutic agent may replace at least a portion of a CDR2. The therapeutic agent may replace at least a portion of a CDR3. The therapeutic agent may replace at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more of the immunoglobulin or a portion thereof.

[00325] The one or more therapeutic agents may be based on or derived from a protein. The protein may be a growth factor, cytokine, hormone or toxin. The growth factor may be GCSF, GMCSF, GDF11 or FGF21. The GCSF may be a bovine GCSF. The GCSF may be a human GCSF. The GMCSF may be a bovine GMCSF or a human GMCSF. The FGF21 may be a bovine FGF21. The FGF21 may be a human FGF21. The protein may be elafin. The protein may be a peptidase inhibitor. The protein may be a skin-derived antileukoprotease (SKALP).

[00326] The cytokine may be an interferon or interleukin. The cytokine may be stromal cell-derived factor 1 (*SDF-1*). The interferon may be interferon-beta. The interferon may be interferon-alpha. The interleukin may be interleukin 11 (IL-11). The interleukin may be interleukin 8 (IL-8) or interleukin 21 (IL-21).

[00327] The hormone may be exendin-4, GLP-1, relaxin, oxyntomodulin, hLeptin, betatrophin, bovine growth hormone (bGH), human growth hormone (hGH), erythropoietin (EPO), or parathyroid hormone. The hormone may be somatostatin. The parathyroid hormone may be a human parathyroid hormone. The erythropoietin may be a human erythropoietin.

[00328] The toxin may be Moka1, VM24 or Mamba1. The toxin may be ziconotide or chlorotoxin. In one embodiment, the toxin is mu-SLPTX-Ssm6a (Ssam6).

[00329] The protein may be angiopoietin-like 3 (ANGPTL3). The angiopoietin-like 3 may be a human angiopoietin-like 3.

[00330] The therapeutic agent may be glucagon-like peptide 2 (GLP2).

[00331] In some embodiments, the therapeutic agent is a glucagon analog.

[00332] In some embodiments, the therapeutic agent is a dual agonist.

[00333] In some embodiments, one or more regions of the therapeutic agent is configured to treat diabetes and/or diabetes related conditions. In some embodiments, 2, 3, 4, 5 or more regions of the therapeutic agent are configured to treat diabetes and/or diabetes related conditions. Diabetes may include, type I diabetes, type 2 diabetes, gestational diabetes, and prediabetes. In some embodiments, one

or more regions of the therapeutic agent is configured to treat obesity and/or obesity related conditions. In some embodiments, 2, 3, 4, 5 or more regions of the therapeutic agent are configured to treat obesity and/or obesity related conditions. Conditions may include complications and diseases. Examples of diabetes related conditions include, but are not limited to, diabetic retinopathy, diabetic nephropathy, diabetic heart disease, diabetic foot disorders, diabetic neuropathy, macrovascular disease, diabetic cardiomyopathy, infection and diabetic ketoacidosis. Diabetic neuropathy may include, but is not limited to symmetric polyneuropathy, autonomic neuropathy, radiculopathy, cranial neuropathy, and mononeuropathy. Obesity related conditions include, but are not limited to, heart disease, stroke, high blood pressure, diabetes, osteoarthritis, gout, sleep apnea, asthma, gallbladder disease, gallstones, abnormal blood fats (e.g., abnormal levels of LDL and HDL cholesterol), obesity hypoventilation syndrome, reproductive problems, hepatic steatosis, and mental health conditions.

[00334] In some embodiments, one or more regions of the therapeutic agent is a glucagon-like protein-1 (GLP-1) receptor agonist or formulation thereof. In some embodiments, one or more regions of the therapeutic agent is an incretin mimetic. In some embodiments, one or more regions of the therapeutic agent comprises an amino acid sequence based on or derived from an amino acid sequence of exendin-4, exenatide, or synthetic thereof. In some embodiments, one or more regions of the therapeutic agent is a glucagon analog or formulation thereof. In some embodiments, one or more regions of the therapeutic agent comprises an amino acid sequence based on or derived from an amino acid sequence of insulin. In some embodiments, one or more regions of the therapeutic agent is dual-specific. In some embodiments, the therapeutic agent has specificity for a GLP-1 receptor and a glucagon receptor. In some embodiments, one or more regions of the therapeutic agent comprises an amino acid sequence based on or derived from an amino acid sequence of oxyntomodulin.

[00335] In some embodiments, one or more regions of the therapeutic agent is configured to treat short bowel syndrome and/or short bowel syndrome related conditions. In some embodiments, 2, 3, 4, 5 or more regions of the therapeutic agent are configured to treat short bowel syndrome and/or short bowel syndrome related conditions. Short bowel syndrome related conditions may include, but are not limited to, bacterial overgrowth in the small intestine, metabolic acidosis, gallstones, kidney stones, malnutrition, osteomalacia, intestinal failure, and weight loss. In some embodiments, one or more regions of the therapeutic agent is configured to treat inflammatory bowel disease and/or an inflammatory bowel related conditions. In some embodiments, 2, 3, 4, 5 or more regions of the therapeutic agent are configured to treat inflammatory bowel disease and/or an inflammatory bowel related conditions. Inflammatory bowel disease and/or inflammatory bowel disease related conditions may include, but are not limited to, ulcerative colitis, Crohn's disease, collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behcet's disease, intermediate colitis, anemia, arthritis, pyoderma gangrenosum, primary sclerosing cholangitis, non-thyroidal illness syndrome; and abdominal pain, vomiting, diarrhea, rectal bleeding, internal cramps or muscle spasms, and weight loss in individual with an inflammatory bowel disease.

[00336] In some embodiments, one or more regions of the therapeutic agent comprises an amino acid sequence based on or derived from an amino acid sequence of glucagon, glucagon analog, glucagon like peptide, and/or a glucagon like peptide analog. In some embodiments, one or more regions of the therapeutic agent comprises an amino acid sequence based on or derived from an amino acid sequence of a glucagon like peptide-2 (GLP2).

[00337] In some embodiments, one or more regions of the therapeutic agent is configured to treat an autoimmune disease and/or autoimmune disease related conditions. In some embodiments, 2, 3, 4, 5 or more regions of the therapeutic agent are configured to treat autoimmune disease and/or autoimmune disease related conditions. Autoimmune disease and/or autoimmune disease related conditions may include, but are not limited to, acute disseminated encephalomyelitis, alopecia areata, antiphospholipid syndrome, autoimmune cardiomyopathy, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease, autoimmune lymphoproliferative syndrome, autoimmune peripheral neuropathy, autoimmune pancreatitis, autoimmune polyendocrine syndrome, autoimmune progesterone dermatitis, autoimmune thrombocytopenic purpura, autoimmune urticaria, autoimmune uveitis, Behcet's disease, Celiac disease, cold agglutinin disease, Crohn's disease, dermatomyositis, diabetes mellitus type 1, eosinophilic fasciitis, gastrointestinal pemphigoid, Goodpasture's syndrome, Grave's disease, Guillain-Barre syndrome, Hashimoto's encephalopathy, Hashimoto's thyroiditis, idiopathic thrombocytopenic purpura, lupus erythematosus, Miller-Fisher syndrome, mixed connective tissue disease, multiple sclerosis, myasthenia gravis, narcolepsy, pemphigus vulgaris, pernicious anemia, polymyositis, primary biliary cirrhosis, psoriasis, psoriatic arthritis, relapsing polychondritis, rheumatoid arthritis, rheumatic fever, Sjogren's syndrome, temporal arteritis, transverse myelitis, ulcerative colitis, undifferentiated connective tissue disease, vasculitis, and Wegener's granulomatosis.

[00338] In some embodiments, one or more regions of the therapeutic agent comprises an amino acid sequence based on or derived from an amino acid sequence which binds to potassium channels. In some embodiments, one or more regions of the therapeutic agent comprises an amino acid sequence based on or derived from an amino acid sequence of a Mokatoxin-1 (Moka).

[00339] In some embodiments, one or more regions of the therapeutic agent is configured to treat pain. In some embodiments, 2, 3, 4, 5 or more regions of the therapeutic agent are configured to treat pain.

[00340] In some embodiments, one or more regions of the therapeutic agent comprises an amino acid sequence based on or derived from an amino acid sequence which is a neurotoxin. In some embodiments, one or more regions of the therapeutic agent comprises an amino acid sequence based on or derived from an amino acid sequence of a neurotoxin mu-SLPTX-Ssm6a (Ssam6). In some embodiments, one or more regions of the therapeutic agent comprises an amino acid sequence based on or derived from an amino acid sequence of mambalign-1.

[00341] In some embodiments, one or more regions of the therapeutic agent is configured to treat heart failure and/or fibrosis. In some embodiments, one or more regions of the therapeutic agent is configured to treat heart failure and/or fibrosis related conditions. In some embodiments, 2, 3, 4, 5 or more regions of the therapeutic agent are configured to treat heart failure and/or fibrosis. In some embodiments, 2, 3, 4, 5

or more regions of the therapeutic agent are configured to treat heart failure and/or fibrosis related conditions. Heart failure related conditions may include coronary heart disease, high blood pressure, diabetes, cardiomyopathy, heart valve disease, arrhythmias, congenital heart defects, obstructive sleep apnea, myocarditis, hyperthyroidism, hypothyroidism, emphysema, hemochromatosis, and amyloidosis. Heart failure may be left-sided heart failure, right-sided heart failure, systolic heart failure, and diastolic heart failure. Fibrosis may include, but is not limited to, pulmonary fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, cirrhosis, endomyocardial fibrosis, myocardial infarction, atrial fibrosis, mediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, progressive massive fibrosis, nephrogenic systemic fibrosis, Crohn's disease, keloid, scleroderma/systemic sclerosis, arthrofibrosis, Peyronie's disease, Dupuytren's contracture, and adhesive capsulitis.

[00342] In some embodiments, one or more regions of the therapeutic agent comprises an amino acid sequence based on or derived from an amino acid sequence which belongs to the insulin superfamily. In some embodiments, one or more regions of the therapeutic agent comprises an amino acid sequence based on or derived from an amino acid sequence of insulin.

[00343] In some embodiments, amino acids of the therapeutic agent, in whole or in part, are based on or derived from any one of SEQ ID NOs: 227-267. The therapeutic agent may comprise an amino acid sequence that is at least about 50% homologous to any one of SEQ ID NOs: 227-267. The therapeutic agent may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% homologous to any one of SEQ ID NOs: 227-267. The therapeutic agent may comprise an amino acid sequence that is at least about 70% homologous to any one of SEQ ID NOs: 227-267. The therapeutic agent may comprise an amino acid sequence that is at least about 80% homologous to any one of SEQ ID NOs: 227-267. The therapeutic agent may comprise an amino acid sequence that is at least about 50% identical to any one of SEQ ID NOs: 227-267. The therapeutic agent may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% identical to any one of SEQ ID NOs: 227-267. The therapeutic agent may comprise an amino acid sequence that is at least about 70% identical to any one of SEQ ID NOs: 227-267. The therapeutic agent may comprise an amino acid sequence that is at least about 80% identical to any one of SEQ ID NOs: 227-267. The therapeutic agent may comprise an amino acid sequence that is 100% identical to any one of SEQ ID NOs: 227-267. In some embodiments, the therapeutic agent comprises an amino acid sequence that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% homologous to an amino acid sequence of any one of SEQ ID NOs: 227-267. In some embodiments, the therapeutic agent comprises an amino acid sequence that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% identical to an amino acid sequence of any one of SEQ ID NOs: 227-267. In some embodiments, the therapeutic agent comprises an amino acid sequence that is 100% identical to an amino acid sequence of any one of SEQ ID NOs: 227-267.

[00344] The therapeutic agent may comprise an amino acid sequence comprising 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more amino acids based on or derived from any one of SEQ ID NOs: 227-267. The therapeutic agent may comprise an amino acid sequence comprising 125, 150, 175, 200, 225, 250, 275,

300, 325, 350, 375, 400, 425, 450, 475, 450, 500 or more amino acids based on or derived from any one of SEQ ID NOs: 227-267. The therapeutic agent may comprise an amino acid sequence comprising 10 or more amino acids based on or derived from any one of SEQ ID NOs: 227-267. The therapeutic agent may comprise an amino acid sequence comprising 50 or more amino acids based on or derived from any one of SEQ ID NOs: 227-267. The therapeutic agent may comprise an amino acid sequence comprising 100 or more amino acids based on or derived from any one of SEQ ID NOs: 227-267. The therapeutic agent may comprise an amino acid sequence comprising 200 or more amino acids based on or derived from any one of SEQ ID NOs: 227-267. The amino acids may be consecutive. Alternatively, or additionally, the amino acids are nonconsecutive. In some embodiments, the therapeutic agent may comprise amino acids derived from any one of SEQ ID NOs: 227-267 and amino acids not derived from any one of SEQ ID NOs: 227-267. In some embodiments, the therapeutic agent may comprise amino acids derived from one or more of SEQ ID NOs: 227-267 and amino acids not derived from any one of SEQ ID NOs: 227-267. In some embodiments, the therapeutic agent comprises amino acids derived from 1, 2, 3, or 4 of SEQ ID NOs: 227-267.

[00345] The therapeutic agent may be encoded by a nucleic acid sequence based on or derived from any one of SEQ ID NOs: 186-226. The therapeutic agent may be encoded by a nucleic acid sequence that may be at least about 50% homologous to any one of SEQ ID NOs: 186-226. The therapeutic agent may be encoded by a nucleic acid sequence that may be at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more homologous to any one of SEQ ID NOs: 186-226. The therapeutic agent may be encoded by a nucleic acid sequence that may be at least about 70% homologous to any one of SEQ ID NOs: 186-226. The therapeutic agent may be encoded by a nucleic acid sequence that may be at least about 80% homologous to any one of SEQ ID NOs: 186-226.

[00346] The therapeutic agent may comprise a protease cleavage site. The protease cleavage site may be inserted within the therapeutic agent. In some embodiments, the therapeutic agent comprises a first therapeutic agent region and a second therapeutic agent region. In some embodiments, the therapeutic agent comprises a protease cleavage site disposed between the first therapeutic agent region and the second therapeutic agent region. In some embodiments, the first therapeutic agent region and the second therapeutic agent region are derived from the same protein or set of amino acid sequences. In some embodiments, the first therapeutic agent region and the second therapeutic agent regions are derived from different proteins or sets of amino acid sequences. The one or more protease cleavage sites may be attached to the N-terminus, C-terminus or both the N- and C-termini of a region of a therapeutic agent.

[00347] The therapeutic agent may comprise one or more internal linker peptides. The therapeutic agent may comprise two or more internal linker peptides. The therapeutic agent may comprise 3, 4, 5, 6, 7 or more internal linker peptides. The linker peptides may be different. The linker peptides may be the same. The linker peptide may be inserted within the therapeutic agent. In some embodiments, the therapeutic agent comprises a first therapeutic region, a second therapeutic region, an one or more linker peptides positioned between the first therapeutic region and the second therapeutic region. The one or more linker peptides may be attached to the N-terminus, C-terminus or both the N- and C-termini of a

region of a therapeutic agent. In some embodiments, the linker peptide is a protease cleavage site. In some embodiments, the linker peptide is a tag, such as an affinity tag. An example of an affinity tag is a 6X (HHHHHH) histidine tag. In some embodiments, the internal linker comprises amino acids having repeating sequences. In some embodiments, the internal linker has 2, 3, 4, 5, 6, 7, 8, 9, 10 or more repeating sequences. In some embodiments, the internal linker is low immunogenic. In some embodiments, the internal linker is biodegradable.

[00348] The therapeutic agents may be inserted into the antibody region. Insertion of the therapeutic agent into the antibody region may comprise removal or deletion of one or more amino acids from the antibody region.

[00349] In some embodiments, an immunoglobulin fusion protein comprises one or more extender peptides. The one or more extender peptides may be attached to the N-terminus, C-terminus or both the N- and C-termini of a therapeutic agent.

[00350] In some embodiments, an immunoglobulin fusion protein comprises one or more linker peptides. The one or more linkers may be attached to the N-terminus, C-terminus or both the N- and C-termini of a therapeutic agent.

[00351] In some embodiments, an immunoglobulin fusion protein comprises one or more proteolytic cleavage sites. The one or more proteolytic cleavage sites may be attached to the N-terminus, C-terminus or both the N- and C-termini of a therapeutic agent.

[00352] In some embodiments, the therapeutic agent may be connected to the antibody region without the aid of an extender peptide. The therapeutic agent may be connected to the antibody via one or more linkers.

[00353] Linkers

[00354] The immunoglobulin fusion proteins, antibody regions, non-antibody regions and/or extender fusion regions may further comprise one or more linkers. The immunoglobulin fusion proteins, antibody regions, non-antibody regions and/or extender fusion region may further comprise 2, 3, 4, 5, 6, 7, 8, 9, 10 or more linkers. The extender fusion region may further comprise one or more linkers. The extender fusion region may further comprise 2, 3, 4, 5, 6, 7, 8, 9, 10 or more linkers.

[00355] The one or more linkers are attached to the N-terminus, C-terminus or both N- and C-termini of a therapeutic agent. The one or more linkers are attached to the N-terminus, C-terminus or both N- and C-termini of the extender peptide. The one or more linkers are attached to the N-terminus, C-terminus or both N- and C-termini of a proteolytic cleavage site. The one or more linkers may be attached to a therapeutic agent, extender peptide, proteolytic cleavage site, extender fusion region, antibody region, or a combination thereof.

[00356] In some embodiments, the linker peptide is a connecting peptide or part of a connecting peptide.

[00357] The one or more linkers may comprise the sequence $(X^e X^f X^g X^h)_n$ (SEQ ID NO: 176). In one embodiment, n is between about 1 and about 20. In one embodiment n is any one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In one embodiment, n is between about 1 and about 10. In

one embodiment, n is between about 1 and about 5. In one embodiment, n is between about 1 and about 3. In one embodiment, X^e, X^f and X^g are independently selected from a hydrophobic amino acid. X^h may be a polar, uncharged amino acid. The linker sequence may further comprise one or more cysteine (C) residues. The one or more cysteine residues are at the N-terminus, C-terminus, or a combination thereof. The linker peptide may comprise the sequence CX^eX^fX^gX^h (SEQ ID NO: 177). In one embodiment, X^e, X^f and X^g are independently selected from a hydrophobic amino acid. X^h may be a polar, uncharged amino acid. The linker peptide may comprise the sequence X^eX^fX^gX^hC (SEQ ID NO: 178). In one embodiment, X^e, X^f and X^g are independently selected from a hydrophobic amino acid. X^h may be a polar, uncharged amino acid.

[00358] The one or more linkers may comprise the sequence (GGGGS)_n (SEQ ID NO: 179). In one embodiment, n is between about 1 and about 20. In one embodiment n is any one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In one embodiment, n is between about 1 and about 10. In one embodiment, n is between about 1 and about 5. In one embodiment, n is between about 1 and about 3.

[00359] The one or more linkers may comprise an amino acid sequence selected from any one of SEQ ID NOs: 176-181. The one or more linkers may comprise an amino acid sequence that is at least about 50% homologous to any one of SEQ ID NOs: 176-181. The one or more linkers may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more homologous to any one of SEQ ID NOs: 176-181. The one or more linkers may comprise an amino acid sequence that is at least about 70% homologous to any one of SEQ ID NOs: 176-181. The one or more linkers may comprise an amino acid sequence that is at least about 80% homologous to any one of SEQ ID NOs: 176-181.

[00360] Proteolytic cleavage Site

[00361] The immunoglobulin fusion proteins disclosed herein may further comprise one or more proteolytic cleavage sites. The immunoglobulin fusion proteins disclosed herein may further comprise 2 or more proteolytic cleavage sites. The immunoglobulin fusion proteins disclosed herein may further comprise 3 or more proteolytic cleavage sites. The immunoglobulin fusion proteins disclosed herein may further comprise 4, 5, 6, 7 or more proteolytic cleavage sites. The therapeutic agents disclosed herein may further comprise one or more proteolytic cleavage sites.

[00362] The immunoglobulin fusion proteins may comprise a sequence with one or more cleavage sites between the antibody region and the non-antibody region. The immunoglobulin fusion proteins may comprise a sequence with one or more cleavage sites between the antibody region and the extender fusion region. In some embodiments, the proteolytic cleavage site is a connecting peptide or is part of a connecting peptide.

[00363] The one or more proteolytic cleavage sites may be attached to the N-terminus, C-terminus or both N- and C-termini of a therapeutic peptide. The one or more proteolytic cleavage sites may be attached to the N-terminus, C-terminus or both N- and C-termini of an extender peptide. The one or more proteolytic cleavage sites may be attached to the N-terminus, C-terminus or both N- and C-termini of a linker peptide.

The one or more proteolytic cleavage sites may be attached to a therapeutic peptide, extender peptide, linker, extender fusion region, immunoglobulin region, non-immunoglobulin region or a combination thereof.

[00364] Digestion of the proteolytic cleavage site may result in release of the N- or C- terminus of the therapeutic agent from the immunoglobulin fusion protein. The proteolytic cleavage site may be on the N- and C-termini of the therapeutic agent. Digestion of the proteolytic cleavage site may result in release of the therapeutic agent from the immunoglobulin fusion protein.

[00365] Alternatively, or additionally, the proteolytic cleavage site is located within the amino acid sequence of the therapeutic agent, extender peptide, antibody region, or a combination thereof. The therapeutic agent may comprise one or more proteolytic cleavage sites within its amino acid sequence. For example, SEQ ID NO: 89 discloses a relaxin protein comprising two internal proteolytic cleavage sites. Digestion of the proteolytic cleavage sites within the relaxin protein may result in release of an internal fragment of the relaxin protein.

[00366] Two or more proteolytic cleavage sites may surround a therapeutic agent, extender peptide, linker, antibody region, or combination thereof. Digestion of the proteolytic cleavage site may result in release of a peptide fragment located between the two or more proteolytic cleavage sites. For example, the proteolytic cleavage sites may flank a therapeutic agent-linker peptide. Digestion of the proteolytic cleavage sites may result in release of the therapeutic agent-linker.

[00367] The proteolytic cleavage site may be recognized by one or more proteases. The one or more proteases may be a serine protease, threonine protease, cysteine protease, aspartate protease, glutamic protease, metalloprotease, exopeptidases, endopeptidases, or a combination thereof. The proteases may be selected from the group comprising Factor VII or Factor Xa. Additional examples of proteases include, but are not limited to, aminopeptidases, carboxypeptidases, trypsin, chymotrypsin, pepsin, papain, and elastase. The protease may be prohormone convertase 2 (PC2).

[00368] The one or more proteolytic cleavage sites may comprise an amino acid sequence selected from any one of SEQ ID NOs: 182-185. The one or more proteolytic cleavage sites may comprise an amino acid sequence that is at least about 50% homologous to any one of SEQ ID NOs: 182-185. The one or more proteolytic cleavage sites may comprise an amino acid sequence that is at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more homologous to any one of SEQ ID NOs: 182-185. The one or more proteolytic cleavage sites may comprise an amino acid sequence that is at least about 70% homologous to any one of SEQ ID NOs: 182-185. The one or more proteolytic cleavage sites may comprise an amino acid sequence that is at least about 80% homologous to any one of SEQ ID NOs: 182-185.

[00369] Vectors, Host Cells and Recombinant Methods

[00370] Immunoglobulin fusion proteins, as disclosed herein, may be expressed by recombinant methods. Generally, a nucleic acid encoding an immunoglobulin fusion protein may be isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the immunoglobulin fusion protein may be prepared by PCR amplification and sequenced using

conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to nucleotides encoding immunoglobulin fusion proteins). In an exemplary embodiment, nucleic acid encoding an immunoglobulin fusion protein is PCR amplified, restriction enzyme digested and gel purified. The digested nucleic acid may be inserted into a replicable vector. The replicable vector containing the digested immunoglobulin fusion protein insertion may be transformed or transduced into a host cell for further cloning (amplification of the DNA) or for expression. Host cells may be prokaryotic or eukaryotic cells.

[00371] Polynucleotide sequences encoding polypeptide components (e.g., antibody region, extender peptide, therapeutic agent) of the immunoglobulin fusion proteins may be obtained by PCR amplification. Polynucleotide sequences may be isolated and sequenced from cells containing nucleic acids encoding the polypeptide components. Alternatively, or additionally, polynucleotides may be synthesized using nucleotide synthesizer or PCR techniques. Once obtained, sequences encoding the polypeptide components may be inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in prokaryotic and/or eukaryotic hosts.

[00372] In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism may be used as transforming vectors in connection with these hosts. For example, bacteriophage such as λ GEM™-11 may be utilized in making a recombinant vector which may be used to transform susceptible host cells such as *E. coli* LE392.

[00373] Immunoglobulin fusion proteins may be expressed intracellularly (e.g., cytoplasm) or extracellularly (e.g., secretion). For extracellular expression, the vector may comprise a secretion signal which enables translocation of the immunoglobulin fusion proteins to the outside of the cell.

[00374] Suitable host cells for cloning or expression of immunoglobulin fusion proteins-encoding vectors include prokaryotic or eukaryotic cells. The host cell may be a eukaryotic. Examples of eukaryotic cells include, but are not limited to, Human Embryonic Kidney (HEK) cells, Chinese Hamster Ovary (CHO) cells, fungi, yeasts, invertebrate cells (e.g., plant cells and insect cells), lymphoid cells (e.g., YO, NSO, Sp20 cells). Other examples of suitable mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); baby hamster kidney cells (BHK); mouse sertoli cells; monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK); buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TR1 cells; MRC 5 cells; and FS4 cells. The host cell may be a prokaryotic cell (e.g., *E. coli*).

[00375] Host cells may be transformed with vectors containing nucleotides encoding an immunoglobulin fusion proteins. Transformed host cells may be cultured in media. The media may be supplemented with one or more agents for inducing promoters, selecting transformants, or amplifying or expressing the genes encoding the desired sequences. Methods for transforming host cells are known in the art and may include electroporation, calcium chloride, or polyethylene glycol/DMSO.

[00376] Alternatively, host cells may be transfected or transduced with vectors containing nucleotides encoding immunoglobulin fusion proteins. Transfected or transduced host cells may be cultured in media.

The media may be supplemented with one or more agents for inducing promoters, selecting transfected or transduced cells, or expressing genes encoding the desired sequences.

[00377] Host cells may be transfected or transduced with vectors comprising nucleotides encoding one or more proteases. The protease comprising vectors may be co-transfected with vectors encoding any immunoglobulin fusion protein disclosed herein. Proteases include Factor Xa and PC2.

[00378] The expressed immunoglobulin fusion proteins may be secreted into and recovered from the periplasm of the host cells or transported into the culture media. Protein recovery from the periplasm may involve disrupting the host cell. Disruption of the host cell may comprise osmotic shock, sonication or lysis. Centrifugation or filtration may be used to remove cell debris or whole cells. The immunoglobulin fusion proteins may be further purified, for example, by affinity resin chromatography.

[00379] Alternatively, immunoglobulin fusion proteins that are secreted into the culture media may be isolated therein. Cells may be removed from the culture and the culture supernatant being filtered and concentrated for further purification of the proteins produced. The expressed polypeptides may be further isolated and identified using commonly known methods such as polyacrylamide gel electrophoresis (PAGE) and Western blot assay.

[00380] Immunoglobulin fusion proteins production may be conducted in large quantity by a fermentation process. Various large-scale fed-batch fermentation procedures are available for production of recombinant proteins. Large-scale fermentations have at least 1000 liters of capacity, preferably about 1,000 to 100,000 liters of capacity. These fermentors use agitator impellers to distribute oxygen and nutrients, especially glucose (a preferred carbon/energy source). Small scale fermentation refers generally to fermentation in a fermentor that is no more than approximately 100 liters in volumetric capacity, and can range from about 1 liter to about 100 liters.

[00381] In a fermentation process, induction of protein expression is typically initiated after the cells have been grown under suitable conditions to a desired density, *e.g.*, an OD₅₅₀ of about 180-220, at which stage the cells are in the early stationary phase. A variety of inducers may be used, according to the vector construct employed, as is known in the art and described herein. Cells may be grown for shorter periods prior to induction. Cells are usually induced for about 12-50 hours, although longer or shorter induction time may be used.

[00382] To improve the production yield and quality of the immunoglobulin fusion proteins disclosed herein, various fermentation conditions may be modified. For example, to improve the proper assembly and folding of the secreted immunoglobulin fusion proteins polypeptides, additional vectors overexpressing chaperone proteins, such as Dsb proteins (DsbA, DsbB, DsbC, DsbD and or DsbG) or FkpA (a peptidylprolyl *cis*,*trans*-isomerase with chaperone activity) may be used to co-transform the host prokaryotic cells. The chaperone proteins have been demonstrated to facilitate the proper folding and solubility of heterologous proteins produced in bacterial host cells.

[00383] To minimize proteolysis of expressed heterologous proteins (especially those that are proteolytically sensitive), certain host strains deficient for proteolytic enzymes may be used for the present disclosure. For example, host cell strains may be modified to effect genetic mutation(s) in the

genes encoding known bacterial proteases such as Protease III, OmpT, DegP, Tsp, Protease I, Protease Mi, Protease V, Protease VI and combinations thereof. Some *E. coli* protease-deficient strains are available.

[00384] Standard protein purification methods known in the art may be employed. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC, chromatography on silica or on a cation-exchange resin such as DEAE, chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography and gel filtration using, for example, Sephadex™ G-75.

[00385] Immunoglobulin fusion proteins may be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon® ultrafiltration unit.

[00386] Protease inhibitors or protease inhibitor cocktails may be included in any of the foregoing steps to inhibit proteolysis of the immunoglobulin fusion proteins.

[00387] In some cases, an immunoglobulin fusion protein may not be biologically active upon isolation. Various methods for "refolding" or converting a polypeptide to its tertiary structure and generating disulfide linkages, may be used to restore biological activity. Such methods include exposing the solubilized polypeptide to a pH usually above 7 and in the presence of a particular concentration of a chaotrope. The selection of chaotrope is very similar to the choices used for inclusion body solubilization, but usually the chaotrope is used at a lower concentration and is not necessarily the same as chaotropes used for the solubilization. In most cases the refolding/oxidation solution will also contain a reducing agent or the reducing agent plus its oxidized form in a specific ratio to generate a particular redox potential allowing for disulfide shuffling to occur in the formation of the protein's cysteine bridge(s). Some of the commonly used redox couples include cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride, dithiothreitol(DTT)/dithiane DTT, and 2-mercaptoethanol(bME)/di-thio-b(ME). In many instances, a cosolvent may be used to increase the efficiency of the refolding, and common reagents used for this purpose include glycerol, polyethylene glycol of various molecular weights, arginine and the like.

[00388] Compositions

[00389] Disclosed herein are compositions comprising an immunoglobulin fusion protein and/or component of an immunoglobulin fusion protein disclosed herein. The compositions may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more immunoglobulin fusion proteins. The immunoglobulin fusion proteins may be different. Alternatively, the immunoglobulin fusion proteins may be the same or similar. The immunoglobulin fusion proteins may comprise different antibody regions, extender fusion regions, extender peptides, therapeutic agents or a combination thereof.

[00390] The compositions may further comprise one or more pharmaceutically acceptable salts, excipients or vehicles. Pharmaceutically acceptable salts, excipients, or vehicles for use in the present pharmaceutical compositions include carriers, excipients, diluents, antioxidants, preservatives, coloring, flavoring and diluting agents, emulsifying agents, suspending agents, solvents, fillers, bulking agents,

buffers, delivery vehicles, tonicity agents, cosolvents, wetting agents, complexing agents, buffering agents, antimicrobials, and surfactants.

[00391] Neutral buffered saline or saline mixed with serum albumin are exemplary appropriate carriers. The pharmaceutical compositions may include antioxidants such as ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counter ions such as sodium; and/or nonionic surfactants such as TweenTM, pluronics, or polyethylene glycol (PEG). Also by way of example, suitable tonicity enhancing agents include alkali metal halides (preferably sodium or potassium chloride), mannitol, sorbitol, and the like. Suitable preservatives include benzalkonium chloride, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid and the like. Hydrogen peroxide also may be used as preservative. Suitable cosolvents include glycerin, propylene glycol, and PEG. Suitable complexing agents include caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxy-propyl-beta-cyclodextrin. Suitable surfactants or wetting agents include sorbitan esters, polysorbates such as polysorbate 80, tromethamine, lecithin, cholesterol, tyloxapal, and the like. The buffers may be conventional buffers such as acetate, borate, citrate, phosphate, bicarbonate, or Tris-HCl. Acetate buffer may be about pH 4-5.5, and Tris buffer may be about pH 7-8.5. Additional pharmaceutical agents are set forth in Remington's Pharmaceutical Sciences, 18th Edition, A. R. Gennaro, ed., Mack Publishing Company, 1990.

[00392] The composition may be in liquid form or in a lyophilized or freeze-dried form and may include one or more lyoprotectants, excipients, surfactants, high molecular weight structural additives and/or bulking agents (see, for example, U.S. Patent Nos. 6,685,940, 6,566,329, and 6,372,716). In one embodiment, a lyoprotectant is included, which is a non-reducing sugar such as sucrose, lactose or trehalose. The amount of lyoprotectant generally included is such that, upon reconstitution, the resulting formulation will be isotonic, although hypertonic or slightly hypotonic formulations also may be suitable. In addition, the amount of lyoprotectant should be sufficient to prevent an unacceptable amount of degradation and/or aggregation of the protein upon lyophilization. Exemplary lyoprotectant concentrations for sugars (*e.g.*, sucrose, lactose, trehalose) in the pre-lyophilized formulation are from about 10 mM to about 400 mM. In another embodiment, a surfactant is included, such as for example, nonionic surfactants and ionic surfactants such as polysorbates (*e.g.*, polysorbate 20, polysorbate 80); poloxamers (*e.g.*, poloxamer 188); poly(ethylene glycol) phenyl ethers (*e.g.*, TritonTM); sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (*e.g.*, lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl ofeyle-aurate; the MONAQUATTM series (Mona Industries, Inc., Paterson, N.J.), polyethyl glycol, polypropyl glycol, and

copolymers of ethylene and propylene glycol (*e.g.*, Pluronics, PF68 etc). Exemplary amounts of surfactant that may be present in the pre-lyophilized formulation are from about 0.001-0.5%. High molecular weight structural additives (*e.g.*, fillers, binders) may include for example, acacia, albumin, alginate, calcium phosphate (dibasic), cellulose, carboxymethylcellulose, carboxymethylcellulose sodium, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, microcrystalline cellulose, dextran, dextrin, dextrans, sucrose, tylose, pregelatinized starch, calcium sulfate, amylose, glycine, bentonite, maltose, sorbitol, ethylcellulose, disodium hydrogen phosphate, disodium phosphate, disodium pyrosulfite, polyvinyl alcohol, gelatin, glucose, guar gum, liquid glucose, compressible sugar, magnesium aluminum silicate, maltodextrin, polyethylene oxide, polymethacrylates, povidone, sodium alginate, tragacanth microcrystalline cellulose, starch, and zein. Exemplary concentrations of high molecular weight structural additives are from 0.1% to 10% by weight. In other embodiments, a bulking agent (*e.g.*, mannitol, glycine) may be included.

[00393] Compositions may be suitable for parenteral administration. Exemplary compositions are suitable for injection or infusion into an animal by any route available to the skilled worker, such as intraarticular, subcutaneous, intravenous, intramuscular, intraperitoneal, intracerebral (intraparenchymal), intracerebroventricular, intramuscular, intraocular, intraarterial, or intralesional routes. A parenteral formulation typically will be a sterile, pyrogen-free, isotonic aqueous solution, optionally containing pharmaceutically acceptable preservatives.

[00394] Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringers' dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, anti-microbials, anti-oxidants, chelating agents, inert gases and the like. See generally, Remington's Pharmaceutical Science, 16th Ed., Mack Eds., 1980.

[00395] Compositions described herein may be formulated for controlled or sustained delivery in a manner that provides local concentration of the product (*e.g.*, bolus, depot effect) and/or increased stability or half-life in a particular local environment. The compositions may comprise the formulation of immunoglobulin fusion proteins, polypeptides, nucleic acids, or vectors disclosed herein with particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, *etc.*, as well as agents such as a biodegradable matrix, injectable microspheres, microcapsular particles, microcapsules, bioerodible particles beads, liposomes, and implantable delivery devices that provide for the controlled or sustained release of the active agent which then may be delivered as a depot injection. Techniques for formulating such sustained-or controlled-delivery means are known and a variety of polymers have been developed and used for the controlled release and delivery of drugs. Such polymers are typically biodegradable and biocompatible. Polymer hydrogels, including those formed by complexation of enantiomeric polymer or polypeptide segments, and hydrogels with temperature or pH sensitive

properties, may be desirable for providing drug depot effect because of the mild and aqueous conditions involved in trapping bioactive protein agents. See, for example, the description of controlled release porous polymeric microparticles for the delivery of pharmaceutical compositions in WO 93/15722.

[00396] Suitable materials for this purpose include polylactides (see, *e.g.*, U.S. Patent No. 3,773,919), polymers of poly-(α -hydroxycarboxylic acids), such as poly-D(-)-3-hydroxybutyric acid (EP 133,988A), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, *Biopolymers*, 22: 547-556 (1983)), poly(2-hydroxyethyl-methacrylate) (Langer *et al.*, *J. Biomed. Mater. Res.*, 15: 167-277 (1981), and Langer, *Chem. Tech.*, 12: 98-105 (1982)), ethylene vinyl acetate, or poly-D(-)-3-hydroxybutyric acid. Other biodegradable polymers include poly(lactones), poly(acetals), poly(orthoesters), and poly(orthocarbonates). Sustained-release compositions also may include liposomes, which may be prepared by any of several methods known in the art (see, *e.g.*, Eppstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82: 3688-92 (1985)). The carrier itself, or its degradation products, should be nontoxic in the target tissue and should not further aggravate the condition. This may be determined by routine screening in animal models of the target disorder or, if such models are unavailable, in normal animals.

[00397] The immunoglobulin fusion proteins disclosed herein may be microencapsulated.

[00398] A pharmaceutical composition disclosed herein can be administered to a subject by any suitable administration route, including but not limited to, parenteral (intravenous, subcutaneous, intraperitoneal, intramuscular, intravascular, intrathecal, intravitreal, infusion, or local), topical, oral, or nasal administration.

[00399] Formulations suitable for intramuscular, subcutaneous, peritumoral, or intravenous injection can include physiologically acceptable sterile aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and non-aqueous carriers, diluents, solvents, or vehicles including water, ethanol, polyols (propyleneglycol, polyethylene-glycol, glycerol, cremophor and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity is maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. Formulations suitable for subcutaneous injection also contain optional additives such as preserving, wetting, emulsifying, and dispensing agents.

[00400] For intravenous injections, an active agent can be optionally formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer.

[00401] Parenteral injections optionally involve bolus injection or continuous infusion. Formulations for injection are optionally presented in unit dosage form, *e.g.*, in ampoules or in multi dose containers, with an added preservative. The pharmaceutical composition described herein can be in a form suitable for parenteral injection as a sterile suspensions, solutions or emulsions in oily or aqueous vehicles, and contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Pharmaceutical

formulations for parenteral administration include aqueous solutions of an active agent in water soluble form. Additionally, suspensions are optionally prepared as appropriate oily injection suspensions.

[00402] Alternatively or additionally, the compositions may be administered locally via implantation into the affected area of a membrane, sponge, or other appropriate material on to which an immunoglobulin fusion protein disclosed herein has been absorbed or encapsulated. Where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of an immunoglobulin fusion protein, nucleic acid, or vector disclosed herein may be directly through the device via bolus, or via continuous administration, or via catheter using continuous infusion.

[00403] A pharmaceutical composition comprising an immunoglobulin fusion protein disclosed herein may be formulated for inhalation, such as for example, as a dry powder. Inhalation solutions also may be formulated in a liquefied propellant for aerosol delivery. In yet another formulation, solutions may be nebulized. Additional pharmaceutical composition for pulmonary administration include, those described, for example, in WO 94/20069, which discloses pulmonary delivery of chemically modified proteins. For pulmonary delivery, the particle size should be suitable for delivery to the distal lung. For example, the particle size may be from 1 μm to 5 μm ; however, larger particles may be used, for example, if each particle is fairly porous.

[00404] Certain formulations comprising an immunoglobulin fusion protein disclosed herein may be administered orally. Formulations administered in this fashion may be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents may be included to facilitate absorption of a selective binding agent. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders also may be employed.

[00405] Another preparation may involve an effective quantity of an immunoglobulin fusion protein in a mixture with non-toxic excipients which are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or another appropriate vehicle, solutions may be prepared in unit dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

[00406] Suitable and/or preferred pharmaceutical formulations may be determined in view of the present disclosure and general knowledge of formulation technology, depending upon the intended route of administration, delivery format, and desired dosage. Regardless of the manner of administration, an effective dose may be calculated according to patient body weight, body surface area, or organ size.

[00407] Further refinement of the calculations for determining the appropriate dosage for treatment involving each of the formulations described herein are routinely made in the art and is within the ambit of tasks routinely performed in the art. Appropriate dosages may be ascertained through use of appropriate dose-response data.

[00408] The compositions disclosed herein may be useful for providing prognostic or providing diagnostic information.

[00409] "Pharmaceutically acceptable" may refer to approved or approvable by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, including humans.

[00410] "Pharmaceutically acceptable salt" may refer to a salt of a compound that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound.

[00411] "Pharmaceutically acceptable excipient, carrier or adjuvant" may refer to an excipient, carrier or adjuvant that may be administered to a subject, together with at least one antibody of the present disclosure, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the compound.

[00412] "Pharmaceutically acceptable vehicle" may refer to a diluent, adjuvant, excipient, or carrier with which at least one antibody of the present disclosure is administered.

[00413] Kits

[00414] Further disclosed herein are kits which comprise one or more immunoglobulin fusion proteins or components thereof. The immunoglobulin fusion proteins may be packaged in a manner which facilitates their use to practice methods of the present disclosure. For example, a kit comprises an immunoglobulin fusion protein described herein packaged in a container with a label affixed to the container or a package insert that describes use of the immunoglobulin fusion protein in practicing the method. Suitable containers include, for example, bottles, vials, syringes, *etc.* The containers may be formed from a variety of materials such as glass or plastic. The container may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The kit may comprise a container with an immunoglobulin fusion protein contained therein. The kit may comprise a container with (a) an antibody region of an immunoglobulin fusion protein; (b) an extender fusion region of an immunoglobulin fusion protein; (c) an extender peptide of the extender fusion region; (d) a therapeutic agent of the extender fusion region; or (e) a combination of a-d. The kit may further comprise a package insert indicating that the first and second compositions may be used to treat a particular condition. Alternatively, or additionally, the kit may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer (e.g., bacteriostatic water for injection (BWI), phosphate-buffered saline, Ringer's solution and dextrose solution). It may further comprise other materials desirable from a commercial and user standpoint, including, but not limited to, other buffers, diluents, filters, needles, and syringes. The immunoglobulin fusion protein may be packaged in a unit dosage form. The kit may further comprise a device suitable for administering the immunoglobulin fusion protein according to a specific route of administration or for practicing a screening assay. The kit may contain a label that describes use of the immunoglobulin fusion protein composition.

[00415] The composition comprising the immunoglobulin fusion protein may be formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous

administration to mammals, such as humans, bovines, felines, canines, and murines. Typically, compositions for intravenous administration comprise solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and/or a local anaesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients may be supplied either separately or mixed together in unit dosage form. For example, the immunoglobulin fusion protein may be supplied as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the immunoglobulin fusion protein. Where the composition is to be administered by infusion, it may be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline may be provided so that the ingredients may be mixed prior to administration.

[00416] The amount of the composition described herein which will be effective in the treatment, inhibition and/or prevention of a disease or disorder associated with aberrant expression and/or activity of a therapeutic agent may be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation may also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro, animal model test systems or clinical trials.

[00417] Therapeutic Use

[00418] Further disclosed herein are immunoglobulin fusion proteins for and methods of treating, alleviating, inhibiting and/or preventing one or more diseases and/or conditions. The method may comprise administering to a subject in need thereof a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region. The non-antibody region comprises one or more therapeutic agents. The extender fusion region comprises one or more therapeutic agents. In some embodiments, the non-immunoglobulin region comprises one or more extender peptides. In some embodiments, the extender fusion region comprises one or more extender peptides. In one embodiment, the extender peptide comprises an amino acid sequence having an alpha helix secondary structure. In one embodiment, the extender peptide does not comprise an amino acid sequence having a beta strand secondary structure. In some embodiments, the non-immunoglobulin region comprises one or more linker peptides. In some embodiments, the extender fusion region comprises one or more linker peptides. In one embodiment, the linker peptide does not comprise an amino acid sequencing having an alpha helix or beta strand secondary structure.

[00419] The composition may further comprise a pharmaceutically acceptable carrier. The subject may be a mammal. The mammal may be a human. Alternatively, the mammal is a bovine. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small

molecule. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The therapeutic agent may be GCSF, bovine GCSF, human GCSF, Moka1, Vm24, Mamba1, human GLP-1, exendin-4, human EPO, human FGF21, human GMCSF, human interferon-beta, human interferon-alpha, relaxin, oxyntomodulin, hLeptin, betatrophin, growth differentiation factor 11 (GDF11), parathyroid hormone, angiopoietin-like 3 (ANGPTL3), IL-11, human growth hormone (hGH), elafin or derivative or variant thereof. Alternatively, or additionally, therapeutic agent is interleukin 8 (IL-8), IL-21, ziconotide, somatostatin, chlorotoxin, SDF1 alpha or derivative or variation thereof. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The disease or condition may be an autoimmune disease, heteroimmune disease or condition, inflammatory disease, pathogenic infection, thromboembolic disorder, respiratory disease or condition, metabolic disease, central nervous system (CNS) disorder, bone disease or cancer. In other instances, the disease or condition is a blood disorder. In some instances, the disease or condition is obesity, diabetes, osteoporosis, anemia, or pain. The therapeutic agent may be hGCSF and the disease or condition may be neutropenia. The therapeutic agent may be hLeptin and the disease or condition may be diabetes. The therapeutic agent may be hGH and the disease or condition may be a growth disorder. The therapeutic agent may be IFN-alpha and the disease or condition may be a viral infection. The therapeutic agent may be Mamba1 and the disease or condition may be pain. The therapeutic agent may be elafin and the disease or condition may be inflammation. The therapeutic agent may be IFN-alpha and the disease or condition may be an elastase inhibitor peptide and the disease or condition may be chronic obstructive pulmonary disease (COPD).

[00420] The disease and/or condition may be a chronic disease or condition. Alternatively, the disease and/or condition is an acute disease or condition. The disease or condition may be recurrent, refractory, accelerated, or in remission. The disease or condition may affect one or more cell types. The one or more

diseases and/or conditions may be an autoimmune disease, inflammatory disease, cardiovascular disease, metabolic disorder, pregnancy, and cell proliferative disorder.

[00421] The disease or condition may be an autoimmune disease. In some cases, the autoimmune disease may be scleroderma, diffuse scleroderma or systemic scleroderma.

[00422] The disease or condition may be an inflammatory disease. In some cases, the inflammatory disease may be hepatitis, fibromyalgia or psoriasis.

[00423] The disease or condition may be a rheumatic disease. In some cases, the rheumatic disease may be Ankylosing spondylitis, back pain, bursitis, tendinitis, shoulder pain, wrist pain, bicep pain, leg pain, knee pain, ankle pain, hip pain, Achilles pain, Capsulitis, neck pain, osteoarthritis, systemic lupus, erythematosus, rheumatoid arthritis, juvenile arthritis, Sjögren syndrome, Polymyositis, Behçet's disease, Reiter's syndrome, or Psoriatic arthritis. The rheumatic disease may be chronic. Alternatively, the rheumatic disease is acute.

[00424] The disease or condition may be a cardiovascular disease. In some cases, the cardiovascular disease may be acute heart failure, congestive heart failure, compensated heart failure, decompensated heart failure, hypercholesterolemia, atherosclerosis, coronary heart disease or ischemic stroke. The cardiovascular disease may be cardiac hypertrophy.

[00425] The disease or condition may be a metabolic disorder. In some cases, the metabolic disorder may be hypercholesterolemia, hypobetalipoproteinemia, hypertriglyceridemia, hyperlipidemia, dyslipidemia, ketosis, hypolipidemia, refractory anemia, appetite control, gastric emptying, non-alcoholic fatty liver disease, obesity, type I diabetes mellitus, type II diabetes mellitus, gestational diabetes mellitus, metabolic syndrome. The metabolic disorder may be type I diabetes. The metabolic disorder may be type II diabetes.

[00426] The disease or condition may be pregnancy. The immunoglobulin fusion proteins may be used to treat preeclampsia or induce labor.

[00427] The disease or condition may be a cell proliferative disorder. The cell proliferative disorder may be a leukemia, lymphoma, carcinoma, sarcoma, or a combination thereof. The cell proliferative disorder may be a myelogenous leukemia, lymphoblastic leukemia, myeloid leukemia, myelomonocytic leukemia, neutrophilic leukemia, myelodysplastic syndrome, B-cell lymphoma, burkitt lymphoma, large cell lymphoma, mixed cell lymphoma, follicular lymphoma, mantle cell lymphoma, hodgkin lymphoma, recurrent small lymphocytic lymphoma, hairy cell leukemia, multiple myeloma, basophilic leukemia, eosinophilic leukemia, megakaryoblastic leukemia, monoblastic leukemia, monocytic leukemia, erythroleukemia, erythroid leukemia, hepatocellular carcinoma, solid tumors, lymphoma, leukemias, liposarcoma (advanced/metastatic), myeloid malignancy, breast cancer, lung cancer, ovarian cancer, uterine cancer, kidney cancer, pancreatic cancer, and malignant glioma of brain.

[00428] Disclosed herein are methods of treating a disease or condition in a subject in need thereof, the method comprising administering to the subject a composition comprising an immunoglobulin fusion protein disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region comprising a therapeutic agent. In some instances, the immunoglobulin fusion

protein comprises an antibody region attached to an extender fusion region comprising a therapeutic agent. In one embodiment, the therapeutic agent is oxyntomodulin. The disease or condition may be a metabolic disorder. The metabolic disorder may be diabetes. Diabetes may be type II diabetes mellitus. Diabetes may be type I diabetes. The metabolic disorder may be obesity. Additional metabolic disorders include, but are not limited to, metabolic syndrome, appetite control or gastric emptying.

[00429] Disclosed herein are methods of treating a disease or condition in a subject in need thereof, the method comprising administering to the subject a composition comprising an immunoglobulin fusion protein disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region comprising a therapeutic agent. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region comprising a therapeutic agent. In one embodiment, the therapeutic agent is relaxin. The disease or condition may be a cardiovascular disease. The cardiovascular disease may be acute heart failure. Additional cardiovascular diseases include, but are not limited to, congestive heart failure, compensated heart failure or decompensated heart failure. The disease or condition may be an autoimmune disorder. The autoimmune disorder may be scleroderma, diffuse scleroderma or systemic scleroderma. The disease or condition may be an inflammatory disease. The inflammatory disease may be fibromyalgia. The disease or condition may be fibrosis. Alternatively, the disease or condition is pregnancy. The immunoglobulin fusion protein may be used to treat preeclampsia or induce labor.

[00430] Disclosed herein are methods of treating a disease or condition in a subject in need thereof, the method comprising administering to the subject a composition comprising an immunoglobulin fusion protein disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region comprising a therapeutic agent. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region comprising a therapeutic agent. In one embodiment, the therapeutic agent is beta-trophin. The disease or condition may be a metabolic disorder. The metabolic disorder may be obesity. Alternatively, the metabolic disorder is diabetes. Diabetes may be type I diabetes mellitus or type II diabetes mellitus.

[00431] Disclosed herein are methods of treating a disease or condition in a subject in need thereof, the method comprising administering to the subject a composition comprising an immunoglobulin fusion protein disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region comprising a therapeutic agent. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region comprising a therapeutic agent. In one embodiment, the therapeutic agent is FGF 21. The disease or condition may be a metabolic disorder. The metabolic disorder may be obesity. The metabolic disorder may be diabetes. Diabetes may be type 2 diabetes mellitus, type I diabetes mellitus or gestational diabetes mellitus. Additional metabolic disorders include, but are not limited to, appetite control and non-alcoholic fatty liver disease. The disease or condition may be a cell proliferative disorder. The cell proliferative disorder may be breast cancer.

[00432] Disclosed herein are methods of treating a disease or condition in a subject in need thereof, the method comprising administering to the subject a composition comprising an immunoglobulin fusion

protein disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region comprising a therapeutic agent. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region comprising a therapeutic agent. In one embodiment, the therapeutic agent is GDF11. The disease or condition may be a cell proliferative disorder. The cell proliferative disorder may be acute, chronic, recurrent, refractory, accelerated, in remission, stage I, stage II, stage III, stage IV, juvenile or adult. The cell proliferative disorder may be a myelogenous leukemia, lymphoblastic leukemia, myeloid leukemia, myelomonocytic leukemia, neutrophilic leukemia, myelodysplastic syndrome, B-cell lymphoma, burkitt lymphoma, large cell lymphoma, mixed cell lymphoma, follicular lymphoma, mantle cell lymphoma, hodgkin lymphoma, recurrent small lymphocytic lymphoma, hairy cell leukemia, multiple myeloma, basophilic leukemia, eosinophilic leukemia, megakaryoblastic leukemia, monoblastic leukemia, monocytic leukemia, erythroleukemia, erythroid leukemia, hepatocellular carcinoma, solid tumors, lymphoma, leukemias, liposarcoma (advanced/metastatic), myeloid malignancy, breast cancer, lung cancer, ovarian cancer, uterine cancer, kidney cancer, pancreatic cancer, and malignant glioma of brain. The disease or condition may be a cardiovascular disease. The cardiovascular disease may be age-related cardiac disease. The disease or condition may be cardiac hypertrophy.

[00433] Disclosed herein are methods of treating a disease or condition in a subject in need thereof, the method comprising administering to the subject a composition comprising an immunoglobulin fusion protein disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region comprising a therapeutic agent. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region comprising a therapeutic agent. In one embodiment, the therapeutic agent is angiopoietin-like 3. The metabolic disorder may be hypercholesterolemia, hypobetalipoproteinemia, hypertriglyceridemia, hyperlipidemia, dyslipidemia, hypolipidemia or ketosis. The disease or condition may be a cardiovascular disease. The cardiovascular disease may be atherosclerosis, coronary heart disease or ischemic stroke. The disease or condition may be a rheumatic disease. The rheumatic disease may be ankylosing spondylitis, back pain, bursitis, tendinitis, shoulder pain, wrist pain, bicep pain, leg pain, knee (patellar) pain, ankle pain, hip pain, Achilles pain, Capsulitis, Neck pain, osteoarthritis, systemic lupus, erythematosus, rheumatoid arthritis, juvenile arthritis, Sjögren syndrome, scleroderma, Polymyositis, Behçet's disease, Reiter's syndrome, Psoriatic arthritis. In some cases, the disease or condition may be a cell proliferative disorder. The cell proliferative disorder may be hepatocellular carcinoma or ovarian cancer. The disease or condition may be an inflammatory disease. The inflammatory disease may be hepatitis.

[00434] Disclosed herein are methods of preventing or treating a disease or condition in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise one or more immunoglobulin heavy chains, light chains, or a combination thereof.

[00435] The immunoglobulin fusion protein may comprise a sequence which shares 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 99%, or more amino acid sequence identity to an amino acid sequence of any

of SEQ ID NOs: 68-99, and 122-143. The nucleotide sequence encoding the immunoglobulin fusion protein may comprise a sequence which shares 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 99%, or more nucleotide sequence identity to a nucleotide sequence of any of SEQ ID NOs: 37-67, and 100-121.

[00436] The immunoglobulin fusion protein may comprise a sequence which shares 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 99%, or more amino acid sequence identity to a heavy chain sequence provided by SEQ ID NOs: 69-79, 81-93, 95-97, 99, and 123-143. The antibody region may comprise an immunoglobulin heavy chain. The immunoglobulin heavy chain polypeptide may comprise a sequence which shares 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 99%, or more amino acid sequence identity to a heavy chain sequence provided by SEQ ID NOs: 22-27 and 29-35. The antibody region may comprise an immunoglobulin light chain.

[00437] The immunoglobulin fusion protein may comprise a sequence which shares 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 99%, or more amino acid sequence identity to a light chain sequence provided by SEQ ID NOs: 68, 80, 94, 98, and 122. The antibody region may comprise an immunoglobulin light chain. The immunoglobulin light chain polypeptide may comprise a sequence which shares 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 99%, or more amino acid sequence identity to a light chain sequence provided by SEQ ID NOs: 19-21, 28, and 36. The antibody region may comprise an immunoglobulin heavy chain.

[00438] The immunoglobulin fusion protein may be encoded by a nucleotide sequence that is at least about 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 99% or more homologous to a nucleotide sequence of any one of SEQ ID NOs: 68-99, and 122-143. The immunoglobulin heavy chain may be encoded by a nucleotide sequence that is at least about 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 99%, or more homologous to SEQ ID NOs: 22-27, 29-35, 69-79, 81-93, 95-97, 99, and 123-143. The immunoglobulin light chain may be encoded by a nucleotide sequence that is at least about 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 99%, or more homologous to SEQ ID NOs: 19-21, 28, 36, 68, 80, 94, 98, and 122.

[00439] The immunoglobulin fusion protein may comprise one or more extender peptides. The immunoglobulin fusion protein may comprise one or more linkers. The immunoglobulin fusion protein may comprise one or more proteolytic cleavage sites. The disease or condition may be an autoimmune disease, heteroimmune disease or condition, inflammatory disease, pathogenic infection, thromboembolic disorder, respiratory disease or condition, metabolic disease, central nervous system (CNS) disorder, bone disease or cancer. The disease or condition may be a blood disorder. In some instances, the disease or condition may be obesity, diabetes, osteoporosis, anemia, or pain.

[00440] Disclosed herein is a method of preventing or treating an autoimmune disease in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region.

The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The composition may further comprise a pharmaceutically acceptable carrier. The subject may be a mammal. The mammal may be a human. Alternatively, the mammal may be a bovine. The therapeutic agent may be Moka1 or a derivative or variant thereof. The therapeutic agent may be VM24 or a derivative or variant thereof. The therapeutic agent may be beta-interferon or a derivative or variant thereof. The immunoglobulin fusion protein or antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. The immunoglobulin domain may be from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. The mammalian antibody may be a murine antibody. The antibody, antibody region or extender fusion region may further comprise a linker. The linker may attach Moka1, VM24, beta-interferon, or a derivative or variant thereof to the extender peptide. The linker may attach the antibody region to the extender fusion region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The autoimmune disease may be a T-cell mediated autoimmune disease. T-cell mediated autoimmune diseases include, but are not limited to, multiple sclerosis, type-1 diabetes, and psoriasis. In other instances, the autoimmune disease lupus, Sjogren's syndrome, scleroderma, rheumatoid arthritis, dermatomyositis, Hashimoto's thyroiditis, Addison's disease, celiac disease, Crohn's disease, pernicious anemia, pemphigus vulgaris, vitiligo, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, myasthenia gravis, Ord's thyroiditis, Graves' disease, Guillain-Barre syndrome, acute disseminated encephalomyelitis, opsoclonus-myoclonus syndrome, ankylosing spondylitis, antiphospholipid antibody syndrome, aplastic anemia, autoimmune hepatitis, Goodpasture's syndrome, Reiter's syndrome, Takayasu's arteritis, temporal arteritis, Wegener's granulomatosis, alopecia universalis, Behcet's disease, chronic fatigue, dysautonomia, endometriosis, interstitial cystitis, neuromyotonia, scleroderma, and vulvodynia. Lupus can include, but may be not limited to, acute cutaneous lupus erythematosus, subacute cutaneous lupus erythematosus, chronic cutaneous lupus erythematosus, discoid lupus erythematosus, childhood discoid lupus erythematosus, generalized discoid lupus erythematosus, localized discoid lupus erythematosus, chilblain lupus erythematosus (hutchinson), lupus erythematosus-lichen planus overlap syndrome, lupus erythematosus panniculitis (lupus

erythematosus profundus), tumid lupus erythematosus, verrucous lupus erythematosus (hypertrophic lupus erythematosus), complement deficiency syndromes, drug-induced lupus erythematosus, neonatal lupus erythematosus, and systemic lupus erythematosus. The disease or condition may be multiple sclerosis. The disease or condition may be diabetes.

[00441] Further disclosed herein is a method of preventing or treating a disease or condition which would benefit from the modulation of a potassium voltage-gated channel in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The composition may further comprise a pharmaceutically acceptable carrier. The potassium voltage-gated channel may be a KCNA3 or K_v1.3 channel. The subject may be a mammal. The mammal may be a human. Alternatively, the mammal may be a bovine. The therapeutic agent may be Moka1 or a derivative or variant thereof. The therapeutic agent may be VM24 or a derivative or variant thereof. The immunoglobulin fusion protein or antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. The immunoglobulin domain may be from a mammalian antibody. Alternatively, the immunoglobulin domain may be from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody may be a murine antibody. The immunoglobulin fusion protein, antibody region, and/or extender fusion region may further comprise one or more linkers. The linker may attach Moka1, VM24, or a derivative or variant thereof to the extender peptide. The linker may attach the antibody region to the extender fusion region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The disease or condition may be an autoimmune disease. The autoimmune disease may be a T-cell mediated autoimmune disease. The disease or condition may be episodic ataxia, seizure, or neuromyotonia. Modulating a potassium voltage-gated channel may comprise inhibiting or

blocking a potassium voltage-gated channel. Modulating a potassium voltage-gated channel may comprise activating a potassium voltage-gated channel.

[00442] Provided herein is a method of preventing or treating a metabolic disease or condition in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The composition may further comprise a pharmaceutically acceptable carrier. The subject may be a mammal. The mammal may be a human. Alternatively, the mammal may be a bovine. The therapeutic agent may be GLP-1, exendin-4, FGF21 or a derivative or variant thereof. The GLP-1 may be a human GLP-1. The FGF21 may be a human FGF21. The antibody or antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. The immunoglobulin domain may be from a mammalian antibody. Alternatively, the immunoglobulin domain may be from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody may be a murine antibody. The immunoglobulin fusion protein, antibody region, and/or extender fusion region may further comprise one or more linkers. The linker may attach GLP-1, exendin-4, FGF21, or a derivative or variant thereof to the extender peptide. The linker may attach the antibody region to the extender fusion region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. Metabolic diseases and/or conditions may include disorders of carbohydrate metabolism, amino acid metabolism, organic acid metabolism (organic acidurias), fatty acid oxidation and mitochondrial metabolism, porphyrin metabolism, purine or pyrimidine metabolism, steroid metabolism, mitochondrial function, peroxisomal function, urea cycle disorder, urea cycle defects or lysosomal storage disorders. The metabolic disease or condition may be diabetes. In other instances, the metabolic disease or condition may be glycogen storage disease, phenylketonuria, maple syrup urine disease, glutaric acidemia

type 1, Carbamoyl phosphate synthetase I deficiency, alcaptonuria, Medium-chain acyl-coenzyme A dehydrogenase deficiency (MCADD), acute intermittent porphyria, Lesch-Nyhan syndrome, lipid congenital adrenal hyperplasia, congenital adrenal hyperplasia, Kearns-Sayre syndrome, Zellweger syndrome, Gaucher's disease, or Niemann Pick disease.

[00443] Provided herein is a method of preventing or treating a central nervous system (CNS) disorder in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The composition may further comprise a pharmaceutically acceptable carrier. The subject may be a mammal. The mammal may be a human. Alternatively, the mammal may be a bovine. The therapeutic agent may be GLP-1, exendin-4 or a derivative or variant thereof. The GLP-1 may be a human GLP-1. The antibody may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. The immunoglobulin domain may be from a mammalian antibody. Alternatively, the immunoglobulin domain may be from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody may be a murine antibody. The immunoglobulin fusion protein, antibody region, and/or extender fusion region may further comprise one or more linkers. The linker may attach GLP-1, exendin-4, or a derivative or variant thereof to the immunoglobulin domain or fragment thereof. The linker may attach the antibody region to the extender fusion region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The CNS disorder may be Alzheimer's disease (AD). Additional CNS disorders include, but are not limited to, encephalitis, meningitis, tropical spastic paraparesis, arachnoid cysts, Huntington's disease, locked-in syndrome, Parkinson's disease, Tourette's, and multiple sclerosis.

[00444] Provided herein is a method of preventing or treating a disease or condition which benefits from a GLP-1R and/or glucagon receptor (GCGR) agonist in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The composition may further comprise a pharmaceutically acceptable carrier. The subject may be a mammal. The mammal may be a human. Alternatively, the mammal may be a bovine. The therapeutic agent may be GLP-1, exendin-4 or a derivative or variant thereof. The GLP-1 may be a human GLP-1. The immunoglobulin fusion protein or antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. The immunoglobulin domain may be from a mammalian antibody. Alternatively, the immunoglobulin domain may be from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody may be a murine antibody. The immunoglobulin fusion protein, antibody region, and/or extender fusion region may further comprise one or more linkers. The linker may attach GLP-1, exendin-4, or a derivative or variant thereof to the extender peptide. In other instances, the linker attaches the extender fusion region to the antibody region. The disease or condition may be a metabolic disease or disorder. The disease or condition may be diabetes. In other instances, the disease or condition may be obesity. Additional diseases and/or conditions which benefit from a GLP-1R and/or GCGR agonist include, but are not limited to, dyslipidemia, cardiovascular and fatty liver diseases.

[00445] Provided herein is a method of preventing or treating a blood disorder in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker

peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The composition may further comprise a pharmaceutically acceptable carrier. The subject may be a mammal. The mammal may be a human. Alternatively, the mammal may be a bovine. The therapeutic agent may be erythropoietin, GMCSF or a derivative or variant thereof. The erythropoietin may be a human erythropoietin. The GMCSF may be a human GMCSF. The antibody may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. The immunoglobulin domain may be from a mammalian antibody. Alternatively, the immunoglobulin domain may be from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody may be a murine antibody. The immunoglobulin fusion protein, antibody region, and/or extender fusion region may further comprise one or more linkers. The linker may attach erythropoietin, GMCSF, or a derivative or variant thereof to the extender peptide. The linker may attach the antibody region to the extender fusion region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The blood disorder may be anemia. Examples of anemia include, but are not limited to, hereditary xerocytosis, congenital dyserythropoietic anemia, Rh null disease, infectious mononucleosis related anemia, drugs-related anemia, aplastic anemia, microcytic anemia, macrocytic anemia, normocytic anemia, hemolytic anemia, poikilocytic anemia, spherocytic anemia, drepanocytic anemia, normochromic anemia, hyperchromic anemia, hypochromic anemia, macrocytic-normochromic anemia, microcytic-hypochromic anemia, normocytic-normochromic anemia, iron-deficiency anemia, pernicious anemia, folate-deficiency anemia, thalassemia, sideroblastic anemia, posthemorrhagic anemia, sickle cell anemia, chronic anemia, achrestic anemia, autoimmune haemolytic anemia, Cooley's anemia, drug-induced immune haemolytic anemia, erythroblastic anemia, hypoplastic anemia, Diamond-Blackfan anemia, Pearson's anemia, transient anemia, Fanconi's anemia, Lederer's anemia, myelopathic anemia, nutritional anemia, spur-cell anemia, Von Jaksh's anemia, sideroblastic anemia, sideropenic anemia, alpha thalassemia, beta thalassemia, hemoglobin h disease, acute acquired hemolytic anemia, warm autoimmune hemolytic anemia, cold autoimmune hemolytic anemia, primary cold autoimmune hemolytic anemia, secondary cold autoimmune

hemolytic anemia, secondary autoimmune hemolytic anemia, primary autoimmune hemolytic anemia, x-linked sideroblastic anemia, pyridoxine-responsive anemia, nutritional sideroblastic anemia, pyridoxine deficiency-induced sideroblastic anemia, copper deficiency-induced sideroblastic anemia, cycloserine-induced sideroblastic anemia, chloramphenicol-induced sideroblastic anemia, ethanol-induced sideroblastic anemia, isoniazid-induced sideroblastic anemia, drug-induced sideroblastic anemia, toxin-induced sideroblastic anemia, microcytic hyperchromic anemia, macrocytic hyperchromic anemia, megalocytic-normochromic anemia, drug-induced immune hemolytic anemia, non-hereditary spherocytic anemia, inherited spherocytic anemia, and congenital spherocytic anemia. In other instances, the blood disorder may be malaria. Alternatively, the blood disorder may be lymphoma, leukemia, multiple myeloma, or myelodysplastic syndrome. The blood disorder may be neutropenia, Shwachmann-Diamond syndrome, Kostmann syndrome, chronic granulomatous disease, leukocyte adhesion deficiency, myeloperoxidase deficiency, or Chediak Higashi syndrome.

[00446] Provided herein is a method of preventing or treating a disease or disorder which benefits from stimulating or increasing white blood cell production in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The composition may further comprise a pharmaceutically acceptable carrier. The subject may be a mammal. The mammal may be a human. Alternatively, the mammal may be a bovine. The therapeutic agent may be GMCSF or a derivative or variant thereof. The GMCSF may be a human GMCSF. The immunoglobulin fusion protein or antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. The immunoglobulin domain may be from a mammalian antibody. Alternatively, the immunoglobulin domain may be from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody may be a

murine antibody. The immunoglobulin fusion protein, antibody region, and/or extender fusion region may further comprise one or more linkers. The linker may attach the antibody region to the extender fusion region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The disease or disorder may be neutropenia, Shwachmann-Diamond syndrome, Kostmann syndrome, chronic granulomatous disease, leukocyte adhesion deficiency, myeloperoxidase deficiency, or Chediak Higashi syndrome.

[00447] Provided herein is a method of preventing or treating a disease or disorder which benefits from stimulating or increasing red blood cell production in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The composition may further comprise a pharmaceutically acceptable carrier. The subject may be a mammal. The mammal may be a human. Alternatively, the mammal may be a bovine. The therapeutic agent may be erythropoietin or a derivative or variant thereof. The erythropoietin may be a human erythropoietin. The antibody may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. The immunoglobulin domain may be from a mammalian antibody. Alternatively, the immunoglobulin domain may be from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody may be a murine antibody. The immunoglobulin fusion protein, antibody region, and/or extender fusion region may further comprise one or more linkers. The linker may attach erythropoietin, or a derivative or variant thereof to the extender peptide. The linker may attach the antibody region to the extender fusion region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The disease or disorder may be anemia.

[00448] Provided herein is a method of preventing or treating obesity in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The composition may further comprise a pharmaceutically acceptable carrier. The subject may be a mammal. The mammal may be a human. Alternatively, the mammal may be a bovine. The therapeutic agent may be GLP-1 or a derivative or variant thereof. The GLP-1 may be a human GLP-1. The therapeutic agent may be FGF21 or a derivative or variant thereof. The FGF21 may be a human FGF21. The therapeutic agent may be exendin-4 or a derivative or variant thereof. The antibody may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. The immunoglobulin domain may be from a mammalian antibody. Alternatively, the immunoglobulin domain may be from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody may be a murine antibody. The immunoglobulin fusion protein, antibody region, and/or extender fusion region may further comprise one or more linkers. The linker may attach GLP-1, exendin-4, FGF21, or a derivative or variant thereof to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent.

[00449] Provided herein is a method of preventing or treating a pain in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may

comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The subject may be a mammal. In certain instances, the mammal may be a human. Alternatively, the mammal may be a bovine. The therapeutic agent may be a Mambal or a derivative or variant thereof. The immunoglobulin fusion proteins, antibody regions, and/or extender fusion regions may further comprise one or more linkers. The linker may attach the Mambal or a derivative or variant thereof to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent.

[00450] Provided herein is a method of preventing or treating a disease or condition which benefits from modulating a sodium ion channel in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The subject may be a mammal. In certain instances, the mammal may be a human. Alternatively, the mammal may be a bovine. The one or more antibodies, antibody fragments, or immunoglobulin constructs further comprise a linker. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent.

[00451] Provided herein is a method of preventing or treating a disease or condition which benefits from modulating an acid sensing ion channel (ASIC) in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-

antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The subject may be a mammal. In certain instances, the mammal may be a human. Alternatively, the mammal may be a bovine. The therapeutic agent may be Mamba 1 or a derivative or variant thereof. The therapeutic agent may be neutrophil elastase inhibitor or a derivative or variant thereof. The one or more antibodies, antibody fragments, or immunoglobulin constructs further comprise a linker. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. Modulating an ASIC may comprise inhibiting or blocking the ASIC. Modulating an ASIC may comprise activating the ASIC. The disease or condition may be a central nervous system disorder. In other instances, the disease or condition is pain.

[00452] Provided herein is a method of preventing or treating a pathogenic infection in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The composition may further comprise a pharmaceutically acceptable carrier. The subject may be a mammal. The mammal may be a human. Alternatively, the mammal may be a bovine. The therapeutic agent may be alpha-interferon or a derivative or variant thereof. The therapeutic agent may be beta-interferon or a derivative or variant thereof. The antibody may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. The immunoglobulin domain may be from a mammalian

antibody. Alternatively, the immunoglobulin domain may be from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody may be a murine antibody. The immunoglobulin fusion protein, antibody region, and/or extender fusion region may further comprise one or more linkers. The linker may attach alpha-interferon, beta-interferon, or a derivative or variant thereof to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The pathogenic infection may be a bacterial infection. The pathogenic infection may be a fungal infection. The pathogenic infection may be a parasitic infection. The pathogenic infection may be a viral infection. The viral infection may be a herpes virus.

[00453] Provided herein is a method of preventing or treating a cancer in a subject in need thereof comprising administering to the subject a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The composition may further comprise a pharmaceutically acceptable carrier. The subject may be a mammal. The mammal may be a human. Alternatively, the mammal may be a bovine. The therapeutic agent may be beta-interferon or a derivative or variant thereof. The antibody may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. The immunoglobulin domain may be from a mammalian antibody. Alternatively, the immunoglobulin domain may be from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody may be a murine antibody. The immunoglobulin fusion protein, antibody region, and/or extender fusion region may further comprise one or more linkers. The linker may

attach a therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The cancer may be a hematological malignancy. The hematological malignancy may be a leukemia or lymphoma. The hematological malignancy may be a B-cell lymphoma, T-cell lymphoma, follicular lymphoma, marginal zone lymphoma, hairy cell leukemia, chronic myeloid leukemia, mantle cell lymphoma, nodular lymphoma, Burkitt's lymphoma, cutaneous T-cell lymphoma, chronic lymphocytic leukemia, or small lymphocytic leukemia.

[00454] Provided herein is a method of preventing or treating a disease or condition which would benefit from modulation of a receptor in a subject in need thereof comprising administering to the subject a composition disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises one or more immunoglobulin fusion proteins comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The subject may be a mammal. In certain instances, the mammal may be a human. Alternatively, the mammal may be a bovine. The therapeutic agent may be hGCSF or a derivative or variant thereof and the receptor may be GCSFR. The therapeutic agent may be erythropoietin or a derivative or variant thereof and the receptor may be EPOR. The therapeutic agent may be exendin-4 or a derivative or variant thereof and the receptor may be GLP1R. The therapeutic agent may be GLP-1 or a derivative or variant thereof and the receptor may be GLP1R. The therapeutic agent may be hLeptin or a derivative or variant thereof and the receptor may be LepR. The therapeutic agent may be hGH or a derivative or variant thereof and the receptor may be GHR. The therapeutic agent may be interferon-alpha or a derivative or variant thereof and the receptor may be IFNR. The therapeutic agent may be interferon-beta or a derivative or variant thereof and the receptor may be IFNR. The therapeutic agent may be relaxin or a derivative or variant thereof and the receptor may be LGR7. The therapeutic agent may be GMCSF or a derivative or variant thereof and the receptor may be GMCSFR. The one or more antibodies, antibody fragments, or immunoglobulin constructs further comprise a linker. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The disease or condition may be an autoimmune disease. The autoimmune disease may be a T-cell mediated autoimmune disease. The disease or condition may be a metabolic disorder. The metabolic

disorder may be diabetes. The disease or condition may be an inflammatory disorder. The inflammatory disorder may be multiple sclerosis. The disease or condition may be a cell proliferative disorder. The disease or condition may be a blood disorder. The blood disorder may be neutropenia. The blood disorder may be anemia. The disease or condition may be a pathogenic infection. The pathogenic infection may be a viral infection. The disease or condition may be a growth disorder. The disease or condition may be a cardiovascular condition. The cardiovascular condition may be acute heart failure. Modulating the receptor may comprise inhibiting or blocking the receptor. Modulating the receptor may comprise activating the receptor. The therapeutic agent may act as a receptor agonist. The therapeutic agent may act as a receptor antagonist.

[00455] Provided herein is a method of preventing or treating a disease in a mammal in need thereof comprising administering a pharmaceutical composition described herein to said mammal. In some embodiments, the disease may be an infectious disease. In certain embodiments, the infectious disease may be mastitis. In some embodiments, the infectious disease may be a respiratory disease. In certain embodiments, the respiratory disease may be bovine respiratory disease of shipping fever. In certain embodiments, the mammal in need may be a dairy animal selected from a list comprising cow, camel, donkey, goat, horse, reindeer, sheep, water buffalo, moose and yak. In some embodiments, the mammal in need may be bovine.

[00456] Provided may be a method of preventing or treating mastitis in a dairy animal, comprising providing to said dairy animal an effective amount of a composition comprising one or more immunoglobulin fusion proteins disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. In some instances, the immunoglobulin fusion protein comprises an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising an amino acid sequence comprising an alpha helix and (i) an amino acid sequence comprising 7 or fewer amino acids based on or derived from an ultralong CDR3 or (ii) an amino acid sequence that does not comprise an ultralong CDR3; and (b) a therapeutic agent. The therapeutic agent may be GCSF. The GCSF may be a bovine GCSF. The GCSF may be a human GCSF. In some embodiments, the dairy animal may be a cow or a water buffalo.

[00457] Provided are methods of treatment, inhibition and prevention of a disease or condition in a subject in need thereof by administration to the subject of an effective amount of an immunoglobulin fusion protein or pharmaceutical composition described herein. The immunoglobulin fusion protein may be substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject may be an animal, including but not limited to animals such as cows, pigs,

sheep, goats, rabbits, horses, chickens, cats, dogs, mice, etc. The subject may be a mammal. The subject may be a human. The subject may be a non-human primate. Alternatively, the subject may be a bovine. The subject may be an avian, reptile or amphibian.

[00458] Additional uses

[00459] Further disclosed herein are uses of an immunoglobulin fusion protein (IFP) in the manufacture of a medicament for the treatment of a disease or condition. The IFP may be any of the IFPs disclosed herein. Disclosed herein is the use of an immunoglobulin fusion protein in the manufacture of a medicament for the treatment of a disease or condition, the immunoglobulin fusion protein comprising an antibody region attached to a non-antibody region. Further disclosed herein is the use of an immunoglobulin fusion protein in the manufacture of a medicament for the treatment of a disease or condition, the IFP comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The non-antibody region may be inserted within the antibody region. The non-antibody region may be inserted within an immunoglobulin heavy chain of the antibody region. The non-antibody region may be inserted within an immunoglobulin light chain of the antibody region. The non-antibody region may be conjugated to the antibody region. The non-antibody may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may comprise GCSF. The GCSF may be a human GCSF. The therapeutic agent may be Moka1. The therapeutic agent may be VM24. The therapeutic agent may be exendin-4. The therapeutic agent may be

erythropoietin. The erythropoietin may be a human erythropoietin. The therapeutic agent may be hLeptin. The therapeutic agent may be a growth hormone (GH). The growth hormone may be a human growth hormone (hGH). The therapeutic agent may be interferon-alpha. The therapeutic agent may be interferon-beta. The therapeutic agent may be GLP-1. The therapeutic agent may be neutrophil elastase inhibitor. The therapeutic agent may be relaxin. The therapeutic agent may be Mambal. The therapeutic agent may be elafin. The therapeutic agent may be betatrophin. The therapeutic agent may be GDF11. The therapeutic agent may be GMCSF. The disease or condition may be an autoimmune disease, heteroimmune disease or condition, inflammatory disease, pathogenic infection, thromboembolic disorder, respiratory disease or condition, metabolic disease, central nervous system (CNS) disorder, bone disease or cancer. In other instances, the disease or condition is a blood disorder. In some instances, the disease or condition is obesity, diabetes, osteoporosis, anemia, or pain. The disease or condition may be a growth disorder.

[00460] Disclosed herein is the use of an immunoglobulin fusion protein in the manufacture of a medicament for the treatment of a cell proliferative disorder. The IFP may be any of the IFPs disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The cell proliferative disorder may be cancer. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The non-antibody region may be inserted within the antibody region. The non-antibody region may be inserted within an immunoglobulin heavy chain of the antibody region. The non-antibody region may be inserted within an immunoglobulin light chain of the antibody region. The non-antibody region may be conjugated to the antibody region. The non-antibody region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or

fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule.

[00461] Disclosed herein is the use of an immunoglobulin fusion protein in the manufacture of a medicament for the treatment of a metabolic disorder. The metabolic disorder may be diabetes. Diabetes may be type I diabetes. Diabetes may be type II diabetes. The IFP may be any of the IFPs disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In

other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be exendin-4. The therapeutic agent may be GLP-1. The therapeutic agent may be hLeptin. The therapeutic agent may be betatrophin.

[00462] Disclosed herein is the use of an immunoglobulin fusion protein in the manufacture of a medicament for the treatment of an autoimmune disease or condition. The IFP may be any of the IFPs disclosed herein. The IFP may be any of the IFPs disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may

attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be Moka1. The therapeutic agent may be VM24.

[00463] Disclosed herein is the use of an immunoglobulin fusion protein in the manufacture of a medicament for the treatment of an inflammatory disease or condition. The inflammatory disease or condition may be multiple sclerosis. The IFP may be any of the IFPs disclosed herein. The IFP may be any of the IFPs disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be elafin. The therapeutic agent may be interferon-beta.

[00464] Disclosed herein is the use of an immunoglobulin fusion protein in the manufacture of a medicament for the treatment of a disease or condition of the central nervous system. The IFP may be any of the IFPs disclosed herein. The disease or condition of the central nervous system may be pain. The IFP may be any of the IFPs disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be Mambal.

[00465] Disclosed herein is the use of an immunoglobulin fusion protein in the manufacture of a medicament for the treatment of a cardiovascular disease or condition. The IFP may be any of the IFPs disclosed herein. The cardiovascular disease or condition may be acute heart failure. The cardiovascular disease or condition may be cardiac hypertrophy. The IFP may be any of the IFPs disclosed herein. The

immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be relaxin. The therapeutic agent may be GDF11.

[00466] Disclosed herein is the use of an immunoglobulin fusion protein in the manufacture of a medicament for the treatment of a hematological disease or condition. The IFP may be any of the IFPs disclosed herein. The hematological disease or condition may be anemia. The hematological disease or condition may be neutropenia. The IFP may be any of the IFPs disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a

proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be GCSF. The GCSF may be a human GCSF. The therapeutic agent may be erythropoietin. The erythropoietin may be a human erythropoietin. The therapeutic agent may be GMCSF.

[00467] Disclosed herein is the use of an immunoglobulin fusion protein in the manufacture of a medicament for the treatment of a pathogenic infection. The IFP may be any of the IFPs disclosed herein. The pathogenic infection may be a viral infection. The IFP may be any of the IFPs disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may

comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be interferon-alpha.

[00468] Disclosed herein is the use of an immunoglobulin fusion protein in the manufacture of a medicament for the treatment of a growth disorder. The IFP may be any of the IFPs disclosed herein. Examples of growth disorders included, but are not limited to, achondroplasia, achondroplasia in children, acromegaly, adiposogenital dystrophy, dwarfism, gigantism, Brooke Greenberg, hemihypertrophy, hypochondroplasia, Jansen's metaphyseal chondrodysplasia, Kowarski syndrome, Léri-Weill dyschondrosteosis, local gigantism, macrodystrophia lipomatosa, Majewski's polydactyly syndrome, microcephalic osteodysplastic primordial dwarfism type II, midget, overgrowth syndrome, parastremmatic dwarfism, primordial dwarfism, pseudoachondroplasia, psychosocial short stature, Seckel syndrome, short rib – polydactyly syndrome and Silver–Russell syndrome. The IFP may be any of the IFPs disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may

comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be a growth hormone. The growth hormone may be a human growth hormone (hGH).

[00469] Further disclosed herein are uses of an immunoglobulin fusion protein for the treatment of a disease or condition. The IFP may be any of the IFPs disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to

an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may comprise GCSF. The GCSF may be a human GCSF. The therapeutic agent may be Moka1. The therapeutic agent may be VM24. The therapeutic agent may be exendin-4. The therapeutic agent may be erythropoietin. The erythropoietin may be a human erythropoietin. The therapeutic agent may be hLeptin. The therapeutic agent may be a growth hormone (GH). The growth hormone may be a human growth hormone (hGH). The therapeutic agent may be interferon-alpha. The therapeutic agent may be interferon-beta. The therapeutic agent may be GLP-1. The therapeutic agent may be relaxin. The therapeutic agent may be neutrophil elastase inhibitor. The therapeutic agent may be Mamba1. The therapeutic agent may be elafin. The therapeutic agent may be betatrophin. The therapeutic agent may be GDF11. The therapeutic agent may be GMCSF. The disease or condition may be an autoimmune disease, heteroimmune disease or condition, inflammatory disease, pathogenic infection, thromboembolic disorder, respiratory disease or condition, metabolic disease, central nervous system (CNS) disorder, bone disease or cancer. In other instances, the disease or condition is a blood disorder. In some instances, the disease or condition is obesity, diabetes, osteoporosis, anemia, or pain. The disease or condition may be a growth disorder.

[00470] Disclosed herein is the use of an immunoglobulin fusion protein for the treatment of a cell proliferative disorder in a subject in need thereof. The IFP may be any of the IFPs disclosed herein. The cell proliferative disorder may be cancer. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a

linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule.

[00471] Disclosed herein is the use of an immunoglobulin fusion protein for the treatment of a metabolic disorder in a subject in need thereof. The IFP may be any of the IFPs disclosed herein. The metabolic disorder may be diabetes. Diabetes may be type I diabetes. Diabetes may be type II diabetes. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP

may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be exendin-4. The therapeutic agent may be GLP-1. The therapeutic agent may be hLeptin. The therapeutic agent may be betatrophin.

[00472] Disclosed herein is the use of an immunoglobulin fusion protein for the treatment of an autoimmune disease or condition in a subject in need thereof. The IFP may be any of the IFPs disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic

agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be Moka1. The therapeutic agent may be VM24.

[00473] Disclosed herein is the use of an immunoglobulin fusion protein for the treatment of an inflammatory disease or condition in a subject in need thereof. The IFP may be any of the IFPs disclosed herein. The inflammatory disease or condition may be multiple sclerosis. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody

region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be elafin. The therapeutic agent may be interferon-beta.

[00474] Disclosed herein is the use of an immunoglobulin fusion protein for the treatment of a disease or condition of the central nervous system in a subject in need thereof. The IFP may be any of the IFPs disclosed herein. The disease or condition of the central nervous system may be pain. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody.

Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be Mambal.

[00475] Disclosed herein is the use of an immunoglobulin fusion protein for the treatment of a cardiovascular disease or condition in a subject in need thereof. The IFP may be any of the IFPs disclosed herein. The cardiovascular disease or condition may be acute heart failure. The cardiovascular disease or condition may be cardiac hypertrophy. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian

antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be relaxin. The therapeutic agent may be GDF11.

[00476] Disclosed herein is the use of an immunoglobulin fusion protein for the treatment of a hematological disease or condition in a subject in need thereof. The IFP may be any of the IFPs disclosed herein. The hematological disease or condition may be anemia. The hematological disease or condition may be neutropenia. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion

region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be GCSF. The GCSF may be a human GCSF. The therapeutic agent may be erythropoietin. The erythropoietin may be a human erythropoietin. The therapeutic agent may be GMCSF.

[00477] Disclosed herein is the use of an immunoglobulin fusion protein for the treatment of a pathogenic infection in a subject in need thereof. The IFP may be any of the IFPs disclosed herein. The pathogenic infection may be a viral infection. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively, therapeutic agent is a small molecule. The therapeutic agent may be interferon-alpha.

[00478] Disclosed herein is the use of an immunoglobulin fusion protein for the treatment of a growth disorder in a subject in need thereof. Examples of growth disorders included, but are not limited to, achondroplasia, achondroplasia in children, acromegaly, adiposogenital dystrophy, dwarfism, gigantism, Brooke Greenberg, hemihypertrophy, hypochondroplasia, Jansen's metaphyseal chondrodysplasia, Kowarski syndrome, Léri-Weill dyschondrosteosis, local gigantism, macrodystrophia lipomatosa, Majewski's polydactyly syndrome, microcephalic osteodysplastic primordial dwarfism type II, midget, overgrowth syndrome, parastremmatic dwarfism, primordial dwarfism, pseudoachondroplasia, psychosocial short stature, Seckel syndrome, short rib- polydactyly syndrome and Silver-Russell syndrome. The IFP may be any of the IFPs disclosed herein. The immunoglobulin fusion protein may comprise an antibody region attached to a non-antibody region. The non-antibody region may comprise a therapeutic agent. The non-antibody region may comprise an extender peptide. The non-antibody region may comprise a linker peptide. The non-antibody region may comprise a proteolytic cleavage site. The immunoglobulin fusion protein may comprise an antibody region attached to an extender fusion region. The extender fusion region may comprise a therapeutic agent. The extender fusion region may comprise an extender peptide. The extender fusion region may comprise a linker peptide. The extender fusion region may comprise a proteolytic cleavage site. The IFP may comprise a non-antibody region attached to an antibody region, wherein the antibody region comprises 6 or fewer amino acids of an ultralong CDR3. The non-antibody region may comprise one or more therapeutic agents. In some instances, the immunoglobulin fusion protein comprising an antibody region attached to an extender fusion region, wherein the extender fusion region comprises (a) an extender peptide comprising at least one secondary structure; and (b) a therapeutic agent. The extender fusion region may be inserted within the antibody region. The extender fusion region may be inserted within an immunoglobulin heavy chain of the antibody region. The extender fusion region may be inserted within an immunoglobulin light chain of the antibody region. The extender fusion region may be conjugated to the antibody region. The extender fusion region may be conjugated to a position within the antibody region. The antibody region may comprise one or more immunoglobulin domains. The immunoglobulin domain may be an immunoglobulin A, an immunoglobulin D, an immunoglobulin E, an immunoglobulin G, or an immunoglobulin M. The immunoglobulin domain may be an immunoglobulin heavy chain region or fragment thereof. In some instances, the immunoglobulin domain is from a mammalian antibody. Alternatively, the immunoglobulin domain is from a chimeric antibody. The immunoglobulin domain may be from an engineered antibody or recombinant antibody. The immunoglobulin domain may be from a humanized, human engineered or fully human antibody. The mammalian antibody may be a bovine antibody. The mammalian antibody may be a human antibody. In other instances, the mammalian antibody is a murine antibody. The immunoglobulin fusion protein, antibody region and/or extender fusion region may further comprise one or more linkers. The linker may attach therapeutic agent to the extender peptide. The linker may attach the extender fusion region to the antibody region. The linker may attach a proteolytic cleavage site to the antibody region, extender fusion region, extender peptide, or therapeutic agent. The therapeutic agent may be a peptide or derivative or variant thereof. Alternatively,

therapeutic agent is a small molecule. The therapeutic agent may be a growth hormone. The growth hormone may be a human growth hormone (hGH).

[00479] Pharmacological Properties

[00480] Further disclosed herein are methods of improving one or more pharmacological properties of a therapeutic agent. The method may comprise producing an immunoglobulin fusion protein disclosed herein. Examples of pharmacological properties may include, but are not limited to, half-life, stability, solubility, immunogenicity, toxicity, bioavailability, absorption, liberation, distribution, metabolism, and excretion. Liberation may refer to the process of releasing of a therapeutic agent from the pharmaceutical formulation. Absorption may refer to the process of a substance entering the blood circulation. Distribution may refer to the dispersion or dissemination of substances throughout the fluids and tissues of the body. Metabolization (or biotransformation, or inactivation) may refer to the recognition by an organism that a foreign substance is present and the irreversible transformation of parent compounds into daughter metabolites. Excretion may refer to the removal of the substances from the body.

[00481] The half-life of a therapeutic agent may greater than the half-life of the non-conjugated therapeutic agent. The half-life of the therapeutic agent may be greater than 4 hours, greater than 6 hours, greater than 12 hours, greater than 24 hours, greater than 36 hours, greater than 2 days, greater than 3 days, greater than 4 days, greater than 5 days, greater than 6 days, greater than 7 days, greater than 8 days, greater than 9 days, greater than 10 days, greater than 11 days, greater than 12 days, greater than 13 days, or greater than 14 days when administered to a subject. The half-life of the therapeutic agent may be greater than 4 hours when administered to a subject. The half-life of the therapeutic agent may be greater than 6 hours when administered to a subject.

[00482] The half-life of the therapeutic agent may increase by at least about 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20 or more hours. The half-life of the therapeutic agent may increase by at least about 2 hours. The half-life of the therapeutic agent may increase by at least about 4 hours. The half-life of the therapeutic agent may increase by at least about 6 hours. The half-life of the therapeutic agent may increase by at least about 8 hours.

[00483] The half-life of a therapeutic agent may be at least about 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10-fold greater than the half-life of the non-conjugated therapeutic peptide. The half-life of a therapeutic agent an antibody described herein may be at least about 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50-fold greater than the half-life of the non-conjugated therapeutic peptide. The half-life of a therapeutic agent an antibody described herein may be at least about 2-fold greater than the half-life of the non-conjugated therapeutic peptide. The half-life of a therapeutic agent an antibody described herein may be at least about 5-fold greater than the half-life of the non-conjugated therapeutic peptide. The half-life of a therapeutic agent an antibody described herein may be at least about 10-fold greater than the half-life of the non-conjugated therapeutic peptide.

[00484] The half-life of a therapeutic agent an antibody described herein may be at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97%

greater than the half-life of the non-conjugated therapeutic peptide. The half-life of a therapeutic agent an antibody described herein may be at least about 10% greater than the half-life of the non-conjugated therapeutic peptide. The half-life of a therapeutic agent an antibody described herein may be at least about 20% greater than the half-life of the non-conjugated therapeutic peptide. The half-life of a therapeutic agent an antibody described herein may be at least about 30% greater than the half-life of the non-conjugated therapeutic peptide. The half-life of a therapeutic agent an antibody described herein may be at least about 40% greater than the half-life of the non-conjugated therapeutic peptide. The half-life of a therapeutic agent an antibody described herein may be at least about 50% greater than the half-life of the non-conjugated therapeutic peptide.

EXAMPLES

Example 1: Construction of trastuzumab-coil-bGCSF fusion protein vectors for expression in mammalian cells

[00485] A gene encoding bovine GCSF (bGCSF) (SEQ ID NO: 186) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). To optimize the folding and stability of the immunoglobulin fusion protein, flexible linkers of GGGGS (SEQ ID NO: 179, n=1) were added on both ends of the bGCSF fragments. Then, sequences encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the bGCSF-linker fragment. Subsequently, PCR fragments encoding the bGCSF gene with the extender peptides and linkers was grafted into the complementarity determining region 3 of the heavy chain (CDR3H) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace the Trp99–Met107 loop. The trastuzumab-coil based bGCSF fusion protein was further modified to replace the hIgG1 CH1-CH3 constant region of trastuzumab with hIgG4 CH1-CH3 constant region containing triple mutants (S228P, F234A and L235A) to generate trastuzumab-coil bGCSF HC (SEQ ID NO: 38). The expression vectors of trastuzumab-coil based fusion proteins were generated by in-frame ligation of the amplified fusion genes to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain of trastuzumab IgG antibody (SEQ ID NO: 1) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 2: Construction of bovine-coil-bGCSF fusion protein vectors for expression in mammalian cells

[00486] A gene encoding bovine GCSF (bGCSF) (SEQ ID NO: 186) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). To optimize the folding and stability of the immunoglobulin fusion protein, flexible linkers of GGGGS (SEQ ID NO: 179, n=1) were added on both ends of the bGCSF fragments. Then, sequences encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the bGCSF-linker fragment. Subsequently, PCR fragments encoding the bGCSF gene with the extender peptides and linkers was grafted into the complementarity determining region 3 of the heavy chain (CDR3H) of bovine IgG antibody (BLV1H12) by exploiting overlap extension PCR to generate bovine-coil bGCSF HC (SEQ ID

NO: 39). The expression vectors of bovine-coil based fusion proteins were generated by in-frame ligation of the amplified fusion genes to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain of bovine IgG antibody (SEQ ID NO: 18) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 3: Expression and purification of trastuzumab-coil-bGCSF and bovine-coil-bGCSF based fusion proteins

[00487] Trastuzumab-coil-bGCSF based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil-bGCSF fusion protein heavy chain (SEQ ID NO: 38) and the trastuzumab light chain (SEQ ID NO: 1). Bovine-coil-bGCSF based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding bovine-coil-bGCSF fusion protein heavy chain (SEQ ID NO: 39) and the bovine light chain (SEQ ID NO: 18). Expressed fusion proteins were secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins were purified by Protein A/G chromatography (Thermo Fisher Scientific, IL), and analyzed by SDS-PAGE gel. As shown in FIG.4, Lane 1 depicts the protein ladder, Lane 2 depicts bovine-coil IgG, Lane 3 depicts bovine-coil IgG treated with DTT, Lane 4 depicts bovine-coil-bGCSF IgG, Lane 5 depicts bovine-coil-bGCSF IgG treated with DTT, Lane 6 depicts trastuzumab-coil-bGCSF IgG, Lane 7 depicts trastuzumab-coil-bGCSF IgG treated with DTT, Lane 8 depicts trastuzumab IgG and Lane 9 depicts trastuzumab IgG treated with DTT.

Example 4: In vitro study of trastuzumab-coil bGCSF fusion protein and bovine-coil bGCSF fusion protein proliferative activity on mouse NFS-60 cells

[00488] Mouse NFS-60 cells were obtained from American Type Culture Collection (ATCC), VA, and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 0.05 mM 2-mercaptoethanol and 62 ng/ml human macrophage colony stimulating factor (M-CSF). For proliferation assays, mouse NFS-60 cells were washed three times with RPMI-1640 medium and resuspended in RPMI-1640 medium with 10% FBS and 0.05 mM 2-mercaptoethanol at a density of 1.5×10^5 cells/ml. In 96-well plates, 100 μ l of cell suspension was added into each well, followed by the addition of varied concentrations of trastuzumab IgG (SEQ ID NOs: 22 and 19), trastuzumab-coil-bGCSF IgG (SEQ ID NOs: 69 and 19), bovine-coil IgG (SEQ ID NOs: 36 and 271), bovine-coil-bGCSF IgG (SEQ ID NOs: 70 and 36), and bGCSF (SEQ ID NO: 227). The plates were incubated at 37°C in a 5% CO₂ incubator for 72 hours. Cells were then treated with AlamarBlue (Invitrogen) (1/10 volume of cell suspension) for 4 hours at 37°C. Fluorescence at 595 nm for each well was read to indicate the cell viability and is displayed in Table 13. FIG. 6 depicts a graphical representation of the data. The EC₅₀ of trastuzumab-coil-bGCSF IgG was 2.49 ± 0.26 ng/mL. The EC₅₀ of bovine-coil-bGCSF IgG was 2.55 ± 0.38 ng/mL. The EC₅₀ of bGCSF was 4.87 ± 0.29 ng/mL.

Table 13			
trastuzumab IgG (ng/mL)	Fluorescence Intensity	trastuzumab-coil bGCSF IgG (ng/mL)	Fluorescence Intensity
1000	1465.7345	1000	7392.629
333.33333	1464.256	333.33333	8058.969
111.11111	1497.443	111.11111	8386.5135
37.03704	1533.4505	37.03704	7799.397
12.34568	1546.9655	12.34568	7649.2075
4.11523	1613.3125	4.11523	6019.7085
1.37174	1909.983	1.37174	3517.689
0.45725	1751.1505	0.45725	2359.373
0.15242	1596.733	0.15242	1863.8285
0.05081	1674.4565	0.05081	1823.8255
0.01694	1729.6545	0.01694	1834.7485
0.00565	1929.9635	0.00565	1873.0145

Example 5: Binding of trastuzumab-coil-bGCSF to Her2 receptor

[00489] The binding affinity of trastuzumab-coil-bGCSF fusion proteins to Her2 receptor was examined by ELISA. Human Her2-Fc chimera (5 ug/mL) (R&D Systems) was coated on 96-well ELISA plate overnight at 4°C, followed by blocking with 1% BSA in PBS (pH7.4) for 2 hours at 37°C. After washing with 0.05% Tween-20 in PBS (pH7.4), varied concentrations of trastuzumab IgG (SEQ ID NOs: 22 and 19) and trastuzumab-coil-bGCSF (SEQ ID NOs: 69 and 19) fusion proteins were added to each well and incubated for 2 hours at 37°C. Subsequently, goat polyclonal anti-human kappa light chain antibody with HRP conjugate (Sigma) was added and incubated for 2 hours at 37°C. Wells were subsequently washed and binding affinities were examined on the basis of fluorescence intensity at 425 nm by adding fluoregenic peroxidase substrate to each well. Table 2 displays the fluorescence intensity at 425 nm of the trastuzumab IgG and trastuzumab-coil-bGCSF IgG. FIG. 7 depicts a graphical representation of the data in Table 14. As shown in FIG. 7, Line 1 represents trastuzumab IgG and Line 2 represents trastuzumab-coil-bGCSF IgG. The EC₅₀ of trastuzumab IgG was 110 ± 14 pM.

Table 14			
trastuzumab IgG (pM)	Fluorescence Intensity	trastuzumab-coil bGCSF IgG (pM)	Fluorescence Intensity
4074.07407	13113.5475	4074.07407	1216.3565
1358.02469	11544.1275	1358.02469	591.2115
452.6749	10776.7925	452.6749	342.6245
150.89163	7846.828	150.89163	240.7235
50.29721	4164.892	50.29721	215.4655
16.76574	1994.7745	16.76574	215.9255
5.58858	1023.4985	5.58858	208.08
1.86286	566.8795	1.86286	198.5575

Example 6: Construction of BLV1H12 betatrophin based fusion protein vectors for expression in mammalian cells

[0490] A gene encoding betatrophin (SEQ ID NO: 198) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). To optimize the folding and stability of fusion proteins,

flexible linkers of GGGGS (SEQ ID NO: 179, n=1) were added on both ends of the betatrophin fragment. Subsequently, PCR fragments encoding genes of interest are grafted into the complementarity determining region 3 of the heavy chain (CDR3H) of a bovine IgG antibody (BLV1H12) by exploiting overlap extension PCR to generate BLV1H12-direct betatrophin fusion (SEQ ID NO: 118). To generate a BLV1H12-coil betatrophin based fusion protein, sequences encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAALEAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, are added at the ends of the N- and C-terminal of the betatrophin-linker fragment. Subsequently, the PCR fragment comprising betatrophin, linkers, and extender peptides is grafted into the complementarity determining region 3 of the heavy chain (CDR3H) of a BLV1H12 antibody by exploiting overlap extension PCR to generate trastuzumab-coil betatrophin (CDRH3) HC (SEQ ID NO: 66). The expression vectors of BLV1H12- betatrophin based fusion proteins were generated by in-frame ligation of the amplified fusion genes to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain of BLV1H12 antibody (SEQ ID NO: 18) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 7: Expression and purification of BLV1H12 betatrophin fusion proteins

[0491] BLV1H12-direct betatrophin fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding BLV1H12-direct betatrophin fusion protein heavy chain (SEQ ID NO: 140) and the BLV1H12 light chain (SEQ ID NO: 36). BLV1H12-coil betatrophin fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding BLV1H12-coil betatrophin fusion protein heavy chain (SEQ ID NO: 97) and the BLV1H12 light chain (SEQ ID NO: 36). Expressed fusion proteins were secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins were purified by Protein A/G chromatography (Thermo Fisher Scientific, IL), and analyzed by Western blot (FIG. 8). As shown in FIG. 8, Lane 1 contains the protein ladder; Lane 2 contains BLV1H12-coil betatrophin fusion protein (SEQ ID NOs: 97 and 36) treated with DTT; and Lane 3 contains BLV1H12-coil betatrophin fusion protein (SEQ ID NOs: 97 and 36).

Example 8: Construction of trastuzumab-direct bGCSF protein vectors for expression in mammalian cells

[0492] A gene encoding bGCSF (SEQ ID NO: 186) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). To optimize the folding and stability of fusion proteins, flexible linkers of GGGGS (SEQ ID NO: 179, n=1) were added on both ends of the bGCSF fragment. Subsequently, PCR fragments encoding genes of interest are grafted into the complementarity determining region 3 of the heavy chain (CDR3H) of a trastuzumab IgG antibody by exploiting overlap extension PCR to generate trastuzumab-direct bGCSF fusion (SEQ ID NO: 101). The expression vectors of trastuzumab-bGCSF based fusion proteins were generated by in-frame ligation of the amplified fusion genes to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain of trastuzumab antibody (SEQ ID NO: 1) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 9: Expression and purification of trastuzumab-direct bGCSF fusion protein

[00493] Trastuzumab-direct bGCSF fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-direct bGCSF fusion protein heavy chain (SEQ ID NO: 123) and the trastuzumab light chain (SEQ ID NO: 19). Expressed fusion proteins were secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins were purified by Protein A/G chromatography (Thermo Fisher Scientific, IL), and analyzed by SDS-PAGE gel (FIG. 10). As shown in FIG. 10, Lane 1 contains the protein ladder; Lane 2 contains trastuzumab-direct bGCSF fusion protein (SEQ ID NOs: 123 and 19); and Lane 3 contains trastuzumab-direct bGCSF fusion protein (SEQ ID NOs: 123 and 19) treated with DTT.

Example 10: In vitro study of trastuzumab-direct bGCSF fusion protein proliferative activity on mouse NFS-60 cells

[00494] Mouse NFS-60 cells were obtained from American Type Culture Collection (ATCC), VA, washed three times with RPMI-1640 medium, and resuspended in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 0.05 mM 2-mercaptoethanol at a density of 1.5×10^5 cells/mL. In 96-well plates, 100 μ l of cell suspension was added into each well, followed by the addition of varied concentrations of trastuzumab-direct bGCSF IgG (SEQ ID NOs: 123 and 19) and bGCSF (SEQ ID NO: 227). The plates were incubated at 37°C in a 5% CO₂ incubator for 72 hours. Cells were then treated with AlamarBlue (Invitrogen) (1/10 volume of cell suspension) for 4 hours at 37°C. Fluorescence at 595 nm for each well was read to indicate the cell viability. FIG. 11 depicts a graphical representation of the data. The EC₅₀ of trastuzumab-direct-bGCSF IgG was 1.8 ± 0.4 ng/mL. The EC₅₀ of bGCSF was 1.3 ± 0.2 ng/mL.

Example 11: Construction of trastuzumab-coil-exendin-4 fusion protein vectors for expression in mammalian cells

[00495] A gene encoding exendin-4 (Ex-4) (SEQ ID NO: 188) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). A cleavage site of Factor Xa (SEQ ID NO: 182) was placed in front of the N-terminal of Ex-4. A flexible CGGGGS linker (SEQ ID NO: 177) was added immediately before the Factor Xa protease cleavage site and a GGGGSC linker (SEQ ID NO: 178) was added at the end of C-terminal of Ex-4 gene fragment to increase folding and stability of the fusion protein. Then, sequences encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAALEAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the exendin-4 linker fragment. Subsequently, PCR fragments encoding genes of interest were grafted into the complementarity determining region 3 of the heavy chain (CDR3H) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace the Trp99–Met107 loop. The trastuzumab-coil-exendin-4 based fusion protein was modified with human hIgG1 CH1-CH3 constant region containing seven mutations (E233P, L234V, L235A, Δ G236, A327G, A330S, and P331S) to generate trastuzumab-coil-Ex4 HC fusion (SEQ ID NO: 40). The expression vectors of trastuzumab-coil-exendin-4 based fusion proteins were generated by in-frame ligation of the amplified fusion genes to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain

of trastuzumab IgG antibody (SEQ ID NO: 1) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 12: Expression and purification of trastuzumab-coil-exendin-4 based fusion proteins

[00496] Trastuzumab-coil-exendin-4 based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil-exendin-4 fusion protein heavy chain (SEQ ID NO: 71) and the trastuzumab light chain (SEQ ID NO: 19). Expressed fusion proteins were secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins were purified by Protein A/G chromatography (Thermo Fisher Scientific, IL), and analyzed by SDS-PAGE gel. trastuzumab-coil based Ex-4 fusion protein was further treated with Factor Xa protease (GE Healthcare) following manufacture's protocol to release N-terminal of fused peptide. After treatment, fusion proteins were re-purified by Protein A/G affinity column to remove protease and analyzed by SDS-PAGE gel as shown in Fig. 12. Lane 1 is a protein marker. Lane 2 is trastuzumab-coil-Ex-4 IgG (SEQ ID NOs: 71 and 19). Lane 3 is trastuzumab-coil-Ex-4 IgG (SEQ ID NOs: 71 and 19) treated with DTT. Lane 4 is a protein marker. Lane 5 is trastuzumab-coil-Ex-4 IgG (SEQ ID NOs: 71 and 19) after cleavage with Factor Xa, releasing the N-terminus of Ex-4 peptide to generate trastuzumab-coil-Ex-4 RN IgG, wherein RN is an abbreviation for released N-terminus. Lane 6 is trastuzumab-coil-Ex-4 RN IgG treated with DTT.

Example 13: Electrospray Ionization Mass Spectrometry (ESI-MS) of trastuzumab-coil-exendin-4 IgG

[00497] 10 µg of purified trastuzumab-coil-exendin-4 heavy chain (HC) fusion (SEQ ID NOs: 71 and 19), in PBS (pH 7.4) was treated overnight at 37 °C with 1 µL (500 units) of peptide-N-glycosidase (NEB), followed by the addition of 50 mM DTT. The fusion protein was analyzed by ESI-MS using a 6520 Q-TOF LC/MS from Agilent Technology. The chromatograph is shown in Fig. 13. The expected molecular weight for trastuzumab-coil-exendin-4 HC is 56,880 Da. The observed molecular weight for trastuzumab-coil-exendin-4 HC was 56,748 Da. The observed molecular weight correlates to the expected molecular weight without the first amino acid glutamic acid (E).

Example 14: Electrospray Ionization Mass Spectrometry (ESI-MS) of trastuzumab-coil-exendin-4 RN IgG

[00498] 10 µg of purified Factor Xa cleaved trastuzumab-coil-exendin-4 heavy chain (HC) fusion (SEQ ID NOs: 71 and 19) in PBS (pH 7.4) was treated overnight at 37 °C with 1 µL (500 units) of peptide-N-glycosidase (NEB), followed by the addition of 50 mM DTT. The cleaved fusion protein fragments were analyzed by ESI-MS using a 6520 Q-TOF LC/MS from Agilent Technology. The chromatograph of the N-terminal fragment is shown in Fig. 14 (A) and the chromatograph of the C-terminal fragment is shown in Fig. 14 (B). The expected molecular weight for trastuzumab-coil-exendin-4 HC RN N-terminal fragment is 13,309 Da. The observed molecular weight for trastuzumab-coil-exendin-4 HC RN N-terminal fragment was 13,307 Da. The expected molecular weight for trastuzumab-coil-exendin-4 HC RN C-terminal fragment is 43,589 Da. The observed molecular weight for trastuzumab-coil-exendin-4 HC RN C-terminal fragment was 43,458 Da.

Example 15: In vitro study of trastuzumab-coil exendin-4 fusion protein activation activities on GLP-1 receptor (GLP-1R)

[00499] HEK293 cells overexpressing surface GLP-1R and cAMP responsive luciferase reporter gene were seeded in 384 well plates at a density of 5,000 cells per well. After 24 h incubation at 37°C with 5% CO₂, cells were treated with various concentrations of exendin-4 peptide (SEQ ID NO: 228), trastuzumab (SEQ ID NOs: 19 and 22), trastuzumab-coil exendin-4 (SEQ ID NOs: 71 and 19), and trastuzumab-coil exendin-4 (SEQ ID NOs: 71 and 19) RN; and incubated for another 24 h. Subsequently, a luciferase assay was performed using One-Glo luciferase reagent according manufacture's instruction (Promega). FIG. 15 depicts a graphical representation of the data. The EC₅₀ of exendin-4 was 0.03 ± 0.004 nM. The EC₅₀ of trastuzumab-coil exendin-4 was 3.8 ± 0.2 nM. The EC₅₀ of trastuzumab-coil exendin-4 RN was 0.01 ± 0.001 nM.

Example 16: In Vitro study of trastuzumab-coil-exendin-4 fusion protein glucagon receptor activation assay

[00500] HEK 293 cells overexpressing glucagon receptor (GCGR) and CRE-Luc reporter were grown in DMEM with 10% FBS at 37°C with 5% CO₂. Cells were seeded in 384-well plates at a density of 5,000 cells per well and treated with various concentrations of glucagon, exendin-4 peptide (SEQ ID NO: 228), trastuzumab (SEQ ID NOs: 19 and 22), trastuzumab-coil exendin-4 (SEQ ID NOs: 71 and 19), and trastuzumab-coil exendin-4 (SEQ ID NOs: 71 and 19) RN fusion proteins for 24 hours at 37°C with 5% CO₂. Luminescence intensities were then measured using One-Glo (Promega, WI) luciferase reagent by following manufacturer's instruction. Fig. 16 depicts a graphical representation of the data.

Example 17: Pharmacokinetics of trastuzumab-coil-Ex-4 RN Fusion Protein in Mice

[00501] Ex-4 (SEQ ID NO: 228) (1.6 mg/kg) and trastuzumab-coil-Ex-4 (SEQ ID NOs: 71 and 19) RN fusion protein (2.8 mg/kg) were administrated by intravenous (i.v.) or subcutaneous (s.c.) injection into CD1 mice (N=3). Blood samples were collected from day 0 to day 8 for Ex-4 peptide and day 0 to day 14 for trastuzumab-coil-Ex-4 RN fusion protein. The remaining activities were analyzed using HEK 293-GLP-1R-CRE-Luc cells. Data were normalized by taking the maximal concentration at the first time point (30 minutes) for the intravenous injection. Data were normalized by taking the maximal concentration at the second time point (1 day) for the subcutaneous injection. Percentages of the maximal concentration were plotted versus time points of blood sample collection, and half-lives were determined by fitting data into the first-order equation, $A=A_0e^{-kt}$, where A_0 is the initial concentration, t is the time, and k is the first-order rate constant. Fig. 17 depicts a graphical representation of the data. Figure 17 (A) depicts intravenous inject. Figure 17 (B) depicts subcutaneous inject. The $t_{1/2}$ of exendin-4 (i.v.) was 1.5 ± 0.2 hours. The $t_{1/2}$ of trastuzumab-coil exendin-4 RN (i.v.) was 2.4 ± 0.1 days. The $t_{1/2}$ of trastuzumab-coil exendin-4 RN (s.c.) was 3.9 ± 1.2 days.

Example 18: Pharmacodynamics of trastuzumab-coil-Ex-4 RN Fusion Protein in Mice

[00502] Single doses of Ex-4 (SEQ ID NO: 228) (20 µg/kg), trastuzumab (SEQ ID NOs: 19 and 22) (8 mg/kg), and varied concentrations of trastuzumab-coil-Ex-4 RN (SEQ ID NOs: 71 and 19) fusion protein were administrated by subcutaneous (s.c.) injection into CD1 mice (N=5). Glucose (3 g/kg, p.o.) were

given at 30 minutes, 24, 48, 72, 96, 120, 144, 168, and 216 hours post single-dose treatments, followed by blood glucose measurements immediately prior to and at 15, 30, 45, 60, and 120 minutes post glucose load. Fig. 18 depicts a graphical representation of the data at 30 minutes, 24, 48, 72, 96, 120, 144, 168, and 216 hours post single-dose treatments.

Example 19: Pharmacodynamics of trastuzumab-coil-Ex-4 Fusion Protein in Mice

[00503] Single doses of Ex-4 (SEQ ID NO: 228) (20 µg/kg), trastuzumab (SEQ ID NOs: 19 and 22) (8 mg/kg), and varied concentrations of trastuzumab-coil-Ex-4 (SEQ ID NOs: 71 and 19) fusion protein were administered by subcutaneous (s.c.) injection into CD1 mice (N=5). Glucose (3 g/kg, p.o.) were given at 2, 24, 48, 72, 96, 120, and 144 hours post single-dose treatments, followed by blood glucose measurements immediately prior to and at 15, 30, 45, 60, and 120 minutes post glucose load. Fig. 19 depicts a graphical representation of the data at 2, 24, 48, 72, 144, 168, and 216 hours post single-dose treatments.

Example 20: Binding of trastuzumab-coil-exendin-4 to Her2 receptor

[00504] The binding affinity of trastuzumab-coil- exendin-4 fusion proteins to Her2 receptor is examined by ELISA. Human Her2-Fc chimera (5 ug/mL) (R&D Systems) is coated on 96-well ELISA plate overnight at 4°C, followed by blocking with 1% BSA in PBS (pH7.4) for 2 hours at 37°C. After washing with 0.05% Tween-20 in PBS (pH7.4), varied concentrations of trastuzumab IgG (SEQ ID NOs: 19 and 22) and trastuzumab-coil- exendin-4 (SEQ ID NOs: 71 and 19) fusion proteins are added to incubate for 2 hours at 37°C. Subsequently, goat polyclonal anti-human kappa light chain antibody with HRP conjugate (Sigma) is added and incubated for 2 hours at 37°C. Wells are subsequently washed and binding affinities are examined on the basis of fluorescence intensity at 425 nm by adding fluoregenic peroxidase substrate to each well.

Example 21: Flow Cytometric Analysis of trastuzumab-coil-exendin-4 binding to HER2 Receptor

[00505] HER2-overexpressing SKBR3 cells are grown in DMEM with 10% FBS and 1% penicillin and streptomycin. Cells are washed with cold PBS for three times, blocked with 2% BSA in PBS, and incubated with 10 or 100 nM of trastuzumab (SEQ ID NOs: 19 and 22) and trastuzumab-CDR fusion proteins (SEQ ID NOs: 71 and 19) for 2 hours at 4°C with gentle mixing. Unbound antibody is removed by washing with 2% BSA in PBS. Cells are then stained with FITC anti-human IgG Fc (KPL, Inc., MD) for 1 hour at 4°C with gentle mixing, followed by washing with PBS and analysis by flow cytometry.

Example 22: Construction of trastuzumab-coil-Moka fusion protein vectors for expression in mammalian cells

[00506] A gene encoding Moka (SEQ ID NO: 189) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). To optimize the folding and stability of fusion proteins, flexible linkers of GGGGS (SEQ ID NO: 179, n=1) were added on both ends of the Moka fragment. Then, sequences encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the Moka-Linker fragment. Subsequently, PCR fragments encoding the Moka gene with the extender peptides and linkers was grafted into the complementarity determining

region 3 of the heavy chain (CDR3H) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace the Trp99–Met107 loop. The trastuzumab-coil-Moka-based fusion protein was further modified to replace the hIgG1 CH1-CH3 constant region of trastuzumab with hIgG4 CH1-CH3 constant region containing triple mutants (S228P, F234A and L235A) to generate trastuzumab-coil-Moka IgG (SEQ ID NO: 41). The expression vectors of trastuzumab-coil-Moka-based fusion proteins were generated by in-frame ligation of the amplified fusion genes to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain of trastuzumab IgG antibody (SEQ ID NO: 1) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 23: Expression and purification of trastuzumab-coil-Moka based fusion proteins

[00507] Trastuzumab-coil-Moka based fusion proteins are expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil-Moka fusion protein heavy chain (SEQ ID NO: 72) and the trastuzumab light chain (SEQ ID NO: 19). Expressed fusion proteins are secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins are purified by Protein A/G chromatography (Thermo Fisher Scientific, IL), and analyzed by SDS-PAGE gel as shown in Fig. 20. Lane 1 shows a protein molecular weight marker. Lane 2 shows purified trastuzumab-coil-Moka IgG. Lane 3 shows purified trastuzumab-coil-Moka IgG treated with DTT.

Example 24: Construction of trastuzumab-coil-VM24 fusion protein vectors for expression in mammalian cells

[00508] A gene encoding VM24 (SEQ ID NO: 190) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). To optimize the folding and stability of the fusion proteins, flexible linkers of GGGGS (SEQ ID NO: 179, n=1) were added on both ends of VM24 fragments. Then, sequences encoding encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAALEAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the VM24-linker fragments. Subsequently, PCR fragments encoding genes of interest were grafted into the complementarity determining region 3 of the heavy chain (CDR3H) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace the Trp99–Met107 loop. The trastuzumab-coil-VM24 based fusion protein was further modified to replace the hIgG1 CH1-CH3 constant region of trastuzumab with hIgG4 CH1-CH3 constant region containing triple mutants (S228P, F234A and L235A) to generate SEQ ID NO: 42. The expression vectors of trastuzumab-coil-VM24 based fusion proteins were generated by in-frame ligation of the amplified fusion genes to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain of trastuzumab IgG antibody (SEQ ID NO: 1) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 25: Expression and purification of trastuzumab-coil-VM24 based fusion proteins

[00509] Trastuzumab-coil-VM24 based fusion proteins are expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil-VM24 fusion protein heavy chain (SEQ ID NO: 73) and the trastuzumab light chain (SEQ ID NO: 19). Expressed fusion proteins are secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins are purified by

Protein A/G chromatography (Thermo Fisher Scientific, IL), and analyzed by SDS-PAGE gel as shown in Fig. 20. Lane 1 shows a protein molecular weight marker. Lane 4 shows purified trastuzumab-coil-Vm24 IgG. Lane 5 shows purified trastuzumab-coil-Vm24 IgG treated with DTT.

Example 26: In vitro study of trastuzumab-coil-Moka fusion protein and trastuzumab-coil-Vm24 fusion protein inhibitory activities on human peripheral blood mononuclear cells (PBMCs)/T cells activation

[00510] Human PBMCs were isolated from fresh venous blood of healthy donors through ficoll™ gradient centrifugation, followed by resuspension in RPMI1640 medium with 10% FBS and plating in 96-well plates at a density of 1×10^6 cells/mL. Human T cells were purified from the isolated PBMCs using T cell enrichment kit. Purified PBMCs and T cells were pretreated for 1 h at 37°C with 5% CO₂ with various concentrations of purified trastuzumab-coil Moka (SEQ ID NOs: 72 and 19) and trastuzumab-coil Vm24 (SEQ ID NOs: 73 and 19) fusion proteins and then activated by anti-CD3 and CD28 antibodies. After 24 h treatment, supernatant was collected for measurement of the levels of secreted TNF- α using ELISA kit. Fig. 21 depicts a graphical representation of the in vitro inhibition on T-cell activation data. The EC₅₀ of trastuzumab-coil Moka IgG (SEQ ID NOs: 72 and 19) was 269 ± 46 nM. The EC₅₀ of trastuzumab-coil Vm24 IgG (SEQ ID NOs: 73 and 19) was 178 ± 104 nM.

Example 27: Construction of trastuzumab-coil-hGCSF fusion protein vectors for expression in mammalian cells

[00511] A gene encoding human GCSF (hGCSF) (SEQ ID NO: 187) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). To optimize the folding and stability of the fusion proteins, flexible linkers of GGGGS (SEQ ID NO: 179, n=1) were added on both ends of hGCSF fragments. Then, sequences encoding extender peptides GSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAALEAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the hGCSF-linker fragments. To generate a CDRH3 fusion, PCR fragments encoding genes of interest were grafted into the complementarity determining region 3 of the heavy chain (CDRH3) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace the Trp99–Met107 loop. To generate a CDRH2 fusion, PCR fragments encoding genes of interest were grafted into the complementary determining region 2 of the heavy chain (CDRH2) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace Thr54–Asn55. To generate a CDRL3 fusion, PCR fragments encoding genes of interest were grafted into the complementary determining region 3 of the light chain (CDRL3) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace Thr93–Pro95. The trastuzumab-coil- hGCSF based fusion proteins were modified with human hIgG1 CH1-CH3 constant region containing seven mutations (E233P, L234V, L235A, Δ G236, A327G, A330S, and P331S). The expression vectors of trastuzumab-coil- hGCSF based fusion proteins were generated by in-frame ligation of the amplified fusion genes to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain of trastuzumab IgG antibody (SEQ ID NO: 1) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 28: Expression and purification of trastuzumab-coil-hGCSF based fusion proteins

[00512] Trastuzumab-coil-hGCSF based fusion proteins are expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil-hGCSF fusion protein (CDRH3) heavy chain (SEQ ID NO: 74) and the trastuzumab light chain (SEQ ID NO: 9), trastuzumab-coil-hGCSF fusion protein (CDRH2) heavy chain and the trastuzumab light chain, and trastuzumab-coil-hGCSF fusion protein (CDRL3) light chain and the trastuzumab heavy chain. Expressed fusion proteins are secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins are purified by Protein A/G chromatography (Thermo Fisher Scientific, IL), and analyzed by SDS-PAGE gel. Fig. 22 depicts purified trastuzumab-coil-hGCSF based fusion proteins. Lane 1 shows purified trastuzumab-coil hGCSF (CDRH2) IgG. Lane 2 shows purified trastuzumab-coil hGCSF (CDRH2) IgG treated with DTT. Lane 3 shows a protein molecular weight marker. Lane 4 shows purified trastuzumab-coil hGCSF (CDRL3) IgG. Lane 5 shows purified trastuzumab-coil hGCSF (CDRL3) IgG treated with DTT.

Example 29: Electrospray Ionization Mass Spectrometry (ESI-MS) of trastuzumab-coil-hGCSF based fusion proteins

[00513] 10 µg of purified trastuzumab-coil-hGCSF (CDRH2), in PBS (pH 7.4) was treated overnight at 37 °C with 1 µL (500 units) of peptide-N-glycosidase (NEB), followed by the addition of 50 mM DTT. The fusion protein was analyzed by ESI-MS using a 6520 Q-TOF LC/MS from Agilent Technology. The chromatograph is shown in Fig. 23 (A). The expected molecular weight for trastuzumab-coil-hGCSF (CDRH2) HC is 71,472 Da. The observed molecular weight for trastuzumab-coil-hGCSF (CDRH2) HC was 72,300 Da. The observed molecular weight correlates to O-glycosylation on hGCSF. 10 µg of purified trastuzumab-coil-hGCSF (CDRL3), in PBS (pH 7.4) was treated overnight at 37 °C with 1 µL (500 units) of peptide-N-glycosidase (NEB), followed by the addition of 50 mM DTT. The fusion protein was analyzed by ESI-MS using a 6520 Q-TOF LC/MS from Agilent Technology. The chromatograph is shown in Fig. 23 (B). The expected molecular weight for trastuzumab-coil-hGCSF (CDRL3) LC is 45,746 Da. The observed molecular weight for trastuzumab-coil-hGCSF (CDRL3) LC was 46,692 Da. The observed molecular weight correlates to O-glycosylation on hGCSF.

Example 30: Binding of trastuzumab-coil hGCSF based fusion proteins to HER2 receptor

[00514] HER2-overexpressing SKBR3 cells were grown in DMEM with 10% FBS and 1% penicillin and streptomycin. Cells were washed with cold PBS for three times, blocked with 2% BSA in PBS, and incubated with 10 or 100 nM of trastuzumab and trastuzumab-CDR fusion proteins, trastuzumab-coil-hGCSF (CDRH2) and trastuzumab-coil-hGCSF (CDRL3), for 2 hours at 4°C with gentle mixing. Unbound antibody was removed by washing with 2% BSA in PBS. Cells were then stained with FITC anti-human IgG Fc (KPL, Inc., MD) for 1 hour at 4°C with gentle mixing, followed by washing with PBS and analysis by flow cytometry. Fig. 24 depicts the flow cytometry histogram.

Example 31: In vitro study of trastuzumab-coil hGCSF fusion protein proliferative activity on mouse NFS-60 cells

[00515] Mouse NFS-60 cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 0.05 mM 2-mercapoethanol and 62 ng/ml human macrophage colony stimulating factor (M-CSF). To examine

the proliferative activity of trastuzumab-hGCSF fusion proteins, cells were washed three times with RPMI-1640 medium with 10% FBS, resuspended in RPMI-1640 medium with 10% FBS and 0.05 mM 2-mercapoethanol at a density of 1.5×10^5 cells/ml, plated in 96-well plates (1.5×10^4 cells per well) with various concentrations of hGCSF, trastuzumab, trastuzumab-coil-hGCSF (CDRH2), and trastuzumab-coil-hGCSF (CDRL3), and incubated for 72 hours at 37°C with 5% CO₂. Cells were then treated with AlamarBlue (Life Technologies, CA) for 4 hours at 37°C. Fluorescence intensity measured at 595 nm is proportional to cell viability. FIG. 25 depicts a graphical representation of the data. The EC₅₀ of hGCSF was 1.7 ± 0.3 ng/mL. The EC₅₀ of trastuzumab-coil hGCSF (CDRH2) was 0.4 ± 0.1 ng/mL. The EC₅₀ of trastuzumab-coil hGCSF (CDRL3) was 0.9 ± 0.1 ng/mL.

Example 32: Construction of trastuzumab-coil-hEPO fusion protein vectors for expression in mammalian cells

[00516] A gene encoding hEPO (SEQ ID NO: 192) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). To optimize the folding and stability of the immunoglobulin fusion protein, flexible linkers of GGGGS (SEQ ID NO: 179, n=1) were added on both ends of the hEPO fragments. Then, sequences encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the hEPO-linker fragment. Subsequently, PCR fragments encoding the hEPO gene with the extender peptides and linkers was grafted into the complementarity determining region 3 of the heavy chain (CDR3H) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace the Trp99–Met107 loop. The CH1-CH3 constant regions of trastuzumab-coil hEPO (CDRH3) heavy chain were replaced with human IgG4 CH1-CH3 constant region containing triple mutations (S228P, F234A and L235A) to generate trastuzumab-coil hEPO (CDRH3) HC. The expression vectors of trastuzumab-coil based fusion proteins were generated by in-frame ligation of the amplified fusion genes to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain of trastuzumab IgG antibody (SEQ ID NO: 1) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 33: Expression and purification of trastuzumab-coil-hEPO fusion proteins

[00517] Trastuzumab-coil-hEPO based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil-hEPO fusion protein heavy chain and the trastuzumab light chain (SEQ ID NO: 1). Expressed fusion proteins were secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins were purified by Protein A/G chromatography (Thermo Fisher Scientific, IL), and analyzed by SDS-PAGE gel. As shown in FIG.26, Lane 1 depicts a protein ladder, Lane 2 depicts purified trastuzumab-coil-hEPO (CDRH3) and trastuzumab LC, and Lane 3 depicts trastuzumab-coil-hEPO (CDRH3) and trastuzumab LC treated with DTT.

Example 34: Electrospray Ionization Mass Spectrometry (ESI-MS) of trastuzumab-coil-hEPO based fusion proteins

[00518] 10 µg of purified trastuzumab-coil-hEPO (CDRH3), in PBS (pH 7.4) was treated overnight at 37 °C with 1 µL (500 units) of peptide-N-glycosidase (NEB), followed by the addition of 50 mM DTT. The fusion protein was analyzed by ESI-MS using a 6520 Q-TOF LC/MS from Agilent Technology. The chromatograph is shown in Fig. 27. The expected molecular weight for trastuzumab-coil-hEPO (CDRH3) HC is 70,307 Da. The observed molecular weight for trastuzumab-coil-hEPO (CDRH3) HC was 70,177 Da. The observed molecular weight correlates to O-glycosylation on hEPO and the absence of the first amino acid glutamic acid (E).

Example 35: Binding of trastuzumab-coil hEPO based fusion proteins to HER2 receptor

[00519] HER2-overexpressing SKBR3 cells were grown in DMEM with 10% FBS and 1% penicillin and streptomycin. Cells were washed with cold PBS for three times, blocked with 2% BSA in PBS, and incubated with 10 or 100 nM of trastuzumab and trastuzumab-coil-hEPO (CDRH3), for 2 hours at 4°C with gentle mixing. Unbound antibody was removed by washing with 2% BSA in PBS. Cells were then stained with FITC anti-human IgG Fc (KPL, Inc., MD) for 1 hour at 4°C with gentle mixing, followed by washing with PBS and analysis by flow cytometry. Fig. 28 depicts the flow cytometry histogram.

Example 36: In vitro proliferative activity assay of trastuzumab-coil-hEPO fusion protein on TF-1 cells

[00520] Human TF-1 cells were cultured at 37°C with 5% CO₂ in RPMI-1640 medium containing 10% fetal bovine serum (FBS), penicillin and streptomycin (50 U/mL), and 2 ng/ml human granulocyte macrophage colony stimulating factor (GM-CSF). To examine the proliferative activity of trastuzumab-hEPO fusion proteins, cells were washed three times with RPMI-1640 medium with 10% FBS, resuspended in RPMI-1640 medium with 10% FBS at a density of 1.5×10^5 cells/ml, plated in 96-well plates (1.5×10^4 cells per well) with various concentrations of hEPO, trastuzumab, and trastuzumab-coil hEPO (CDRH3) fusion protein, and then incubated for 72 hours at 37°C with 5% CO₂. Cells were then treated with Alamar Blue (Life Technologies, CA) for 4 hours at 37°C. Fluorescence intensity measured at 595 nm is proportional to cell viability. The EC₅₀ values were determined by fitting data into a logistic sigmoidal function: $y = A_2 + (A_1 - A_2)/(1 + (x/x_0)^p)$, where A₁ is the initial value, A₂ is the final value, x₀ is the inflection point of the curve, and p is the power. FIG. 29 depicts a graphical representation of the data. The EC₅₀ of hEPO was 0.1 ± 0.02 nM. The EC₅₀ of trastuzumab-coil hEPO (CDRH3) was 0.1 ± 0.01 nM.

Example 37: Construction of trastuzumab hGCSF/EPO dual fusion protein

[00521] A gene encoding hEPO (SEQ ID NO: 192) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). To optimize the folding and stability of the immunoglobulin fusion protein, flexible linkers of GGGGS (SEQ ID NO: 179, n=1) were added on both ends of the hEPO fragments. Then, sequences encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the hEPO-linker fragment. Subsequently, PCR fragments encoding the hEPO gene with the extender peptides and linkers was grafted into the

complementarity determining region 3 of the heavy chain (CDR3H) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace the Trp99–Met107 loop. The CH1-CH3 constant regions of trastuzumab-coil hEPO (CDRH3) heavy chain were replaced with human IgG4 CH1-CH3 constant region containing triple mutations (S228P, F234A and L235A) to generate trastuzumab-coil hEPO (CDRH3) HC (SEQ ID NO: 62).

[00522] A gene encoding human GCSF (hGCSF) (SEQ ID NO: 187) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). To optimize the folding and stability of the fusion proteins, flexible linkers of GGGGS (SEQ ID NO: 179, n=1) were added on both ends of hGCSF fragments. Then, sequences encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELEAAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the hGCSF-linker fragments. The PCR fragment encoding the hGCSF-linker-extender was grafted into the complementary determining region 3 of the light chain (CDRL3) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace Thr93-Pro95. The trastuzumab-coil- hGCSF based fusion protein was modified with human hIgG1 CH1-CH3 constant region containing seven mutations (E233P, L234V, L235A, ΔG236, A327G, A330S, and P331S) to generate trastuzumab-coil hGCSF (CDRL3) LC (SEQ ID NO: 63). The expression vectors of trastuzumab-coil based fusion proteins were generated by in-frame ligation of the amplified fusion genes to the pFuse backbone vector (InvivoGen, CA).

Example 38: Expression and purification of trastuzumab hGCSF/EPO dual fusion protein

[00523] Trastuzumab-coil-hGCSF/EPO dual fusion protein was expressed through transient transfections of free style HEK293 cells with a vector encoding trastuzumab-coil hEPO (CDRH3) HC (SEQ ID NO: 62) and trastuzumab-coil hGCSF (CDRL3) LC (SEQ ID NO: 63). Expressed dual fusion proteins were secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins were purified by Protein A/G chromatography (Thermo Fisher Scientific, IL), and analyzed by SDS-PAGE gel. As shown in FIG. 30, Lane 1 depicts the protein ladder, Lane 2 depicts trastuzumab-coil-hGCSF/EPO dual fusion protein, and Lane 3 depicts trastuzumab-coil-hGCSF/EPO dual fusion protein treated with DTT.

Example 39: Electrospray Ionization Mass Spectrometry (ESI-MS) of trastuzumab hGCSF/hEPO based fusion proteins

[00524] 10 µg of purified Trastuzumab-coil-hGCSF/EPO dual fusion protein (SEQ ID NOs: 62, 63), in PBS (pH 7.4) was treated overnight at 37 °C with 1 µL (500 units) of peptide-N-glycosidase (NEB), followed by the addition of 50 mM DTT. The fusion protein was analyzed by ESI-MS using a 6520 Q-TOF LC/MS from Agilent Technology. The chromatograph is shown in Fig. 31 (A). The expected molecular weight for trastuzumab-coil hGCSF (CDRL3) LC is 45,746 Da. The observed molecular weight for trastuzumab-coil hGCSF (CDRL3) LC was 46,690 Da. The observed molecular weights correlates to O-glycosylation on hGCSF.

[00525] 10 µg of purified Trastuzumab-coil-hGCSF/EPO dual fusion protein (SEQ ID NOs: 62, 63), in PBS (pH 7.4) was treated overnight at 37 °C with 1 µL (500 units) of peptide-N-glycosidase (NEB),

followed by the addition of 50 mM DTT. The fusion protein was analyzed by ESI-MS using a 6520 Q-TOF LC/MS from Agilent Technology. The chromatograph is shown in Fig. 31 (B). The expected molecular weight for trastuzumab-coil hEPO (CDRH3) HC is 70,307 Da. The observed molecular weights for trastuzumab-coil-hEPO (CDRH3) HC was 70,179 Da (correlating to the mass of trastuzumab-coil hEPO (CDRH3) HC without the first amino acid glutamic acid) and 71,126 Da (correlating to O-glycosylation on hEPO).

Example 40: Binding of trastuzumab hGCSF/hEPO based fusion proteins to HER2 receptor

[00526] HER2-overexpressing SKBR3 cells were grown in DMEM with 10% FBS and 1% penicillin and streptomycin. Cells were washed with cold PBS for three times, blocked with 2% BSA in PBS, and incubated with 10 or 100 nM of trastuzumab and trastuzumab-coil-hGCSF/EPO dual fusion protein (SEQ ID NOs: 62, 63) for 2 hours at 4°C with gentle mixing. Unbound antibody was removed by washing with 2% BSA in PBS. Cells were then stained with FITC anti-human IgG Fc (KPL, Inc., MD) for 1 hour at 4°C with gentle mixing, followed by washing with PBS and analysis by flow cytometry. Fig. 32 depicts the flow cytometry histogram.

Example 41: In vitro proliferative activity assay of trastuzumab hGCSF/hEPO fusion protein on TF-1 cells

[00527] Human TF-1 cells were cultured at 37°C with 5% CO₂ in RPMI-1640 medium containing 10% fetal bovine serum (FBS), penicillin and streptomycin (50 U/mL), and 2 ng/ml human granulocyte macrophage colony stimulating factor (GM-CSF). To examine the proliferative activity of trastuzumab hGCSF/hEPO fusion proteins, cells were washed three times with RPMI-1640 medium with 10% FBS, resuspended in RPMI-1640 medium with 10% FBS at a density of 1.5×10^5 cells/ml, plated in 96-well plates (1.5×10^4 cells per well) with various concentrations of hEPO, trastuzumab, and trastuzumab hGCSF/hEPO (CDRH3) fusion protein (SEQ ID NOs: 62, 63), and then incubated for 72 hours at 37°C with 5% CO₂. Cells were then treated with Alamar Blue (Life Technologies, CA) for 4 hours at 37°C. Fluorescence intensity measured at 595 nm is proportional to cell viability. FIG. 33 depicts a graphical representation of the data. The EC₅₀ of hEPO was 0.1 ± 0.02 nM. The EC₅₀ of trastuzumab-coil hGCSF/hEPO was 0.2 ± 0.03 nM.

Example 42: In vitro study of trastuzumab-coil hGCSF/hEPO fusion protein proliferative activity on mouse NFS-60 cells

[00528] Mouse NFS-60 cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 0.05 mM 2-mercapoethanol and 62 ng/ml human macrophage colony stimulating factor (M-CSF). To examine the proliferative activity of trastuzumab hGCSF/hEPO fusion proteins, cells were washed three times with RPMI-1640 medium with 10% FBS, resuspended in RPMI-1640 medium with 10% FBS and 0.05 mM 2-mercapoethanol at a density of 1.5×10^5 cells/ml, plated in 96-well plates (1.5×10^4 cells per well) with various concentrations of hGCSF, trastuzumab, and trastuzumab hGCSF/hEPO (CDRH3) fusion protein (SEQ ID NOs: 62, 63), and incubated for 72 hours at 37°C with 5% CO₂. Cells were then treated with AlamarBlue (Life Technologies, CA) for 4 hours at 37°C. Fluorescence intensity measured at 595 nm is

proportional to cell viability. FIG. 34 depicts a graphical representation of the data. The EC_{50} of hGCSF was 1.7 ± 0.3 nM. The EC_{50} of trastuzumab-coil hGCSF/hEPO was 3.1 ± 0.1 nM.

Example 43: Construction of Herceptin hGH fusion protein vectors for expression in mammalian cells

[00529] A gene encoding hGH (SEQ ID NO: 201) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). To optimize the folding and stability of the immunoglobulin fusion protein, flexible linkers of GGGGS (SEQ ID NO: 179, n=1) were added on both ends of the hGH fragments. To generate Herceptin-coil hGH fusion proteins, sequences encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAALEAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the hGH-linker fragment. To generate a trastuzumab-direct hGH (CDRH2) fusion protein (SEQ ID NO: 128), a PCR fragment encoding the hGH gene with the linkers was grafted into the complementarity determining region 2 of the heavy chain (CDRH2) of Herceptin IgG antibody by exploiting overlap extension PCR. To generate a trastuzumab-coil hGH (CDRH3) fusion protein (SEQ ID NO: 75), a PCR fragment encoding the hGH gene with the extender peptides and linkers was grafted into the complementary determining region 3 of the heavy chain (CDRH3) of Herceptin IgG antibody by exploiting overlap extension PCR. To generate a trastuzumab-coil hGH (CDRH2) fusion protein (SEQ ID NO: 76), a PCR fragment encoding the hGH gene with the extender peptides and linkers was grafted into the complementary determining region 2 of the heavy chain (CDRH2) of Herceptin IgG antibody by exploiting overlap extension PCR. The expression vectors of trastuzumab hGH based fusion proteins were generated by in-frame ligation of the amplified fusion genes to the pFuse backbone vector (InvivoGen, CA). The obtained expression vectors were confirmed by DNA sequencing.

Example 44: Expression and purification of trastuzumab hGH based fusion proteins

[00530] Trastuzumab-direct hGH based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-direct hGH (CDRH2) HC (SEQ ID NO: 128), and the trastuzumab light chain (SEQ ID NO: 19). trastuzumab-coil hGH (CDRH3) based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil hGH (CDRH3) HC (SEQ ID NO: 75), and the trastuzumab light chain (SEQ ID NO: 19). Trastuzumab-coil hGH (CDRH2) based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil hGH (CDRH2) HC (SEQ ID NO: 76), and the trastuzumab light chain (SEQ ID NO: 19). Expressed fusion proteins were secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins were purified by Protein A/G chromatography (Thermo Fisher Scientific, IL), and analyzed by SDS-PAGE gel. As shown in FIG.35, Lane 1 depicts a protein ladder, Lane 2 depicts trastuzumab-coil hGH (CDRH3) (SEQ ID NOs: 75, 19), Lane 3 depicts trastuzumab-coil hGH (CDRH3) (SEQ ID NOs: 75, 19) treated with DTT, Lane 4 depicts a protein ladder, Lane 5 depicts trastuzumab-direct hGH (CDRH2) (SEQ ID NOs: 128 and 19), Lane 6 depicts trastuzumab-direct hGH (CDRH2) (SEQ ID NOs: 128 and 19) treated with DTT, Lane 7 depicts trastuzumab-coil hGH (CDRH2) (SEQ ID NOs: 76, 19), and Lane 8 depicts trastuzumab-coil hGH (CDRH2) (SEQ ID NOs: 76, 19) treated with DTT.

Example 45: Trastuzumab hGH based fusion protein activity assays

[00531] hGHR-Ba/F3 proliferation assay: Murine Ba/F3 cell lines were stably transduced with hGH receptor (hGHR) under a EF1 α promoter. Clonally selected hGHR-Ba/F3 were maintained in 10% FBS, RPMI1640, and 50 ng/mL of hGH. The proliferation assay was performed in 96 well culture plates comprising 20,000 cells in 200 μ L assay medium (10% FBS in RPMI1640) per well. Increasing concentrations of hGH, trastuzumab-coil hGH (CDRH3), trastuzumab-coil hGH (CDRH2), and trastuzumab-direct (CDRH2) were incubated with the cells for 72 hours. At the end of the incubation period, 20 μ L of Prestoblu^e was added to each well, and the fluorescent signal recorded on a Spectramax fluorescence plate reader at 590 nm with 550 nm excitation. The EC₅₀ values for hGH and trastuzumab hGH fusions are shown in Table 15.

[00532] NB2 proliferation assay: Rat Nb2-11 cell lines (Sigma) were maintained in 10% FBS, 10% horse serum (HS) in RPMI with 55 μ M β -ME. The proliferation assay was performed in 96 well culture plates comprising 50,000 cells in 200 μ L assay medium (10% HS in RPMI with 55uM β -ME) per well. Increasing concentrations of hGH, trastuzumab-coil hGH (CDRH3), trastuzumab-coil hGH (CDRH2), and trastuzumab-direct (CDRH2) were incubated with the cells for 72 hours. At the end of the incubation period, 20 μ L of Prestoblu^e was added to each well, and the fluorescent signal recorded on a Spectramax fluorescence plate reader at 590 nm with 550 nm excitation. The EC₅₀ values for hGH and trastuzumab hGH fusions are shown in Table 15.

[00533] Stat5 phosphorylation assay: Human IM9 cells (ATCC) were maintained in 10% FBS in RPMI1640. The night before the phosphorylation assay, 2 x 10⁵ IM9 cells were seeded into V bottom 96 well plates in 200 μ L assay medium (1% charcoal stripped FBS in RPMI) and starved overnight. On the day of the phosphorylation experiment, starved cells were stimulated with hGH, trastuzumab-coil hGH (CDRH3), trastuzumab-coil hGH (CDRH2), and trastuzumab-direct (CDRH2) at various concentration for 10 min at 37°C. After stimulation, cells were fixed by 4% formaldehyde at 37°C for 10 min, and then permeabilized with 90% methanol. Cells were then blocked with 5% BSA at room temperature for 10 min and stained with Alexa Fluor[®] 488 conjugated anti-pStat5 (Tyr694) (C71E5) Rabbit mAb (Cell Signaling Technology, Inc.) following the manufacturer's suggested protocol. Cells were washed with PBS and analyzed by a flow cytometer. The EC₅₀ values for hGH and trastuzumab hGH fusions are shown in Table 15. ND = not determined.

Table 15: hGH Activity Assays			
Analyte	NB2 proliferation assay (EC ₅₀)	hGHR-Ba/F3 proliferation assay (EC ₅₀)	Stat5 phosphorylation assay (EC ₅₀)
hGH	0.084 $\pm$ 0.011	0.926 $\pm$ 0.059	0.353 $\pm$ 0.090
trastuzumab-coil hGH (CDRH3)	0.153 $\pm$ 0.044	1.792 $\pm$ 0.448	1.065 $\pm$ 0.116
trastuzumab-coil hGH (CDRH2)	ND	ND	0.524 $\pm$ 0.046
trastuzumab-direct hGH (CDRH2)	ND	ND	0.539 $\pm$ 0.034

Example 46: Trastuzumab-coil hGH (CDRH3) Pharmacokinetics Studies

[00534] Trastuzumab-coil hGH (CDRH3) and genotropin were injected intravenously (i.v) or subcutaneously (s.c.) into two separate experiment groups at 2 mg/kg in PBS into SD female rats with three rats per treatment. Plasma samples were collected at the following time points: 30 min, 1 hr, 2 hr, 4 hr, 6 hr, 24 hr, 48 hr, 3 days, 4 days, 7 days, 10 days, and 14 days. The amount of genotropin was quantified by hGH Human Direct ELISA Kit (Life Technologies). Trastuzumab-coil hGH (CDRH3) was quantified using a sandwich ELISA assay. Briefly, maxisorb ELISA plates were coated with Goat Anti-Human IgG Fc (Abcam, ab98616) for 1 hour at 37°C, and then blocked with 5%BSA. A proper dilution of plasma was added to the blocked wells and the wells incubated for 1 hour at room temperature. After washing the wells, biotinylated polyclonal anti-hGH antibodies (R&D systems, BAF1067) were applied to the wells for 1 hour. The plates were washed and incubated with high sensitivity Streptavidin-HRP conjugate (Pierce, 21130) for 1 hour at room temperature. QuantaBlu fluorogenic ELISA substrate was applied after extensive washing, and signals were obtained with Spectramax fluorescence plate reader. The amount of trastuzumab-coil hGH (CDRH3) fusion in plasma samples was quantified by extrapolating the signal into a linear range (signal vs concentration) of a standard curve. The concentrations of genotropin and trastuzumab-coil hGH (CDRH3) at each collection time point were plotted and shown in Fig. 36. Fig. 36 A shows the pharmacokinetics by intravenous injection. Fig. 36 B shows the pharmacokinetics by subcutaneous injection.

Example 47: Trastuzumab-coil hGH (CDRH3) Pharmacodynamics Studies

[00535] The pharmacodynamics performance of the trastuzumab-coil hGH (CDRH3) fusion was assessed in a standard hypophysectomized rat assay. Hypophysectomized male rats were purchased from Harlan, and pre-screened for several days prior to the study to monitor body weight normalization post-surgery/travel. The rats matched by initial weights were treated with one of several therapies: daily subcutaneous injection of genotropin for 14 days (0.1 mg/kg); or biweekly administration of genotropin (0.3 mg/kg) or trastuzumab-coil hGH (CDRH3) (1.0 mg/kg). The animals were weighed daily. At the end the treatment period animals were sacrificed and epiphyses thickness was measured. The percent change in body weight from day 1 was plotted per day and is shown in Fig. 37.

Example 48: Construction of trastuzumab-coil hLeptin based fusion protein vectors for expression in mammalian cells

[00536] A gene encoding hLeptin (SEQ ID NO: 197) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). To optimize the folding and stability of the immunoglobulin fusion protein, flexible linkers of GGGGS (SEQ ID NO: 179, n=1) were added on both ends of the hLeptin fragments. Then, sequences encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the hLeptin-linker fragment. The PCR fragment encoding the hLeptin gene with the extender peptides and linkers was grafted into the complementarity determining region 3 of the heavy chain (CDRH3) of trastuzumab IgG antibody by exploiting overlap extension PCR. The constant regions of trastuzumab were modified with

human IgG1 CH1-CH3 constant region containing seven mutations (E233P, L234V, L235A, ΔG236, A327G, A330S, and P331S) to generate trastuzumab-coil hLeptin (CDRH3) HC (SEQ ID NO: 78). To generate a CDRL3 fusion, the PCR fragment encoding the hLeptin gene with the extender peptides and linkers was grafted into the complementary determining region 3 of the light chain (CDRL3) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace Thr93-Pro95 and generate trastuzumab-coil hLeptin (CDRL3) (SEQ ID NO: 49). The PCR fragment encoding the hLeptin gene with the extender peptides and linkers was grafted into the complementarity determining region 2 of the heavy chain (CDRH2) of trastuzumab IgG antibody by exploiting overlap extension PCR to generate trastuzumab-coil hLeptin (CDRH2) HC (SEQ ID NO: 79). The expression vectors of the trastuzumab-coil hLeptin based fusion proteins were generated by in-frame ligation of the amplified fusion genes to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain of trastuzumab IgG antibody (SEQ ID NO: 1) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 49: Expression and purification of trastuzumab-coil hLeptin based fusion proteins

[00537] Trastuzumab-coil hLeptin (CDRH3) based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil hLeptin (CDRH3) HC (SEQ ID NO: 78) and the trastuzumab light chain (SEQ ID NO: 19). Trastuzumab-coil hLeptin (CDRH2) based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil hLeptin (CDRH2) HC (SEQ ID NO: 79) and the trastuzumab light chain (SEQ ID NO: 19). Trastuzumab-coil hLeptin (CDRL3) based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil hLeptin (CDRL3) LC (SEQ ID NO: 49) and the trastuzumab heavy chain (SEQ ID NO: 4). Expressed fusion proteins were secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins were purified by Protein A/G chromatography (Thermo Fisher Scientific, IL), and analyzed by SDS-PAGE gel. As shown in FIG.38, Lane 1 depicts trastuzumab-coil hLeptin (CDRH2) (SEQ ID NOs: 79, 19), Lane 2 depicts trastuzumab-coil hLeptin (CDRH2) (SEQ ID NOs: 79, 19) treated with DTT, Lanes 3, 4 and 7 depict protein molecular weight markers, Lane 5 depicts trastuzumab-coil hLeptin (CDRH3) (SEQ ID NOs: 78, 19), Lane 6 depicts trastuzumab-coil hLeptin (CDRH3) (SEQ ID NOs: 78, 19) treated with DTT, Lane 8 depicts trastuzumab-coil hLeptin (CDRL3) (SEQ ID NOs: 49, 4), and Lane 9 depicts trastuzumab-coil hLeptin (CDRL3) (SEQ ID NOs: 49, 4) treated with DTT.

Example 50: In vitro activity of trastuzumab-coil hLeptin based fusion proteins in activating human leptin receptor (LepR)

[00538] Baf3 stable cells overexpressing human Leptin receptor (LepR) were seeded in a 96-well plate, treated with different doses of hLeptin (SEQ ID NO: 238), trastuzumab-coil hLeptin (CDRH2) (SEQ ID NOs: 79, 19), and trastuzumab-coil hLeptin (CDRH3) (SEQ ID NOs: 78, 19) for 72hours. AlamarBlue reagent was added at 1/10 volume, incubated for 2 hrs, and the fluorescent measured at 590nm under excitation at 560nm. The data was analyzed using GraphPad Prism 6. Fig. 39 depicts a graphical representation of the data. The EC₅₀ of hLeptin was 129.4 ± 46.09 pM (Fig. 39 A). The EC₅₀ of

trastuzumab-coil hLeptin (CDRH3) was 55.38 ± 14.04 μ M. The EC_{50} of trastuzumab-coil hLeptin (CDRH2) was 99.41 ± 18.91 μ M. The EC_{50} of hLeptin was 58.19 ± 10.88 μ M (Fig. 39 B). The EC_{50} of trastuzumab-coil hLeptin (CDRL3) was 665.1 ± 62.70 μ M.

Example 51: SKBR3 binding of trastuzumab-coil hLeptin based fusion proteins

[00539] SKBR3 cells were grown in DMEM with 10% FBS and 1% penicillin and streptomycin. Cells were washed with cold PBS for three times, blocked with 2% BSA in PBS, and incubated with 10 or 100 nM of trastuzumab, trastuzumab-coil hLeptin (CDRH2) (SEQ ID NOs: 79, 19), and trastuzumab-coil hLeptin (CDRH3) (SEQ ID NOs: 78, 19) for 2 hours at 4°C with gentle mixing. Unbound antibody was removed by washing with 2% BSA in PBS. Cells were then stained with FITC anti-human IgG Fc (KPL, Inc., MD) for 1 hour at 4°C with gentle mixing, followed by washing with PBS and analysis by flow cytometry. Fig. 40 depicts the flow cytometry histogram of (A) trastuzumab, (B) trastuzumab-coil hLeptin (CDRH2), and (C) trastuzumab-coil hLeptin (CDRH3).

Example 52: Construction of trastuzumab-coil-elafin fusion protein vectors for expression in mammalian cells

[00540] A gene encoding elafin (SEQ ID NO: 217) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). A flexible GGGGS linker (SEQ ID NO: 179) was added to the N-terminus and C-terminus of the elafin gene fragment to increase folding and stability of the fusion protein. Then, sequences encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAALEAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the elafin linker fragment. Subsequently, the PCR fragment encoding elafin with the linker and extender fragments were grafted into the complementarity determining region 3 of the heavy chain (CDR3H) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace the Trp99–Met107 loop. The trastuzumab-coil-elafin based fusion protein was modified with human hIgG1 CH1-CH3 constant region containing seven mutations (E233P, L234V, L235A, Δ G236, A327G, A330S, and P331S) to generate trastuzumab-coil-elafin HC fusion (SEQ ID NO: 54). The expression vector of trastuzumab-coil-elafin (CDRH3) was generated by in-frame ligation of the amplified fusion gene to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain of trastuzumab IgG antibody (SEQ ID NO: 1) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 53: Expression and purification of trastuzumab-coil-elafin based fusion proteins

[00541] Trastuzumab-coil-elafin (CDRH3) based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil elafin fusion protein heavy chain (SEQ ID NO: 54) and the trastuzumab light chain (SEQ ID NO: 19). Expressed fusion proteins were secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins were purified by Protein A/G chromatography (Thermo Fisher Scientific, IL), and analyzed by SDS-PAGE gel as shown in Fig. 41. Lane 1 is a protein marker. Lane 2 is trastuzumab-coil-elafin (CDRH3) IgG (SEQ ID NOs: 85 and 19). Lane 3 is trastuzumab-coil-elafin (CDRH3) IgG (SEQ ID NOs: 85 and 19) treated with DTT.

Example 54: Elastase inhibition assay

[00542] Human elastase was purchased from Elastin Products Company, Inc. Increasing concentrations of elafin (SEQ ID NO: 258) and trastuzumab-coil elafin (CDRH3) IgG (SEQ ID NOs: 85 and 19) were incubated with elastase at room temperature, the residue activity of elastase was analyzed by the addition of fluorogenic elastase substrate MeOSuc-AAPV-AMC (EMD Millipore). The slope of the reactions were obtained by monitoring at 420 nm wavelength with 325 nm excitation on a Spectramax fluorescence plate reader. Each data point was triplicated and fit into the equation:

$Q = (K_i * (1 + (S/K_m)))$. $Y = V_o * (1 - (((Et + X + Q) - (((Et + X + Q)^2 - 4 * Et * X)^{0.5})) / (2 * Et)))$. Figure 42 shows the inhibition of elastase by elafin (A) and trastuzumab-coil elafin (CDRH3) IgG (B).

Example 55: Construction of trastuzumab-coil GLP2 fusion protein vectors for expression in mammalian cells

[00543] A gene encoding GLP2 (SEQ ID NO: 222) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). A flexible CGGGGS linker (SEQ ID NO: 177) was added to the N-terminus of GLP2 and a flexible GGGGSC (SEQ ID NO: 178) was added to the C-terminus of GLP2. Then, sequences encoding extender peptides GSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the GLP2 linker fragment. Subsequently, the PCR fragment encoding GLP2 with the linker and extender fragments were grafted into the complementarity determining region 3 of the heavy chain (CDRH3) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace the Trp99–Met107 loop. The trastuzumab-coil GLP2 based fusion protein was modified with human hIgG1 CH1-CH3 constant region containing seven mutations (E233P, L234V, L235A, ΔG236, A327G, A330S, and P331S) to generate trastuzumab-coil GLP2 (CDRH3) HC fusion (SEQ ID NO: 65). The expression vector of trastuzumab-coil GLP2 (CDRH3) was generated by in-frame ligation of the amplified fusion gene to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain of trastuzumab IgG antibody (SEQ ID NO: 1) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 56: Expression and purification of trastuzumab-coil GLP2 based fusion proteins

[00544] Trastuzumab-coil GLP2 (CDRH3) based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil GLP2 fusion protein heavy chain (SEQ ID NO: 65) and the trastuzumab light chain (SEQ ID NO: 19). Expressed fusion proteins were secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins were purified by Protein A/G chromatography (Thermo Fisher Scientific, IL), and analyzed by SDS-PAGE gel as shown in Fig. 43. Lane 1 is a protein marker. Lane 2 is trastuzumab-coil GLP2 (CDRH3) IgG (SEQ ID NOs: 96 and 19). Lane 3 is trastuzumab-coil GLP2 (CDRH3) IgG (SEQ ID NOs: 96 and 19) treated with DTT.

Example 57: Construction of trastuzumab-coil relaxin (insulin c-peptide) fusion protein vectors for expression in mammalian cells

[00545] A gene encoding relaxin (insulin c peptide) (SEQ ID NO: 225) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). A flexible GGGGS linker (SEQ ID NO: 179) was added to the N-terminus of relaxin and a flexible GGGGS (SEQ ID NO: 179) was added to the C-terminus of relaxin. Then, sequences encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the relaxin linker fragment. Subsequently, the PCR fragment encoding relaxin (insulin c-peptide) with the linker and extender fragments were grafted into the complementarity determining region 3 of the heavy chain (CDR3H) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace the Trp99–Met107 loop. The trastuzumab-coil relaxin (insulin c-peptide) based fusion protein was modified with human hIgG1 CH1-CH3 constant region containing seven mutations (E233P, L234V, L235A, ΔG236, A327G, A330S, and P331S) to generate trastuzumab-coil relaxin (insulin c-peptide) (CDRH3) HC fusion. The expression vector of trastuzumab-coil relaxin (insulin c-peptide) (CDRH3) was generated by in-frame ligation of the amplified fusion gene to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain of trastuzumab IgG antibody (SEQ ID NO: 1) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 58: Expression and purification of trastuzumab-coil relaxin (insulin c-peptide) based fusion proteins

[00546] Trastuzumab-coil relaxin (insulin c-peptide) (CDRH3) based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil relaxin (insulin c-peptide) fusion protein heavy chain and the trastuzumab light chain. Expressed fusion proteins were secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins were purified by Protein A/G chromatography (Thermo Fisher Scientific, IL), and analyzed by SDS-PAGE gel as shown in Fig. 44. Lane 1 is trastuzumab-coil relaxin (insulin c-peptide) (CDRH3) IgG treated with DTT. Lane 2 is trastuzumab-coil relaxin (insulin c-peptide) (CDRH3) IgG. Lane 3 is a protein marker.

Example 59: Construction of trastuzumab-coil relaxin fusion protein vectors for expression in mammalian cells

[00547] A gene encoding relaxin comprising internal protease cleavage sites for Factor Xa and PC2 (IEGRKKR) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). A flexible GGGGS linker (SEQ ID NO: 179) was added to the N-terminus of relaxin and a flexible GGGGS (SEQ ID NO: 179) was added to the C-terminus of relaxin. Then, sequences encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the relaxin linker fragment. Subsequently, the PCR fragment encoding relaxin with the protease cleavage sites, linkers and extender fragments were grafted into the complementarity determining region 3 of the heavy chain

(CDR3H) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace the Trp99–Met107 loop. The trastuzumab-coil relaxin based fusion protein was modified with human hIgG1 CH1-CH3 constant region containing seven mutations (E233P, L234V, L235A, ΔG236, A327G, A330S, and P331S) to generate a trastuzumab-coil relaxin (CDRH3) HC fusion. The expression vector of trastuzumab-coil relaxin (CDRH3) was generated by in-frame ligation of the amplified fusion gene to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain of trastuzumab IgG antibody (SEQ ID NO: 1) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 60: Expression and purification of trastuzumab-coil relaxin based fusion proteins

[00548] Trastuzumab-coil relaxin (CDRH3) based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil relaxin fusion protein heavy chain (SEQ ID NO: 90) and the trastuzumab light chain (SEQ ID NO: 18). Expressed fusion proteins were secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins were purified by Protein A/G chromatography (Thermo Fisher Scientific, IL) and analyzed by SDS-PAGE gel as shown in Fig. 45. Lane 1 is a protein marker. Lane 2 is trastuzumab-coil relaxin (CDRH3) IgG. Lane 3 is trastuzumab-coil relaxin (CDRH3) IgG treated with DTT. Lane 4 is trastuzumab-coil relaxin (CDRH3) IgG co-expressed with protease PC2. Lane 5 is trastuzumab-coil relaxin (CDRH3) IgG co-expressed with PC2 and treated with DTT.

Example 61: Construction of trastuzumab-coil relaxin (XTEN35) fusion protein vectors for expression in mammalian cells

[00549] A gene encoding relaxin (XTEN35) (SEQ ID NO: 224) comprising an internal 6x HIS and a protease cleavage site for PC2 (RKRR) was synthesized by Genscript or IDT, and amplified by polymerase chain reaction (PCR). A flexible GGGGS linker (SEQ ID NO: 179) was added to the N-terminus of relaxin (XTEN35) and a flexible GGGGS (SEQ ID NO: 179) was added to the C-terminus of relaxin (XTEN35). Then, sequences encoding extender peptides GGSGAKLAALKAKLAALK (SEQ ID NO: 151) and ELAALEAELAAGGSG (SEQ ID NO: 161), which form antiparallel coiled coils, were added at the ends of the N- and C-terminal of the relaxin (XTEN35) linker fragment. Subsequently, the PCR fragment encoding relaxin (XTEN35) with the linkers and extender fragments were grafted into the complementarity determining region 3 of the heavy chain (CDR3H) of trastuzumab IgG antibody by exploiting overlap extension PCR, to replace the Trp99–Met107 loop. The trastuzumab-coil relaxin (XTEN35) based fusion protein was modified with human hIgG1 CH1-CH3 constant region containing seven mutations (E233P, L234V, L235A, ΔG236, A327G, A330S, and P331S) to generate a trastuzumab-coil relaxin (CDRH3) HC fusion. The expression vector of trastuzumab-coil relaxin (CDRH3) was generated by in-frame ligation of the amplified fusion gene to the pFuse backbone vector (InvivoGen, CA). Similarly, the gene encoding the light chain of trastuzumab IgG antibody (SEQ ID NO: 1) was cloned into the pFuse backbone vector. The obtained expression vectors were confirmed by DNA sequencing.

Example 62: Expression and purification of trastuzumab-coil relaxin (XTEN35) based fusion proteins

[00550] Trastuzumab-coil relaxin (XTEN35) (CDRH3) based fusion proteins were expressed through transient transfections of free style HEK293 cells with vectors encoding trastuzumab-coil relaxin (XTEN35) fusion protein heavy chain and the trastuzumab light chain. Expressed fusion proteins were secreted into the culture medium and harvested at 48 and 96 hours after transfection. The fusion proteins were purified by Protein A/G chromatography (Thermo Fisher Scientific, IL), cleaved with protease PC2, and analyzed by SDS-PAGE gel as shown in Fig. 46. Lane 1 is trastuzumab-coil relaxin (XTEN35) (CDRH3) IgG treated with DTT. Lane 2 is trastuzumab-coil relaxin (XTEN35) (CDRH3) IgG. Lane 3 is trastuzumab-coil relaxin (XTEN35) (CDRH3) IgG co-expressed with PC2 and treated with DTT. Lane 4 is trastuzumab-coil relaxin (XTEN35) (CDRH3) IgG co-expressed with PC2. Lane 5 is a protein molecular weight marker.

Example 63: Binding of trastuzumab-coil-hGCSF protein to Her2 receptor

[00551] The binding affinity of trastuzumab-coil-hGCSF fusion proteins to Her2 receptor is examined by ELISA. Human Her2-Fc chimera (5 µg/mL) (R&D Systems) is coated on 96-well ELISA plate overnight at 4°C, followed by blocking with 1% BSA in PBS (pH 7.4) for 2 hours at 37°C. After washing with 0.05% Tween-20 in PBS (pH 7.4), various concentrations of trastuzumab IgG and trastuzumab-coil-hGCSF fusion proteins are added to the plate for 2 hours of incubation at 37°C. Subsequently, goat polyclonal anti-human kappa light chain antibody with HRP conjugate (Sigma) is added to the plate and the plate is incubated for 2 hours at 37°C. Wells are subsequently washed and binding affinities are examined on the basis of fluorescence intensity at 425 nm by adding fluoregenic peroxidase substrate to each well.

Example 64: Binding of trastuzumab-coil-VM24 to Her2 receptor

[00552] The binding affinity of trastuzumab-coil-VM24 fusion proteins to Her2 receptor is examined by ELISA. Human Her2-Fc chimera (5 µg/mL) (R&D Systems) is coated on 96-well ELISA plate overnight at 4°C, followed by blocking with 1% BSA in PBS (pH 7.4) for 2 hours at 37°C. After washing with 0.05% Tween-20 in PBS (pH 7.4), various concentrations of trastuzumab IgG and trastuzumab-coil-VM24 fusion proteins are added to the plate for 2 hours at 37°C. Subsequently, goat polyclonal anti-human kappa light chain antibody with HRP conjugate (Sigma) is added to the plate and the plate is incubated for 2 hours at 37°C. Wells are subsequently washed and binding affinities are examined on the basis of fluorescence intensity at 425 nm by adding fluoregenic peroxidase substrate to each well.

Example 65: Construction of trastuzumab-coil hLeptin-exendin-4 dual fusion protein

[0553] Leptin and exendin-4 are fused to the CDR-3H and CDR-3L regions in the trastuzumab backbone with an engineered coiled coil stalk. The generated humanized biologically active fusion proteins may improve pharmacological properties for treatment of relevant diseases. In addition, the combination of hLeptin and Ex-4 may have synergistic effects. Trastuzumab-coil hLeptin/Ex4 fusions contain GGGGS linkers at each terminal of the fused hLeptin and Ex-4 fragments and a GGSG linker to connect the coiled coils to the base of antibody.

Example 66: Binding of trastuzumab-coil-Moka IgG to Her2 receptor

[00554] The binding of trastuzumab-coil-Moka fusion protein to Her2 receptor is examined by ELISA. Human Her2-Fc chimera (5 µg/mL) (R&D Systems) is coated on 96-well ELISA plate overnight at 4°C, followed by blocking with 1% BSA in PBS (pH 7.4) for 2 hours at 37°C. After washing with 0.05% Tween-20 in PBS (pH 7.4), various concentrations of trastuzumab IgG and trastuzumab-coil-Moka fusion proteins are added to each well and the plate is incubated for 2 hours at 37°C. Subsequently, goat polyclonal anti-human kappa light chain antibody with HRP conjugate (Sigma) is added to the plate and the plate is incubated for 2 hours at 37°C. Wells are subsequently washed and binding affinities are examined on the basis of fluorescence intensity at 425 nm by adding fluoregenic peroxidase substrate to each well.

[00555] The preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of the present invention is embodied by the appended claims.

[00556] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

What is claimed is:

1. An immunoglobulin fusion protein comprising an extender fusion region positioned within a complementarity-determining region (CDR) of a first antibody variable domain comprising a variable region of an antibody heavy chain and a variable region of an antibody light chain, wherein the extender fusion region comprises a first extender peptide comprising a first alpha helical secondary structure, a first therapeutic agent, and a second extender peptide comprising a second alpha helical secondary structure; wherein the first therapeutic agent is attached to the first antibody variable domain by the first extender peptide and the second extender peptide.
2. The immunoglobulin fusion protein of claim 1, wherein the first extender peptide does not comprise more than 7 consecutive amino acids that are based on or derived from a bovine ultralong CDR3 amino acid sequence.
3. The immunoglobulin fusion protein of claim 1, further comprising a first linking peptide.
4. The immunoglobulin fusion protein of claim 3, wherein the first linking peptide does not comprise alpha helical or beta strand secondary structure.
5. The immunoglobulin fusion protein of any one of claims 1-4, wherein the first therapeutic agent replaces one or more amino acids of the first antibody variable domain.
6. The immunoglobulin fusion protein of any one of claims 1-5, further comprising a second therapeutic agent.
7. The immunoglobulin fusion protein of any one of claims 1-6, wherein the first antibody variable domain comprises an amino acid sequence selected from SEQ ID NOs: 19-36 and 271-273.

8. The immunoglobulin fusion protein of any one of claims 1-6, wherein the first antibody variable domain comprises a trastuzumab immunoglobulin variable domain, and wherein the extender fusion region optionally replaces at least a portion of a complementarity determining region of the trastuzumab immunoglobulin variable domain.
9. The immunoglobulin fusion protein of any one of claims 1-6, wherein the first antibody variable domain comprises a palivizumab immunoglobulin variable domain, and wherein the extender fusion region optionally replaces at least a portion of a complementarity determining region of the palivizumab immunoglobulin variable domain.
10. The immunoglobulin fusion protein of any one of claims 1-9, further comprising a second antibody variable domain comprising a second variable region of an antibody heavy chain and a second variable region of an antibody light chain.
11. The immunoglobulin fusion protein of any one of claims 1-10, wherein the first extender peptide comprises an amino acid sequence selected from SEQ ID NOs: 148-153 and 158-175.
12. The immunoglobulin fusion protein of claim 11, wherein the first extender peptide comprises SEQ ID NO: 151.
13. The immunoglobulin fusion protein of claim 11, wherein the first extender peptide comprises SEQ ID NO: 161.
14. An immunoglobulin fusion protein comprising an antibody domain and a first extender fusion region positioned within the antibody domain, wherein the extender fusion region comprises a therapeutic agent positioned between a first extender peptide and a second extender peptide, and the first extender peptide comprises an amino acid sequence selected from SEQ ID NOs: 148-153 and 158-175, the second extender peptide comprises an amino acid sequence selected from SEQ ID NOs: 148-153 and 158-175.

15. The immunoglobulin fusion protein of claim 14, wherein the first extender peptide comprises SEQ ID NO: 151.
16. The immunoglobulin fusion protein of claim 14, wherein the first extender peptide comprises SEQ ID NO: 161.
17. The immunoglobulin fusion protein of any one of claims 1-16, wherein the second extender peptide comprises an amino acid sequence selected from SEQ ID NOs: 148-153 and 158-175.
18. The immunoglobulin fusion protein of claim 17, wherein the second extender peptide comprises SEQ ID NO: 161.
19. The immunoglobulin fusion protein of claim 17, wherein the second extender peptide comprises SEQ ID NO: 151.
20. The immunoglobulin fusion protein of claim 11, wherein the first extender peptide comprises SEQ ID NO: 148.
21. The immunoglobulin fusion protein of claim 17 or claim 20, wherein the second extender peptide comprises SEQ ID NO: 158.
22. The immunoglobulin fusion protein of claim 11, wherein the first extender peptide comprises SEQ ID NO: 150.
23. The immunoglobulin fusion protein of claim 17 or claim 22, wherein the second extender peptide comprises SEQ ID NO: 160.
24. The immunoglobulin fusion protein of any one of claims 1-23, further comprising a protease cleavage site.

25. The immunoglobulin fusion protein of claim 24, further comprising a second protease cleavage site.
26. The immunoglobulin fusion protein of any one of claims 1-23, wherein the first therapeutic agent comprises a protease cleavage site.
27. The immunoglobulin fusion protein of claim 6, wherein the second therapeutic agent comprises a protease cleavage site.
28. The immunoglobulin fusion protein of claim 1, wherein the first therapeutic agent comprises an amino acid sequence selected from SEQ ID NOs: 227-267.
29. The immunoglobulin fusion protein of any one of claims 1-6, comprising an amino acid sequence selected from SEQ ID NOs: 68-99.
30. A first genetic construct comprising nucleic acids encoding the immunoglobulin fusion protein of any one of claims 1-29.
31. A first expression vector comprising the genetic construct of claim 30.
32. A mammalian expression host cell comprising the first expression vector of claim 31.
33. A method of producing an immunoglobulin fusion protein comprising
- a) transfecting the first expression vector of claim 31 transiently in a mammalian cell culture,
 - b) growing the cell culture in an expression medium at a controlled temperature and percentage CO₂, and
 - c) harvesting the secreted immunoglobulin fusion protein.

34. The method of claim 33, further comprising purifying the immunoglobulin fusion protein.
35. Use of the immunoglobulin fusion protein of any one of claims 1-29 for treating a disease or condition in a subject in need thereof.
36. A pharmaceutical composition comprising the immunoglobulin fusion protein of any one of claims 1-29, and a pharmaceutically acceptable salt, excipient, vehicle, or combination thereof.
37. The pharmaceutical composition of claim 36, comprising the pharmaceutically acceptable excipient.
38. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat neutropenia.
39. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat diabetes.
40. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat obesity.
41. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat an autoimmune disease.
42. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat anemia.
43. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat growth hormone deficiency.
44. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat chronic obstructive pulmonary disease (COPD).
45. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat pain.
46. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat irritable bowel syndrome (IBS).

47. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat Crohn's disease.
48. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat a metabolic disorder.
49. The use of claim 48, wherein the metabolic disorder includes lipodystrophy, diabetes, and hypertriglyceridemia.
50. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat short bowel syndrome.
51. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat a patient with heart failure.
52. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat fibrosis.
53. Use of the immunoglobulin fusion protein of any one of claims 1-27 to treat pulmonary arterial hypertension, ventilator-induced injury of an immature lung, or lung transplant rejection.
54. The immunoglobulin fusion protein of claim 3, wherein the first linking peptide comprises from 1 to about 50 amino acids.
55. The immunoglobulin fusion protein of claim 1, wherein the first extender peptide comprises from 1 to about 50 amino acids.
56. The immunoglobulin fusion protein of any one of claims 1-29, wherein the first extender peptide and the second extender peptide are configured to form a coiled-coil.
57. Use of the immunoglobulin fusion protein of any one of claims 1-29 in the manufacture of a medicament for treating a disease or condition in a subject in need thereof.

58. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat neutropenia.
59. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat diabetes.
60. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat obesity.
61. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat an autoimmune disease.
62. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat anemia.
63. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat growth hormone deficiency.
64. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat chronic obstructive pulmonary disease (COPD).
65. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat pain.
66. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat irritable bowel syndrome (IBS).
67. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat Crohn's disease.

68. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat a metabolic disorder.
69. The use of claim 68, wherein the metabolic disorder includes lipodystrophy, diabetes, and hypertriglyceridemia.
70. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat short bowel syndrome.
71. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat a patient with heart failure.
72. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat fibrosis.
73. Use of the immunoglobulin fusion protein of any one of claims 1-27 in the manufacture of a medicament to treat pulmonary arterial hypertension, ventilator-induced injury of an immature lung, or lung transplant rejection.
74. The immunoglobulin fusion protein of any one of claims 1-29 for use in treating a disease or condition in a subject in need thereof.
75. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat neutropenia.
76. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat diabetes.
77. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat obesity.
78. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat an autoimmune disease.

79. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat anemia.
80. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat growth hormone deficiency.
81. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat chronic obstructive pulmonary disease (COPD).
82. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat pain.
83. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat irritable bowel syndrome (IBS).
84. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat Crohn's disease.
85. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat a metabolic disorder.
86. The immunoglobulin fusion protein of claim 85, wherein the metabolic disorder includes lipodystrophy, diabetes, and hypertriglyceridemia.
87. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat short bowel syndrome.
88. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat a patient with heart failure.
89. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat fibrosis.

90. The immunoglobulin fusion protein of any one of claims 1-27 for use to treat pulmonary arterial hypertension, ventilator-induced injury of an immature lung, or lung transplant rejection.

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FIG. 1

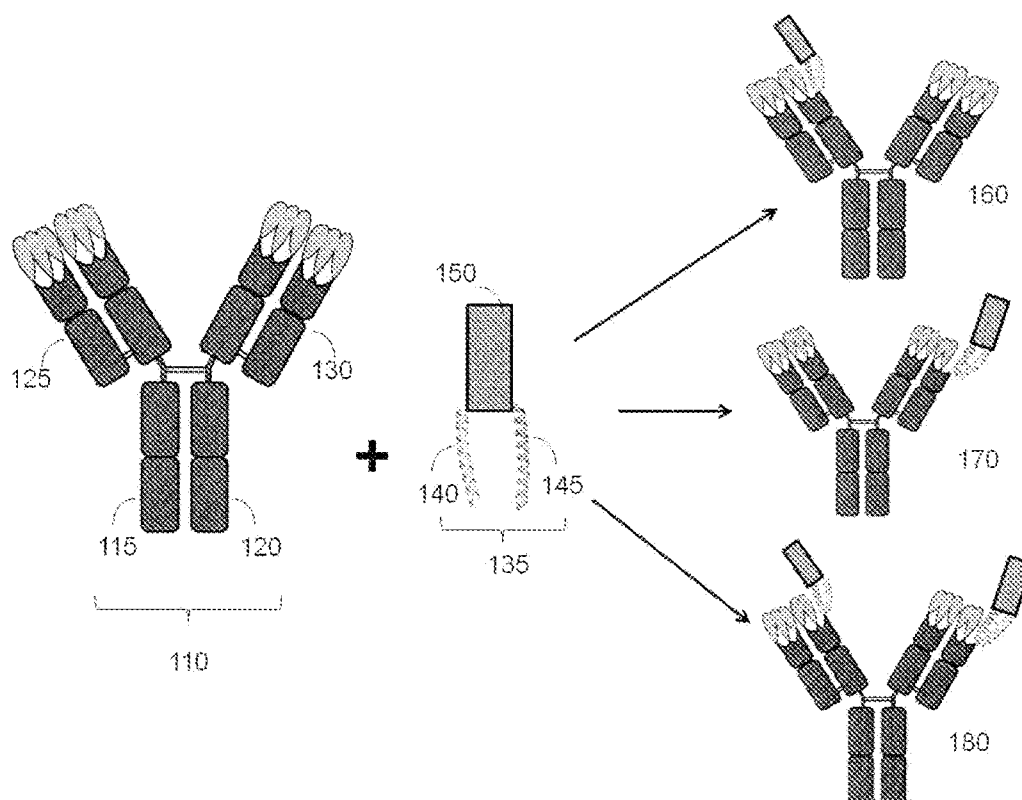
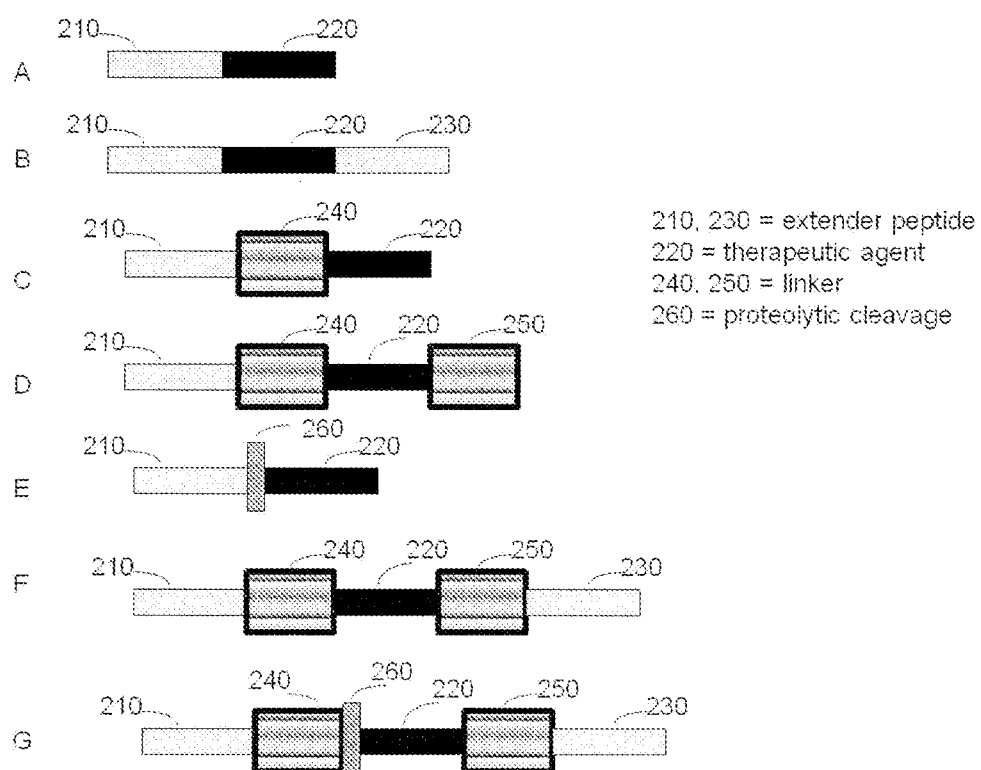


FIG. 2



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FIG. 3

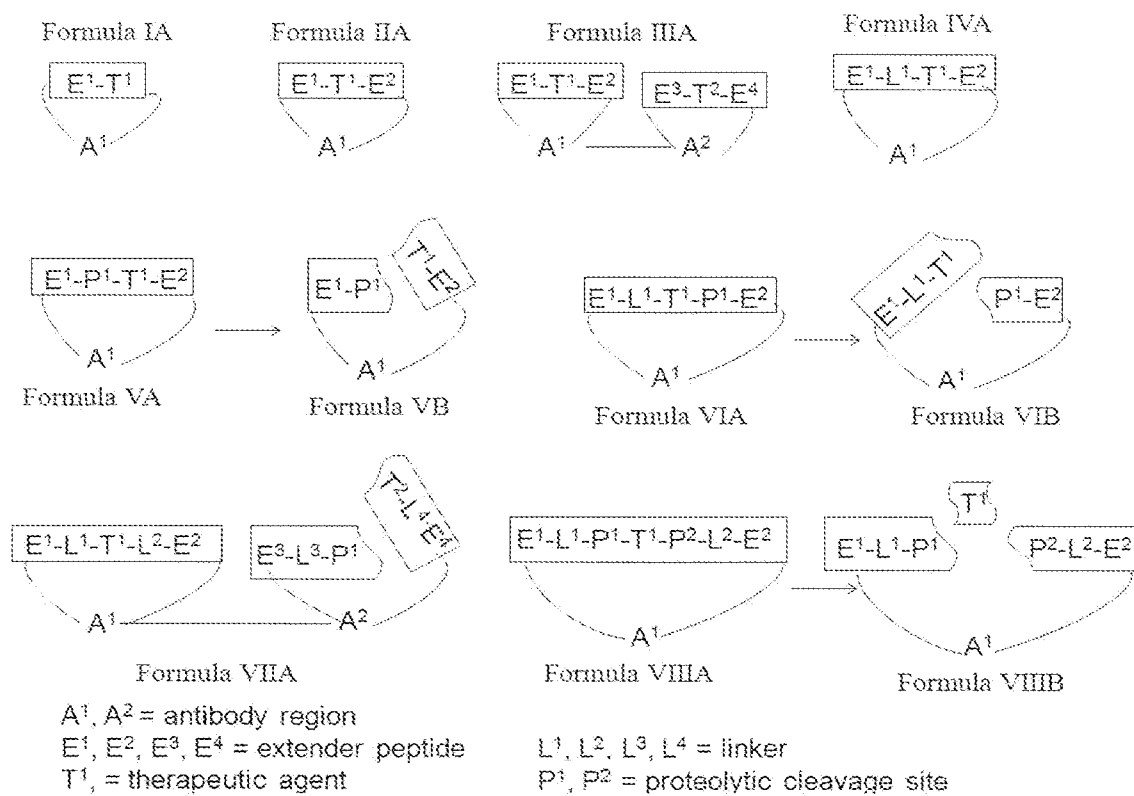
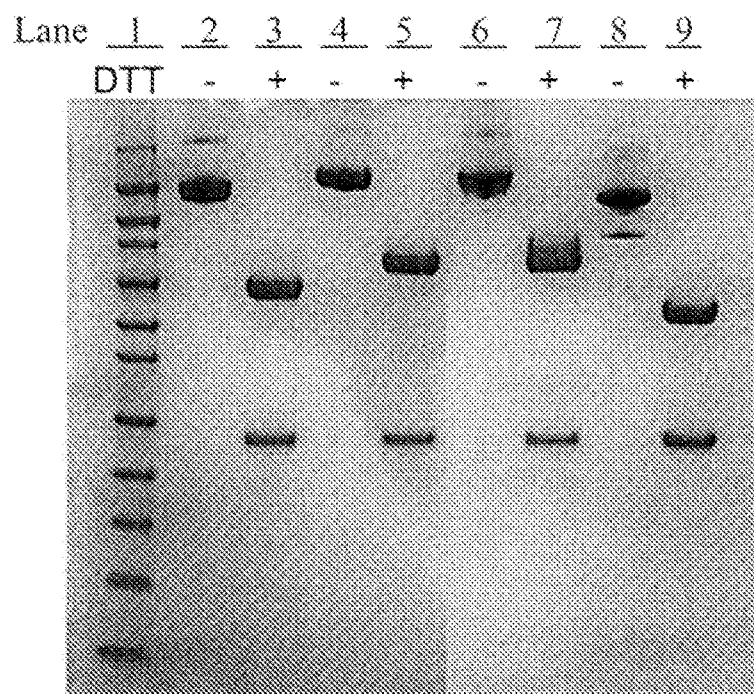


FIG. 4



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FIG. 5

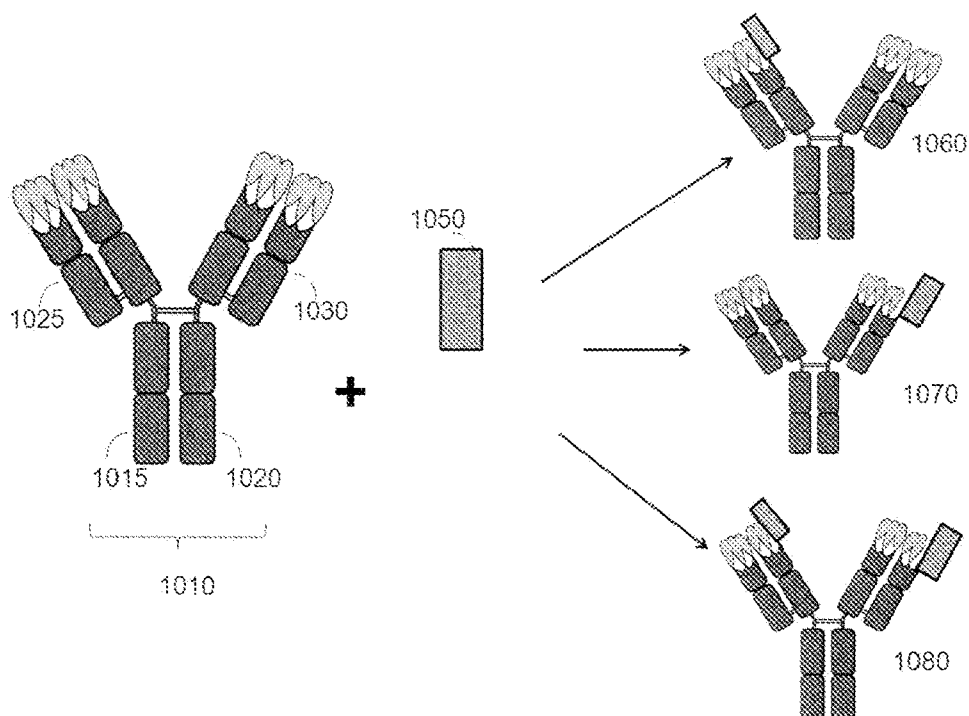
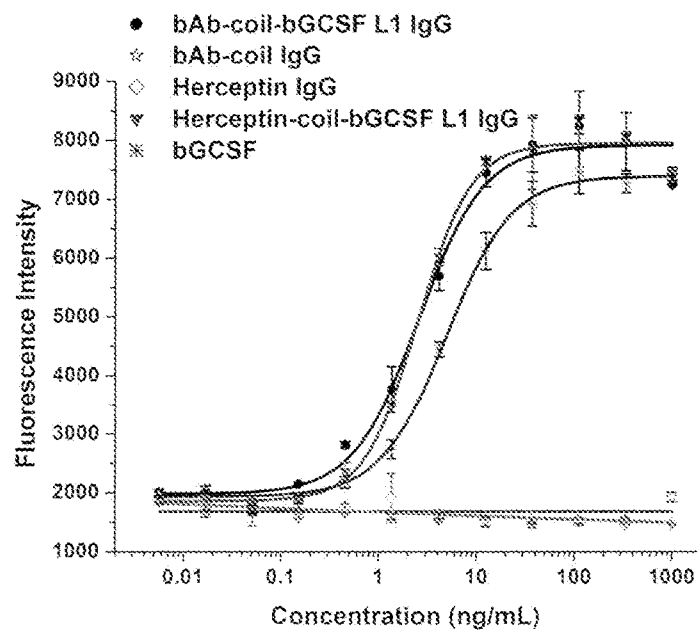


FIG. 6



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FIG. 7

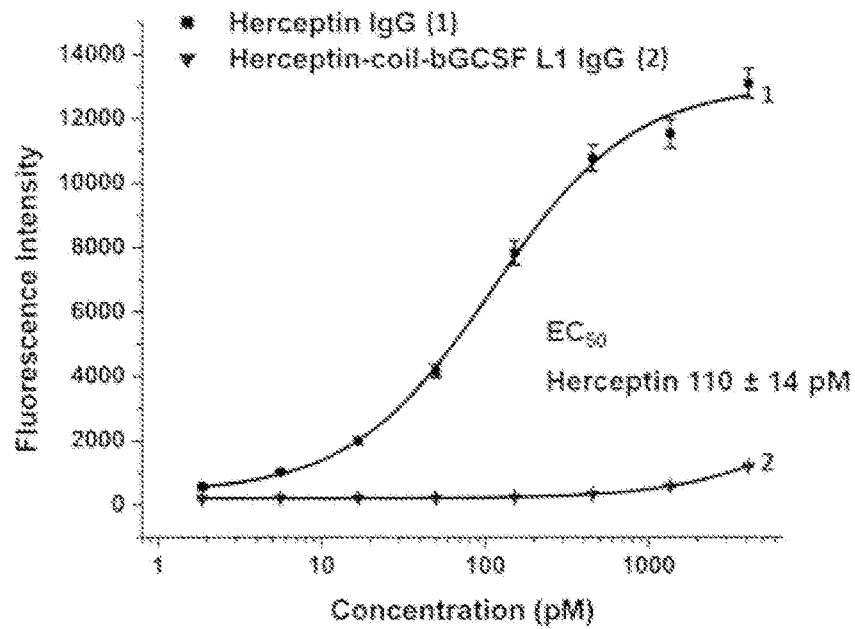
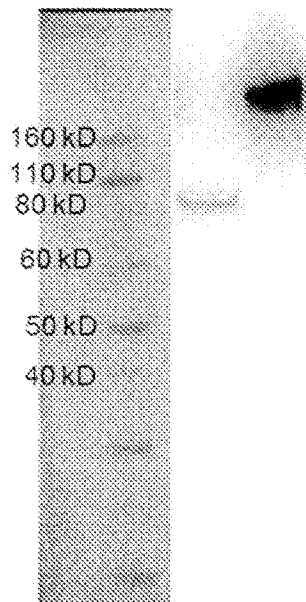


FIG. 8

Lane	1	2	3
DTT		+	-



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FIG. 9

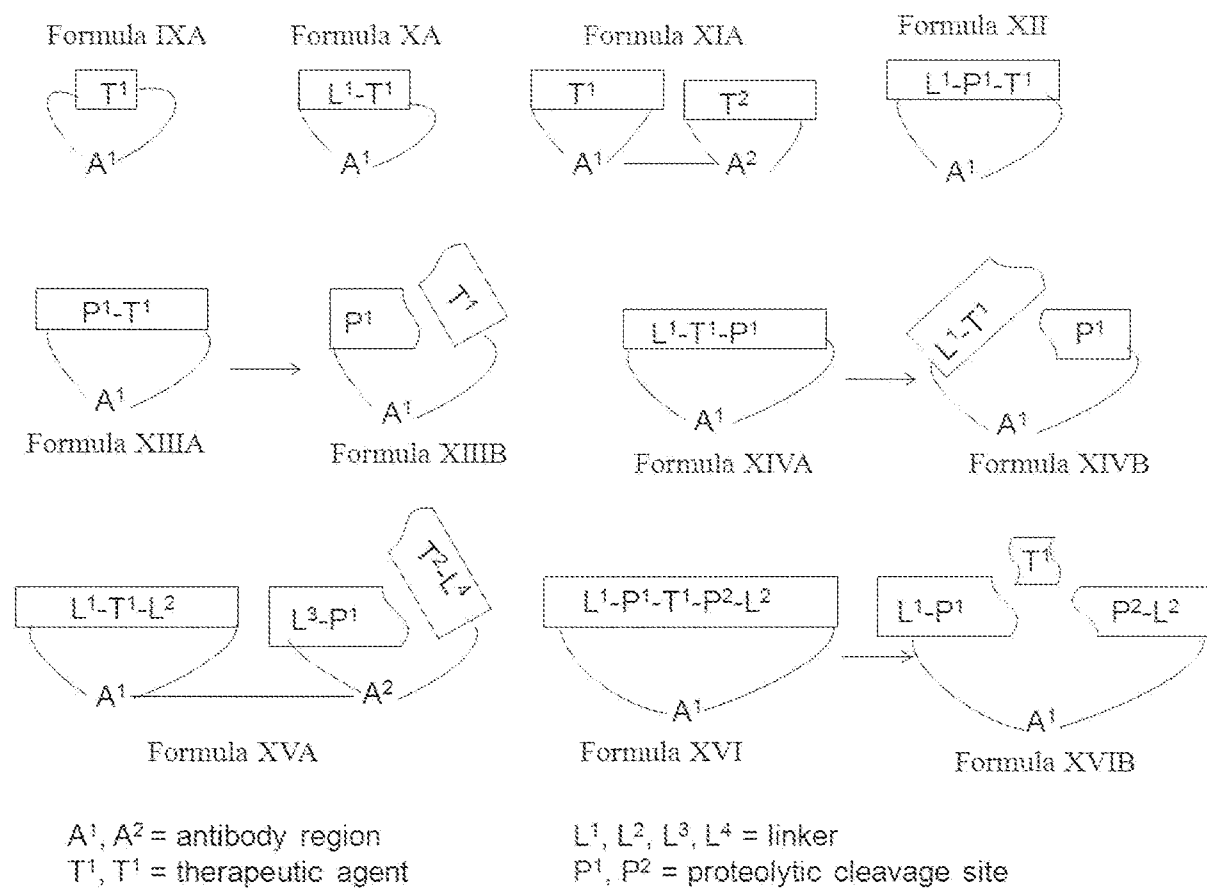
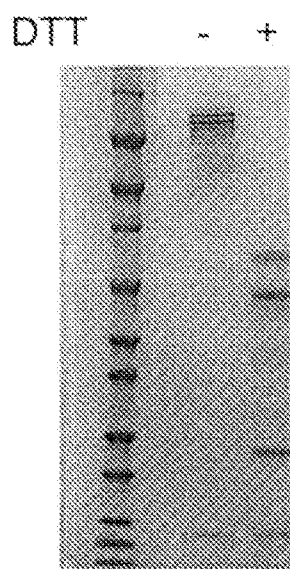


FIG. 10



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FIG. 11

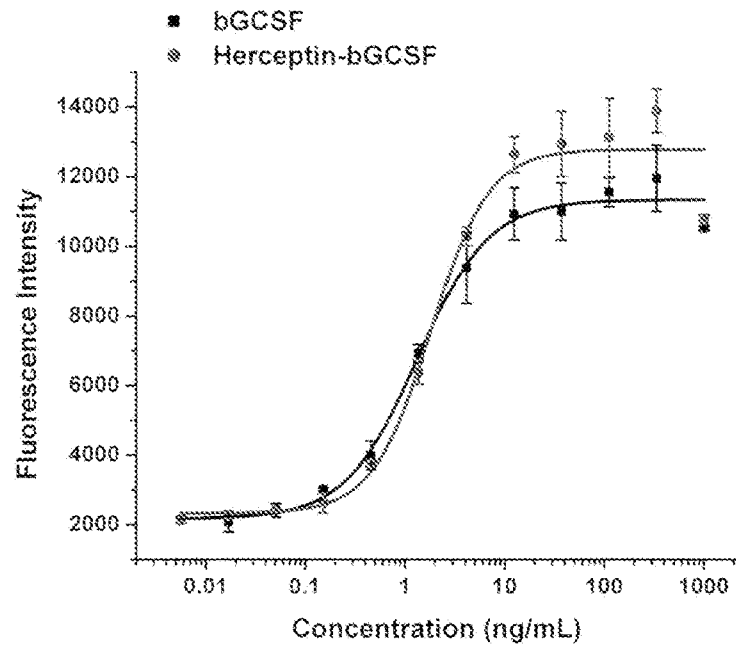
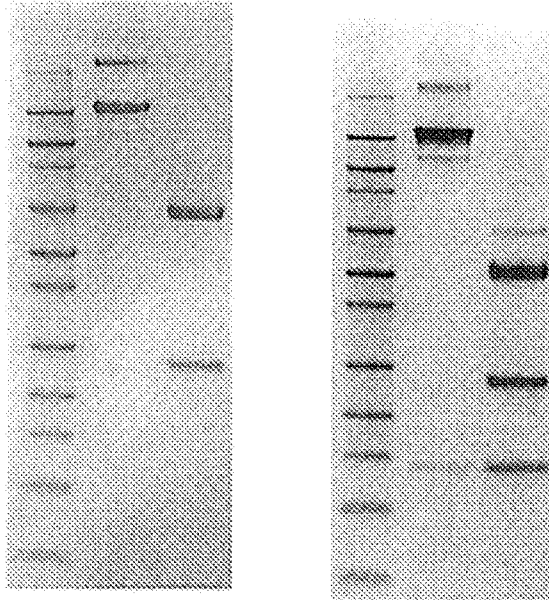


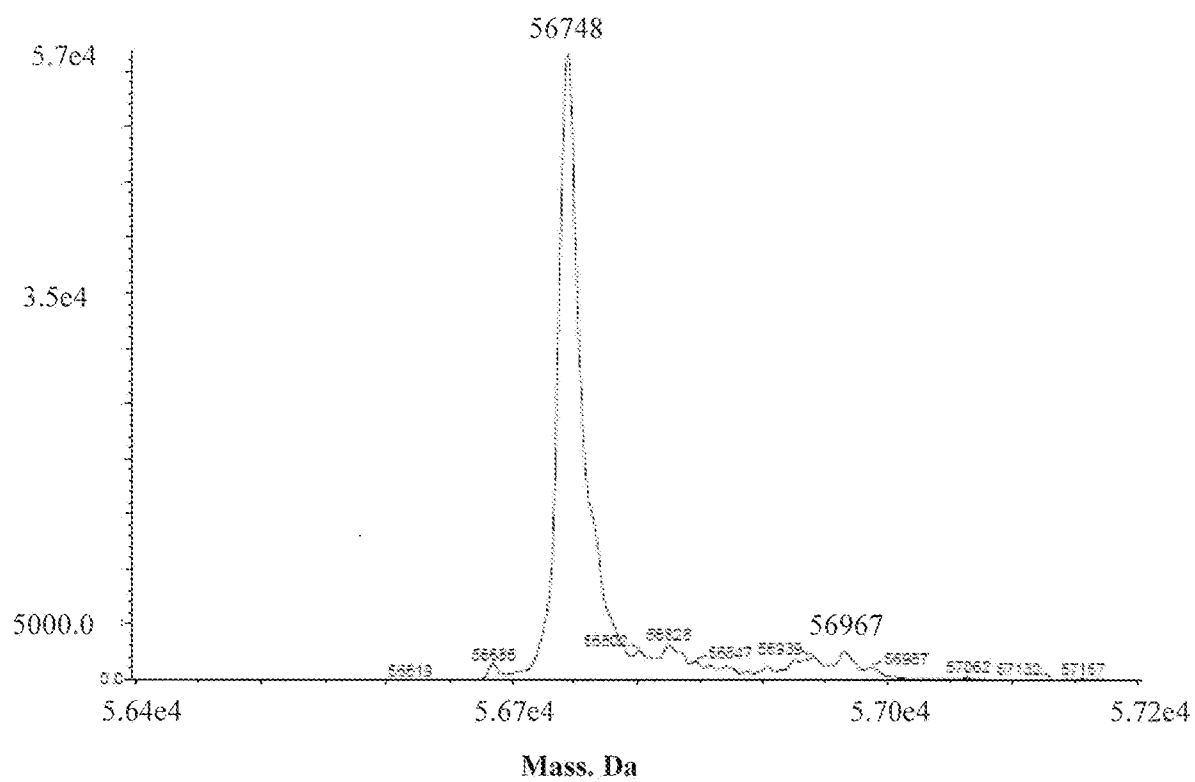
FIG. 12

Lane	1	2	3	4	5	6
DTT	-	-	+	DTT	-	+



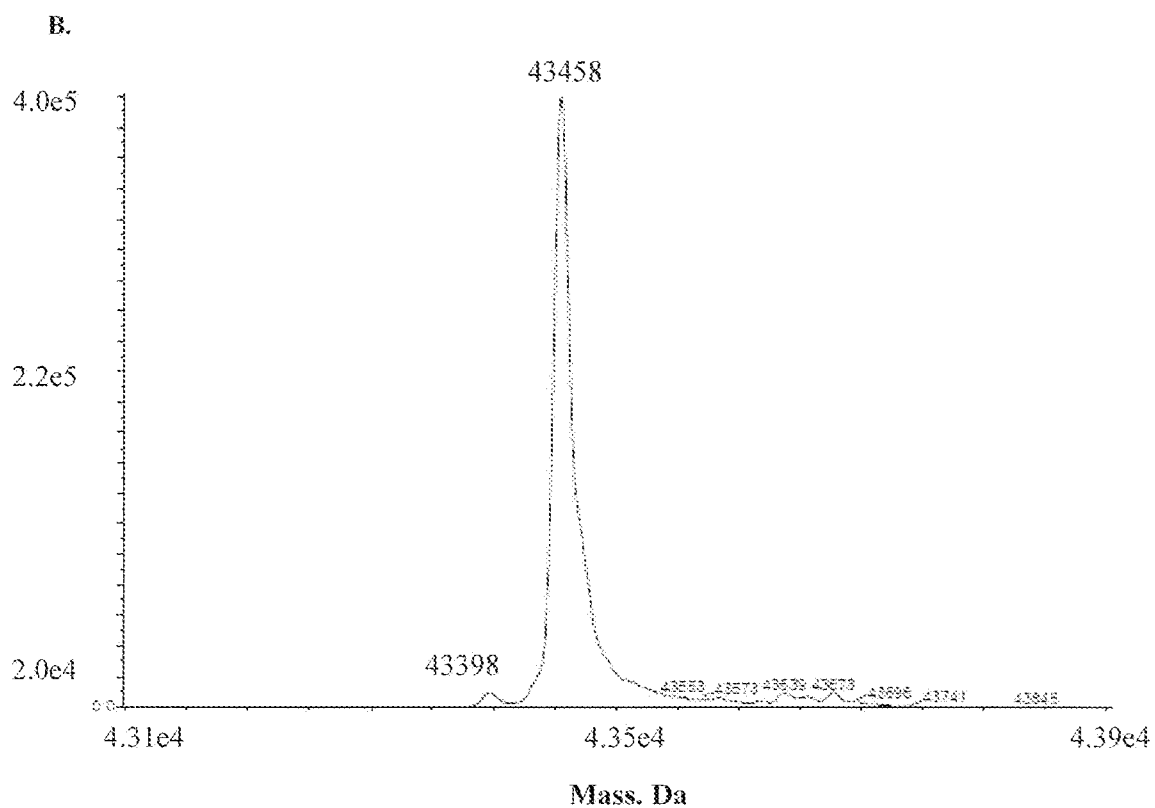
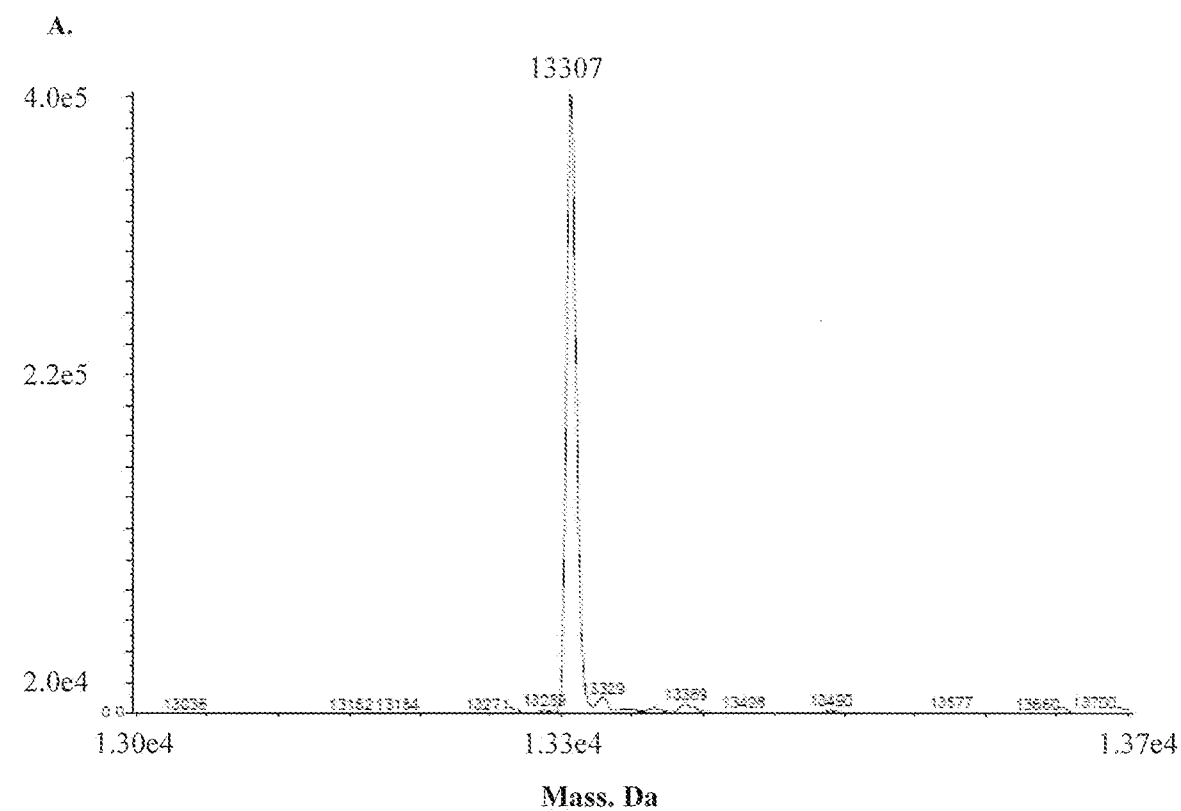
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FIG. 13



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FIG. 14



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FIG. 15

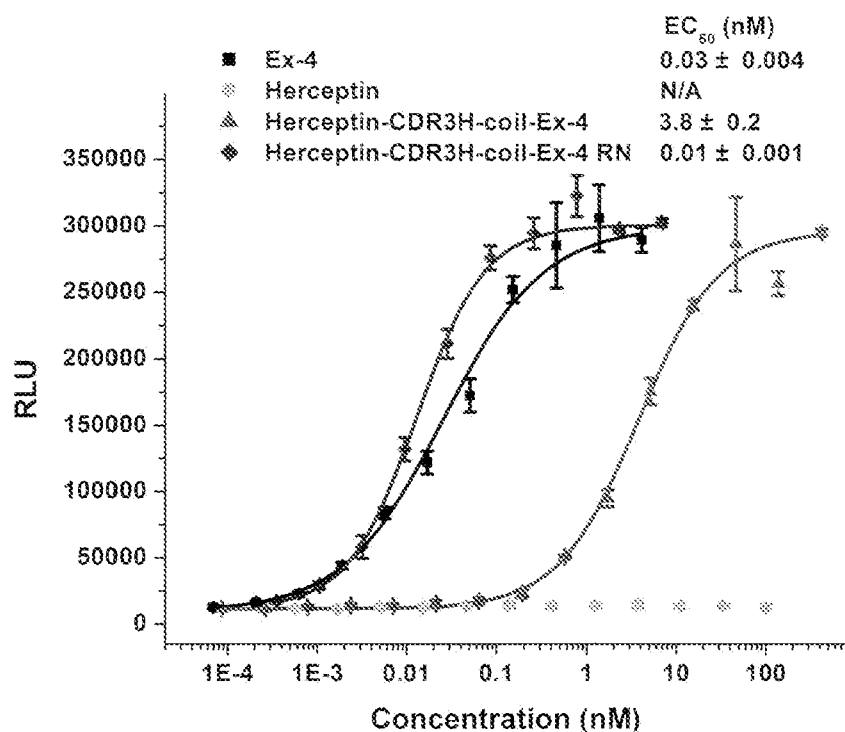
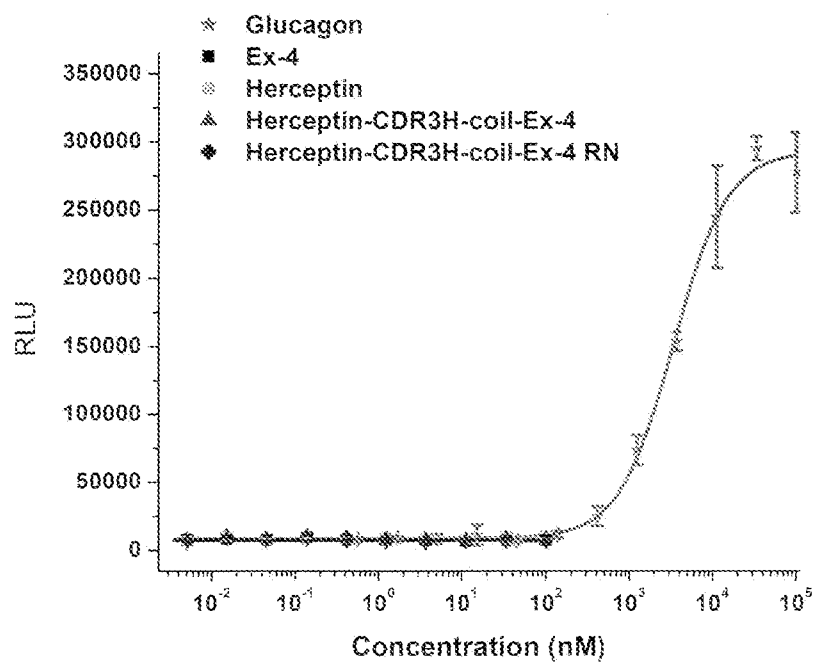
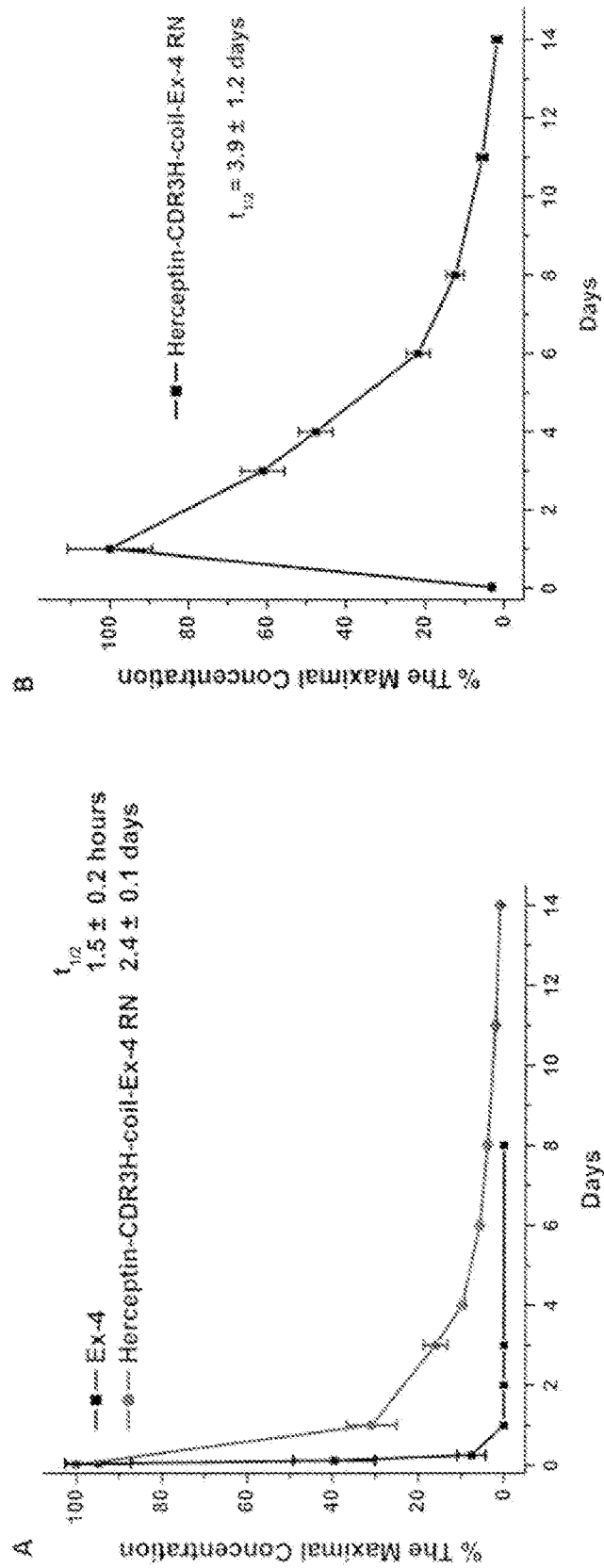


FIG. 16



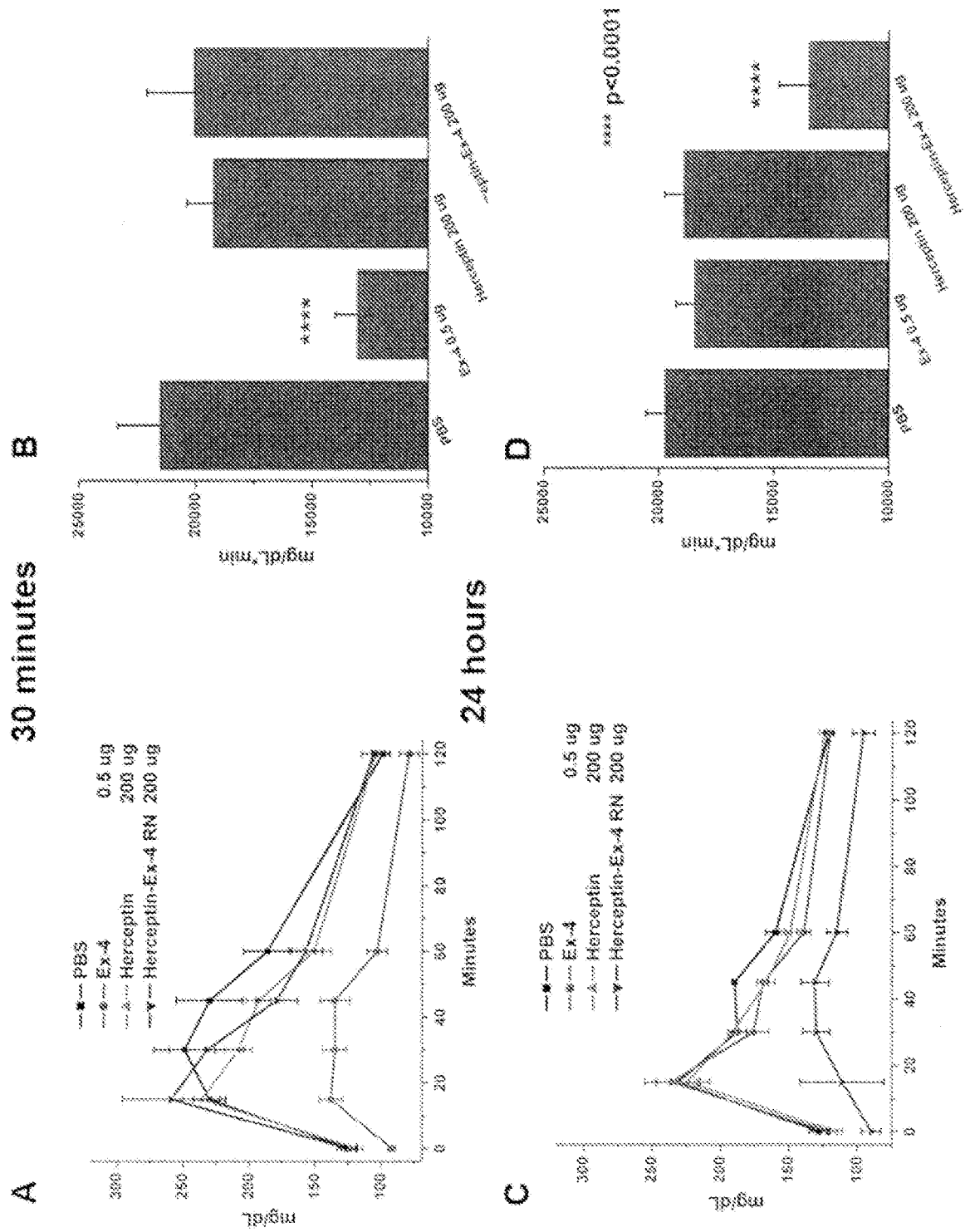
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FIG. 17



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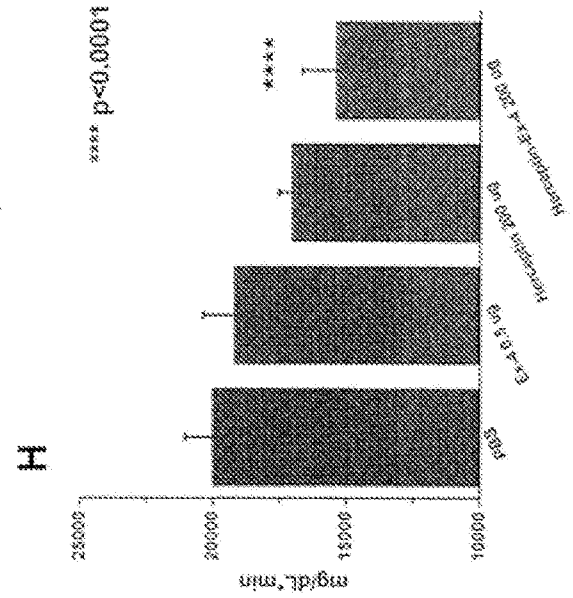
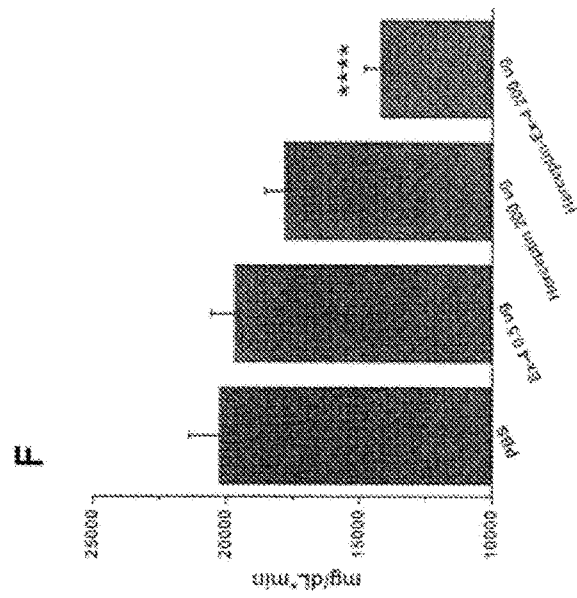
FIG. 18



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FIG. 18 Cont.

48 hours



72 hours

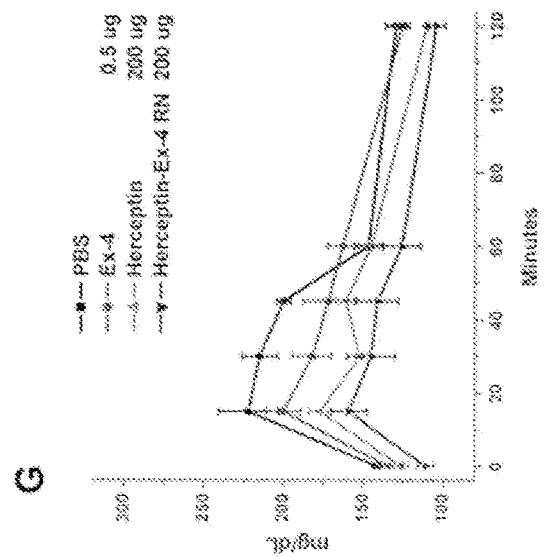
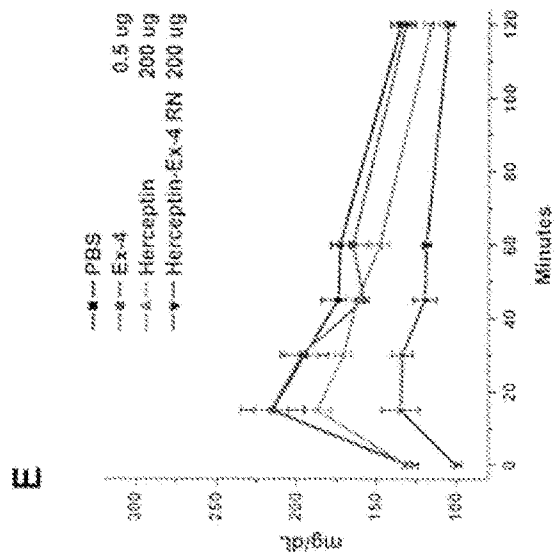
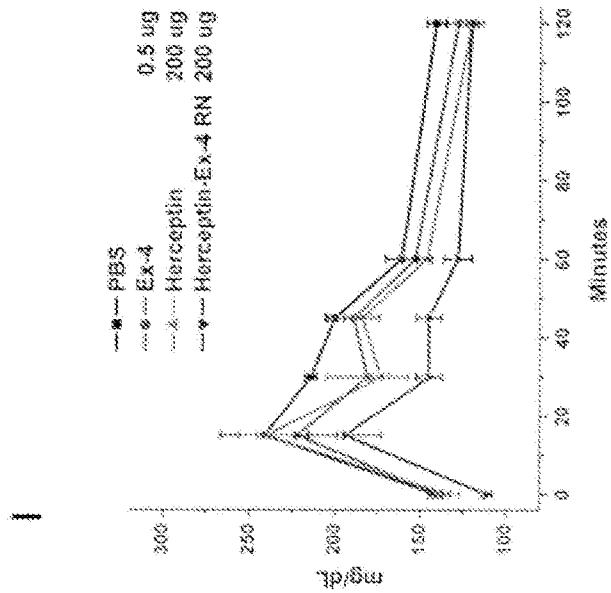
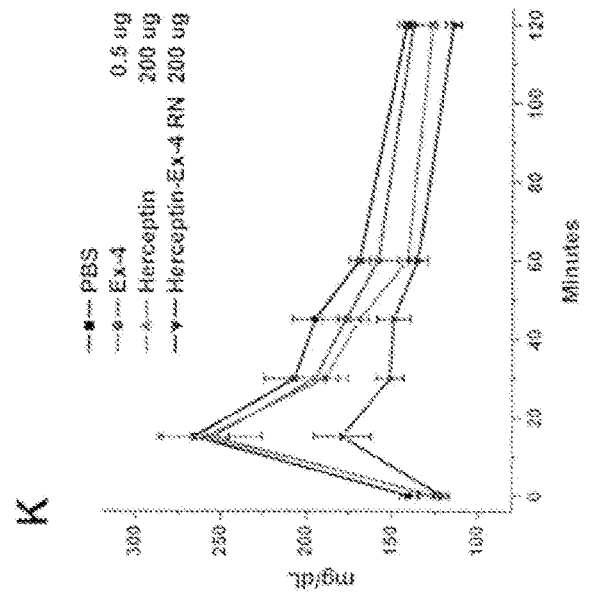
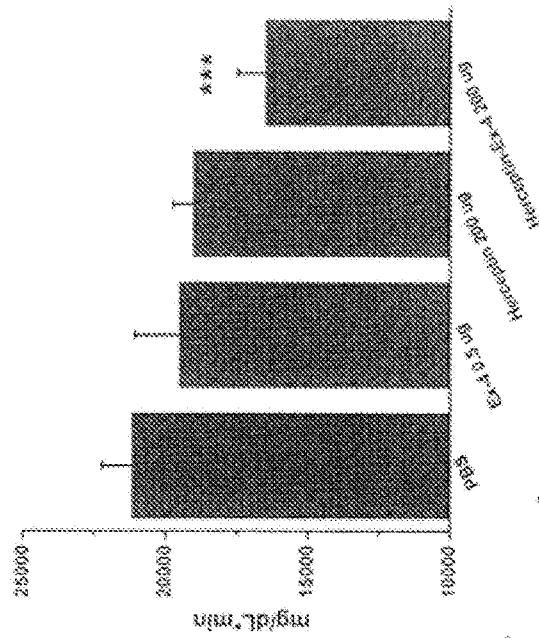
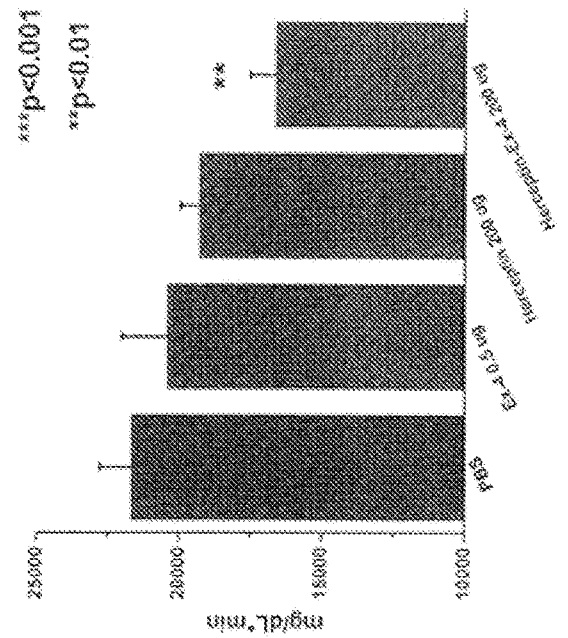


FIG. 18 Cont.

96 hours



120 hours

**J****L**

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FIG. 18 Cont.

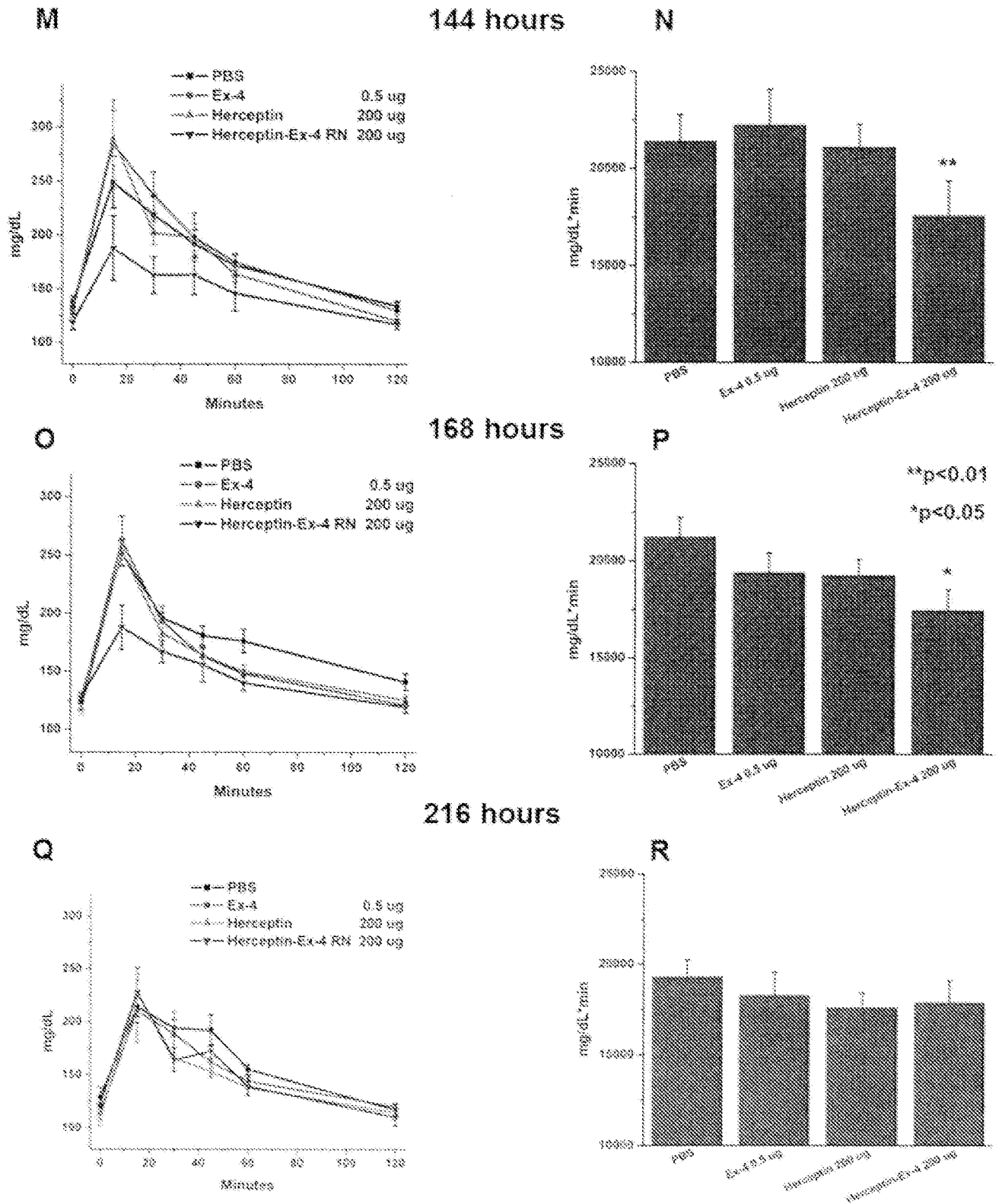
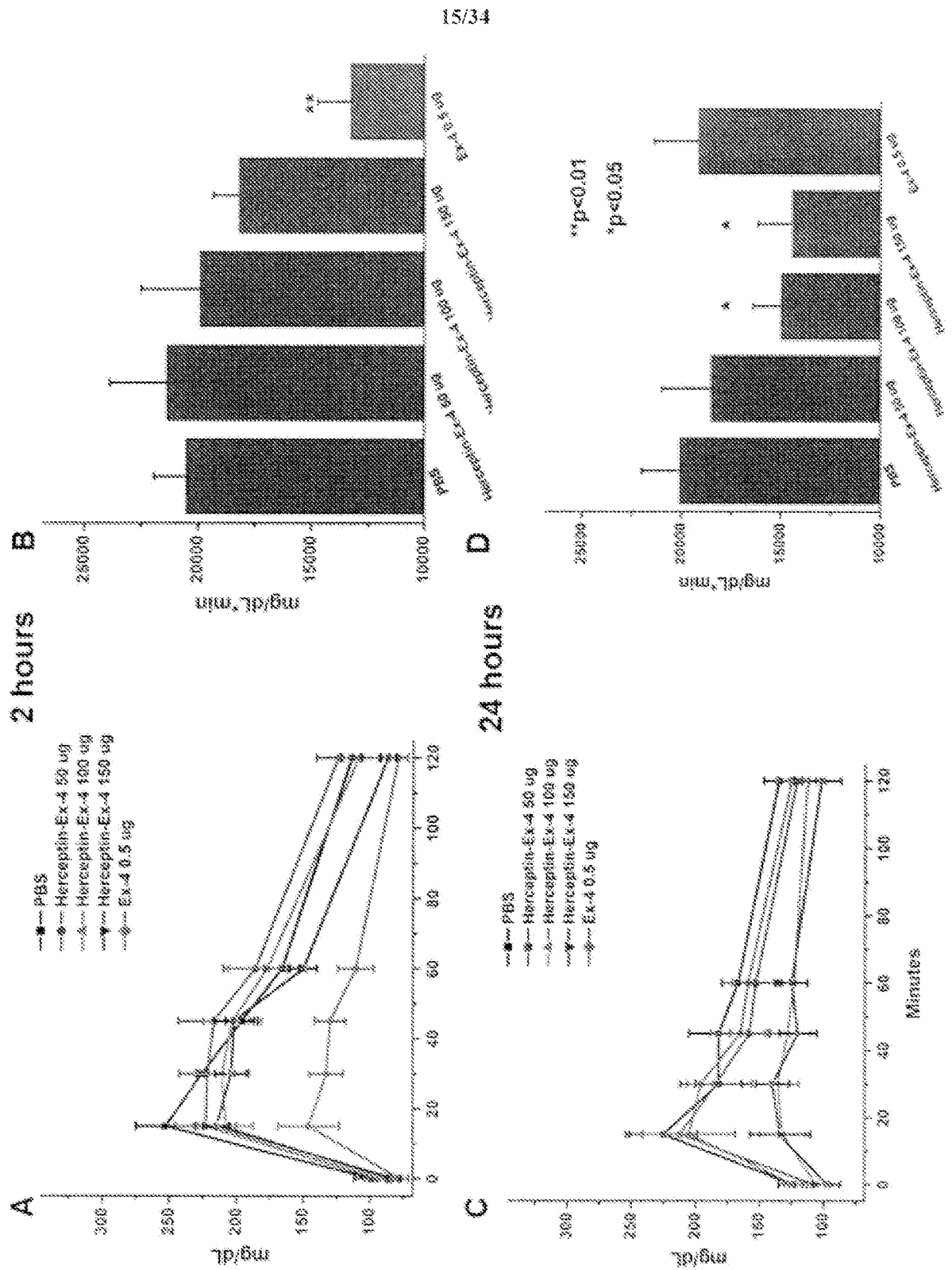


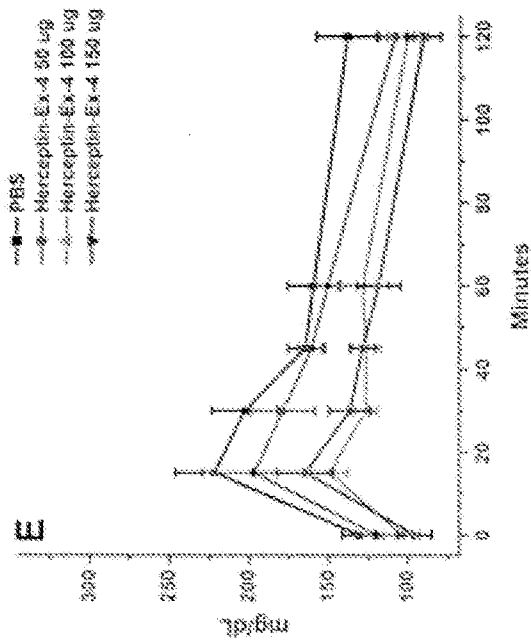
FIG. 19



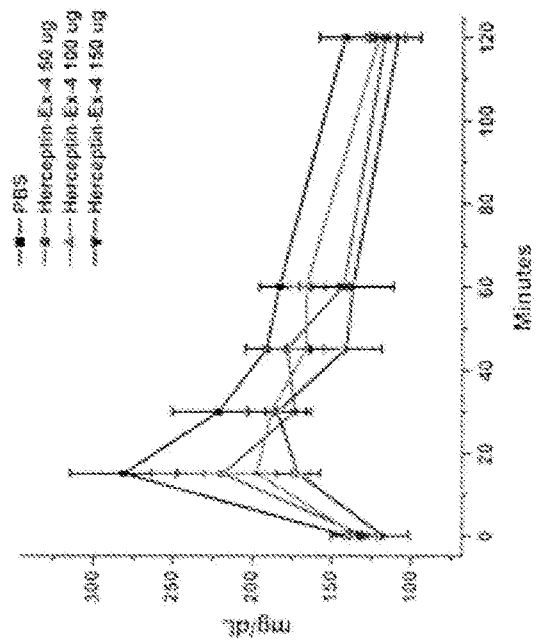
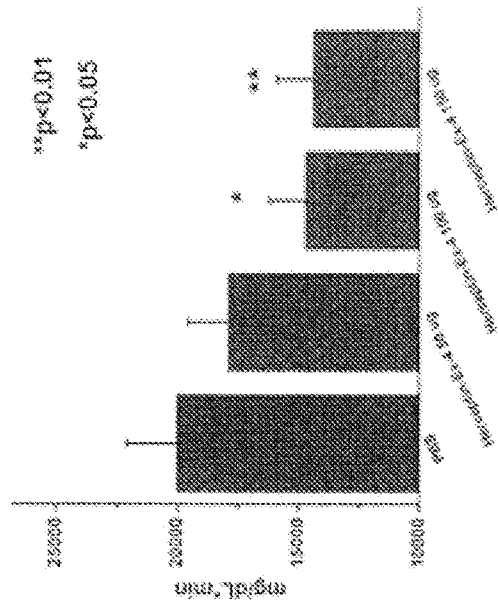
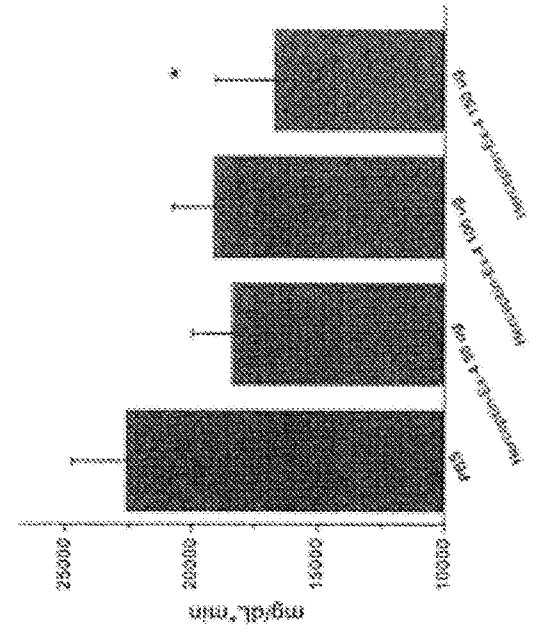
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FIG. 19 Cont.

48 hours

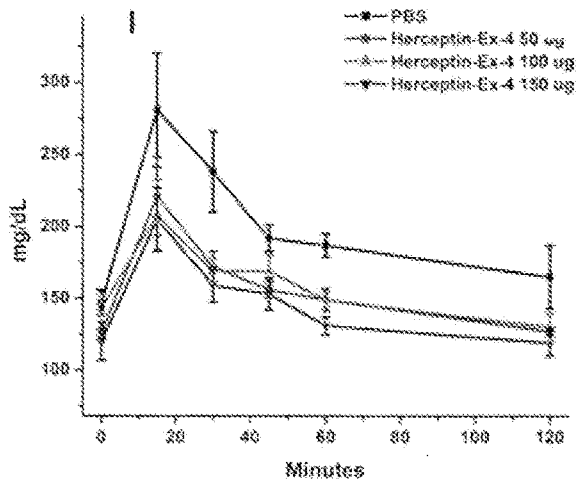


72 hours

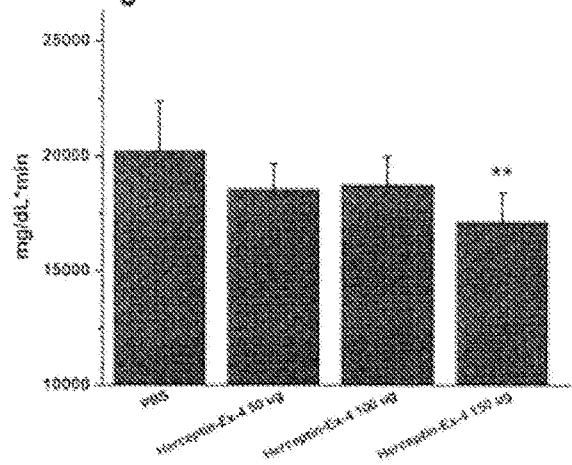
G**F****H**

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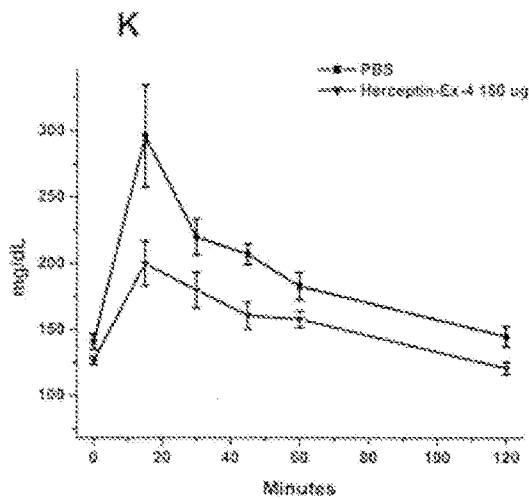
FIG. 19 Cont.



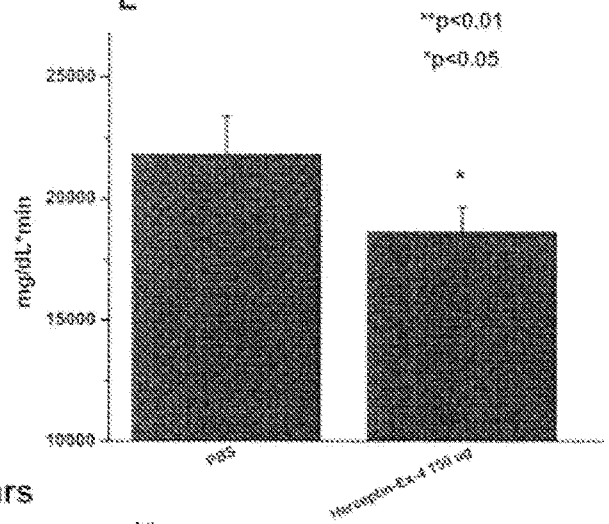
96 hours



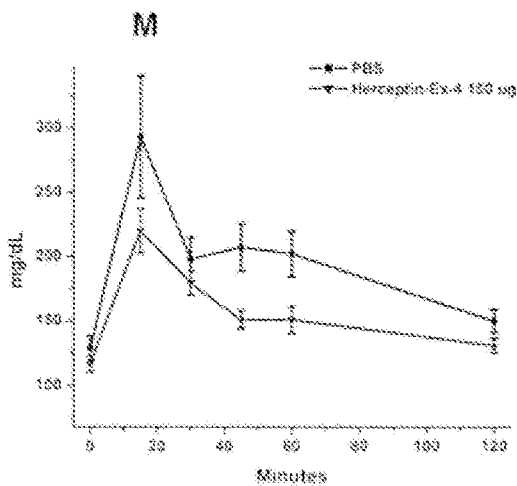
120 hours



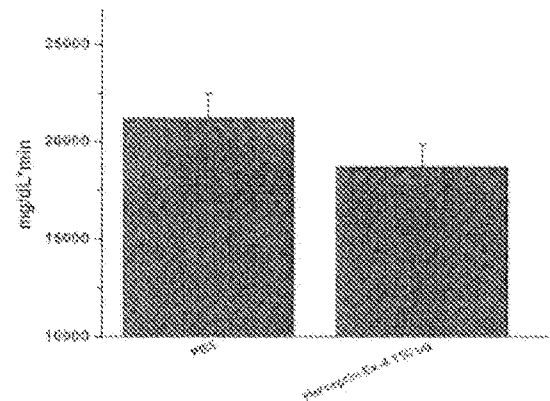
L



144 hours



N



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FIG. 20

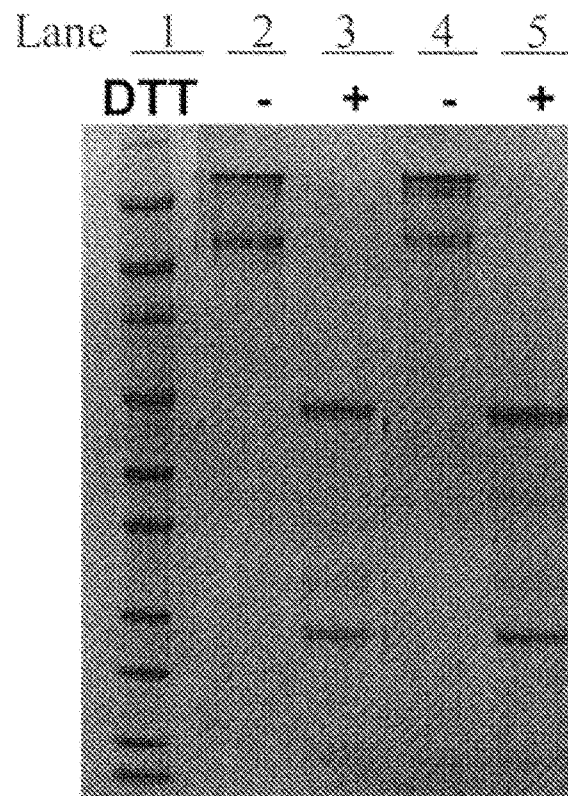
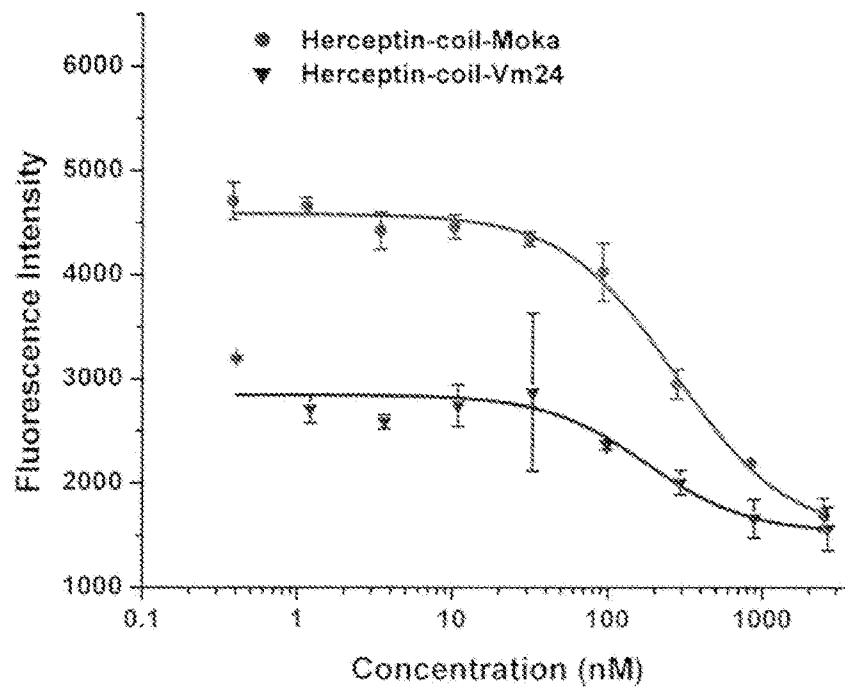
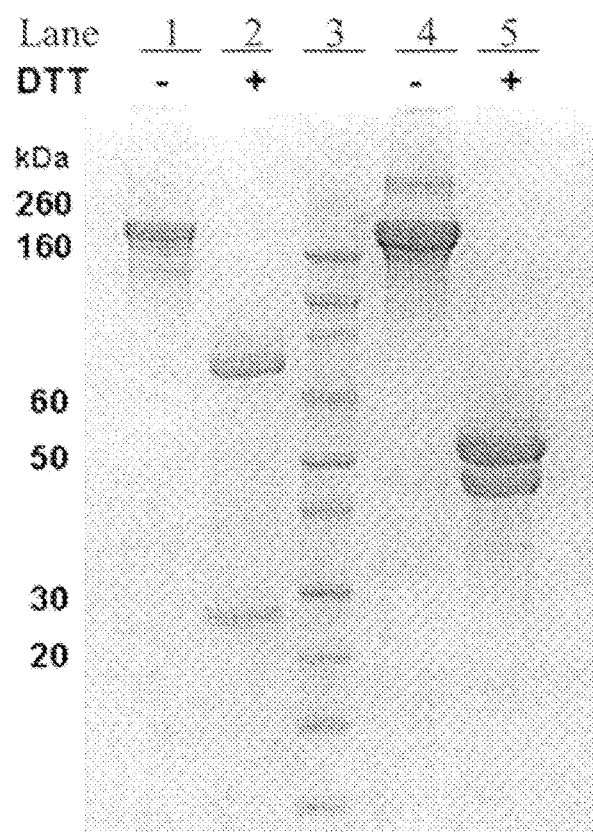


FIG. 21



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FIG. 22



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FIG. 23

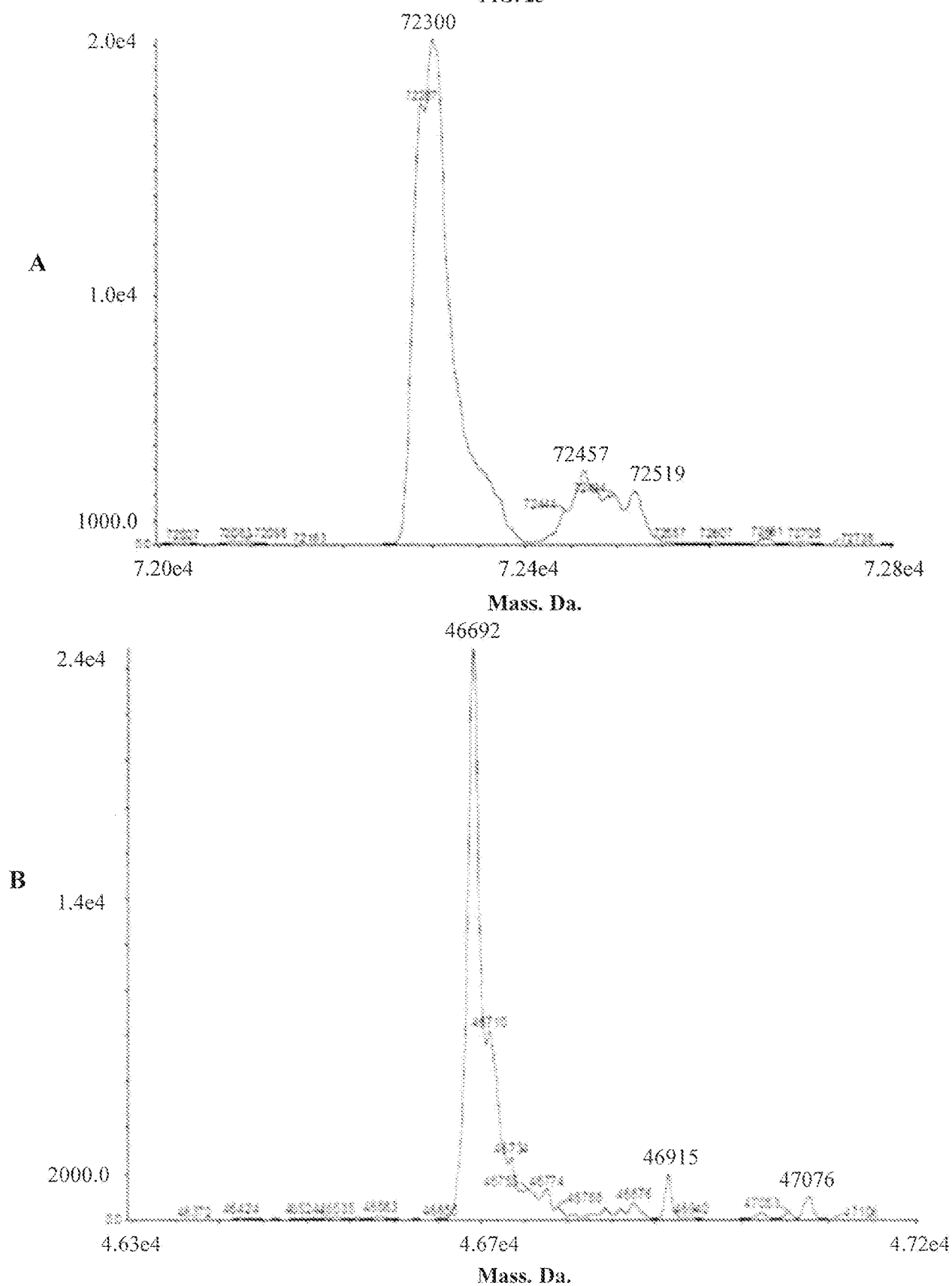
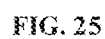


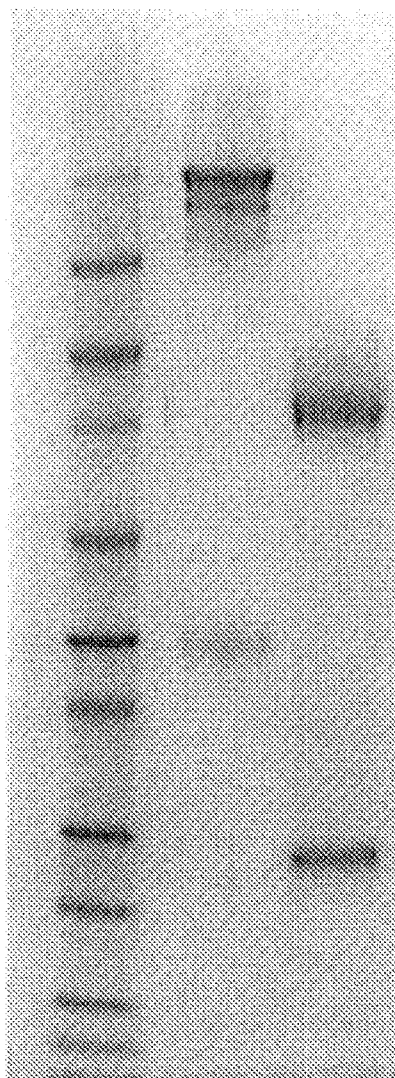
FIG. 24



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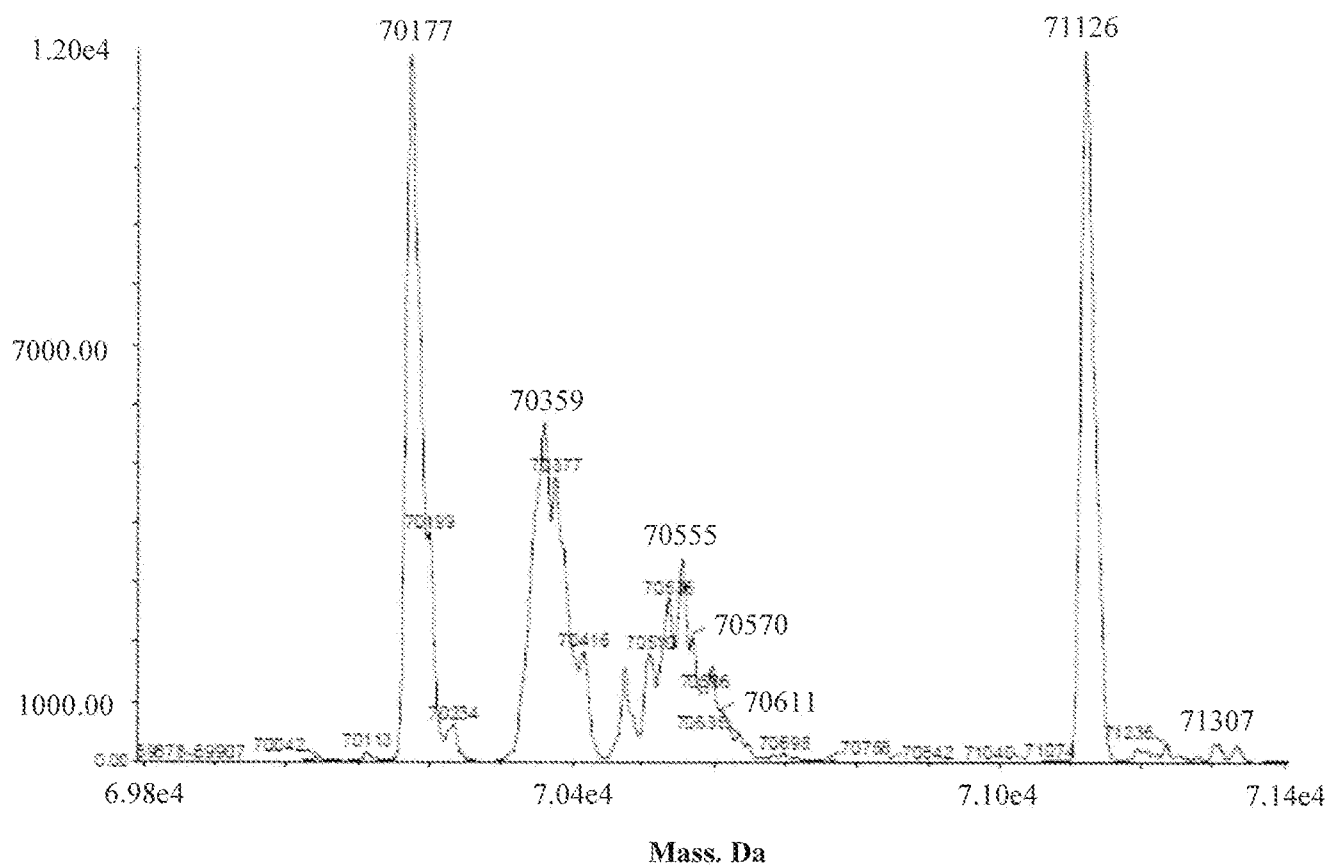
FIG. 26

Lane	<u>1</u>	<u>2</u>	<u>3</u>
DTT		-	+



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FIG. 27



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FIG. 28

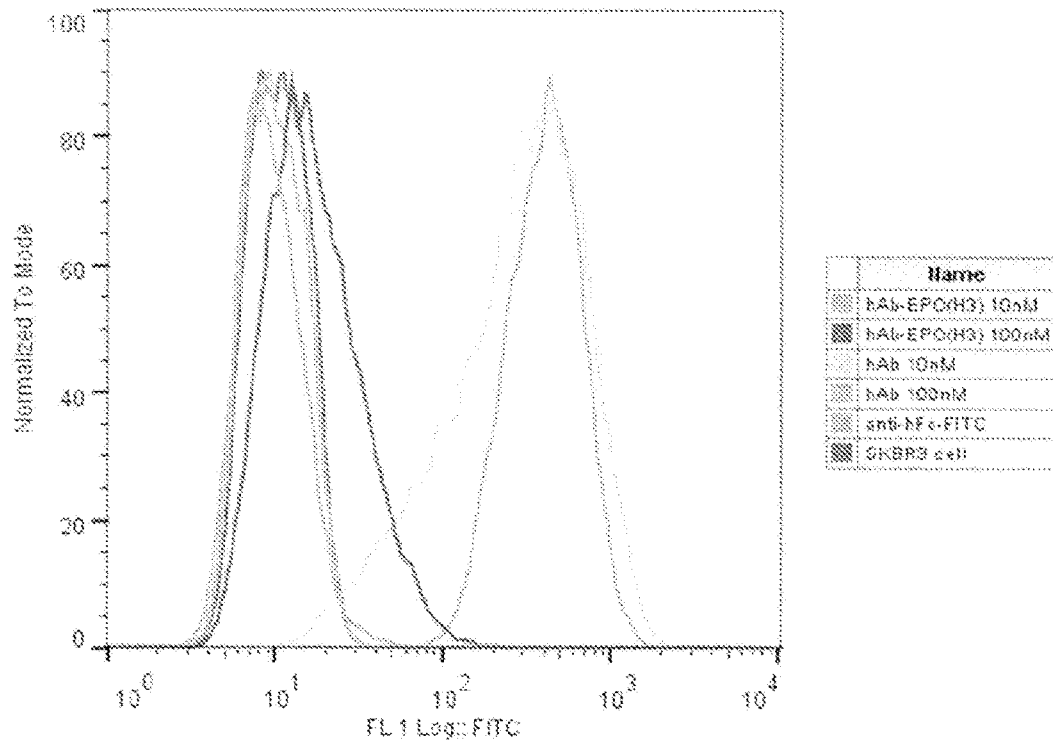
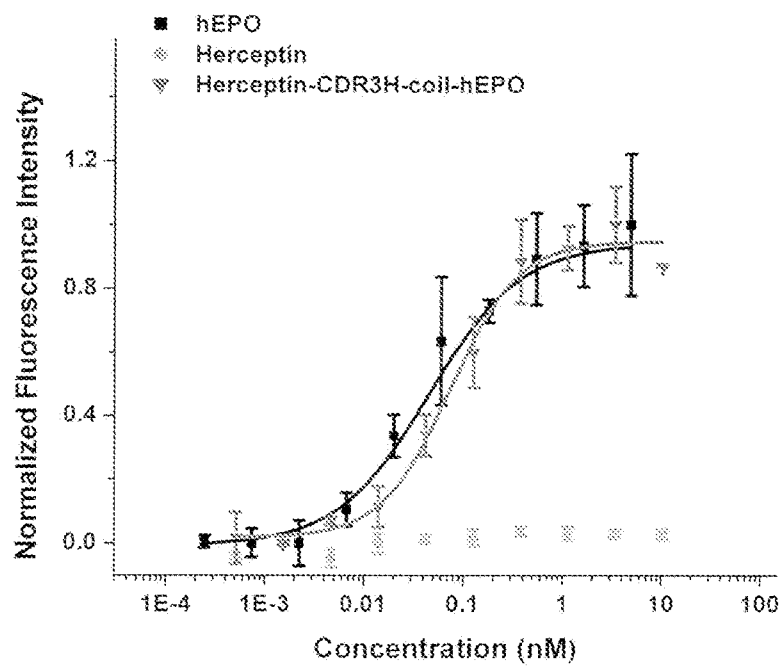
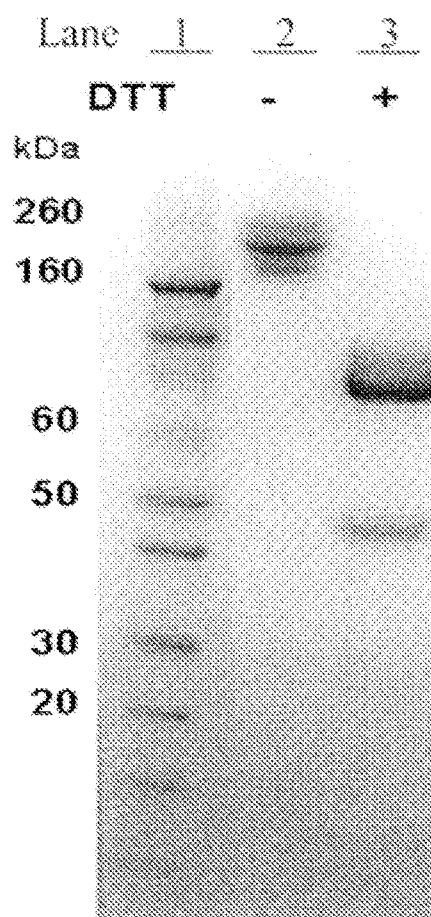


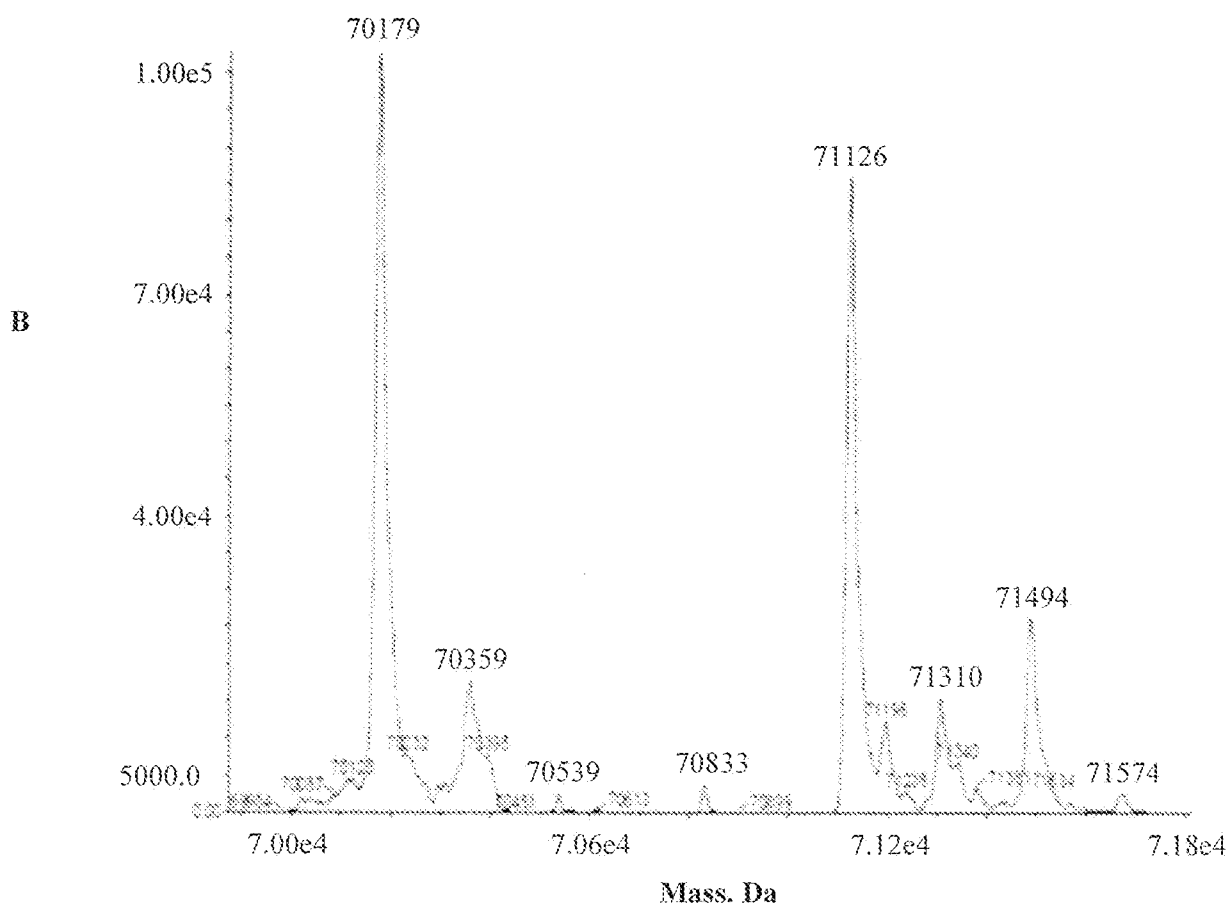
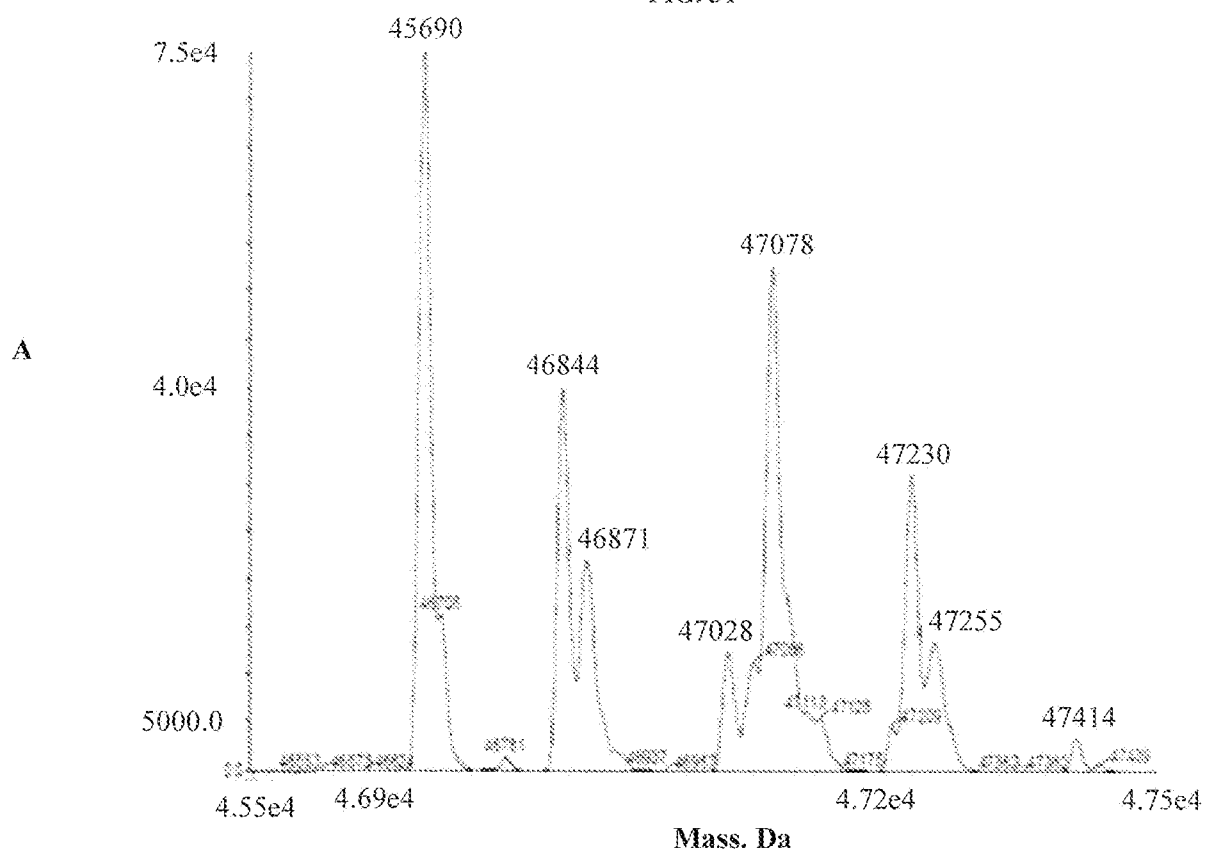
FIG. 29



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FIG. 30



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FIG. 31

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FIG. 32

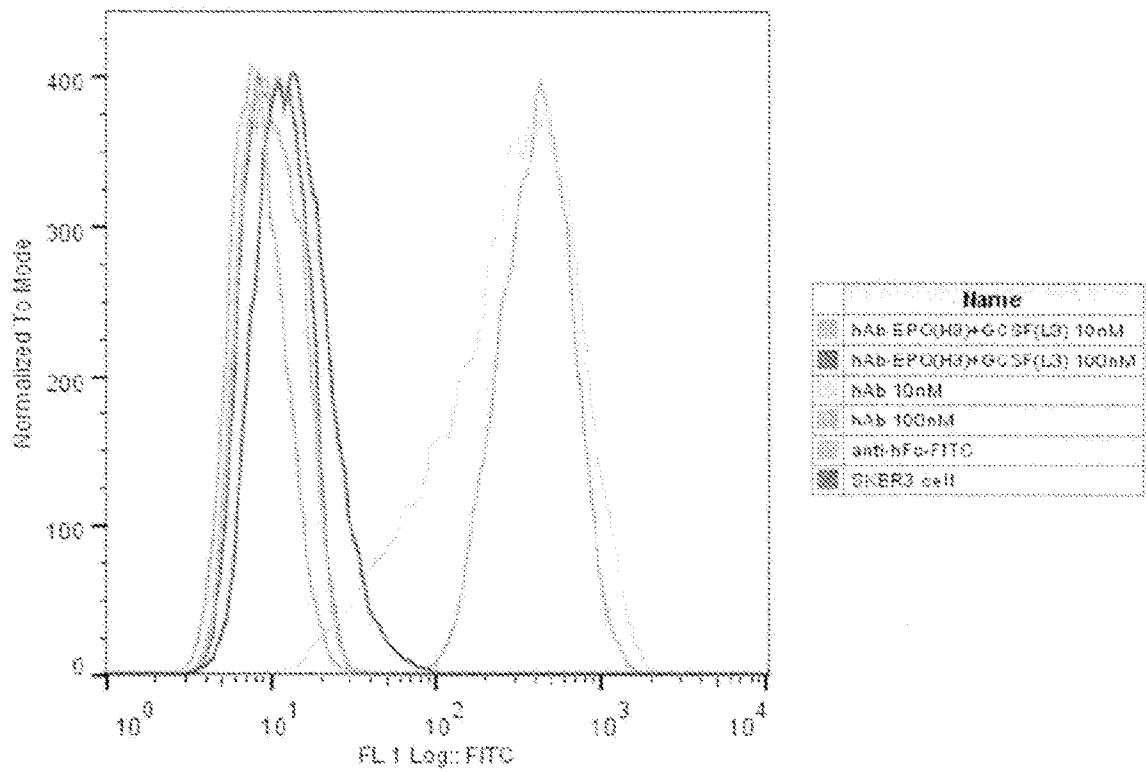
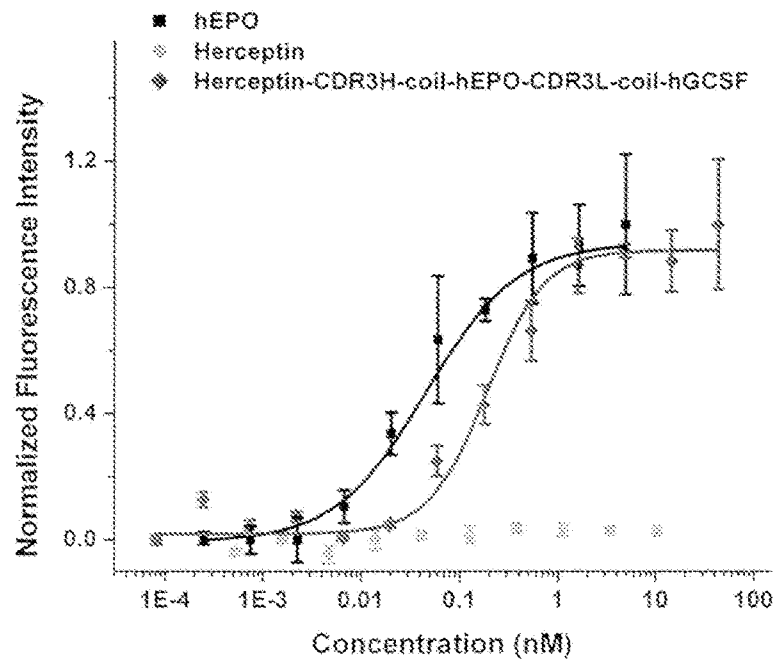


FIG. 33



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FIG. 34

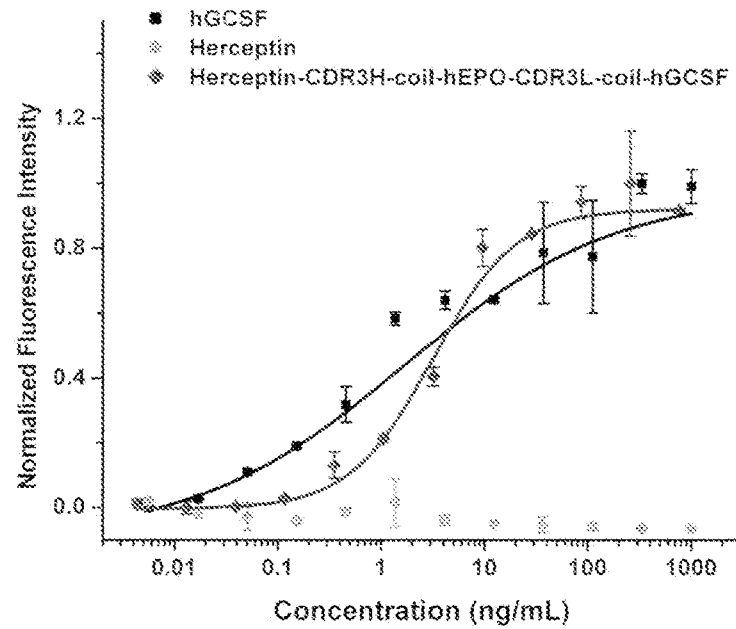
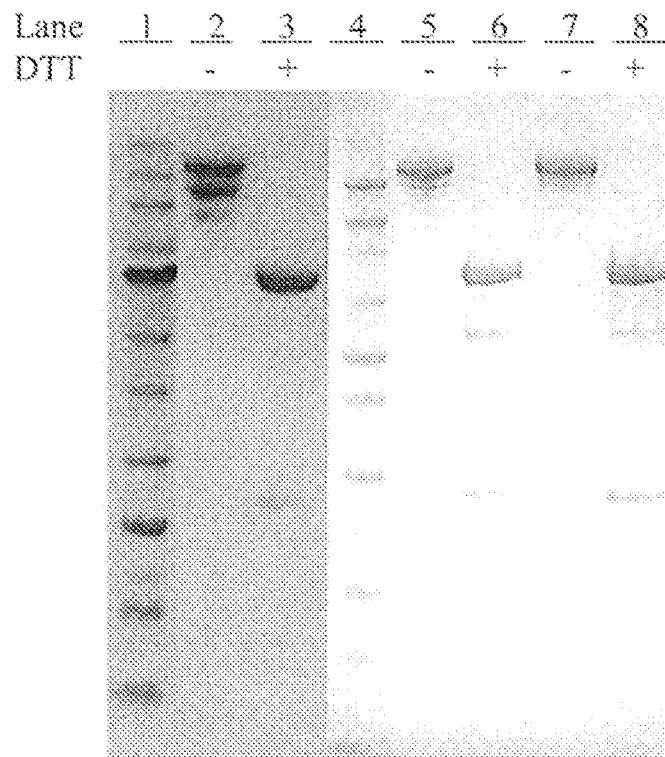


FIG. 35



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FIG. 36

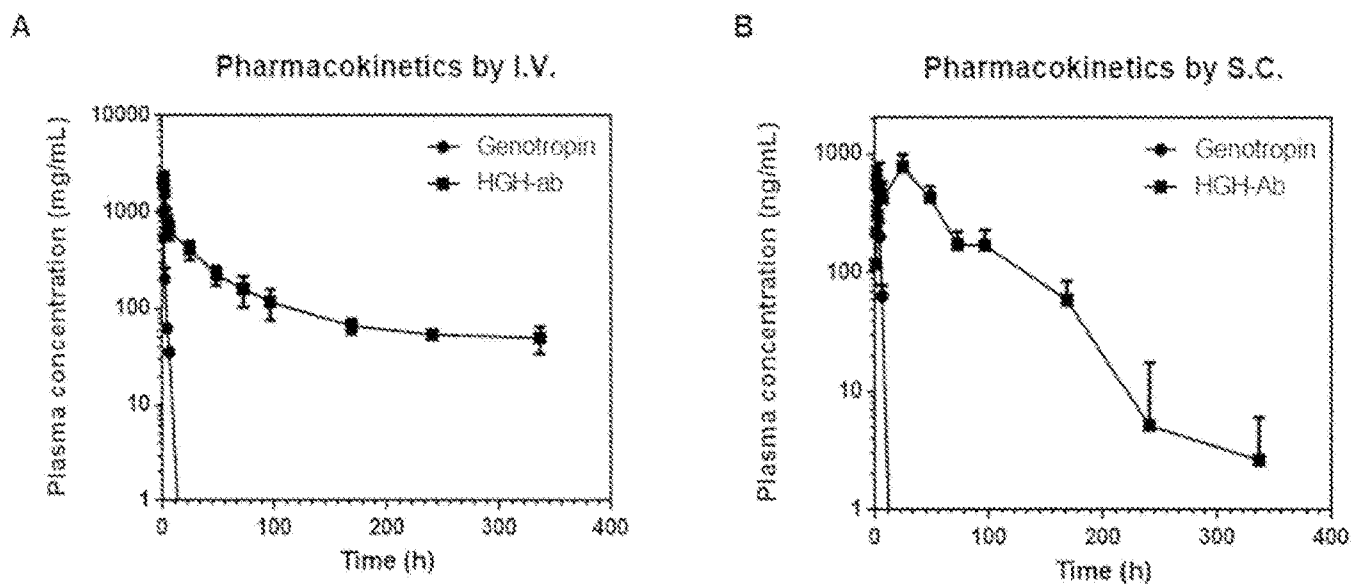
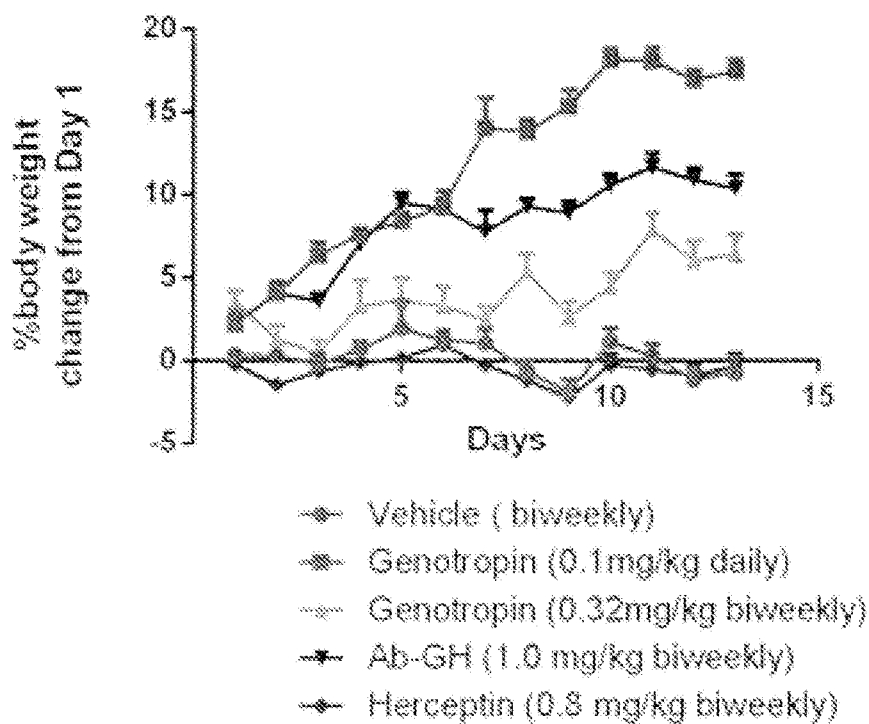


FIG. 37

% body weight change from the first day of study in hypophysectomized male rats



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FIG. 38

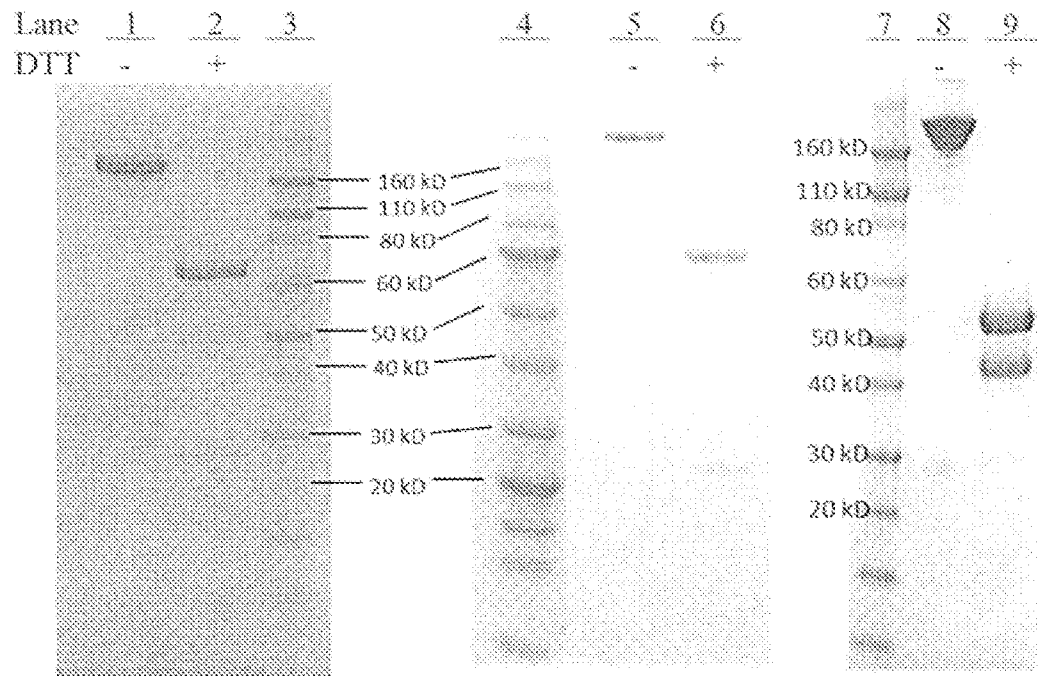
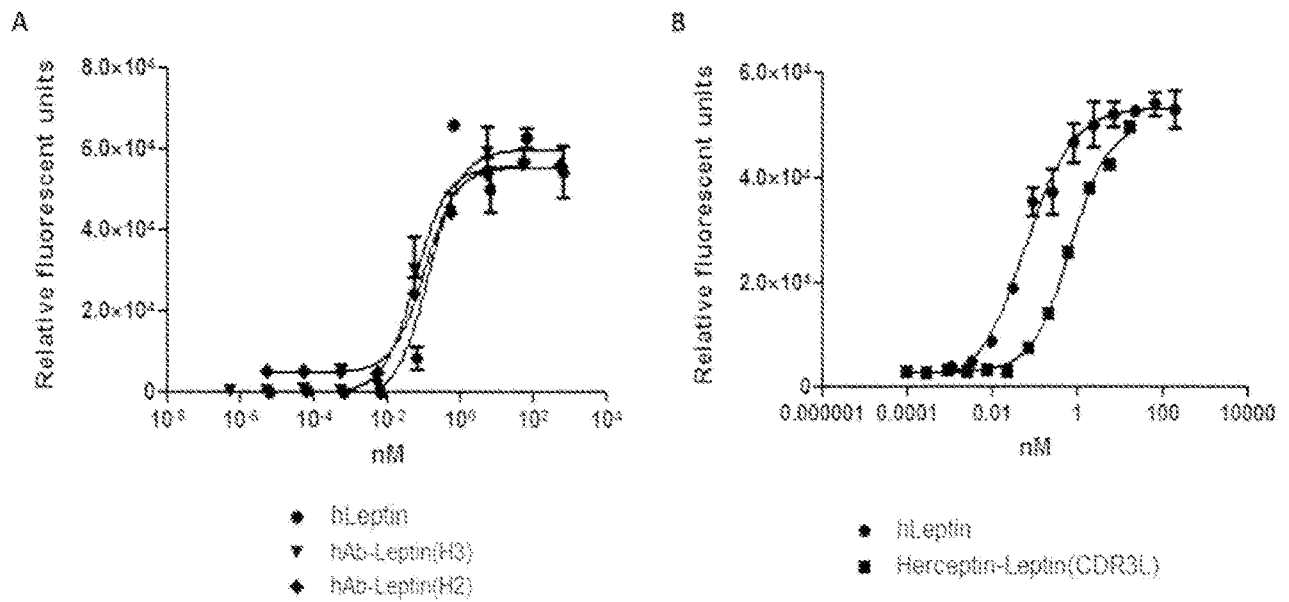
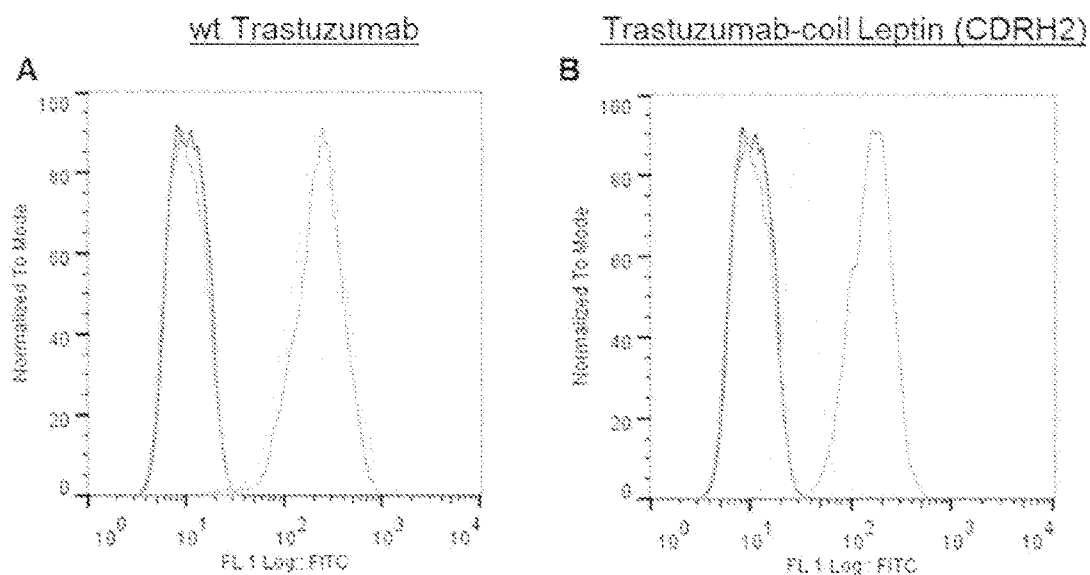


FIG. 39



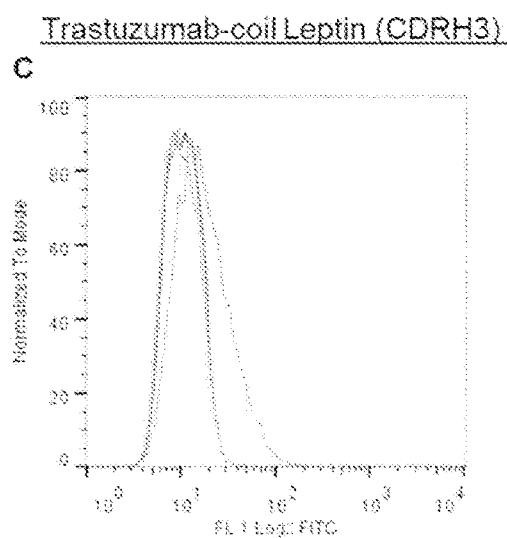
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FIG. 40



	Name
	wt Herceptin IgG 10nM
	wt Herceptin IgG 100nM
	anti-hFc-FITC
	SKBR3 cell

	Name
	Trastuzumab-coil Leptin (CDRH2) IgG 10nM
	Trastuzumab-coil Leptin (CDRH2) IgG 100nM
	Anti-hFc-FITC
	SKBR3 cell



	Name
	Trastuzumab-coil Leptin (CDRH3) IgG 10nM
	Trastuzumab-coil Leptin (CDRH3) IgG 100nM
	Anti-hFc-FITC
	SKBR3 cell

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FIG. 41

Lane	1	2	3
DTT		-	+

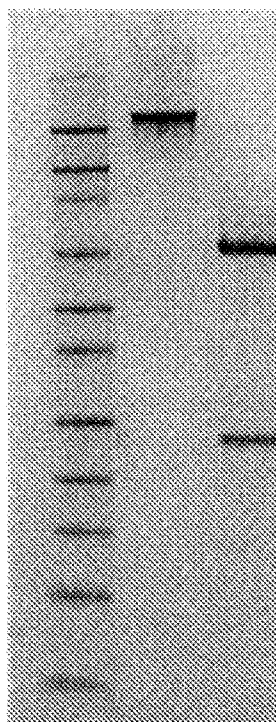
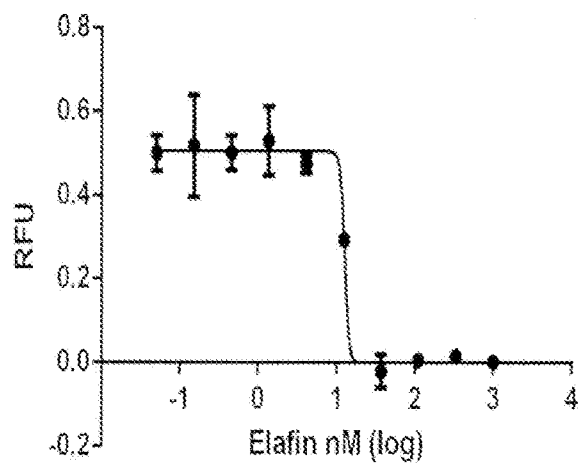


FIG. 42

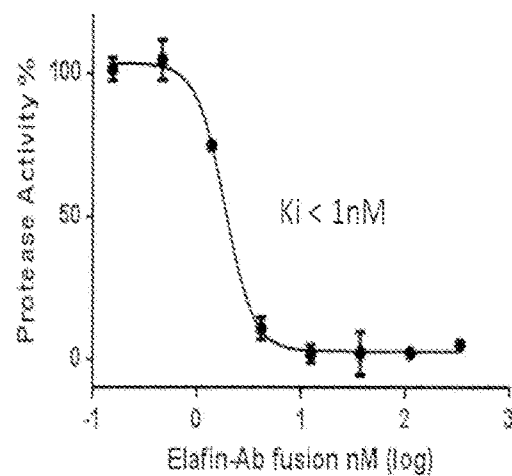
A

Elastase Inhibition



B

Human Leucocyte Elastase Inhibition



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FIG. 43

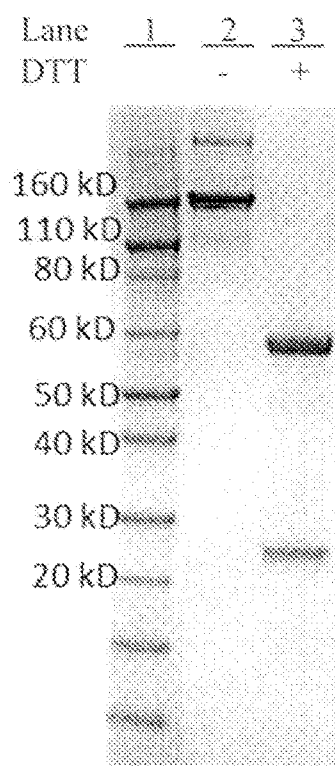
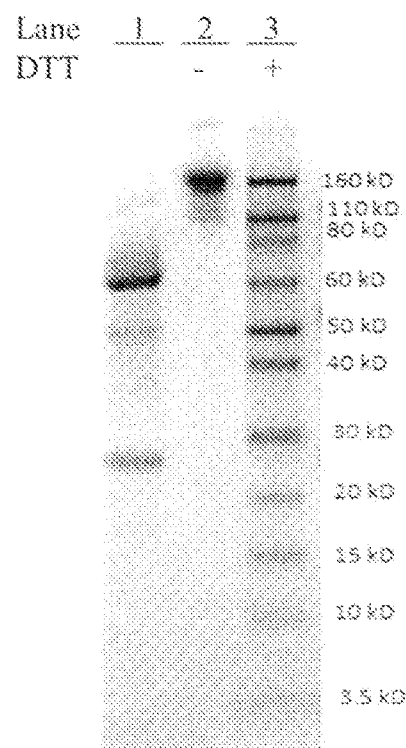


FIG. 44



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FIG. 45

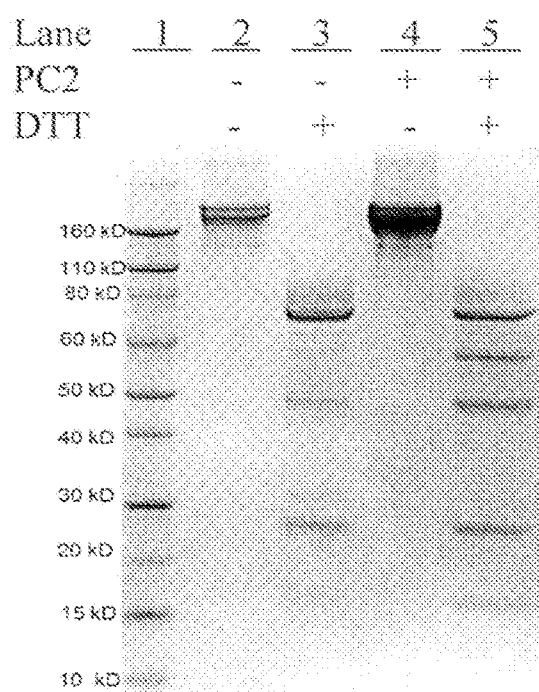
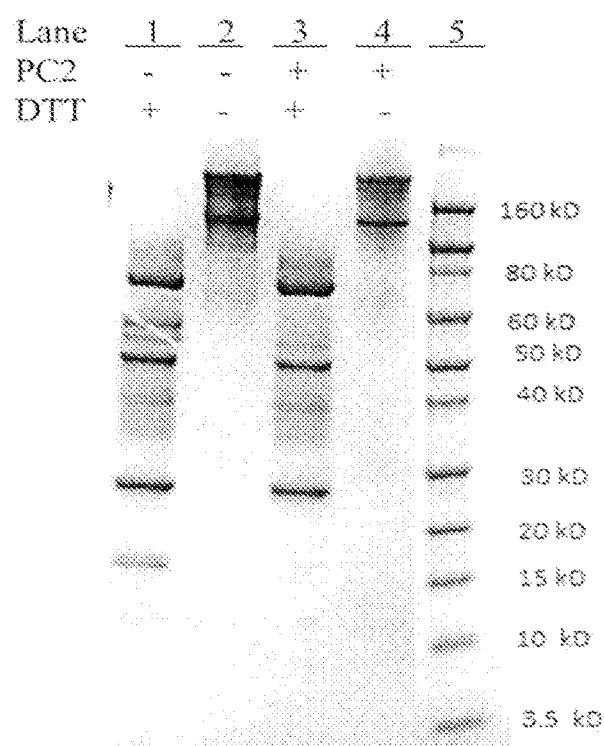
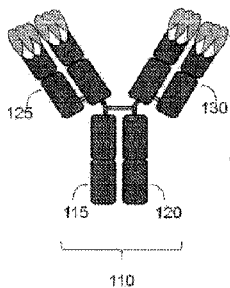


FIG. 46





+

