

COMPOUNDS AND METHODS TARGETING HUMAN AND MOUSE INSL5

The present invention relates to the field of medicine. More particularly, the present invention relates to compounds, diagnostics, and methods which include an antibody or
5 fragment thereof directed against human or mouse insulin-like peptide-5 (INSL5). The compounds and methods of the present invention are expected to be useful in the field of oncology, reproductive, and metabolic diseases, *e.g.*, diabetes and obesity, including diagnostics relating thereto.

The gut hormone INSL5 belongs to the Relaxin/Insulin-like family of peptides.
10 Like the other INSL family members, INSL5 is composed of B- and A-chains connected by two disulfide bonds. INSL5 is expressed in various tissues; however, it is primarily co-secreted with other hormones from specialized enteroendocrine cells (EECs), called L cells, in the distal intestine, particularly in the colon and rectum. These cells regulate metabolic and physiological processes such as intestinal motility, hormone secretion,
15 glucose homeostasis and appetite. INSL5 is the ligand for the receptor RXFR4/GPCR142/GPR100 and its receptor-ligand interaction results in the inhibition of intracellular cAMP levels. While INSL5 is considered an orexigenic (appetite-stimulating) hormone and its secretion is elevated by calorie restriction, the biological consequences of the INSL5-RXFP4 activated signaling pathway remain largely elusive.

20 In order to study INSL5, reliable and sensitive assays for INSL5 are needed. INSL5 has been studied in patients with metabolic dysfunction, polycystic ovarian syndrome (PCOS), colorectal cancer (CRC), and infertility, and preclinical models of these diseases. However, there remains a need to provide antibodies that bind human and mouse INSL5 to study these effects further. In particular, there remains a need for
25 INSL5 antibodies with high affinities to both human and mouse INSL5. Currently available assays have sensitivity, reproducibility, performance, and/or quantitative issues. Thus, additional methods of testing human or mouse samples are needed.

In a specific embodiment of the present disclosure, antibodies are provided which bind INSL5 comprising a heavy chain variable region (VH) and a light chain variable
30 region (VL), wherein the VH comprises heavy chain complementarity determining

regions (HCDR) HCDR1, HCDR2, and HCDR3, and the VL comprises light chain complementarity determining regions (LCDR) LCDR1, LCDR2, and LCDR3, wherein the HCDR1 comprises T ASGFSLSSYDMG (SEQ ID NO:10), the HCDR2 comprises TISAGGYTY (SEQ ID NO:11), the HCDR3 comprises

- 5 ARERWNYDRSGGAGAGYFDL (SEQ ID NO:12), the LCDR1 comprises QASQSITSSYLS (SEQ ID NO:14), the LCDR2 comprises YPAANLAS (SEQ ID NO:15), and the LCDR3 comprises LYG YFSSSIDFA (SEQ ID NO:16).

- According to some embodiments, antibodies of the present disclosure comprise a VH that comprises SEQ ID NO:9 and a VL which comprises SEQ ID NO:13. According to some
10 embodiments, the antibodies of the present disclosure comprise a heavy chain (HC) comprising SEQ ID NO:5 and a light chain (LC) comprising SEQ ID NO:7. According to some embodiments, antibodies of the present disclosure comprise a heavy chain (HC) comprising amino acids 2-446 of SEQ ID NO:5, and a light chain (LC) comprising SEQ ID NO:7. According to some embodiments, the antibodies of the present disclosure
15 comprise a HC consisting of SEQ ID NO:5 and a LC consisting of SEQ ID NO:7.

- According to some embodiments, the present disclosure provides a nucleic acid comprising a sequence encoding SEQ ID NO:5 or SEQ ID NO:7, or both. According to some embodiments, one or more vectors comprise one or more nucleic acids encoding SEQ ID NO:5 or SEQ ID NO:7, or both. According to some embodiments, the present
20 disclosure provides a composition comprising a first vector comprising a nucleic acid sequence encoding SEQ ID NO:5 and a second vector comprising a nucleic acid sequence encoding SEQ ID NO:7. According to other embodiments, the present disclosure provides a composition comprising a vector comprising a nucleic acid encoding SEQ ID NO:5 and SEQ ID NO:7. In another embodiment, the present disclosure provides a cell
25 comprising one or more vectors comprising one or more nucleic acids encoding SEQ ID NO:5 or SEQ ID NO:7, or both. According to some embodiments, the present disclosure provides a cell comprising a first vector comprising a nucleic acid sequence encoding SEQ ID NO:5 and a second vector comprising a nucleic acid sequence encoding SEQ ID NO:7. According to other embodiments, the present disclosure provides a cell
30 comprising a vector comprising a nucleic acid encoding SEQ ID NO:5 and a second

nucleic acid sequence encoding SEQ ID NO:7. In further embodiments, the cell is a mammalian cell.

In a specific embodiment of the present disclosure, antibodies are provided which bind INSL5 comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises heavy chain complementarity determining regions (HCDR) HCDR1, HCDR2, and HCDR3, and the VL comprises light chain complementarity determining regions (LCDR) LCDR1, LCDR2, and LCDR3, wherein the HCDR1 comprises TVSGIDLTYYAMG (SEQ ID NO:22), the HCDR2 comprises IIGGGGRITY (SEQ ID NO:23), the HCDR3 comprises VRGGDFFDL (SEQ ID NO:24), the LCDR1 comprises QASEDISKYLS (SEQ ID NO:26), the LCDR2 comprises YYVSNLEF (SEQ ID NO:27), and the LCDR3 comprises HQGYTGNNVENV (SEQ ID NO:28).

According to some embodiments, antibodies of the present disclosure comprise a VH that comprises SEQ ID NO:21 and a VL that comprises SEQ ID NO:25. In a further embodiment, the antibodies of the present disclosure comprise a heavy chain (HC) comprising SEQ ID NO:17 and a light chain (LC) comprising SEQ ID NO:19. In some embodiments, antibodies of the present disclosure comprise a heavy chain (HC) comprising amino acids 2-435 of SEQ ID NO:17, and a light chain (LC) comprising SEQ ID NO:19. According to some embodiments, antibodies of the present disclosure comprise a HC consisting of SEQ ID NO:17 and a LC consisting of SEQ ID NO:19.

According to some embodiments, the present disclosure provides a nucleic acid comprising a sequence encoding SEQ ID NO:17 or SEQ ID NO:19, or both. According to some embodiments, one or more vectors comprise one or more nucleic acids encoding SEQ ID NO:17 or SEQ ID NO:19, or both. According to some embodiments, the present disclosure provides a composition comprising a first vector comprising a nucleic acid sequence encoding SEQ ID NO:17 and a second vector comprising a nucleic acid sequence encoding SEQ ID NO:19. According to some embodiments, the present disclosure provides a composition comprising a vector comprising a nucleic acid encoding SEQ ID NO:17 and SEQ ID NO:19. In another embodiment, the present disclosure provides a cell comprising one or more vectors comprising one or more nucleic acids encoding SEQ ID NO:17 or SEQ ID NO:19, or both. According to some

embodiments, the present disclosure provides a cell comprising a first vector comprising a nucleic acid sequence encoding SEQ ID NO:17 and a second vector comprising a nucleic acid sequence encoding SEQ ID NO:19. According to other embodiments, the present disclosure provides a cell comprising a vector comprising a nucleic acid encoding
5 SEQ ID NO:17 and a second nucleic acid sequence encoding SEQ ID NO:19. In further embodiments, the cell is a mammalian cell.

According to some embodiments, antibodies of the present disclosure bind to human INSL5. According to some embodiments, antibodies of the present disclosure bind to the A chain (SEQ ID NO:1) and the B chain (SEQ ID NO:2) of human INSL5.
10 According to some embodiments, antibodies of the present disclosure bind to mouse INSL5. According to some embodiments, antibodies of the present disclosure bind to the A chain (SEQ ID NO:3) and the B chain (SEQ ID NO:4) of mouse INSL5.

According to some embodiments, the present invention provides a process of producing an antibody comprising culturing the cell under conditions such that the
15 antibody is expressed and recovering the expressed antibody from the culture medium. According to some embodiments, the present invention provides a process of producing an antibody produced by culturing the cell under conditions such that the antibody is expressed and recovering the expressed antibody from the culture medium.

According to further embodiments of the present disclosure, a method of detecting
20 human or mouse INSL5 in a sample is provided. Such methods comprise the steps of contacting the sample with an antibody of the present disclosure that specifically binds human or mouse INSL5 and detecting a signal by said step of contacting.

According to some embodiments, a method of quantifying human or mouse INSL5 in a sample is provided. Such methods comprise the steps of contacting said
25 sample with an antibody of the present disclosure that specifically binds INSL5 and detecting a signal by said step of contacting. In some embodiments, such methods further comprise the steps of contacting a control standard with the antibody and detecting a signal provided by said step of contacting the control standard.

According to some embodiments of the methods of the present disclosure, such
30 methods further comprise the step of quantifying INSL5 in the sample. In such

embodiments, the step of quantifying INSL5 comprises quantifying INSL5 in the sample when compared to a reference standard.

According to some embodiments of the present disclosure, the sample is one of blood, plasma, serum, or cerebrospinal fluid (CSF).

5 According to some embodiments of the methods of the present disclosure, the methods further comprise the step of contacting the sample with an antibody that specifically binds human or mouse INSL5 and a second antibody, said second antibody also binds human or mouse INSL5. In some such methods, one of the antibody or the second antibody comprises a detectable label and said step of detecting comprises
10 detecting a signal provided by the detectable label upon formation of a complex comprising the antibody, the second antibody, and human or mouse INSL5. According to some such embodiments, one of the antibody and the second antibody are immobilized on a substrate. In some embodiments of the methods of the present disclosure, the steps of contacting the sample with the antibody and contacting the sample with the second
15 antibody happen simultaneously. According to some more specific embodiments, the second antibody comprises an antibody of the present disclosure that specifically binds human or mouse INSL5 as disclosed herein.

 According to some embodiments of the methods of the present disclosure, the methods further comprise the step of contacting the sample with an antibody that
20 specifically binds INSL5 and a second antibody, and said second antibody specifically also binds INSL5. In some such methods, one of the antibody or the second antibody comprises a detectable label and said step of detecting comprises detecting a signal provided by the detectable label upon formation of a complex comprising the antibody, the second antibody and INSL5. According to some such embodiments, one of the
25 antibody and the second antibody are immobilized on a substrate. In other embodiments, the second antibody binds a different epitope of INSL5. In some embodiments of the methods of the present disclosure, the steps of contacting the sample with the antibody and contacting the patient sample with the second antibody occurs simultaneously. In any such embodiments, the antibody or the second antibody, or both, is an antibody that binds
30 INSL5 as disclosed herein.

The term “antibody,” as used herein, refers to an immunoglobulin molecule that binds an antigen. Embodiments of an antibody include a monoclonal antibody, polyclonal antibody, human antibody, humanized antibody, or chimeric antibody. The antibodies can be of any class (e.g., IgG, IgE, IgM, IgD, IgA), and any subclass (e.g., IgG1, IgG2, IgG3, IgG4).

An exemplary antibody of the present disclosure is an immunoglobulin G (IgG) type antibody comprised of four polypeptide chains: two heavy chains (HC) and two light chains (LC) that are cross-linked via inter-chain disulfide bonds. The amino-terminal portion of each of the four polypeptide chains includes a variable region of about 100-125 or more amino acids primarily responsible for antigen recognition. The carboxyl-terminal portion of each of the four polypeptide chains contains a constant region primarily responsible for effector function. Each heavy chain is comprised of a heavy chain variable region (BH) and a heavy chain constant region. Each light chain is comprised of a light chain variable region (VL) and a light chain constant region. The IgG isotype may be further divided into subclasses (e.g., IgG1, IgG2, IgG3, and IgG4).

The VH and VL regions can be further subdivided into regions of hyper-variability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). The CDRs are exposed on the surface of the protein and are important regions of the antibody for antigen binding specificity. Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Herein, the three CDRs of the heavy chain are referred to as “HCDR1, HCDR2, and HCDR3” and the three CDRs of the light chain are referred to as “LCDR1, LCDR2 and LCDR3.” The CDRs contain most of the residues that form specific interactions with the antigen. Assignment of amino acid residues to the CDRs may be done according to the well-known schemes, including those described in Kabat (Kabat et. al, “Sequences of Proteins of Immunological Interest,” National Institutes of Health, Bethesda, Md. (1991)), Chothia (Chothia et. al., “Canonical structures for the hypervariable regions of immunoglobulins”, Journal of Molecular Biology, 196, 901-917 (1987); Al-Lazikani et. al., “Standard Conformations for the canonical structures of immunoglobulins”, Journal of Molecular Biology, 273, 927-948 (1997)), North (North et.

al, "A New Clustering of Antibody CDR Loop Conformations", Journal of Molecular Biology, 406, 228-256 (2011)), or IMGT (the international ImMunoGeneTics database available at www.imgt.org; see Lefranc et. al., Nucleic Acids Res. 1999; 27:209-212). Assignment of amino acids to CDR domains within the LCVR and HCVR regions of the antibodies of the present invention is based on North. LCs, according to some embodiments of the present disclosure, are classified as kappa or lambda and are each characterized by a particular constant region as known in the art. HCs, according to some embodiments of the present disclosure, are classified as gamma, mu, alpha, delta, or epsilon, and define the isotype of an antibody as IgG, IgM, IgA, IgD, or IgE, respectively.

According to some embodiments, the antibodies include IgG HCs, which can be further divided into subclasses, e.g., IgG1, IgG2, IgG3, IgG4. The carboxy-terminal portion of each HC defines a constant region primarily responsible for effector function. In a particular embodiment, the antibodies of the present invention have one or more modifications in the constant region of each HC that reduces effector function.

The antibodies of the present invention are monoclonal antibodies. Monoclonal antibodies are antibodies derived from a single copy or clone including, for example, any eukaryotic, prokaryotic or phage clone, and not the method by which it is produced. Monoclonal antibodies can be produced, for example, by hybridoma technologies, recombinant technologies, phage display technologies, synthetic technologies, e.g., CDR-grafting, or combinations of such or other technologies known in the art.

Methods of producing and purifying antibodies are well known in the art and can be found, for example, in Harlow and Lane (1988), *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring harbor, N.Y., chapters 5-8 and 15, ISBN 0-87969-314-2. For example, mice or rabbits may be immunized with human or mouse INSL5 and the resulting antibodies can be recovered, purified, and the amino acid sequences determined using conventional methods well known in the art. Likewise, a phage library may be screened, whereby thousands of Fab fragments are screened for interaction with human or mouse INSL5 and resulting interactions can be recovered, purified, and the amino acid sequences determined using conventional methods well known in the art, whereby initial lead antibodies can be constructed.

In particular embodiments of the present invention, the antibody, or the nucleic acid encoding same, is provided in isolated form. As used herein, the term “isolated” refers to a protein, peptide, or nucleic acid that is free or substantially free from other macromolecular species found in a cellular environment.

5 The term “bind” and “binds” as used herein are intended to mean, unless indicated otherwise, the ability of a protein or molecule to form a chemical bond or attractive interaction with another protein or molecule, which results in proximity of the two proteins or molecules as determined by common methods known in the art.

 Anti-INS_L5 antibodies of the present disclosure that bind to human or mouse
10 INS_L5 can be used to isolate and/or detect isoforms of human or murine INS_L5 by techniques such as affinity chromatography, immunoprecipitation, immunohistochemistry or ELISA-based assay. Such assay can be used to detect and/or evaluate the abundance and/or patterns of INS_L5 for diagnostic, prognostic, or theranostic purposes to monitor polypeptide levels, for example in serum, plasma, blood or CSF as part of a clinical
15 testing procedure, e.g., to determine the efficacy of a given treatment regimen. As understood in the art, an antibody of the present invention may be coupled to a detectable substance or label to facilitate its detection. Examples of detectable substances or labels include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, chemiluminescent materials and radioactive materials.
20 Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a
25 luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, ruthenium and aequorin, and examples of suitable radioactive material include ¹²⁵I, ¹³¹I, ³⁵S or ³H. Antibodies of the present invention can also be useful in pharmacogenomic analysis. Such embodiments may be used to identify individuals that can benefit from specific or modified treatment modalities and/or monitor efficacy of
30 present treatment regimens.

A “control standard,” as used herein, refers to a sample that can be used to compare the results obtained from a sample in the methods of the invention. Control standards can be cells, blood, plasma, CSF, tissue or known protein concentrations spiked into a media. The concentration levels in a control standard can be an absolute or relative amount, a range of amount, or a minimum amount, a mean amount, and/or a median amount of INSL5. A control standard can also serve as a baseline of INSL5 to which the patient sample is compared. The control standard can include a concentration value from the same patient or a known, normal reference of INSL5. Further, in some embodiments, a control standard may express INSL5 concentrations in the form of a standard curve.

As used herein, the term capture antibody refers to an antibody that will bind INSL5. In such embodiments, the capture antibody is capable of binding and capturing INSL5, for example specifically binding INSL5 in a sample under suitable conditions, such that the capture antibody- INSL5 complex can be separated from the rest of the sample. In some embodiments, the capture antibody may be an antibody that specifically binds to INSL5 and an antibody that specifically binds INSL5 is used as the second (or detection) antibody. In further embodiments, the capture antibody is an antibody provided herein. In further embodiments, the capture antibody is an antibody with HCDRs corresponding to SEQ ID NOs:10-12 and LCDRs corresponding to SEQ ID NOs:14-16, respectively. In further embodiments, the capture antibody is an antibody with HCDRs corresponding to SEQ ID NOs:22-24 and LCDRs corresponding to SEQ ID NOs:26-28, respectively. In some embodiments, the capture antibody is immobilized. In some embodiments, the detection antibody is labeled with a detectable label. In some embodiments, the second (or detection) antibody is an antibody provided herein. In further embodiments, the second (or detection) antibody is an antibody with HCDRs corresponding to SEQ ID NOs:10-12 and LCDRs corresponding to SEQ ID NOs:14-16, respectively. In further embodiments, the second (or detection) antibody is an antibody with HCDRs corresponding to SEQ ID NOs:22-24 and LCDRs corresponding to SEQ ID NOs:26-28, respectively.

In some embodiments, the capture antibody is immobilized in a “sandwich” immunoassay, and the capture or first antibody specifically binds to human or mouse INSL5. In some such embodiments, the capture antibody is an antibody of the present

invention that specifically binds human or mouse INSL5. In further embodiments, the capture antibody is an antibody with HCDRs corresponding to SEQ ID NOs:10-12 and LCDRs corresponding to SEQ ID Nos:14-16. In further embodiments, the capture antibody is an antibody with HCDRs corresponding to SEQ ID NOs:22-24 and LCDRs corresponding to SEQ ID NOs:26-28. In such sandwich immunoassays, a detection (or second) antibody is also utilized. According to some embodiments, a detection or second antibody may bind specifically to the capture antibody and may be labelled with a detectable label. In some embodiments, the detection of second antibody specifically binds to human or mouse INSL5 already bound, or captured, by the capture or first antibody. In some such embodiments, the second antibody is an antibody of the present invention that specifically binds human or mouse INSL5. In some embodiments, the second (or detection) antibody is an antibody provided herein. In further embodiments, the second (or detection) antibody is an antibody with HCDRs corresponding to SEQ ID NOs:10-12 and LCDRs corresponding to SEQ ID NOs:14-16. In further embodiments, the second (or detection) antibody is an antibody with HCDRs corresponding to SEQ ID NOs:22-24 and LCDRs corresponding to SEQ ID NOs:26-28.

As used herein, a “detectable label” is a moiety, composition or technique that can be used to detect the formation of a complex between an antibody of the present invention that specifically binds to the A and B chains of human or mouse INSL5. According to some embodiments, the detectable label may be conjugated to the antibody (either capture or detection, as the case may be) directly or indirectly. Exemplary embodiments of detectable labels include biotin; radioisotopes; fluorophores or other fluorescent moieties; and enzymatic moieties.

Antigen-binding fragments of such antibodies include, for example, Fab fragments, Fab’ fragments, F(ab’)₂ fragments, and single chain Fv fragments.

“Framework region” or “framework sequence” refers to any one of framework regions 1 to 4. Humanized antibodies and antigen-binding fragments thereof encompassed by the present invention include molecules wherein any one or more of framework regions 1 to 4 is humanized, i.e., wherein any of the possible combinations of individual humanized regions 1 to 4, is present. For example, this includes molecules in which framework region 1 and framework region 2, framework region 1 and framework

region 3, framework region 1, 2, and 3, etc., are humanized. Humanized frameworks are those that have at least about 80% sequence identity to a known human germline framework sequence. Human framework germline sequences can be obtained from ImMunoGeneTics (IMGT) or from *The 20 Immunoglobulin FactsBook* by Marie-Paule Lefranc and Gerard Lefranc, Academic Press, 2001, ISBN 012441351. For example, germline light chain frameworks can be selected from the group consisting of A11, A17, A18, A19, A20, A27, A30, L1, L11, L12, L2, L5, L15, L6, L8, O12, O2, and O8, and germline heavy chain framework regions can be selected from the group consisting of VH2-5, VH2-26, VH2-70, VH3-20, 25 VH3-72, VH1-46, VH3-9, VH3-66, VH3-74, VH4-31, VHI-18, VHI-69, VI-13-7, VH3-11, VH3-15, VH3-21, VH3-23, VH3-30, VH3-48, VH4-39, VH4-59, and VH5-51.

Protein “INSL5” (also known as insulin-like peptide-5) refers to a gut hormone encoded by the *Ins15* gene. INSL5 is a peptide produced in enteroendocrine cells of the distal colon in humans and mice.

The results of the following assays demonstrate that the exemplified monoclonal antibodies and antigen-binding fragments thereof of the present invention bind and/or neutralize INSL5 and therefore may be used for diagnostic assays, including, but not limited to sandwich ELISA assays in order to study the role of INSL5 in metabolic diseases including, but not limited to, diabetes and obesity.

Disclosed herein are antibodies for use in diagnostics, such as diagnostic assays to detect the presence or level of INSL5.

EXAMPLES

The following non-limiting examples are offered for purposes of illustration, not limitation.

Example 1: Recombinant Expression of Antibody I

Antibody I is an antibody having a heavy chain amino acid sequence of SEQ ID NO:5 and a light chain amino acid sequence of SEQ ID NO:7.

Antibody I is generated in a mammalian cell expression system using CHO-K1 cell derivatives (Lonza Biologics Inc.). cDNA sequences encoding SEQ ID NO:5 and SEQ ID NO:7 are sub-cloned into GS-containing expression plasmid backbones

(pEE12.4-based plasmids; Lonza Biologics Inc.). Each cDNA sequence is fused in frame with the coding sequence of a signal peptide sequence, METDTLLLWVLLLWVPGSTG (SEQ ID NO:29), to enhance secretion of the antibody into the tissue culture medium. The expression of both cDNA sequences is driven by the viral CMV promoter.

5 For generating the antibody via transient transfection, CHO-K1 cells are transfected with an equal stoichiometric ratio of the recombinant expression plasmids using a PEI-based method. Briefly, the appropriate volume of CHO-K1 suspension cells at a density of 4×10^6 cells/mL is transferred in shake flasks, and both PEI and recombinant plasmid DNA are added to the cells. Cells are incubated in a suspension
10 culture at 32°C for 6 days. At the end of the incubation period, cells are removed by low speed centrifugation and the antibody is purified from the conditioned medium.

 The antibody secreted into the media from the CHO-K1 cells, is purified by Protein A affinity chromatography followed by size-exclusion chromatography (SEC) and/or ion exchange chromatography. Specifically, the antibody from harvested media is
15 captured onto Mab Select Protein A resin (GE). The resin then is briefly washed with a running buffer, such as a phosphate-buffered saline (PBS; pH 7.4) to remove non-specifically bound material. The protein is eluted from the resin with a low pH solution, such as 10 mM citric acid pH 3. Fractions containing the antibody are pooled and may be held at a low pH to inactivate potential viruses. They may be neutralized by adding a base
20 such as 0.1 M Tris pH 8.0. The antibody is further purified by SEC by loading the concentrated Protein A pool on a Superdex200 (GE Healthcare) with isocratic elution in PBS pH 7.4. The antibody may be further purified by an ion exchange chromatography step using resins such as Poros 50 HS (ThermoFisher). In that case the antibody is eluted from the column using a 0 to 500 mM NaCl gradient in 20 mM NaOAc, pH 5.0 over 15
25 column volumes. The purified antibody may be buffer exchanged to PBS pH 7.4 and concentrated for example by using centrifugal filter units (such as Amicon Ultra 50K centrifugal filter units) or by tangential flow ultrafiltration on a regenerated cellulose membranes (Millipore).

 The antibody therefore is prepared in this manner or in a similar manner that
30 would be readily determined by one of skill in the art.

Example 2: Recombinant Expression of Antibody II

Antibody II is an antibody having a heavy chain amino acid sequence of:(SEQ ID NO:17) and a light chain amino acid sequence of:(SEQ ID NO:19).

Here, the antibody of SEQ ID NO:17 and SEQ ID NO:19 is generated essentially
5 as described for Example 1 except that cDNA sequences encoding SEQ ID NOs:17 and 19 are used in the expression plasmids.

IN VITRO FUNCTION**Example 3: Antibody Binding to Human and Mouse INSL5 via SPR**

10 *In vitro* binding of the antibodies of Examples 1 and 2 to human and mouse INSL5 is determined by Surface Plasmon Resonance (SPR) at 25°C and at 37°C. In particular, the affinity of the antibodies of Examples 1 and 2 is summarized below in Tables 1 and 2.

Binding of the antibodies of Examples 1 and 2 to human and mouse INSL5 is
15 carried out on a Biacore 8K (GE Healthcare) instrument. 1x HBS-EP+ (10 mM HEPES pH 7.6, 150 mM NaCl, 3 mM EDTA, 0.05% Polysorbate 20) (Teknova) is used as a running buffer. Protein A (Calbiochem #539202-5mg) immobilization onto a Series S Sensor Chip CM5 (GE Healthcare) is performed according to the manufacturer's instructions (Amine Coupling Kit BR-1000-50) at 25° C. Briefly, carboxyl groups on the
20 sensor chip surfaces (flow cell 1 and 2) are activated by injecting 70 µL of a mixture containing 75 mg/mL EDC and 11.5 mg/mL NHS at 10 µL/min. A solution of 50 µg/mL of Protein A is prepared by diluting 10 µl of a 5 mg/mL stock solution into 1 mL 10 mM sodium acetate pH 4.5. 70 µL of this solution is injected over the activated chip surfaces (flow cell 1 and 2, channels 1 to 8) at 10 µL/min for 7 min. Excess reactive groups on the
25 surfaces (flow cell 1 and 2) are then deactivated by injecting 70 µL of 1 M ETA HCl-NaOH pH 8.5 at 10 µL/min. The chip is conditioned by six 15s injections of 10 mM glycine-HCl pH 1.5 at 30 µL/min.

Human INSL5 (Phoenix Pharmaceuticals Cat #035-70A) and mouse INSL5 (Phoenix Pharmaceuticals 035-40) are reconstituted at 0.5 (99 µM) or 1 mg/mL (195 µM)
30 in DMSO, respectively. A 2-fold dilution series of the peptides is being made in 1x HBS-EP+ buffer at concentrations of 20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, and 0 nM. The

antibodies of Example 1, 2, or a negative control are prepared by diluting them to 1 $\mu\text{g/mL}$ in 1x HBS-EP+ buffer.

The experiment is conducted at 25°C as well as at 37°C and initiated with 5 start-up cycles of injecting 1x HBS-EP+ buffer. Then in each cycle, the antibody is captured onto flow cell 2 of channels 1-8 at 10 $\mu\text{L/min}$ for 20 seconds. Next, 180 μL of the respective INSL5 peptide sample is individually injected across flow cells 1 and 2 for 180 seconds at 60 $\mu\text{L/min}$ and then let dissociate for 1200 seconds at 60 $\mu\text{L/min}$ flow rate. The surface is regenerated by injecting two 15 second pulses of 10 mM glycine-HCl pH 1.5 (BR-1003-54) at 30 $\mu\text{L/min}$. After a stabilization period of 60 seconds at a flow rate of 60 $\mu\text{L/min}$ the next cycle is started.

The resulting sensorgrams are analyzed using Biacore 8K Evaluation Software). 1:1 binding kinetics model fitting is used to calculate the binding kinetic parameters association rate (k_a), dissociation rate (k_d), and equilibrium dissociation constant (K_D).

Table 1: Binding Kinetics of Antibody I and Antibody II to Human and Mouse INSL5 at 25°C.

25°C	Human INSL5			Mouse INSL5		
	k_a (1/Ms)	k_d (1/s)	K_D (M)	k_a (1/Ms)	k_d (1/s)	K_D (M)
Antibody I	8.8E+06	3.6E-04	4.1E-11	1.2E+07	5.4E-05	4.6E-12
Antibody II	1.6E+07	3.4E-04	2.1E-11	1.8E+07	1.1E-04	6.1E-12

For Antibody I, K_D at 25°C is determined as 41 pM to human INSL5 and 4.6 pM to mouse INSL5. For Antibody II, K_D at 25°C is determined as 21 pM to human INSL5 and 6.1 pM to mouse INSL5.

Table 2: Binding Kinetics of Antibody I and Antibody II to Human and Mouse INSL5 at 37°C.

37°C	Human INSL5			Mouse INSL5		
	k_a (1/Ms)	k_d (1/s)	K_D (M)	k_a (1/Ms)	k_d (1/s)	K_D (M)
Antibody I	2.4E+07	2.6E-03	1.1E-10	2.3E+07	1.5E-04	6.7E-12
Antibody II	4.0E+07	3.3E-03	8.1E-11	5.1E+07	7.2E-04	1.4E-11

For Antibody I, K_D at 37°C is determined as 110 pM to human INSL5 and 6.7 pM to mouse INSL5. For Antibody II, K_D at 37°C is determined as 81 pM to human INSL5 and 14 pM to mouse INSL5.

5 **Example 4: In Vitro Neutralization of INSL5 Activity Increases cAMP Production**

In vitro neutralization of human and mouse INSL5 activity with Antibody I and Antibody II is determined with a cAMP dynamic 2 Assay (Cisbio Cat# 62AM4PEJ). The kit is based on HTRF technology that measures the cAMP accumulation in cells. Cells stimulated with a fixed concentration of forskolin increase cAMP production.

- 10 Introducing a fixed concentration of human and/or mouse INSL5 to the cells inhibits the cAMP production by binding to the RXFP4 receptor. Antibody I and Antibody II bind and neutralize the effects of human and mouse INSL5 prior to binding to the RXFP4 receptor. EC_{50} values derived from antibody serial dilution data of Antibody I and Antibody II are summarized below in Table 3 and demonstrate the increase of cAMP
- 15 production as the concentration of antibodies increases. Preparation of cell plates: Mouse RXFP4 CHO-K1 cells (DiscoverX Part# 93-0929E2) and human CHO RXFP4 cells (DiscoverX 93-0701E2) are thawed quickly in a 37°C water bath and reconstituted with 500 μ L of pre-warmed (in 37°C water bath) Cell Plating Reagent 2 (DiscoverX 93-0563R Series). Cells are mixed by pipetting up and down and then added to 11.5 mL of pre
- 20 warmed (in 37°C water bath) Cell Plating Reagent 2. Cells are plated at 8,000 cells/well at 100 μ L per well in white full area plate (Corning-Costar 3917). The plates are incubated for 48 hours at 37°C to allow cells to adhere to the plate. After 48 hours the growth media is aspirated and 40 μ L of Cell Assay Buffer [HBSS (Hyclone SH30028.03), 20mM HEPES (Hyclone SH30237.01), 1% FBS (Gibco 10438-026), 1mM IBMX (Sigma 15879, diluted in DMSO)] are added to each well. 30 μ L of the Compound
- 25 Assay Buffer (HBSS + 20 mM HEPES + 1% FBS) containing 17 μ M of Forskolin (Sigma Aldrich- CAS #F3917 (10mM in DMSO)) are diluted into each well.

- Preparation of samples (antibody-INSL5 peptide mixtures): Human INSL5 peptide (Phoenix Pharmaceuticals, Cat# 035-70) or mouse INSL5 peptide (Phoenix
- 30 Pharmaceuticals, Cat# 035-40) are prepared at their respective IC_{80} concentrations in Cell Plating Reagent 2. 10 μ L of the respective INSL5 peptide (16 times the final

concentration in the assay; i.e. 144nM for mouse INSL5 and 1280nM for human INSL5) are then mixed with 10 μ L of a serial dilution of antibodies (starting at 160nM) of Examples 1 or 2 (final antibody starting concentration is 10 nM). Samples are incubated at room temperature for two hours with moderate shaking in a PCR plate (Fisherbrand Cat# 14230244).

Conducting the cell assay: 10 μ L of the antibody-INSL5 peptide mixtures are added to the 70 μ L of buffer mixture in the assay plates (containing the cells) and incubated at room temperature for 1 hour with moderate shaking. During the incubation, the cAMP detection reagents are prepared: 500 μ L of cAMP-d2 and Anti-cAMP-Cryptate stock reagent solutions (cAMP dynamic 2 100,000 tests Kit Cat# 62AM4PEJ) are diluted into 9.5 mL of Conjugate/Lysis Buffer each. After 1 hour incubation, 40 μ L of the cAMP-d2 detection reagent is added to each well of the cell assay plate. Then immediately 40 μ L of the Anti-cAMP-Cryptate detection reagent is added to the cell assay plate. The plate is incubated at room temperature for 1 hour with aluminum seal. Then the plate is read on Perkin Elmer Envision and the results are calculated from the machine's 665nm/620nm ratio.

Statistical analysis of data: Data is imported from the Perkin Elmer Envision reader into GraphPad Prism® software (GraphPad Software, LLC; La Jolla, CA). EC₅₀ values are generated by variable slope-four parameter dose response curve analysis. SEM is calculated by taking the EC₅₀ standard deviation between independent experiments (n) divided by the square root of independent experiments.

Table 3: Neutralization of Human and Mouse INSL5 Activity Prior to Binding to the RXFP4 Receptor by Antibody I and Antibody II as Monitored by Increase in cAMP Production.

Neutralization	Human INSL5			Mouse INSL5		
	EC ₅₀ (nM)	SEM	n	EC ₅₀ (nM)	SEM	n
Antibody I	4.35	0.49	2	0.84	0.12	3
Antibody II	1.27	N/A	1	0.45	0.01	2

For Antibody I, EC₅₀ is determined as 4.35 nM to human INSL5 and 0.84 nM to mouse INSL5. For Antibody II, EC₅₀ is determined as 1.27 nM to human INSL5 and 0.45 pM to mouse INSL5.

Example 5: Thermal Stability by TDF

Thermostability of Antibody I and Antibody II is determined by Thermal Denaturation Fluorimetry (TDF). Particularly, the melting temperature (T_m) for the Fc and Fab domains of antibodies of Examples 1 and 2 are reported in Table 4.

A thermofluor assay is run on a LightCycler 480II PCR machine using SYPRO Orange dye (5000× concentrate, Invitrogen S6651). Excitation and emissions filters are set at 465 nm and 580 nm respectively and the temperature is ramped continuously from 25°C to 95°C at a rate of 1°C/s. Final assay conditions contain Examples 1 or 2 at 0.2 mg/mL and 10× SYPRO Orange dye in PBS pH 7.4 (Corning 21-040-CV).

The experiment is performed by diluting Examples 1 or 2 to 0.4 mg/mL and mixing it in equal parts with 20× SYPRO Orange dye in PBS pH 7.4 to reach a final volume of 30 μ L per sample (BE05746-005). The mixture is dispensed as 6 μ L aliquots in triplicate into a 384-multiwell assay plate (Roche 04-729-749-001). The T_m values for Examples 1 and 2 are determined using the Thermal Shift Analysis Software (Roche) by the first derivative method. The analysis software smooths the raw fluorescence data and the T_m is collected by determining the temperature where the upward slope of fluorescence vs. temperature is maximal (inflection point). Mean T_m values and SD are calculated for each triplicate set.

Table 4: Thermal Stability of Antibody I and Antibody II by TDF.

	Fc Domain		Fab Domain	
	T_m (°C)	SD	T_m (°C)	SD
Antibody I	76.1	0.02	87.9	0.03
Antibody II	76.4	0.05	82.5	0.03

For Antibody I, the T_m is determined as 76.1°C for the Fc domain and 87.9°C for the Fab domain. For Antibody II, the T_m is determined as 76.4°C for the Fc domain and 82.5°C for the Fab domain.

Example 6: INSL5 Detection Assay

Antibody I and Antibody II are used to detect and quantify human and mouse INSL5 concentrations by sandwich ELISA. In particular, the detection range of this assay

approximates between 30 pg/mL for the lower limit of detection and 100,000 pg/mL for the upper limit of detection as summarized below in Table 5.

- Labeling of Antibody I with MSD SulfoTag (NHS Ester 150 nmole, Cat#R91AN-1): MSD Sulfo-Tag powder is resuspended in the original vial with 50 μ L of ice cold
- 5 Milli Q water, for a concentration of 3 nmol/ μ L. Using a labeling challenge ratio of 12, 2.7 μ L of reagent are added to 100 μ L of 1 mg/mL of antibody. The solution is mixed well and kept mixture out of light while shaking at room temperature for 2 hours. Towards the end of the incubation, a Zeba Spin Desalting column (Thermo Scientific Cat# 87766) is prepared. The column is inverted until all resin is a homogenous mixture.
- 10 The valve on bottom of column is opened and liquid is drained through centrifugation for 1 min at 1500xg. The column is washed three times with 500 μ L of 1X PBS using 1 min 1500xg spins. After 2 hour incubation the antibody labeling reaction solution is added dropwise into the column resin. The column is placed into an Eppendorf tube to collect the labeled antibody after a 2 minute 1500xg spin. The labeled antibody is stored at 4°C
- 15 in the dark.

- For the sandwich ELISA assay, on day 1, Antibody I is diluted to 4 μ g/mL into 1x PBS (Gibco Life Technologies Cat#14190-144). 35 μ L/well of this solution is applied to MesoScale Discovery (MSD) Plates (Cat#L15XA-3). The plates are tapped to evenly coat the well. The plate is sealed and incubated overnight at room temperature. On day 2, the
- 20 plates are washed three times using an automatic plate washer (Biotek ELx405) with 1x PBS with 0.05% Tween 20 (20x PBS Tween20 Thermo Scientific Cat#28352). The plates are tapped dry on a paper towel. 100 μ L of Superblock buffer (Superblock (T20) Thermo Scientific Cat# 37536) are added to each well and shaken moderately for two hours at room temperature. Plates are washed three times and tapped dry as described above. 25
- 25 μ L of Superblock buffer are added to all wells of the plates. Then 25 μ L of four-fold serial dilutions (made in Superblock (T20)) starting at 500 ng/mL of either human INSL5 (Phoenix Pharmaceuticals Cat#035-70 made in DMSO) or mouse INSL5 (Phoenix Pharmaceuticals Cat#035-40 made in DMSO) are added to the respective wells in the plates. The plates are sealed and shaken moderately for 2 hours at room temperature.
- 30 Plates are washed three times and tapped dry as described above. MSD Sulfoltag labeled antibody of Example 2 is diluted to 1 μ g/mL (i.e. about 1000-fold) into 0.2x Superblock

(T20) buffer in 1x PBS/ Tween 0.05%. 25 μ L of this solution is added to each well of the plate and incubated for 1 hour at room temperature. Plates are washed three times and tapped dry as described above. 150 μ L of 1X MSD Read buffer (MSD Read Buffer (4X) Cat#R92TC-2) are added to each well and read on MSD Sector plate reader. Signal is

5 measured in Electrochemiluminescence units (ECLUs).

Statistical analysis of data: Data is imported from the Mesoscale Discovery Sector reader into Microsoft Excel and GraphPad Prism® software (GraphPad Software, LLC; La Jolla, CA) to determine the approximate linear range for accurate INSL5 detection. Detection range approximates between 30 pg/mL and 100,000 pg/mL for both,

10 human and mouse INSL5.

Table 5: Mouse and Human INSL5 Detection by Sandwich ELISA using Antibody I for Capture and Antibody II for Detection.

Dilution No.	Human INSL5		Mouse INSL5	
	Conc. (pg/mL)	ECLU	Conc. (pg/mL)	ECLU
1	504800	218430	511700	244857
2	126200	238191	127925	248527
3	31550	93393	31981	124617
4	7887	19907	7995	21301
5	1972	4570	1999	6823
6	493	1231	450	1902
7	123	579	125	735
8	30.8	433	31.2	491
9	7.70	413	7.81	420
10	1.93	394	1.95	552
11	0.48	771	0.49	385
Buffer only	0	306	0	382

Amino Acid and Nucleotide Sequences

SEQ ID NO:1: Human INSL5 A-chain

QDLQTLCCCTDGCSMTDLSALC

5

SEQ ID NO:2: Human INSL5 B-chain

KESVRLCGLEYIRTVIYICASSRW

SEQ ID NO:3: Mouse INSL5 A-chain

10 RDLQALCCREGCSMKELSTLC

SEQ ID NO:4: Mouse INSL5 B-chain

RQTVKLCGLDYVRTVIYICASSRW

15 SEQ ID NO:5: HC of Antibody I

QSVEESGGRLVTPGTPLTLTCTASGFSLSYDMGWVRQTPGEGLEWVGTISAGG
YTTYAHWAKGRFTISKSSSTTVDLKMTSLTTEDTATYFCARERWNYDRSGGAGA
GYFDLWGPGLVTVSSGQPKAPSVFPLAPCCGDTSPSTVTLGCLVKGYLPEPVTV
TWNSTLTNGVRTFPSVRQSSGLYSLSSVSVTSSSQPVTCNV AHPATNTKVDKT
20 VAPSTCSKPTCPPPELLGGPSVFI FPPKPKDTLMISRTPEVTCVVVDVSQDDPEVQF
TWYINNEQVRTARPLREQQFNSTIRVVSTLPITHQDWLRGKEFKCKVHNKALPA
PIEKTISKARGQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDISVEWEKNGK
AEDNYKTTPAVLDSGYSFLYNKLSVPTSEWQRGDVFTCSVMHEALHNHYTQK
SISRSPGK

25

SEQ ID NO:6: HC DNA of Antibody I

CAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGA
CACTCACCTGCACAGCCTCTGGATTCTCCCTCAGTAGCTACGACATGGGCTGG
GTCCGCCAGACTCCAGGGGAGGGGCTGGAATGGGTCGGAACCATAGTGCTG
30 GTGGTTACACGTACTACGCGCACTGGGCGAAAGGCCGATTACCATCTCCAA
ATCCTCGACCACGGTGGATCTGAAAATGACCAGTCTGACGACCGAGGACACG

GCCACCTATTTCTGTGCCAGAGAAAGATGGAATTACGATAGGTCTGGTGGTG
CTGGTGCTGGCTACTTTGACTTGTGGGGCCCAGGCACCCTGGTCACCGTCTCC
TCAGGGCAACCTAAGGCTCCATCAGTCTTTCCCCTCGCACCTTGCTGTGGTGA
CACGCCCTCATCCACGGTAACACTGGGCTGTCTTGTCAAAGGATACCTTCCGG
5 AGCCAGTCACAGTAACGTGGAACCTCGGGAACATTGACAAACGGCGTAAGAAC
GTTTCCGTCGGTACGTCAAAGTTCAGGCCTCTACTCGCTCAGCTCCGTAGTAT
CGGTGACCTCATCCAGCCAGCCGGTGACTTGCAACGTGGCGCATCCCCGCGAC
CAACACAAAAGTGGATAAGACCGTTGCACCCTCAACTTGCTCCAAGCCCACG
TGTCCCCCACCAGAGCTGCTCGGTGGGCCCTCGGTCTTTATCTTCCCTCCGAA
10 ACCCAAAGACACATTGATGATCTCTCGCACGCCGGAAGTCACGTGCGTGGTC
GTGGACGTCAGCCAAGATGACCCGGAAGTGCAATTCACCTGGTATATCAATA
ACGAACAGGTCAGAACGGCTCGGCCTCCTTTGCGAGAACAACAGTTCAATTC
CACTATCAGGGTTGTATCAACACTTCCCATCACACACCAAGATTGGCTTAGGG
GAAAGGAGTTTAAGTGTAAGTGCACAATAAGGCTTTGCCAGCGCCTATTGA
15 GAAAACCATTTCCAAAGCCCGTGGGCAACCGCTTGAACCCAAAGTCTATACA
ATGGGGCCACCCAGAGAGGAACTGTGCGAGCCGCTCCGTGTCACTGACTTGTA
TGATCAATGGGTTCTATCCGTCGGACATTTCCGTGGAATGGGAGAAGAATGG
AAAAGCAGAGGATAACTACAAAACACTACGCCAGCCGTGTTGGACTCTGACGGG
TCATACTTTCTGTACAATAAGCTCTCTGTCCCCACGTCCGAATGGCAGAGGGG
20 AGATGTGTTTACTTGCTCGGTGATGCATGAGGCGCTCCATAATCACTATACCC
AGAAAAGCATCAGTCGAAGCCCTGGGAAA

SEQ ID NO:7: LC of Antibody I

ADVVMTQTASPVSAAVGGTVTINCQASQSITSSYLSWYQQKPGQPPLLIYPAN
25 LASGVPSRFGSGSGTQFTLTISGVQCDDAATYYCLYGYFSSSIDFAFGGGTEVV
VRGDPVAPTVLIFPPAADQVATGTVTIVCVANKYFPDVTVTWEVDGTTQTTGIEN
SKTPQNSADCTYNLSSLTLTSTQYNHKEYTCKVTQGTTSVVQSFNRGDC

SEQ ID NO:8: LC DNA of Antibody I

30 GCCGATGTCGTGATGACCCAGACTGCATCCCCCGTGTCTGCAGCTGTGGGAG
GCACAGTCACCATCAATTGCCAGGCCAGTCAGAGTATTACTAGTAGCTACTTA

TCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATCCTGC
AGCCAATCTGGCATCTGGAGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGG
ACACAGTTCACTCTCACCATCAGCGGCGTGACGTGTGACGATGCTGCCACTTA
CTACTGTCTATACGGTTATTTTAGTTCTAGTATTGATTTTGCTTTCGGCGGAGG
5 GACCGAGGTGGTGGTCAGAGGTGATCCAGTTGCACCTACTGTCCTCATCTTCC
CACCAGCTGCTGATCAAGTCGCAACAGGTACTGTGACGATCGTGTGTGTCGC
GAACAAATACTTTCCCGACGTGACCGTGACGTGGGAAGTCGACGGAACAACC
CAGACGACCGGGATCGAAAACCTCAAAGACCCCGCAAACTCGGCCGATTGCA
CATAACAATTTGTCCTCTACGCTTACACTCACGTCGACGCAGTACAATAGTCAC
10 AAGGAGTATACATGCAAAGTTACTCAAGGAACCTACGAGCGTGGTCCAGTCAT
TCAATAGAGGGGATTGT

SEQ ID NO:9: VH of Antibody I
QSVEESGGRLVTPGTPLTLTCTASGFSLSYDMGWVRQTPGEGLEWVGTISAGG
15 YTYAAHWAKGRFTISKSSSTTVDLKMTSLTTEDTATYFCARERWNYDRSGGAGA
GYFDLWGPGTLVTVSS

SEQ ID NO:10: HCDR1 of Antibody I
TASGFSLSYDMG

20

SEQ ID NO:11: HCDR2 of Antibody I
TISAGGYTY

SEQ ID NO:12: HCDR3 of Antibody I
25 ARERWNYDRSGGAGAGYFDL

SEQ ID NO:13: VL of Antibody I
ADVVMQTASPVSAAVGGTVTINCQASQSITSSYLSWYQQKPGQPPLIYPAAN
LASGVPSRFGSGSGTQFTLTISGVQCDDAATYYCLYGYFSSSIDFAFGGGTEVV
30 VR

SEQ ID NO:14: LCDR1 of Antibody I

QASQSITSSYLS

SEQ ID NO:15: LCDR2 of Antibody I

5 YPAANLAS

SEQ ID NO:16: LCDR3 of Antibody I

LYGYFSSSIDFA

10 SEQ ID NO:17: HC of Antibody II

QSVEESGGGLVTPGGSLTLTCTVSGIDLTTYAMGWVRQAPGEGLEWIGIIGGGGR
TYAAAWAKGRFTISKSTTTVDLRITSPATEDTATYFCVRGGDFDLWGPGTLVTV
SSGQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTF
PSVRQSSGLYSLSSVSVTSSSQPVTCNVAHPATNTKVDKTVAPSTCSKPTCPPPE
15 LLGGPSVFIFPPKPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARP
PLREQQFNSTIRVVSTLPITHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLE
PKVYTMGPPREELSSRSVSLTCMINGFYPSDISVEWEKNGKAEDNYKTTPAVLDS
DGSYFLYNKLSVPTSEWQRGDVFTCSVMHEALHNHYTQKSISRSPGK

20 SEQ ID NO:18: HC DNA of Antibody II

CAGTCGGTGGAGGAGTCCGGAGGAGGCCTGGTAACGCCTGGAGGATCCCTGA
CACTCACCTGCACAGTCTCTGGAATCGACCTCACTACCTATGCAATGGGCTGG
GTCCGCCAGGCTCCAGGGGAGGGGCTGGAATGGATCGGAATTATTGGTGGTG
GTGGTCGAACATACTACGCGGCCTGGGCGAAAGGCCGCTTCACCATCTCCAA
25 AACCTCGACCACGGTGGATCTGAGAATCACCAGTCCGGCAACCGAGGACACG
GCCACCTATTTCTGTGTCAGAGGAGGAGACTTCTTTGACTTGTGGGGCCCAGG
CACCTGGTCACCGTCTCCTCAGGGCAACCTAAGGCTCCATCAGTCTTTCCCC
TCGCACCTTGCTGTGGTGACACGCCCTCATCCACGGTAACACTGGGCTGTCTT
GTCAAAGGATACCTTCCGGAGCCAGTCACAGTAACGTGGAACCTCGGGAACAT
30 TGACAAACGGCGTAAGAACGTTTCCGTCGGTACGTCAAAGTTCAGGCCTCTA
CTCGCTCAGCTCCGTAGTATCGGTGACCTCATCCAGCCAGCCGGTGACTTGCA

ACGTGGCGCATCCCGCGACCAACACAAAAGTGGATAAGACCGTTGCACCCTC
AACTTGCTCCAAGCCCACGTGTCCCCACCAGAGCTGCTCGGTGGGCCCTCGG
TCTTTATCTTCCCTCCGAAACCCAAAGACACATTGATGATCTCTCGCACGCCG
GAAGTCACGTGCGTGGTTCGTGGACGTCAGCCAAGATGACCCGGAAGTGCAAT
5 TCACCTGGTATATCAATAACGAACAGGTCAGAACGGCTCGGCCTCCTTTGCGA
GAACAACAGTTCAATTCCACTATCAGGGTTGTATCAACACTTCCCATCACACA
CCAAGATTGGCTTAGGGGAAAGGAGTTTAAGTGTAAAGTGCACAATAAGGCT
TTGCCAGCGCCTATTGAGAAAACCATTTCCAAAGCCCGTGGGCAACCGCTTG
AACCCAAAGTCTATACAATGGGGCCACCCAGAGAGGAACTGTGCGAGCCGCTC
10 CGTGTCACTGACTTGTATGATCAATGGGTTCTATCCGTCGGACATTTTCGGTGG
AATGGGAGAAGAATGGAAAAGCAGAGGATAACTACAAAACACTACGCCAGCCG
TGTTGGACTCTGACGGGTCATACTTTCTGTACAATAAGCTCTCTGTCCCCACG
TCGGAATGGCAGAGGGGAGATGTGTTTACTTGCTCGGTGATGCATGAGGCGC
TCCATAATCACTATAACCCAGAAAAGCATCAGTCGAAGCCCTGGGAAA

15

SEQ ID NO:19: LC of Antibody II

AQVLQTTPASVSAAVGGTVTIKQASEDISKYLWYQQKPGQRPKLLIYYVSNLE
FGVPSRFKSGSGSTEYTLTISDLECDDAATYYCHQGYTGVNVENVFGGGTEVVV
RGDPVAPTVLIFPPAADQVATGTVTIVCVANKYFPDVTVTWEVDGTTQTTGIENS
20 KTPQNSADCTYNLSSTLTLTSTQYNHKEYTCKVTQGTTSVVQSFNRGDC

SEQ ID NO:20: LC DNA of Antibody II

GCTCAAGTGCTGACCCAGACTCCAGCCTCCGTGTCTGCAGCTGTGGGAGGCA
CAGTCACCATCAAGTGCCAGGCCAGTGAGGATATTAGCAAGTACTTATCCTG
25 GTATCAGCAGAAACCAGGGCAGCGCCCCAACTCCTGATCTATTATGTATCC
AATCTGGAATTTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACAG
AGTACACTCTCACCATCAGCGACCTGGAGTGTGACGATGCTGCCACTTACTAC
TGTCACCAGGGTTATACCGGTGTTAATGTTGAAAATGTTTTCGGCGGAGGGAC
CGAGGTGGTGGTCAGAGGTGATCCAGTTGCACCTACTGTCCTCATCTTCCCAC
30 CAGCTGCTGATCAAGTCGCAACAGGTACTGTGACGATCGTGTGTGTGCGGAA
CAAATACTTTCCCGACGTGACCGTGACGTGGGAAGTCGACGGAACAACCCAG

ACGACCGGGATCGAAAAC TCAAAGACCCCGCAAAACTCGGCCGATTGCACAT
ACAATTTGTCTCTACGCTTACACTCACGTCGACGCAGTACAATAGTCACAAG
GAGTATACATGCAAAGTTACTCAAGGAACTACGAGCGTGGTCCAGTCATTCA
ATAGAGGGGATTGT

5

SEQ ID NO:21: VH of Antibody II

QSVEESGGGLVTPGGSLTLTCTVSGIDLTTYAMGWVRQAPGEGLEWIGIIGGGGR
TYAAAWAKGRFTISKSTTTVDLRITSPATEDTATYFCVRGGDFFDLWGPGTLVTV
SS

10

SEQ ID NO:22: HCDR1 of Antibody II

TVSGIDLTTYAMG

SEQ ID NO:23: HCDR2 of Antibody II

15 IIGGGGRTY

SEQ ID NO:24: HCDR3 of Antibody II

VRGGDFFDL

20 SEQ ID NO:25: VL of Antibody II

AQVLTQTPASVSAAVGGTVTIKCQASEDISKYLSWYQQKPGQRPKLLIYYVSNLE
FGVPSRFKGS GSGTEYTLTISDLECDDAATYYCHQGYTG VNVENVFGGGTEVVV
R

25 SEQ ID NO:26 LCDR1 of Antibody II

QASEDISKYLS

SEQ ID NO:27 LCDR2 of Antibody II

YYVSNLEF

30

SEQ ID NO:28 LCDR3 of Antibody II

HQGYTGNNENV

SEQ ID NO:29 Signal peptide

METDTLLLVLLLWVPGSTG

WE CLAIM:

1. An antibody that binds INSL5 comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises heavy chain complementarity determining regions (HCDR) HCDR1, HCDR2, and HCDR3, and the VL comprises light chain complementarity determining regions (LCDR) LCDR1, LCDR2, and LCDR3,
5 wherein
the HCDR1 comprises TASGFSLSYDMG (SEQ ID NO:10),
the HCDR2 comprises TISAGGYTY (SEQ ID NO:11),
the HCDR3 comprises ARERWNYDRSGGAGAGYFDL (SEQ ID NO:12),
10 the LCDR1 comprises QASQSITSSYLS (SEQ ID NO:14),
the LCDR2 comprises YPAANLAS (SEQ ID NO:15), and
the LCDR3 comprises LYGYSFSSIDFA (SEQ ID NO:16).
2. The antibody of claim 1, wherein the VH comprises SEQ ID NO:9 and the VL
15 comprises SEQ ID NO:13.
3. The antibody of claim 1 or 2, wherein the antibody comprises a heavy chain (HC) comprising SEQ ID NO:5 and a light chain (LC) comprising SEQ ID NO:7.
- 20 4. The antibody of claim 1 or 2, wherein the antibody comprises a heavy chain (HC) comprising amino acids 2-446 of SEQ ID NO:5, and a light chain (LC) comprising SEQ ID NO:7.
5. The antibody of claim 1 or 2, wherein the antibody comprises a HC consisting of SEQ
25 ID NO:5 and a LC consisting of SEQ ID NO:7.
6. The antibody of any one of claims 1-5 that binds to human INSL5.
7. The antibody of any one of claims 1-5 wherein the antibody binds to the A chain (SEQ
30 ID NO:1) and the B chain (SEQ ID NO:2) of human INSL5.

8. The antibody of any one of claims 1-5 that binds to mouse INSL5.
9. The antibody of any one of claims 1-5 wherein the antibody binds to the A chain (SEQ ID NO:3) and the B chain (SEQ ID NO:4) of mouse INSL5.
- 5 10. A nucleic acid comprising a sequence encoding SEQ ID NO:5 or SEQ ID NO:7.
11. A vector comprising the nucleic acid of claim 10.
- 10 12. A composition comprising a first vector comprising a nucleic acid sequence encoding SEQ ID NO:5 and a second vector comprising a nucleic acid sequence encoding SEQ ID NO:7.
13. A cell comprising:
- 15 (a) a first vector comprising a nucleic acid sequence encoding SEQ ID NO:5 and a second vector comprising a nucleic acid sequence encoding SEQ ID NO:7; or
- (b) a vector comprising a first nucleic acid sequence encoding SEQ ID NO: 5 and a second nucleic acid sequence encoding SEQ ID NO: 7.
- 20 14. The cell of claim 13, wherein the cell is a mammalian cell.
15. A process of producing an antibody comprising culturing the cell of any one of claims 13-14 under conditions such that the antibody is expressed and recovering the expressed antibody from the culture medium.
- 25 16. An antibody produced by culturing the cell of any one of claims 13-14 under conditions such that the antibody is expressed and recovering the expressed antibody from the culture medium.

17. An antibody that binds INSL5 comprising a VH and a VL, wherein the VH comprises HCDR1, HCDR2, and HCDR3, and the VL comprises LCDR1, LCDR2, and LCDR3, wherein
- 5 the HCDR1 comprises TVSGIDLTITYAMG (SEQ ID NO:22),
 the HCDR2 comprises IIGGGGRITY (SEQ ID NO:23),
 the HCDR3 comprises VRGGDFFDL (SEQ ID NO:24),
 the LCDR1 comprises QASEDISKYLS (SEQ ID NO:26),
 the LCDR2 comprises YYVSNLEF (SEQ ID NO:27), and
 the LCDR3 comprises HQGYTGVNVENV (SEQ ID NO:28).
- 10
18. The antibody of claim 17, wherein the VH comprises SEQ ID NO:21 and the VL comprises SEQ ID NO:25.
19. The antibody of claim 17 or 18, wherein the antibody comprises a heavy chain (HC) comprising SEQ ID NO:17 and a light chain (LC) comprising SEQ ID NO:19.
- 15
20. The antibody of claim 17 or 18, wherein the antibody comprises a heavy chain (HC) comprising amino acids 2-435 of SEQ ID NO:17, and a light chain (LC) comprising SEQ ID NO:19.
- 20
21. The antibody of claim 17 or 18, wherein the antibody comprises a HC consisting of SEQ ID NO:17 and a LC consisting of SEQ ID NO:19.
22. The antibody of any one of claims 17-21 wherein the antibody binds to human
- 25 INSL5.
23. The antibody of any one of claims 17-21 wherein the antibody binds to the A chain (SEQ ID NO:1) and B chain (SEQ ID NO:2) of human INSL5.
- 30 24. The antibody of any one of claims 17-21 wherein the antibody binds to mouse INSL5.

25. The antibody of any one of claims 17-21 that binds to the A chain (SEQ ID NO:3) and B chain (SEQ ID NO:4) of mouse INSL5.
26. A nucleic acid comprising a sequence encoding SEQ ID NO:17 or SEQ ID NO:19.
- 5 27. A vector comprising the nucleic acid of claim 26.
28. A composition comprising a first vector comprising a nucleic acid sequence encoding SEQ ID NO:17 and a second vector comprising a nucleic acid sequence encoding SEQ ID NO:19.
- 10 29. A cell comprising:
(a) a first vector comprising a nucleic acid sequence encoding SEQ ID NO:17 and a second vector comprising a nucleic acid sequence encoding SEQ ID NO:19; or
(b) a vector comprising a first nucleic acid sequence encoding SEQ ID NO:17 and a second nucleic acid sequence encoding SEQ ID NO:19.
- 15 30. The cell of claim 29, wherein the cell is a mammalian cell.
- 20 31. A process of producing an antibody comprising culturing the cell of any one of claims 29-30 under conditions such that the antibody is expressed and recovering the expressed antibody from the culture medium.
32. An antibody produced by culturing the cell of any one of claims 29-30 under conditions such that the antibody is expressed and recovering the expressed antibody from the culture medium.
- 25 33. A method of detecting INSL5 in a sample comprising the steps of: contacting the sample with an antibody of any one of claims 1-9 or 17-25; and detecting a signal generated by said step of contacting.
- 30

34. A method of quantifying INSL5 in a sample comprising the steps of: contacting the sample with an antibody of any one of claims 1-9 or 17-25; and detecting a signal generated by said step of contacting.

5 35. The method of claim 34 further comprising the steps of: contacting a control standard with the antibody; and detecting a signal provided by said step of contacting the control standard.

10 36. The method of any one of claims 33-35, further comprising the step of contacting the sample with a second antibody, wherein one of the antibody or the second antibody comprises a detectable label and said step of contacting comprises detecting a signal provided by the detectable label upon formation of a complex comprising the antibody, the second antibody, and INSL5.

15