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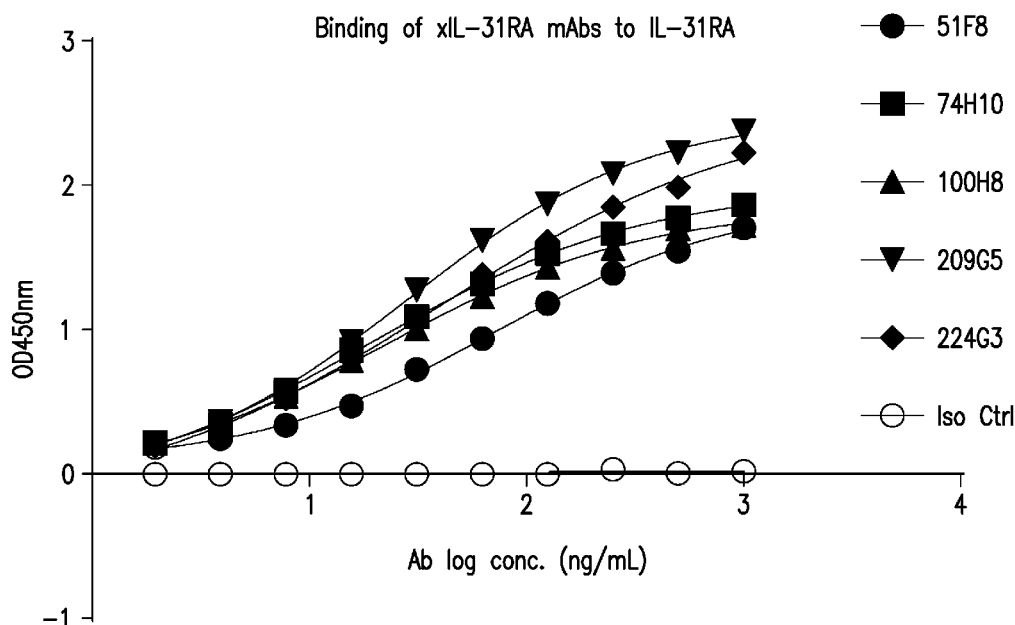


FIG.2A

(57) Abstract: The present invention provides caninized mouse antibodies to canine IL-31 receptor alpha that have a high binding affinity for canine IL-31 receptor alpha, and that can block the binding of canine IL-31 to canine IL-31 receptor alpha. The present invention further provides the use of the antibodies for the treatment of atopic dermatitis in dogs.

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CANINIZED ANTIBODIES TO CANINE INTERLEUKIN-31 RECEPTOR *ALPHA* II

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. The XML file, created on December 9, 2022, is named 25365.xml. This sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) of provisional applications U.S. Serial No. 63/341,443, filed on May 13, 2022, U.S. Serial No. 63/290,259 filed on December 16, 2021, and U.S. Serial No. 63/290,256, filed on December 16, 2021. The subject matter of which are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

The present invention relates to antibodies to canine IL-31 receptor *alpha* that have a high binding affinity for canine IL-31 receptor *alpha*, and that can block the binding of canine IL-31 to the canine IL-31 receptor *alpha*. The present invention also relates to use of the antibodies of the present invention in the treatment of atopic dermatitis in dogs.

BACKGROUND OF THE INVENTION

The immune system comprises a network of resident and recirculating specialized cells that function collaboratively to protect the host against infectious diseases and cancer. The ability of the immune system to perform this function depends to a large extent on the biological activities of a group of proteins secreted by leukocytes and collectively referred to as interleukins. Among the well-studied interleukins are four important molecules identified as interleukin-31 (IL-31), interleukin-4 (IL-4), interleukin-13 (IL-13), and interleukin-22 (IL-22). Although IL-4, IL-13, IL-22, and IL-31, are critical cytokines for the development of immune responses that are required for protection against extracellular pathogens (*e.g.*, tissue or lumen dwelling parasites), these cytokines also have been implicated in the pathogenesis of allergic diseases in humans and animals, including atopic dermatitis.

Atopic dermatitis (AD) is a relapsing pruritic and chronic inflammatory skin disease, that is characterized by immune system dysregulation and epidermal barrier abnormalities in humans.

The pathological and immunological attributes of atopic dermatitis have been the subject of extensive investigations [reviewed in Rahman *et al.* *Inflammation & Allergy-drug target* 10:486-496 (2011) and Harskamp *et al.*, *Seminar in Cutaneous Medicine and Surgery* 32:132-139 (2013)]. Atopic dermatitis is also a common condition in companion animals, especially dogs, where its prevalence has been estimated to be approximately 10-15% of the canine population. The pathogenesis of atopic dermatitis in dogs and cats [reviewed in Nuttall *et al.*, *Veterinary Records* 172(8):201-207 (2013)] shows significant similarities to that of atopic dermatitis in man including skin infiltration by a variety of immune cells and CD4⁺ Th2 polarized cytokine milieu including the preponderance of IL-31, IL-4, and IL-13. In addition, IL-22 has been implicated in the exaggerated epithelial proliferation leading to epidermal hyperplasia that is characteristic of atopic dermatitis.

For example, antibodies against canine IL-31 have been shown to have an effect on pruritus associated with atopic dermatitis in dogs [US 8,790,651 B2; US 10,093,731 B2]. In addition, an antibody against human IL-31 receptor *alpha* (IL-31RA) has been tested and found to have an effect on pruritus associated with atopic dermatitis in humans [Ruzicka, *et al.*, *New England Journal of Medicine*, 376(9),826–835 (2017)].

Pharmaceuticals that have either proven to aid in the treatment of atopic dermatitis and/or have shown promise to do so include: Janus kinase (JAK) inhibitors [see e.g., U.S. 8,133,899; U.S. 8,987,283; WO 2018/108969], spleen tyrosine kinase (SYK) inhibitors [see e.g., U.S. 8,759,366], and antagonists to a chemoattractant receptor-homologous molecule expressed on TH2 cells [see e.g., U.S. 7,696,222, U.S. 8,546,422, U.S. 8,637,541, and U.S. 8,546,422].

However, despite some recent success in treating atopic dermatitis, there remains a need to design alternative and/or better therapies that can address one or more of the symptoms of canine atopic dermatitis.

The citation of any reference herein should not be construed as an admission that such reference is available as "prior art" to the instant application.

SUMMARY OF THE INVENTION

The present invention provides new mammalian antibodies, including caninized murine antibodies, to IL-31 receptor *alpha* (IL-31RA) from canines. In certain embodiments, the mammalian antibodies to canine IL-31 receptor *alpha* (cIL-31RA) are isolated antibodies. In preferred embodiments, the mammalian antibodies or antigen binding fragments thereof bind canine IL-31RA. In more particular embodiments, the mammalian antibodies or antigen binding

fragments also block the binding of canine IL-31RA to canine interleukin-31. In certain embodiments, the mammalian antibodies are antibodies to canine IL-31RA. In more particular embodiments, the mammalian antibodies are caninized antibodies. In even more particular embodiments, the caninized antibodies are caninized murine antibodies to canine IL-31RA.

5 Accordingly, the present invention provides mammalian antibodies or antigen binding fragments thereof that bind canine interleukin-31 receptor *alpha* and that comprise a heavy chain that comprises a set of three heavy chain complementary determining regions (HCDRs), a CDR heavy 1 (HCDR1), a CDR heavy 2 (HCDR2), and a CDR heavy 3 (HCDR3) in which the HCDR1 comprises the amino acid sequence of SEQ ID NO: 3; the HCDR2 comprises the amino
10 acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 13; and the HCDR3 comprises the amino acid sequence of SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, or SEQ ID NO: 23.

 In certain embodiments, the mammalian antibody or antigen binding fragment further comprises a light chain that comprises a set of three light chain complementary determining
15 regions (LCDRs) a CDR light 1 (LCDR1), a CDR light 2 (LCDR2), and a CDR light 3 (LCDR3) in which the LCDR1 comprises the amino acid sequence of SEQ ID NO: 30 or SEQ ID NO: 31; the LCDR2 comprises the amino acid sequence of SEQ ID NO: 38, SEQ ID NO: 39, or SEQ ID NO: 40; and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 45.

 In particular embodiments, when bound to canine IL-31RA the mammalian antibody or
20 antigen binding fragment thereof binds to one or more epitopes comprised by the amino acid sequences of SEQ ID NO: 97, SEQ ID NO: 103, SEQ ID NO: 99, or SEQ ID NO: 102. In more particular embodiments, when bound to canine IL-31RA the mammalian antibody or antigen binding fragment thereof binds to two epitopes, one epitope of which is comprised by the amino acid sequence of SEQ ID NO: 97 and the other epitope is comprised by the amino acid sequence
25 of SEQ ID NO: 103. In other embodiments, when bound to canine IL-31RA the mammalian antibody or antigen binding fragment thereof binds to two epitopes, one epitope of which is comprised by the amino acid sequence of SEQ ID NO: 99 and the other epitope is comprised by the amino acid sequence of SEQ ID NO: 102.

 In particular embodiments, when bound to canine IL-31RA the mammalian antibody or
30 antigen binding fragment thereof binds to an epitope comprised by the amino acid sequence of SEQ ID NO: 97. In other embodiments, when bound to canine IL-31RA the mammalian antibody or antigen binding fragment thereof binds to an epitope comprised by an amino acid sequence of SEQ ID NO: 103. In still other embodiments, when bound to canine IL-31RA the

mammalian antibody or antigen binding fragment thereof binds to an epitope comprised by an amino acid sequence of SEQ ID NO: 99. In yet other embodiments, when bound to canine IL-31RA the mammalian antibody or antigen binding fragment thereof binds to an epitope comprised by an amino acid sequence of SEQ ID NO: 102.

5 In related embodiments, when bound to canine IL-31RA, the antibody binds at least one amino acid residue, preferably one to three amino acid residues, more preferably two to five amino acid residues, and/or more preferably three to eight amino acid residues or more within the amino acid sequence of SEQ ID NO: 97 or SEQ ID NO: 103, or both SEQ ID NO: 97 and SEQ ID NO: 103, and/or SEQ ID NO: 99 or SEQ ID NO: 102, or both SEQ ID NO: 99 and SEQ ID
10 NO: 102.

In particular embodiments, the mammalian antibody or antigen binding fragment thereof comprises an HCDR1 that comprises the amino acid sequence of SEQ ID NO: 3, an HCDR2 that comprises the amino acid sequence of SEQ ID NO: 10, and an HCDR3 that comprises the amino acid sequence of SEQ ID NO: 20. In more particular embodiments of this type, the mammalian
15 antibody or antigen binding fragment thereof further comprises a LCDR1 that comprises the amino acid sequence of SEQ ID NO: 30, a LCDR2 that comprises the amino acid sequence of SEQ ID NO: 40, and the LCDR3 that comprises the amino acid sequence of SEQ ID NO: 45.

In other embodiments, the mammalian antibody or antigen binding fragment thereof comprises an HCDR1 that comprises the amino acid sequence of SEQ ID NO: 3, an HCDR2 that
20 comprises the amino acid sequence of SEQ ID NO: 11, and an HCDR3 that comprises the amino acid sequence of SEQ ID NO: 21. In more particular embodiments of this type, the mammalian antibody or antigen binding fragment thereof further comprises a LCDR1 that comprises the amino acid sequence of SEQ ID NO: 30, a LCDR2 that comprises the amino acid sequence of SEQ ID NO: 40, and the LCDR3 that comprises the amino acid sequence of SEQ ID NO: 45.

25 In still other embodiments, the mammalian antibody or antigen binding fragment thereof comprises an HCDR1 that comprises the amino acid sequence of SEQ ID NO: 3, an HCDR2 that comprises the amino acid sequence of SEQ ID NO: 12, and an HCDR3 that comprises the amino acid sequence of SEQ ID NO: 22. In more particular embodiments of this type, the mammalian antibody or antigen binding fragment thereof further comprises a LCDR1 that comprises the
30 amino acid sequence of SEQ ID NO: 30, a LCDR2 that comprises the amino acid sequence of SEQ ID NO: 38, and the LCDR3 that comprises the amino acid sequence of SEQ ID NO: 45.

In yet other embodiments, the mammalian antibody or antigen binding fragment thereof comprises an HCDR1 that comprises the amino acid sequence of SEQ ID NO: 3, an HCDR2 that

comprises the amino acid sequence of SEQ ID NO: 13, and an HCDR3 that comprises the amino acid sequence of SEQ ID NO: 23. In more particular embodiments of this type, the mammalian antibody or antigen binding fragment thereof further comprises a LCDR1 that comprises the amino acid sequence of SEQ ID NO: 31, a LCDR2 that comprises the amino acid sequence of SEQ ID NO: 39, and the LCDR3 that comprises the amino acid sequence of SEQ ID NO: 45.

In preferred embodiments, the antibody and antigen binding fragment thereof bind canine IL-31RA and block the binding of canine IL-31RA to canine interleukin-31. In specific embodiments, the mammalian antibody to canine IL-31RA is a murine antibody. In particular embodiments, the mammalian antibody to canine IL-31RA is a caninized antibody. In more particular embodiments, the caninized antibody to canine IL-31RA is a caninized murine antibody.

The caninized antibodies of the present invention comprise a canine fragment crystallizable region (cFc region). The caninized antibodies of the present invention also comprise a canine light chain constant region. In particular embodiments the canine light chain constant region is a *kappa* canine light chain constant region. In more specific embodiments, the *kappa* canine light chain constant region comprises the amino acid sequence of SEQ ID NO: 127.

Furthermore the caninized antibody or antigen binding fragment thereof can comprise a heavy chain that comprises a cFc region and a hinge region. The hinge region is preferably a canine hinge region. The canine hinge region can comprise a natural occurring: IgG-A hinge region, IgG-B hinge region, IgG-C hinge region, or IgG-D hinge region. Alternatively, the hinge region is a corresponding modified canine hinge region. In particular embodiments, the hinge region is the IgG-A hinge region comprising an amino acid sequence comprising at least 90%, 95%, or 100% identity with the amino acid sequence of SEQ ID NO: 112. In other embodiments, the hinge region is the IgG-B hinge region comprising an amino acid sequence comprising at least 90%, 95%, or 100% identity with the amino acid sequence of SEQ ID NO: 113. In still other embodiments, the hinge region is the IgG-C hinge region comprising an amino acid sequence comprising at least 90%, 95%, or 100% identity with the amino acid sequence of SEQ ID NO: 114. In yet other embodiments, the hinge region is a modified IgG-D hinge region comprising the amino acid sequence of SEQ ID NO: 115.

Similarly, the canine Fc region can be an IgG-A, IgG-B, IgG-C, an IgG-D or modifications thereof. In particular embodiments, a caninized antibody or antigen binding fragment thereof comprises an IgG-Bm. In certain embodiments, a caninized antibody or antigen binding fragment thereof comprises an IgG-A that comprises an amino acid sequence that has at

least 90%, 95%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 116.

In other embodiments, a caninized antibody or antigen binding fragment thereof comprises an IgG-B that comprises an amino acid sequence that has at least 90%, 95%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 110. In still other embodiments, a

5 caninized antibody or antigen binding fragment thereof comprises an IgG-C that comprises an amino acid sequence that has at least 90%, 95%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 117. In yet other embodiments, a caninized antibody or antigen binding fragment thereof comprises an IgG-D that comprises an amino acid sequence that has at least 90%, 95%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 118.

10 In still other embodiments, a caninized antibody or antigen binding fragment thereof comprises an IgG-Bm that comprises an amino acid sequence that has at least 90%, 95%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 111, wherein both the aspartic acid residue (D) at position 31 of SEQ ID NO: 110 and the asparagine residue (N) at position 63 of SEQ ID NO: 110, remain substituted by an alanine residue (A) in the sequence of IgG-Bm.

15 In particular embodiments, the caninized antibody or antigen binding fragment thereof comprises the canine IgG-D, but the naturally occurring IgG-D hinge region is replaced by a hinge region comprising an amino acid sequence comprising at least 90%, 95%, or 100% identity with the amino acid sequence of SEQ ID NO: 112. In other embodiments, the caninized antibody comprises a heavy chain that comprises an IgG-D, but the naturally occurring IgG-D
20 hinge region is replaced by a hinge region comprising an amino acid sequence has at least 90%, 95%, or 100% identity with the amino acid sequence of SEQ ID NO: 113. In still other embodiments, the caninized antibody comprises a heavy chain that comprises an IgG-D, but the naturally occurring IgG-D hinge region is replaced by a hinge region comprising an amino acid sequence has at least 90%, 95%, or 100% identity with the amino acid sequence of SEQ ID
25 NO: 114. In yet other embodiments, the caninized antibody comprises a heavy chain that comprises an IgG-D, but the naturally occurring IgG-D hinge region is replaced by a hinge region comprising the amino acid sequence of SEQ ID NO: 115.

In certain embodiments of the compositions, the caninized antibody against canine IL-31RA comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 79, SEQ
30 ID NO: 80, or SEQ ID NO: 81 and a light chain comprising the amino acid sequence of SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, or SEQ ID NO: 85. The present invention further provides antigen binding fragments of these caninized antibodies.

In particular embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 84 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 80. In other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 84 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 81. In yet other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 84 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 79.

In still other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 85 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 80. In yet other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 85 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 81. In still other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 85 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 79.

In yet other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 83 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 80. In still other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 83 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 81. In yet other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 83 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 79.

In still other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 82 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 80. In yet other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 82 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 81. In still other embodiments, the caninized antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 82 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 79.

In specific embodiments, when bound to canine IL-31RA, the mammalian antibody (*e.g.*, a caninized antibody) binds to an epitope comprised by the amino acid of SEQ ID NO: 97 or SEQ ID NO: 103, or to both SEQ ID NO: 97 and SEQ ID NO: 103. In particular embodiments, the identification of the epitopes is based on chemical crosslinking and mass spectrometry detection. In related embodiments, when bound to canine IL-31RA, the mammalian antibody

binds at least one amino acid residue, preferably one to three amino acid residues, more preferably two to five amino acid residues, and/or more preferably three to eight amino acid residues or more within the amino acid sequence of SEQ ID NO: 97 or SEQ ID NO: 103, or within both SEQ ID NO: 97 and SEQ ID NO: 103.

5 In particular embodiments, the mammalian antibody that binds to SEQ ID NO: 97, binds to the tyrosine residue at position 94 of SEQ ID NO. 2, *i.e.* Y₉₄. In other embodiments, the mammalian antibody binds to the lysine residue at position 102 of SEQ ID NO. 2, *i.e.* K₁₀₂. In still other embodiments, the mammalian antibody binds to the lysine residue at position 112 of SEQ ID NO. 2, *i.e.* K₁₁₂. In yet other embodiments, the mammalian antibody binds to binds to
10 the tyrosine residue at position 94 of SEQ ID NO. 2, and the lysine residue at position 102 of SEQ ID NO. 2. In still other embodiments, the mammalian antibody binds to the lysine residue at position 102 of SEQ ID NO. 2, and the lysine residue at position 112 of SEQ ID NO. 2. In yet other embodiments, the mammalian antibody binds to binds to the tyrosine residue at position 94 of SEQ ID NO. 2, and the lysine residue at position 112 of SEQ ID NO. 2. In still other
15 embodiments, the mammalian antibody binds to the tyrosine residue at position 94, binds to the lysine residue at position 102 of SEQ ID NO. 2, and the lysine residue at position 112 of SEQ ID NO. 2.

In related embodiments, the mammalian antibody that binds to SEQ ID NO: 103, binds to the arginine residue at position 183 of SEQ ID NO. 2, *i.e.* R₁₈₃. In other embodiments, the
20 mammalian antibody binds to the serine residue at position 193 of SEQ ID NO. 2, *i.e.* S₁₉₃. In still other embodiments, the mammalian antibody binds to the threonine residue at position 202 of SEQ ID NO. 2, *i.e.* T₂₀₂. In yet other embodiments, the mammalian antibody binds to binds to the arginine residue at position 183 of SEQ ID NO. 2, and the serine residue at position 193 of SEQ ID NO. 2. In still other embodiments, the mammalian antibody binds to the serine residue
25 at position 193 of SEQ ID NO. 2, and the threonine residue at position 202 of SEQ ID NO. 2. In yet other embodiments, the mammalian antibody binds to binds to the arginine residue at position 183 of SEQ ID NO. 2, and the threonine residue at position 202 of SEQ ID NO. 2. In still other embodiments, the mammalian antibody binds to the arginine residue at position 183 of SEQ ID NO. 2, binds to the serine residue at position 193 of SEQ ID NO. 2, and the threonine residue at
30 position 202 of SEQ ID NO. 2. The present invention further provides antigen binding fragments of these mammalian antibodies.

The present invention also provides nucleic acids, including isolated nucleic acids, that encode any of: the sets of 3 HCDRs or 3 LCDRs; the heavy chain variable regions of the

caninized antibodies or antigen binding fragments thereof; the heavy chains of the caninized antibodies or antigen binding fragments thereof, the light chain variable regions of the caninized antibodies or antigen binding fragments thereof, and/or the light chains of the caninized antibodies or antigen binding fragments thereof. The present invention further provides a pair of
5 nucleic acids, wherein one of the pair of nucleic acids comprises a nucleotide sequence that encodes the light chain of a specific caninized antibody of any one of the antibodies of the present invention and the other of the pair of nucleic acids comprises a nucleotide sequence that encodes the heavy chain of that (said) specific caninized antibody. The present invention further provides expression vectors that comprise such pairs of nucleic acids, or alternatively individual
10 nucleic acids of the present invention. In addition, the present invention provides pairs of expression vectors, wherein one of the pair of expression vectors comprises a nucleic acid comprising a nucleotide sequence that encodes the light chain of a specific caninized antibody of any one of the antibodies of the present invention, and the other of the pair of expression vectors comprises a nucleic acid comprising a nucleotide sequence that encodes the heavy chain of that
15 (said) specific caninized antibody.

Accordingly, the present invention further provides nucleic acids that encode a set of the three heavy chain complementary determining regions (CDRs), a CDR heavy 1 (HCDR1), a CDR heavy 2 (HCDR2), and a CDR heavy 3 (HCDR3) of a mammalian antibody (including a caninized antibody) of the present invention. In more specific embodiments, the nucleic acid
20 encodes an HCDR1 that comprises the amino acid sequence of SEQ ID NO: 3; the HCDR2 that comprises the amino acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 13; and the HCDR3 that comprises the amino acid sequence of SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, or SEQ ID NO: 23; or any combination thereof.

The present invention further provides nucleic acids that encodes a set of the three light
25 chain complementary determining regions (CDRs), a CDR light 1 (LCDR1), a CDR light 2 (LCDR2), and a CDR light 3 (LCDR3) of a mammalian antibody (including a caninized antibody) or an antigen binding fragment thereof of the present invention. In more specific embodiment of this type, the nucleic acid encodes a LCDR1 that comprises the amino acid sequence of SEQ ID NO: 30 or SEQ ID NO: 31; a LCDR2 that comprises the amino acid
30 sequence of SEQ ID NO: 38, SEQ ID NO: 39, or SEQ ID NO: 40; and a LCDR3 that comprises the amino acid sequence of SEQ ID NO: 45; or any combination thereof.

The present invention further provides nucleic acids that encode any of the heavy chains of a mammalian antibody (including a caninized antibody) or an antigen binding fragment

thereof of the present invention. The present invention also provides nucleic acids that encode any of the light chains of a mammalian antibody (including a caninized antibody) or an antigen binding fragment thereof of the present invention. In addition, the present invention provides expression vectors that comprise and can express one or more of the nucleic acids of the present invention, and host cells that comprise one or more of such expression vectors.

The present invention further provides pharmaceutical compositions that comprise the caninized antibodies and antigen binding fragments thereof of the present invention along with a pharmaceutically acceptable carrier and/or diluent. The present invention further provides pharmaceutical compositions that comprise a nucleic acid of the present invention, along with a pharmaceutically acceptable carrier and/or diluent, and/or an expression vector that comprise one or more of the nucleic acids of the present invention, along with a pharmaceutically acceptable carrier and/or diluent.

The present invention also provides methods of treating atopic dermatitis comprising administering one of the aforesaid pharmaceutical compositions to an animal subject that has atopic dermatitis. In particular embodiments, the animal subject is a canine. The present invention also provides methods of aiding in blocking pruritus associated with atopic dermatitis in an animal subject, comprising administering to an animal subject in need thereof of a therapeutically effective amount of a pharmaceutical composition of the present invention. In particular embodiments, the animal subject is a canine.

In addition, the present invention provides methods of producing a caninized antibody or antigen binding fragment thereof that binds canine IL-31RA. In particular embodiments, the method includes culturing a host cell comprising one or more expression vectors that encode and express the light chain of a caninized antibody of the present invention and the heavy chain of that caninized antibody in a culture medium under conditions in which the nucleic acid is expressed, thereby producing a polypeptide comprising the light chain of a caninized antibody of the present invention, and/or the heavy chain of that caninized antibody. The polypeptides are then recovered from the host cell or culture medium. In certain embodiments, the polypeptides comprising the light chain of a caninized antibody of the present invention and the polypeptides comprising the heavy chain of that caninized antibody are combined with each other under conditions that are conducive to form a caninized antibody.

These and other aspects of the present invention will be better appreciated by reference to the following Brief Description of the Drawings and the Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the binding of IL-31 to IL-31RA. The extracellular domain (ECD) of canine IL-31RA was tested for its ability to bind to canine IL-31. The results indicate that IL-31RA ECD binds in a dose-dependent manner to biotinylated canine IL-31 with an EC₅₀ of 0.55 ng/ml.

Figures 2A-2B show the binding of xIL-31RA monoclonal antibodies (mAbs) to IL-31RA. The selected mouse mAbs were tested for their reactivity to canine IL-31RA. The results indicate that the selected mouse mAbs bind to canine IL-31RA in a dose-dependent manner. All of the 10 mouse monoclonal antibodies have strong binding reactivity to canine IL-31RA.

Figure 2A depicts mouse mAbs: 51F8 (●), 74H10 (■), 100H8(▲), 209G5 (▼), 224G3(◆), and Iso control (○). Figure 2B depicts mouse mAbs: 55B3 (●), 65G9 (■), 85C10 (▲), 218D9 (▼), 227E7(◆), and Iso control (○).

Figure 3 shows the blocking of the binding of IL-31 to IL-31RA by monoclonal antibodies (mAbs) to IL-31RA. The selected mouse mAbs (anti-canine IL-31RA) were tested for their ability to block the binding of IL-31 with IL-31RA/OSMR by Flow Cytometry. The FACS result indicates that the ten mouse mAbs can block the binding of IL-31 with the IL-31RA/OSMR complex presented on CHO- IL-31RA/OSMR cells. Antibodies 51F8, 74H10, 100H8, 209G5, and 218D9 exhibited superior blocking activity.

Figure 4 shows the induction of STAT-3 phosphorylation by IL-31. Ba/f3-OI cells expressing the IL-31 receptor complex were tested for IL-31-induced STAT-3 phosphorylation. The results indicate that STAT-3 phosphorylation was induced by IL 31 in the Baf3-OI cells (■) in a dose-dependent manner, implying that: (i) the canine IL-31 receptor complex is successfully expressed on the cell surface; (ii) that the binding of canine IL-31 to the IL-31 receptor can stimulate the endogenous STAT3 phosphorylation; and (iii) then initiate its downstream signaling pathway. Ba/f3 cells (○) were used as the control.

Figures 5A-5B show the inhibition of IL-31-mediated STAT-3 phosphorylation in Ba/f3-OI cells by the selected xIL-31RA antibodies. The results indicate that the selected mAbs inhibit IL-31 mediated STAT-3 phosphorylation in a dose-dependent manner in Ba/f3-OI cells. Figure 5A depicts mouse mAbs 209G5 (■), 218D9 (▲), 85C10 (▼), and IL-31 protein (◆). Figure 5B depicts mouse mAbs 100H8 (●), 74H10 (■), 85C10 (▲), 51F8 (▼), and cIL-31 protein (◆).

Figures 6A-6E provides the epitopes on canine IL-31RA for the antibodies 100H8, 51F8, 218D9, 85C10, and 224G3, respectively. Figure 6A depicts the epitope for 100H8; the epitope

comprises the amino acid sequences of SEQ ID NO: 97 (within SEQ ID NO: 119) and SEQ ID NO: 103 (within SEQ ID NO: 120), respectively. Figure 6B depicts the epitope for 51F8; the epitope comprises the amino acid sequences of SEQ ID NO: 98 (within SEQ ID NO: 121) and SEQ ID NO: 100 (within SEQ ID NO: 122). Figure 6C depicts the epitope for 218D9; the epitope comprises the amino acid sequences of SEQ ID NO: 104 (within SEQ ID NO: 123) and SEQ ID NO: 105 (within SEQ ID NO: 124), respectively. Figure 6D depicts the epitope for 85C10; the epitope comprises the amino acid sequences of SEQ ID NO: 106 (within SEQ ID NO: 125) and SEQ ID NO: 108 (within SEQ ID NO: 126), respectively. Figure 6E depicts the epitope for 224G3; the epitope comprises the amino acid sequence of SEQ ID NO: 109 (also within SEQ ID NO: 126). The position of binding residues of the amino acid sequence of SEQ ID NO: 2 for the respective epitopes on the cIL-31R ECD antigen are also denoted.

Figures 7A-7E provides plots for the binding activity of the identified murine-canine chimeric or caninized antibodies to canine IL-31RA. The results indicate that the caninized antibodies have similar binding affinity as their corresponding parental antibodies (as represented by the murine-canine chimeric antibodies). Figure 7A depicts the binding plots for monoclonal 51F8 antibodies: Chimeric 51F8 (●) c51F8VH3VL6 (■), c51F8VH3VL7 (▲), c51F8VH4VL6 (▼), and c51F8VH4VL7 (◆); and the iso control (○). Figure 7B depicts the binding plots for monoclonal 100H8 antibodies: Chimeric 100H8 (●), c100H8VH5VL4 (■), and c100H8VH7VL4 (▲). Figure 7C depicts the binding plots for monoclonal 85C10 antibodies: Chimeric 85C10 (●), c85C10VH3VL2 (■), and c85C10VH1VL2 (▲). Figure 7D depicts the binding plots for monoclonal 218D9 antibodies: Chimeric 218D9 (●), c218D9VH3VL2 (■), c218D9VH3VL3 (▲), c218D9VH4VL2 (▼), and c218D9VH4VL3 (◆); and the iso control (○). Figure 7E depicts the binding plots for monoclonal 224G3 antibodies: m224G3 Chim (●), c224G3VH2VL2 (■), and c224G3VH2VL3 (▲). The EC50 is provided in the Tables

The term “Chimeric” before the antibody number signifies that the antibody is a murine-canine chimeric antibody, *e.g.*, Chimeric 218D9 or Chimeric 51F8. In addition, an “m” before the antibody number followed by a “Chim” signifies that the antibody is a murine-canine chimeric antibody, *e.g.*, m224G3 Chim. The lower case “c” before the antibody number signifies that it is a caninized antibody, *e.g.*, c218D9VH4VL2.

Figure 8 shows the blocking of the binding of IL-31 to IL-31RA by the inhibition of the IL-31-mediated STAT-3 phosphorylation in Ba/f3-OI cells. The results indicate that the

caninized 218D9 antibodies can inhibit IL-31 mediated STAT-3 phosphorylation in a dose-dependent manner in Ba/f3-OI cells, and that the constructs c218D9VH3VL3 and c218D9VH4VL3 have the same inhibitory activity as the parental mouse-canine chimeric 218D9 antibody: Chimeric 218D9 (●), c218D9VH3VL2 (■), c218D9VH3VL3 (▲), c218D9VH4VL2 (▼), and c218D9VH4VL3 (◆); and the IL-31 only control (○).

DETAILED DESCRIPTION OF THE INVENTION

In response to need for better therapies for atopic dermatitis, the present invention provides formulations and methodology that can achieve a significant effect on the skin inflammation associated with atopic dermatitis.

ABBREVIATIONS

Throughout the detailed description and examples of the invention the following abbreviations will be used:

ADCC	Antibody-dependent cellular cytotoxicity
15 CDC	Complement-dependent cytotoxicity
CDR	Complementarity determining region in the immunoglobulin variable regions, defined using the Kabat numbering system
EC50	concentration resulting in 50% efficacy or binding
ELISA	Enzyme-linked immunosorbant assay
20 FR	Antibody framework region: the immunoglobulin variable regions excluding the CDR regions
IC50	concentration resulting in 50% inhibition
IgG	Immunoglobulin G
Kabat	An immunoglobulin alignment and numbering system pioneered by Elvin A. Kabat [<i>Sequences of Proteins of Immunological Interest</i> , 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)]
25 mAb	Monoclonal antibody (also Mab or MAb)
V region	The segment of IgG chains which is variable in sequence between different antibodies.
30 VH	Immunoglobulin heavy chain variable region
VL	Immunoglobulin light chain variable region

DEFINITIONS

So that the invention may be more readily understood, certain technical and scientific terms are specifically defined below. Unless specifically defined elsewhere in this document, all other technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this invention belongs.

5 As used herein, including the appended claims, the singular forms of words such as "a," "an," and "the," include their corresponding plural references unless the context clearly dictates otherwise.

"Administration" and "treatment", as it applies to an animal, *e.g.*, a canine subject, cell, tissue, organ, or biological fluid, refers to contact of an exogenous pharmaceutical, therapeutic,
10 diagnostic agent, or composition to the animal *e.g.*, a canine subject, cell, tissue, organ, or biological fluid. Treatment of a cell encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell.

"Administration" and "treatment" also mean *in vitro* and *ex vivo* treatments, *e.g.*, of a cell, by a reagent, diagnostic, binding compound, or by another cell. The term "subject" includes any
15 organism, preferably an animal, more preferably a mammal (*e.g.*, canine or feline) and most preferably a canine.

"Treat" or "treating" means to administer a therapeutic agent, such as a composition containing any of the antibodies of the present invention, internally or externally to *e.g.*, a canine subject or patient having one or more symptoms, or being suspected of having a condition, for
20 which the agent has therapeutic activity. Typically, the agent is administered in an amount effective to alleviate and/or ameliorate one or more disease/condition symptoms in the treated subject or population, whether by inducing the regression of or inhibiting the progression of such symptom(s) by any clinically measurable degree. The amount of a therapeutic agent that is effective to alleviate any particular disease/condition symptom (also referred to as the
25 "therapeutically effective amount") may vary according to factors such as the disease/condition state, age, and weight of the patient (*e.g.*, canine), and the ability of the pharmaceutical composition to elicit a desired response in the subject. Whether a disease/condition symptom has been alleviated or ameliorated can be assessed by any clinical measurement typically used by veterinarians or other skilled healthcare providers to assess the severity or progression status of
30 that symptom. While an embodiment of the present invention (*e.g.*, a treatment method or article of manufacture) may not be effective in alleviating the target disease/condition symptom(s) in every subject, it should alleviate the target disease/condition symptom(s) in a statistically significant number of subjects as determined by any statistical test known in the art such as the

Student's t-test, the χ^2 -test, the U-test according to Mann and Whitney, the Kruskal-Wallis test (H-test), Jonckheere-Terpstra-test and the Wilcoxon-test.

"Treatment," as it applies to a human, veterinary (*e.g.*, canine), or research subject, refers to therapeutic treatment, as well as research and diagnostic applications. "Treatment" as it applies to a human, veterinary (*e.g.*, canine), or research subject, or cell, tissue, or organ, encompasses contact of the antibodies of the present invention to *e.g.*, a canine or other animal subject, a cell, tissue, physiological compartment, or physiological fluid.

As used herein, the term "canine" includes all domestic dogs, *Canis lupus familiaris* or *Canis familiaris*, unless otherwise indicated.

As used herein, the term "feline" refers to any member of the *Felidae* family. Members of this family include wild, zoo, and domestic members, including domestic cats, pure-bred and/or mongrel companion cats, show cats, laboratory cats, cloned cats, and wild or feral cats

As used herein the term "canine frame" refers to the amino acid sequence of the heavy chain and light chain of a canine antibody other than the hypervariable region residues defined herein as CDR residues. With regard to a caninized antibody, in the majority of embodiments the amino acid sequences of the native canine CDRs are replaced with the corresponding foreign CDRs (*e.g.*, those from a mouse or rat antibody) in both chains. Optionally the heavy and/or light chains of the canine antibody may contain some foreign non-CDR residues, *e.g.*, so as to preserve the conformation of the foreign CDRs within the caninized antibody, and/or to modify the Fc function, as exemplified below and/or disclosed in U.S. 10,106,607 B2, hereby incorporated by reference herein in its entirety.

The "Fragment crystallizable region" abbreviated as "Fc" or as used interchangeably "Fc region" corresponds to the CH3-CH2 portion of an antibody that interacts with cell surface receptors called Fc receptors. The canine fragment crystallizable region (cFc region) of each of the four canine IgGs were first described by Tang *et al.* [*Vet. Immunol. Immunopathol.* 80: 259-270 (2001); *see also*, Bergeron *et al.*, *Vet. Immunol. Immunopathol.* 157: 31-41 (2014) and U.S. 10,106,607 B2].

As used herein the canine Fc (cFc) "IgG-Bm" is canine IgG-B Fc comprising two (2) amino acid residue substitutions, D31A and N63A, as in the amino acid sequence of SEQ ID NO: 111 of IgG-B (*see below*) and without the c-terminal lysine ('K'). Both the aspartic acid residue (D) at position 31 of SEQ ID NO: 110 and the asparagine residue (N) at position 63 of SEQ ID NO: 110, are substituted by an alanine residue (A) in IgG-Bm. These two amino acid residue substitutions serve to significantly diminish the antibody-dependent cytotoxicity (ADCC)

and complement-dependent cytotoxicity (CDC) of the naturally occurring canine IgG-B [*see*, U.S. 10,106,607 B2, the contents of which are hereby incorporated by reference in their entirety]. Further amino acid substitutions to the IgG-Bm are also envisioned, which parallel those which can be made in IgG-B and may include amino acid substitutions to favor heterodimer formation in bispecific antibodies. The amino acid sequence of IgG-B, SEQ ID NO: 110 is:

1 50
LGGPSVFIFP PKPKDTLLIA RTPEVTCVVV DLDPEDPEVQ ISWFVDGKQM
↳ CH2

51 100
10 QTAKTQPREE QFNGTYRVVS VLPIGHQDWL KGKQFTCKVN NKALPSPIER

101 150
TISKARGQAH QPSVYVLPPS REELSKNTVS LTCLIKDFFP PDIDVEWQSN
↳ CH3

15 151 200
GQQEPESKYR TTPPQLDEDG SYFLYSKLSV DKSRWQRGDT FICAVMHEAL

201 215
HNHYTQKSLS HSPGK

The amino acid sequence of IgG-Bm, SEQ ID NO: 111, is provided below.

LGGPSVFIFPPKPKDTLLIARTPEVTCVVVALDPEDEPEVQISWFVDGKQMOTAKTQPREEQFAGT
YRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVS
LTCLIKDFFPPDIDVEWQSNQEQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAV
25 MHEALHNHYTQESLSHSPG

As used herein, a “substitution of an amino acid residue” with another amino acid residue in an amino acid sequence of an antibody for example, is equivalent to “replacing an amino acid residue” with another amino acid residue and denotes that a particular amino acid residue at a specific position in the amino acid sequence has been replaced by (or substituted for) by a different amino acid residue. Such substitutions can be particularly designed *i.e.*, purposefully replacing an alanine with a serine at a specific position in the amino acid sequence by *e.g.*, recombinant DNA technology. Alternatively, a particular amino acid residue or string of amino acid residues of an antibody can be replaced by one or more amino acid residues through more natural selection processes *e.g.*, based on the ability of the antibody produced by a cell to bind to a given region on that antigen, *e.g.*, one containing an epitope or a portion thereof, and/or for the

antibody to comprise a particular CDR that retains the same canonical structure as the CDR it is replacing. Such substitutions/replacements can lead to “variant” CDRs and/or variant antibodies.

As used herein, the term "antibody" refers to any form of antibody that exhibits the desired biological activity. An antibody can be a monomer, dimer, or larger multimer. Thus, it is used in the broadest sense and specifically covers, but is not limited to, monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multi-specific antibodies (e.g., bispecific antibodies), caninized antibodies, fully canine antibodies, chimeric antibodies and camelized single domain antibodies. "Parental antibodies" are antibodies obtained by exposure of an immune system to an antigen prior to modification of the antibodies for an intended use, such as caninization of an antibody for use as a canine therapeutic antibody.

As used herein, antibodies of the present invention that "block" or is “blocking” or is “blocking the binding” of e.g., a canine receptor to its binding partner (ligand), is an antibody that blocks (partially or fully) the binding of the canine receptor to its canine ligand and *vice versa*, as determined in standard binding assays (e.g., BIAcore®, ELISA, or flow cytometry).

Typically, an antibody or antigen binding fragment of the invention retains at least 10% of its canine antigen binding activity (when compared to the parental antibody) when that activity is expressed on a molar basis. Preferably, an antibody or antigen binding fragment of the invention retains at least 20%, 50%, 70%, 80%, 90%, 95% or 100% or more of the canine antigen binding affinity as the parental antibody. It is also intended that an antibody or antigen binding fragment of the invention can include conservative or non-conservative amino acid substitutions (referred to as "conservative variants" or "function conserved variants" of the antibody) that do not substantially alter its biologic activity.

"Isolated antibody" refers to the purification status and in such context means the molecule is substantially free of other biological molecules such as nucleic acids, proteins, lipids, carbohydrates, or other material such as cellular debris and growth media. Generally, the term "isolated" is not intended to refer to a complete absence of such material or to an absence of water, buffers, or salts, unless they are present in amounts that substantially interfere with experimental or therapeutic use of the binding compound as described herein.

As used herein, an antibody is said to bind specifically to a polypeptide comprising a given antigen sequence (in this case a portion of the amino acid sequence of canine IL-31RA) if it binds to polypeptides comprising the portion of the amino acid sequence of canine IL-31RA, but does not bind to other canine proteins lacking that portion of the sequence of canine IL-31RA. For example, an antibody that specifically binds to a polypeptide comprising canine

IL-31RA, may bind to a FLAG[®]-tagged form of canine IL-31RA, but will not bind to other FLAG[®]-tagged canine proteins.

As used herein, unless otherwise indicated, "antibody fragment" or "antigen binding fragment" refers to antigen binding fragments of antibodies, *i.e.* antibody fragments that retain the ability to bind specifically to the antigen (*e.g.*, canine IL-31RA) bound by the full-length antibody, *e.g.* fragments that retain one or more CDR regions. Examples of antigen binding fragments include, but are not limited to, Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules, *e.g.*, sc-Fv; nanobodies and multispecific antibodies formed from antibody fragments.

An antibody, or binding compound derived from the antigen-binding site of an antibody, binds to its canine antigen, or a variant or mutein thereof, "with specificity" when it has an affinity for that canine antigen or a variant or mutein thereof which is at least ten-times greater, more preferably at least 20-times greater, and even more preferably at least 100-times greater than its affinity for any other canine antigen tested.

As used herein, a "chimeric antibody" is an antibody having the variable domain from a first antibody and the constant domain from a second antibody, where the first and second antibodies are from different species. [U.S. 4,816,567; and Morrison *et al.*, *Proc. Natl. Acad. Sci. USA* 81: 6851-6855 (1984)]. Typically the variable domains are obtained from an antibody from an experimental animal (the "parental antibody"), such as a rodent, and the constant domain sequences are obtained from the animal subject antibodies, *e.g.*, human or canine so that the resulting chimeric antibody will be less likely to elicit an adverse immune response in a human or canine subject respectively, than the parental (*e.g.*, rodent) antibody.

As used herein, the term "caninized antibody" refers to forms of antibodies that contain sequences from both canine and non-canine (*e.g.*, mouse) antibodies. In general, the caninized antibody will comprise substantially all of at least one or more typically, two variable domains in which all or substantially all of the hypervariable loops correspond to those of a non-canine immunoglobulin (*e.g.*, comprising 6 CDRs as exemplified below), and all or substantially all of the framework (FR) regions (and typically all or substantially all of the remaining frame) are those of a canine immunoglobulin sequence. As exemplified herein, a caninized antibody comprises both the three heavy chain CDRs and the three light chain CDRs from a murine (mouse) anti-canine antigen antibody together with a canine frame or a modified canine frame. A modified canine frame comprises one or more amino acids changes as exemplified herein that further optimize the effectiveness of the caninized antibody, *e.g.*, to increase its binding to its

canine antigen and/or its ability to block the binding of that canine antigen to the canine antigen's natural binding partner.

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, in general, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are, in general, the same. Typically, the variable domains of both the heavy and light chains comprise three hypervariable regions, also called complementarity determining regions (CDRs), located within relatively conserved framework regions (FR). The CDRs are usually aligned by the framework regions, enabling binding to a specific epitope. In general, from N-terminal to C-terminal, both light and heavy chains variable domains comprise FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is, generally, in accordance with the definitions of *Sequences of Proteins of Immunological Interest*, Kabat, *et al.*; National Institutes of Health, Bethesda, Md. ; 5th ed.; NIH Publ. No. 91-3242 (1991); Kabat, *Adv. Prot. Chem.* 32:1-75 (1978); Kabat, *et al.*, *J. Biol. Chem.* 252:6609-6616 (1977); Chothia, *et al.*, *J. Mol. Biol.* 196:901-917 (1987) or Chothia, *et al.*, *Nature* 342:878-883 (1989)].

As used herein, the term "hypervariable region" refers to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.* LCDR1, LCDR2 and LCDR3 in the light chain variable domain and HCDR1, HCDR2 and HCDR3 in the heavy chain variable domain). [See Kabat *et al. Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), defining the CDR regions of an antibody by sequence; *see also* Chothia and Lesk, *J. Mol. Biol.* 196: 901-917 (1987) defining the CDR regions of an antibody by structure]. As used herein, the term "framework" or "FR" residues refers to those variable domain residues other than the hypervariable region residues defined herein as CDR residues.

There are four known IgG heavy chain subtypes of dog IgG and they are referred to as IgG-A, IgG-B, IgG-C, and IgG-D. The two known light chain subtypes are referred to as *lambda* and *kappa*. In specific embodiments of the invention, besides binding canine IL-31RA, a canine or caninized antibody against its antigen of the present invention optimally has two attributes:

- 1) Lack of effector functions such as antibody-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and
- 2) be readily purified on a large scale using industry standard technologies such as that based on protein A chromatography.

None of the naturally occurring canine IgG isotypes satisfy both criteria. For example, IgG-B can be purified using protein A, but has high level of ADCC activity. On the other hand, IgG-A binds weakly to protein A, but also displays ADCC activity. Moreover, neither IgG-C nor IgG-D can be purified on protein A columns, although IgG-D displays no ADCC activity.

5 (IgG-C has considerable ADCC activity). One way the present invention addresses these issues in certain embodiments is by providing modified canine IgG-B antibodies of the present invention specific to an antigen of the present invention that lack the effector functions such as ADCC and can be easily purified using industry standard protein A chromatography.

10 As used herein an “antipruritic agent” is a compound, macromolecule, and/or formulation that tends to inhibit, relieve, and/or prevent itching. Antipruritic agents are colloquially referred to as anti-itch drugs.

As used herein an “antipruritic antibody” is an antibody that can act as an antipruritic agent in an animal, including a mammal such as a human, a canine, and/or a feline, particularly with respect to atopic dermatitis. In particular embodiments, the antipruritic antibody binds to 15 specific proteins in the IL-31 signaling pathway, such as IL-31 or its receptor IL-31RA. The binding of the antipruritic antibody to its corresponding antigen (*e.g.*, IL-31 or IL-31RA) inhibits the binding of *e.g.*, IL-31 with IL-31RA, and interferes with and/or prevents the successful signaling of this pathway, and thereby inhibits, relieves, and/or prevents the itching that is otherwise caused by the IL-31 signaling pathway.

20 “Homology”, as used herein, refers to sequence similarity between two polynucleotide sequences or between two polypeptide sequences when they are optimally aligned. When a position in both of the two compared sequences is occupied by the same base or amino acid residue, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology is the number of 25 homologous positions shared by the two sequences divided by the total number of positions compared $\times 100$. For example, if 6 of 10 of the positions in two sequences are matched or homologous when the sequences are optimally aligned then the two sequences are 60% homologous. Generally, the comparison is made when two sequences are aligned to give maximum percent homology.

30 Sequence identity refers to the degree to which the amino acids of two polypeptides are the same at equivalent positions when the two sequences are optimally aligned. As used herein one amino acid sequence is 100% “identical” to a second amino acid sequence when the amino acid residues of both sequences are identical. Accordingly, an amino acid sequence is 50%

"identical" to a second amino acid sequence when 50% of the amino acid residues of the two amino acid sequences are identical. The sequence comparison is performed over a contiguous block of amino acid residues comprised by a given protein, *e.g.*, a protein, or a portion of the polypeptide being compared. In particular embodiments, selected deletions or insertions that could otherwise alter the correspondence between the two amino acid sequences are taken into account.

Sequence similarity includes identical residues and nonidentical, biochemically related amino acids. Biochemically related amino acids that share similar properties and may be interchangeable.

"Conservatively modified variants" or "conservative substitution" refers to substitutions of amino acids in a protein with other amino acids having similar characteristics (*e.g.* charge, side-chain size, hydrophobicity/hydrophilicity, backbone conformation and rigidity, etc.), such that the changes can frequently be made without altering the biological activity of the protein. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity [*see, e.g.*, Watson *et al.*, *Molecular Biology of the Gene*, The Benjamin/Cummings Pub. Co., p. 224 (4th Ed.; 1987)]. In addition, substitutions of structurally or functionally similar amino acids are less likely to disrupt biological activity. Exemplary conservative substitutions are set forth in Table A directly below.

TABLE A
EXEMPLARY CONSERVATIVE AMINO ACID SUBSTITUTIONS

Original residue	Conservative substitution
Ala (A)	Gly; Ser
Arg (R)	Lys; His
Asn (N)	Gln; His
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln

Original residue	Conservative substitution
Ile (I)	Leu; Val
Leu (L)	Ile; Val
Lys (K)	Arg; His
Met (M)	Leu; Ile; Tyr
Phe (F)	Tyr; Met; Leu
Pro (P)	Ala; Gly
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu

Function-conservative variants of the antibodies of the invention are also contemplated by the present invention. "Function-conservative variants," as used herein, refers to antibodies or fragments in which one or more amino acid residues have been changed without altering a desired property, such as an antigen affinity and/or specificity. Such variants include, but are not limited to, replacement of an amino acid with one having similar properties, such as the conservative amino acid substitutions of Table A above.

"Isolated nucleic acid molecule" means a DNA or RNA of genomic, mRNA, cDNA, or synthetic origin or some combination thereof which is not associated with all or a portion of a polynucleotide in which the isolated polynucleotide is found in nature, or is linked to a polynucleotide to which it is not linked in nature. For purposes of this disclosure, it should be understood that "a nucleic acid molecule comprising" a particular nucleotide sequence does not encompass intact chromosomes. Isolated nucleic acid molecules "comprising" specified nucleic acid sequences may include, in addition to the specified sequences, coding sequences for up to ten or even up to twenty or more other proteins or portions or fragments thereof, or may include operably linked regulatory sequences that control expression of the coding region of the recited nucleic acid sequences, and/or may include vector sequences.

The present invention provides isolated caninized antibodies of the present invention, methods of use of the antibodies in the treatment of a condition *e.g.*, the treatment of atopic dermatitis in canines. In canine, there are four IgG heavy chains referred to as A, B, C, and D. These heavy chains represent four different subclasses of dog IgG, which are referred to as IgG-

A (or IgGA), IgG-B (or IgGB), IgG-C (or IgGC) and IgG-D (or IgGD). Each of the two heavy chains consists of one variable domain (VH) and three constant domains referred to as CH-1, CH-2, and CH-3. The CH-1 domain is connected to the CH-2 domain *via* an amino acid sequence referred to as the “hinge” or alternatively as the “hinge region”.

5 The nucleic acid and amino acid sequences of these four heavy chains were first identified by Tang *et al.* [*Vet. Immunol. Immunopathol.* 80: 259-270 (2001)]. The amino acid and nucleic sequences for these heavy chains are also available from the GenBank data bases. For example, the amino acid sequence of IgGA heavy chain has accession number AAL35301.1, IgGB has accession number AAL35302.1, IgGC has accession number AAL35303.1, and IgGD
10 has accession number (AAL35304.1). Canine antibodies also contain two types of light chains, *kappa* and *lambda*. The DNA and amino acid sequence of these light chains can be obtained from GenBank Databases. For example, the *kappa* light chain amino acid sequence has accession number ABY 57289.1 and the *lambda* light chain has accession number ABY 55569.1.

15 The known amino acid sequences of the four unmodified canine Fcs are:

cIgG-A [SEQ ID NO: 116]

LGGPSVLIFPPKPKDILRITRTPEVTCVVLDLGREDPEVQISWFVDGKEVHTAKTQSREQQFNGT
YRVVSVLPIEHQDWLTGKEFKCRVNHIDLPSPIERTISKARGRAHKPSVYVLPSPKELSSSDTV
SITCLIKDFYPPDIDVEWQSNQQEPPERKHRMTPPQLDEDGSYFLYSKLSVDKSRWQQGDPFTCA
20 VMHETLQNHYTDLSSLHSPGK

cIgG-B [SEQ ID NO: 110]

LGGPSVFIFFPKPKDILLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMOTAKTQPREEQFNGT
YRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPSPREELSKNTVS
25 LTCLIKDFFPDIDVEWQSNQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAV
MHEALHNHYTQESLSHSPGK

cIgG-C [SEQ ID NO: 117]

LGGPSVFIFFPKPKDILVTARTPTVTCVVVDLDPENPEVQISWFVDSKQVQTANTQPREEQSNGT
30 YRVVSVLPIGHQDWLSGKQFKCKVNNKALPSPIEEIISKTPGQAHQPNVYVLPSPRDEMKNVT
LTCLVKDFFPPEIDVEWQSNQQEPESKYRMTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAV
MHEALHNHYTQISLSHSPGK

cIgG-D [SEQ ID NO: 118]

LGGPSVFIFPPKPKDILRITRTPEITCVVLDLGREDPEVQISWFVDGKEVHTAKTQPREQQFNST
YRVVSVLPPIEHQDWLTGKEFKCRVNHIGLPSPIERTISKARGQAHQPSVYVLPPSPKELSSSDTV
TLTCLIKDFFPPEIDVEWQSNGQPEPESESKYHTTAPQLDEDGSYFLYSKLSVDKSRWQQGDTFTCA
5 VMHEALQNHYTDLSSLHSPGK

An amino acid sequence of the *kappa* canine light chain constant region is: [SEQ ID NO: 127]

RNDAPAVYLFQPSPDQLHTGSASVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTY
SLSSTLTMSSTEYLSHELYSCEITHKSLPSTLIKSFORSECQRVD

10

In the present invention, the amino acid sequence for each of the four canine IgG Fc fragments is based on the identified boundary of CH1 and CH2 domains as determined by Tang *et al, supra*. Caninized mouse anti-canine antibodies that bind canine IL-31RA include, but are not limited to: antibodies of the present invention that comprise canine IgG-A, IgG-B, IgG-C, and IgG-D heavy chains and/or canine *kappa* or *lambda* light chains together with mouse anti-canine IL-31RA CDRs. Accordingly, the present invention provides caninized mouse anti-canine antibodies of the present invention, including isolated caninized mouse anti-canine antibodies, that bind to canine IL-31RA and that preferably also block the binding of that canine IL-31RA to canine IL-31.

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Accordingly, the present invention further provides caninized mouse antibodies and methods of use of the antibodies of the present invention in the treatment of a condition *e.g.*, the treatment of atopic dermatitis in canines.

25

The present invention further provides full length caninized heavy chains that can be matched with corresponding light chains to make a caninized antibody. Accordingly, the present invention further provides caninized mouse anti-canine antigen antibodies (including isolated caninized mouse anti-canine antibodies) of the present invention and methods of use of the antibodies of the present invention in the treatment of a condition *e.g.*, the treatment of atopic dermatitis in canines.

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The present invention also provides antibodies of the present invention that comprise a canine fragment crystallizable region (cFc region) in which the cFc region has been genetically modified to augment, decrease, or eliminate one or more effector functions. In one aspect of the present invention, the genetically modified cFc region decreases or eliminates one or more effector functions. In another aspect of the invention the genetically modified cFc region

augments one or more effector function. In certain embodiments, the genetically modified cFc region is a genetically modified canine IgGB Fc region. In another such embodiment, the genetically modified cFc region is a genetically modified canine IgGC Fc region. In a particular embodiment the effector function is antibody-dependent cytotoxicity (ADCC) that is augmented, decreased, or eliminated. In another embodiment the effector function is complement-dependent cytotoxicity (CDC) that is augmented, decreased, or eliminated. In yet another embodiment, the cFc region has been genetically modified to augment, decrease, or eliminate both the ADCC and the CDC.

In order to generate variants of canine IgG that lack effector functions, a number of mutant canine IgGB heavy chains were generated. These variants may include one or more of the following single or combined substitutions in the Fc portion of the heavy chain amino acid sequence: P4A, D31A, N63A, G64P, T65A, A93G, and P95A. Variant heavy chains (*i.e.*, containing such amino acid substitutions) are cloned into expression plasmids and are transfected into HEK 293 cells along with a plasmid containing the gene encoding a light chain. Intact antibodies are expressed and purified from HEK 293 cells and then can be evaluated for binding to Fc γ RI and C1q to assess their potential for mediation of immune effector functions. [See, U.S. 10,106,607 B2, the contents of which are hereby incorporated by reference in its entirety.]

The present invention also provides modified canine IgG-Ds which in place of its natural IgG-D hinge region they comprise a hinge region from:

IgG-A:	FNECRCTDTPPCPVPEP	SEQ ID NO: 112
IgG-B:	PKRENGRVPRPPDCPKCPAPEM	SEQ ID NO: 113; or
IgG-C:	AKECECKCNCNNCPGCGGL	SEQ ID NO: 114.

Alternatively, the IgG-D hinge region can be genetically modified by replacing a serine residue with a proline residue, *i.e.*, PKESTCKC**I**PPCPVPES, SEQ ID NO: 115 (with the proline residue (P) underlined and in bold substituting for the naturally occurring serine residue). Such modifications can lead to a canine IgG-D lacking fab arm exchange. The modified canine IgG-Ds can be constructed using standard methods of recombinant DNA technology [*e.g.*, Maniatis *et al.*, *Molecular Cloning, A Laboratory Manual* (1982)]. In order to construct these variants, the nucleic acids encoding the amino acid sequence of canine IgG-D can be modified so that it encodes the modified IgG-Ds. The modified nucleic acid sequences are then cloned into expression plasmids for protein expression.

The six complementary determining regions (CDRs) of a caninized mouse anti-canine antibody, as described herein can comprise a canine antibody *kappa* (*k*) or *lambda* (*l*) light chain

comprising a mouse light chain LCDR1, LCDR2, and LCDR3 and a canine antibody heavy chain IgG comprising a mouse heavy chain HCDR1, HCDR2, and HCDR3.

NUCLEIC ACIDS

The present invention further comprises the nucleic acids encoding the antibodies of the present invention (*see e.g.*, Examples below).

Also included in the present invention are nucleic acids that encode immunoglobulin polypeptides comprising amino acid sequences that are at least about 70% identical, preferably at least about 80% identical, more preferably at least about 90% identical and most preferably at least about 95% identical (*e.g.*, 95%, 96%, 97%, 98%, 99%, 100%) to the amino acid sequences of the caninized antibodies, with the exception of the CDRs which do not change, provided herein when the comparison is performed by a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences. The present invention further provides nucleic acids that encode immunoglobulin polypeptides comprising amino acid sequences that are at least about 70% similar, preferably at least about 80% similar, more preferably at least about 90% similar and most preferably at least about 95% similar (*e.g.*, 95%, 96%, 97%, 98%, 99%, 100%) to any of the reference amino acid sequences when the comparison is performed with a BLAST algorithm, wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences, are also included in the present invention.

As used herein, nucleotide and amino acid sequence percent identity can be determined using C, MacVector (MacVector, Inc. Cary, NC 27519), Vector NTI (Informax, Inc. MD), Oxford Molecular Group PLC (1996) and the Clustal W algorithm with the alignment default parameters, and default parameters for identity. These commercially available programs can also be used to determine sequence similarity using the same or analogous default parameters. Alternatively, an Advanced Blast search under the default filter conditions can be used, *e.g.*, using the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin) pileup program using the default parameters.

The following references relate to BLAST algorithms often used for sequence analysis:

BLAST ALGORITHMS: Altschul, S.F., *et al.*, *J. Mol. Biol.* 215:403-410 (1990); Gish, W., *et al.*, *Nature Genet.* 3:266-272 (1993); Madden, T.L., *et al.*, *Meth. Enzymol.* 266:131-141(1996); Altschul, S.F., *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997); Zhang, J., *et al.*, *Genome Res.* 7:649-656 (1997); Wootton, J.C., *et al.*, *Comput. Chem.* 17:149-163 (1993); Hancock, J.M. *et al.*,

Comput. Appl. Biosci. 10:67-70 (1994); ALIGNMENT SCORING SYSTEMS: Dayhoff, M.O., *et al.*, "A model of evolutionary change in proteins." in *Atlas of Protein Sequence and Structure*, vol. 5, suppl. 3. M.O. Dayhoff (ed.), pp. 345-352, (1978); *Natl. Biomed. Res. Found.*, Washington, DC; Schwartz, R.M., *et al.*, "Matrices for detecting distant relationships." in *Atlas of Protein Sequence and Structure*, vol. 5, suppl. 3." (1978), M.O. Dayhoff (ed.), pp. 353-358 (1978), *Natl. Biomed. Res. Found.*, Washington, DC; Altschul, S.F., *J. Mol. Biol.* 219:555-565 (1991); States, D.J., *et al.*, *Methods* 3:66-70(1991); Henikoff, S., *et al.*, *Proc. Natl. Acad. Sci. USA* 89:10915-10919 (1992); Altschul, S.F., *et al.*, *J. Mol. Evol.* 36:290-300 (1993); ALIGNMENT STATISTICS: Karlin, S., *et al.*, *Proc. Natl. Acad. Sci. USA* 87:2264-2268 (1990); Karlin, S., *et al.*, *Proc. Natl. Acad. Sci. USA* 90:5873-5877 (1993); Dembo, A., *et al.*, *Ann. Prob.* 22:2022-2039 (1994); and Altschul, S.F. "Evaluating the statistical significance of multiple distinct local alignments." in *Theoretical and Computational Methods in Genome Research* (S. Suhai, ed.), pp. 1-14, Plenum, New York (1997).

Antibody Protein Engineering

By way of example, and not limitation, the canine heavy chain constant region can be from IgGA, IgG-B, IgGC, IgGD, or a modified cFc, such as the IgG-Bm used herein [see, U.S. 10,106,607 B2, hereby incorporated by reference in its entirety] and the canine light chain constant region can be from *kappa* or *lambda*.

The antibodies can be engineered to include modifications to the canine framework and/or the canine frame residues within the variable domains of a parental (*i.e.*, mouse) monoclonal antibody, *e.g.* to improve the properties of the antibody.

The construction of caninized anti-canine IL-31 receptor *alpha* monoclonal antibodies can be performed by determining a DNA sequence that encodes the heavy and light chains of canine IgG were determined. The DNA and protein sequence of the canine heavy and light chains are known in the art and can be obtained by searching of the NCBI gene and protein databases. As indicated above, for canine antibodies there are four known IgG subtypes: IgG-A, IgG-B, IgG-C, and IgG-D, and two types of light chains, *i.e.*, *kappa* and *lambda*.

A caninized mouse anti-canine IL-31RA antibody can be produced recombinantly by methods that are known in the field. Mammalian cell lines available as hosts for expression of the antibodies or fragments disclosed herein are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, *inter alia*, Chinese hamster ovary (CHO) cells, NSO, SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells

(e.g., Hep G2), A549 cells, 3T3 cells, HEK-293 cells and a number of other cell lines.

Mammalian host cells include human, mouse, rat, dog, monkey, pig, goat, bovine, horse and hamster cells. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as Sf9 cells, amphibian cells, bacterial cells, plant cells and fungal cells. When recombinant expression vectors encoding the heavy chain or antigen-binding portion or fragment thereof, the light chain and/or antigen-binding fragment thereof are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown.

Antibodies can be recovered from the culture medium using standard protein purification methods. Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase gene expression system (the GS system) is a common approach for enhancing expression under certain conditions. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

In certain embodiments, the antibody or antigen binding fragment comprises a heavy chain constant region, e.g., a canine constant region, such as IgG-A, IgG-B, IgG-C and IgG-D canine heavy chain constant region or a variant thereof. In certain embodiments, the antibody or antigen binding fragment comprises a light chain constant region, e.g., a canine light chain constant region, such as *lambda* or *kappa* canine light chain region or variant thereof. By way of example, and not limitation, the canine heavy chain constant region can be from IgG-B and the canine light chain constant region can be from *kappa*.

EPITOPE MAPPING

The interaction of antibodies with their cognate protein antigens is mediated through the binding of specific amino acids of the antibodies (paratopes) with specific amino acids (epitopes) of target antigens. An epitope is an antigenic determinant that causes a specific reaction by an immunoglobulin. An epitope consists of a group of amino acids on the surface of the antigen. A protein of interest may contain several epitopes that are recognized by different antibodies. The epitopes recognized by antibodies are classified as linear or conformational epitopes. Linear epitopes are formed by a stretch of a continuous sequence of amino acids in a protein, while

conformational epitopes are composed of amino acids that are discontinuous (*e.g.*, far apart) in the primary amino acid sequence, but are brought together upon three-dimensional protein folding.

Epitope mapping refers to the process of identifying the amino acid sequences (*i.e.*, epitopes) that are recognized by antibodies on their target antigens. Identification of epitopes recognized by monoclonal antibodies (mAbs) on target antigens has important applications. For example, it can aid in the development of new therapeutics, diagnostics, and vaccines. Epitope mapping can also aid in the selection of optimized therapeutic mAbs and help elucidate their mechanisms of action. Epitope information on IL-31 receptor *alpha* can also elucidate unique epitopes and define the protective or pathogenic effects of vaccines. Epitope identification also can lead to development of subunit vaccines based on chemical or genetic coupling of the identified peptide epitope to a carrier protein or other immunostimulating agents.

Epitope mapping can be carried out using polyclonal or monoclonal antibodies and several methods are employed for epitope identification depending on the suspected nature of the epitope (*i.e.*, linear *versus* conformational). Mapping linear epitopes is more straightforward and relatively, easier to perform. For this purpose, commercial services for linear epitope mapping often employ peptide scanning. In this case, an overlapping set of short peptide sequences of the target protein are chemically synthesized and tested for their ability to bind antibodies of interest. The strategy is rapid, high-throughput, and relatively inexpensive to perform. On the other hand, mapping of a discontinuous epitope is more technically challenging and requires more specialized techniques such as x-ray co-crystallography of a monoclonal antibody together with its target protein, Hydrogen-Deuterium (H/D) exchange, Mass Spectrometry coupled with enzymatic digestion as well as several other methods known to those skilled in the art.

Epitope Binding and Cross-Blocking Antibodies

An anti-canine IL-31RA antibody or antigen-binding fragment thereof of the present invention includes any antibody or antigen-binding fragment thereof that binds to the same epitope in canine IL-31RA as the one of the antibodies, disclosed herein, bind, *e.g.*, such as the 218D9 antibody which binds to the epitope comprising the amino acid sequence either SEQ ID NO: 104, SEQ ID NO: 105, or both SEQ ID NO: 104 and SEQ ID NO: 105, or the 51F8 antibody which binds to the epitope comprising the amino acid sequence either SEQ ID NO: 98, SEQ ID NO: 100, or both SEQ ID NO: 98 and SEQ ID NO: 100, including caninized antibodies, and any antibody or antigen-binding fragment that cross-blocks (partially or fully) or is cross-

blocked (partially or fully) by an antibody or fragment discussed herein for canine IL-31RA binding; as well as any variant thereof.

The cross-blocking antibodies and antigen-binding fragments can be identified based on their ability to cross-compete with *e.g.*, the 100H8 or 74H10 antibody in standard binding assays (*e.g.*, BIAcore[®], ELISA, as exemplified below, or flow cytometry). For example, standard ELISA assays can be used in which a recombinant canine IL-31RA protein is immobilized on the plate, one of the antibodies is fluorescently labeled and the ability of non-labeled antibodies to compete off the binding of the labeled antibody is evaluated. Additionally or alternatively, BIAcore[®] analysis can be used to assess the ability of the antibodies to cross-compete. The ability of a test antibody to inhibit the binding of the *e.g.*, 100H8 or 74H10 antibody, to canine IL-31RA demonstrates that the test antibody can compete with the 100H8 or 74H10 antibody for binding to canine IL-31RA and thus, may, in some cases, bind to the same epitope on canine IL-31RA as the 100H8 and/or 74H10 antibody binds.

Antibodies and fragments thereof that bind to the same epitope as any of the anti-canine IL-31RA antibodies or fragments of the present invention also form part of the present invention.

PHARMACEUTICAL COMPOSITIONS AND ADMINISTRATION

To prepare pharmaceutical or sterile compositions comprising the antibodies of the present invention, these antibodies can be admixed with a pharmaceutically acceptable carrier or excipient. [See, *e.g.*, *Remington's Pharmaceutical Sciences* and *U.S. Pharmacopeia: National Formulary*, Mack Publishing Company, Easton, PA (1984)].

Formulations of therapeutic and diagnostic agents may be prepared by mixing with acceptable carriers, excipients, or stabilizers in the form of, *e.g.*, lyophilized powders, slurries, aqueous solutions or suspensions [see, *e.g.*, Hardman, *et al.* (2001) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, McGraw-Hill, New York, NY; Gennaro (2000) *Remington: The Science and Practice of Pharmacy*, Lippincott, Williams, and Wilkins, New York, NY; Avis, *et al.* (eds.) (1993) *Pharmaceutical Dosage Forms: Parenteral Medications*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Tablets*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Disperse Systems*, Marcel Dekker, NY; Weiner and Kotkoskie (2000) *Excipient Toxicity and Safety*, Marcel Dekker, Inc., New York, NY]. In one embodiment, the antibodies of the present invention are diluted to an appropriate concentration in a sodium acetate solution pH 5-6, and

NaCl or sucrose is added for tonicity. Additional agents, such as polysorbate 20 or polysorbate 80, may be added to enhance stability.

Toxicity and therapeutic efficacy of the antibody compositions, administered alone or in combination with another agent, can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index (LD₅₀/ ED₅₀). In particular aspects, antibodies exhibiting high therapeutic indices are desirable. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in canines. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration.

The mode of administration can vary. Suitable routes of administration include oral, rectal, transmucosal, intestinal, parenteral; intramuscular, subcutaneous, intradermal, intramedullary, intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, intraocular, inhalation, insufflation, topical, cutaneous, transdermal, or intra-arterial. In particular embodiments, the antibodies of the present invention can be administered by an invasive route such as by injection. In further embodiments of the invention, the antibodies of the present invention, or pharmaceutical composition thereof, is administered intravenously, subcutaneously, intramuscularly, intraarterially, or by inhalation, aerosol delivery. Administration by non-invasive routes (*e.g.*, orally; for example, in a pill, capsule or tablet) is also within the scope of the present invention.

Compositions can be administered with medical devices known in the art. For example, a pharmaceutical composition of the invention can be administered by injection with a hypodermic needle, including, *e.g.*, a prefilled syringe or autoinjector. The pharmaceutical compositions disclosed herein may also be administered with a needleless hypodermic injection device; such as the devices disclosed in U.S. Patent Nos.: 6,620,135; 6,096,002; 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824 or 4,596,556.

The pharmaceutical compositions disclosed herein may also be administered by infusion. Examples of well-known implants and modules form administering pharmaceutical compositions include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No.

4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent. No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments. Many other such implants, delivery systems, and modules are well known to those skilled in the art.

5 Alternatively, one may administer the antibodies of the present invention in a local rather than systemic manner, often in a depot or sustained release formulation.

The administration regimen depends on several factors, including the serum or tissue turnover rate of the therapeutic antibodies, the level of symptoms, the immunogenicity of the therapeutic antibodies and the accessibility of the target cells in the biological matrix. Preferably,
 10 the administration regimen delivers sufficient therapeutic antibodies to effect improvement in the target disease/condition state, while simultaneously minimizing undesired side effects. Accordingly, the amount of biologic delivered depends in part on the particular therapeutic antibodies and the severity of the condition being treated. Guidance in selecting appropriate doses of therapeutic antibodies is available [*see, e.g.,* Wawrzynczak *Antibody Therapy*, Bios
 15 Scientific Pub. Ltd, Oxfordshire, UK (1996); Kresina (ed.) *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New York, NY (1991); Bach (ed.) *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*, Marcel Dekker, New York, NY (1993); Baert, *et al. New Engl. J. Med.* 348:601-608 (2003); Milgrom *et al. New Engl. J. Med.* 341:1966-1973 (1999); Slamon *et al. New Engl. J. Med.* 344:783-792 (2001); Beniaminovitz *et al. New Engl. J.*
 20 *Med.* 342:613-619 (2000); Ghosh *et al. New Engl. J. Med.* 348:24-32 (2003); Lipsky *et al. New Engl. J. Med.* 343:1594-1602 (2000)].

Determination of the appropriate dose is made by the veterinarian, *e.g.,* using parameters or factors known or suspected in the art to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter
 25 until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of the symptoms.

Antibodies provided herein may be provided by continuous infusion, or by doses administered, *e.g.,* daily, 1-7 times per week, weekly, bi-weekly, monthly, bimonthly, quarterly, semiannually, annually etc. Doses may be provided, *e.g.,* intravenously, subcutaneously,
 30 topically, orally, nasally, rectally, intramuscular, intracerebrally, intraspinally, or by inhalation. A total weekly dose is generally at least 0.05 µg/kg body weight, more generally at least 0.2 µg/kg, 0.5 µg/kg, 1 µg/kg, 10 µg/kg, 100 µg/kg, 0.25 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 5.0 mg/ml, 10 mg/kg, 25 mg/kg, 50 mg/kg or more [*see, e.g.,* Yang, *et al. New Engl. J. Med.* 349:427-434

(2003); Herold, *et al. New Engl. J. Med.* 346:1692-1698 (2002); Liu, *et al. J. Neurol. Neurosurg. Psych.* 67:451-456 (1999); Portielji, *et al. Cancer Immunol. Immunother.* 52:133-144 (2003)].

Doses may also be provided to achieve a pre-determined target concentration of antibodies of the present invention in the canine's serum, such as 0.1, 0.3, 1, 3, 10, 30, 100, 300 µg/ml or more. In
5 other embodiments, antibodies of the present invention is administered subcutaneously or intravenously, on a weekly, biweekly, "every 4 weeks," monthly, bimonthly, or quarterly basis at 10, 20, 50, 80, 100, 200, 500, 1000 or 2500 mg/subject.

As used herein, "inhibit" or "treat" or "treatment" includes a postponement of development of the symptoms associated with a disorder and/or a reduction in the severity of the
10 symptoms of such disorder. The terms further include ameliorating existing uncontrolled or unwanted symptoms, preventing additional symptoms, and ameliorating or preventing the underlying causes of such symptoms. Thus, the terms denote that a beneficial result has been conferred on a vertebrate subject (*e.g.*, a canine) with a disorder, condition and/or symptom, or with the potential to develop such a disorder, disease or symptom.

As used herein, the terms "therapeutically effective amount", "therapeutically effective dose" and "effective amount" refer to an amount of antibodies of the present invention that, when administered alone or in combination with an additional therapeutic agent to a cell, tissue, or subject, *e.g.*, canine, is effective to cause a measurable improvement in one or more symptoms of a disease or condition or the progression of such disease or condition. A therapeutically effective
20 dose further refers to that amount of the antibodies sufficient to result in at least partial amelioration of symptoms, *e.g.*, treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in
25 combination, serially, or simultaneously. An effective amount of a therapeutic will result in an improvement of a diagnostic measure or parameter by at least 10%; usually by at least 20%; preferably at least about 30%; more preferably at least 40%, and most preferably by at least 50%. An effective amount can also result in an improvement in a subjective measure in cases where subjective measures are used to assess severity of the condition.

EXAMPLES

EXAMPLE 1

IL-31 RECEPTOR *alpha**Nucleotide Sequence*

- 5 The nucleotide sequence of SEQ ID NO: 1 encodes the extracellular domain of the canine IL-31 receptor *alpha* (cIL-31RA) fused to a HIS tag. Canine IL-31RA ECD HIS-tagged protein comprises the amino acid sequence of SEQ ID NO: 2. The nucleotide sequence was prepared by chemical synthesis and then cloned into expression plasmids that are suitable for production of the corresponding proteins in eukaryotic cells, either HEK-293 or CHO cells.
- 10 Canine IL-31RA ECD-10His: [SEQ ID NO: 1]
- gtgctgcccgcgaagcccgcgaacatcagctgcatcttctactacgaggagaacttcacctgcacctggag
 cccgcgaagaggaggccagctacacctgggtacaaggtgaagagaacctacagctacggctacaagagcgaca
 tctgcagcaccgacaacagcaccagaggcaaccacgccagctgcagcttcctgccccccaccatcaccaac
 cccgcgaactacaccatccaggtggaggcccagaacgccgacggcatcatgaagagcgacatcacctactg
 15 gaacctggacgccatcatgaagatcgagcccccgagatcttcagcgtgaagagcgtgctgggcatcaaga
 gaatgctgcagatcaagtggatcagaccctgctggccccccacagcagcacctgaagtacaccctgaga
 ttcagaaccatcaacagcgcctactggatggaggtgaacttcaccaaggaggacatcgacagagacgagac
 ctacaacctgaccgagctgcaggccttcaccgagtagctgatgacctgagatgcgcccccgccgagagca
 tgttctggagcggctggagccaggagaaggtgggcaccaccgaggaggaggccccctacggcctggacctg
 20 tggagagtgcctgaagcccgcctatggtggacggcagaagaccctgcagctgatgtggaagaaggccaccgg
 cgccccctgctggagaaggccctgggctacaacatctggtacttccccgcgaacaacaccaacctgaccg
 agaccgtgaacaccaccaaccagaccacgagctgtacctgggcggcaagacctactgggtgtacgtggtg
 agctacaacagcctgggagagagccccgtggccaccctgagaatccccgcctgaacgagaagaccttcca
 gtgcatcgaggccatgcaggcctgcctgaccaggaccagctggtggtggagtggcagagcagcgcccccg
 25 aggtggacacctggatggtggagtgggtccccgacgtggacagcgagccagcagcttcagctgggagagc
 gtgagccaggccagaaaactggaccatccagaaggacgagctgaagcccctgtggtgctacaacatcagcgt
 gtacccctgctgagagacagagtgggcccagccctacagcaccaggcctacgtgcaggagggcatcccca
 gcgcccggccccgtgaccaggccgacagcatcggcgtgaagaccgtgaccatcacctggaaggagatcccc
 aagagcaagagaaaacggcttcatcaagaactacaccatcttctaccaggccgaggacggcaaggagttcag
 30 caagaccgtgaacagcaacatcctgcagtacagactggagagcctgaccagaagaaccagctacagcctgc
 aggtgatggccagcaccaacgccggcgccaccaacggcaccaagatcaacttcaagacctgagcatcagc
 caccaccaccaccaccaccaccaccaccac

EXAMPLE 2

- 35 EXPRESSION AND PURIFICATION OF IL-31 RECEPTOR *alpha* ECD

Plasmids comprising the nucleotide sequence of SEQ ID NO: 1 were transfected into HEK-293 or CHO cells using electroporation *via* the MaxCyte instrument as per the manufacturer's recommendation. Several days following transfection, the supernatants of transfected cells and un-transfected controls were harvested and spun down to remove cellular debris. IL-31RA with the HIS tag was purified from cell culture fluids by passing the clarified harvested fluid from transfected cells over nickel columns as per the manufacturer's recommendation. Purified proteins were quantified by measuring their absorbance of ultraviolet light at 280 nm.

Canine IL-31RA ECD-10His: [SEQ ID NO: 2]

VLPAPENISCIIFYEENFTCTWSPEKEASYTWYKVKRTYSYGYKSDICSTDNSTRGNHASCFL
PPTITNPDNYTIQVEAQNADGIMKSDITYWNLDAIMKIEPPEIFSVKSVLGKRLQIKWIRPVL
APHSSTLKYTLRFRTINSAYWMEVNFTKEDIDRDETYNLTELQAFTEYVMTLRCAPAESMFWSGW
SQEKVGTTEEEAPYGLDLWRVLKPAMVDGRRPVQLMWKKATGAPVLEKALGYNIWYFPENNTNLT
ETVNTTNQTHELYLGGKTYWVYVVSYNLGGESPVATLRIPALNEKTFQCIAMQACLTQDQLVVE
WQSSAPEVDTWMVEWFPDVDSEPSSFWSVSQARNWTIQKDELKPLWCYNISVYPVLRDRVGQP
YSTQAYVQEGIPSAGPVTQADSIGVKTVTITWKEIPKSKRNGFIKNYTIIFYQAEDGKEFSKTVNS
NILQYRLESLTRRTSYSLQVMASNTAGGTNGTKINFKTLSSHHHHHHHHHH

TABLE 1

CANINE IL-31RA EXTRACELLULAR DOMAIN -HIS TAG

	SEQ ID NO:	Nucleic Acid	Amino Acid
canine IL-31RA ECD-10His	1	X	
canine IL-31RA ECD-10His	2		X

EXAMPLE 3

BINDING OF CANINE IL-31RA TO BIOTINYLATED CANINE IL-31:

Protocol

- 1) Coat immunoplate(s) with IL-31RA proteins by diluting to 1 µg/mL in phosphate-buffered saline solution (PBS). Add 100µL/well. Incubate the plate(s) at 2-7° overnight.
- 2) Wash the plates 3 times with 275 µL/well of phosphate-buffered saline solution plus TWEEN 20 (PBST).
- 3) Block the plates with 200 µL/well of blocking buffer [1% nonfat dried milk (NFDM) in PBST] for 30-45 minutes at 36 ± 2°C with gentle shaking (120 ± 20 RPM).

- 4) Wash the plates 3 times with 275 μ L/well of PBST.
- 5) 3-fold dilute biotinylated IL-31 (at 10 μ g/mL) in 1% NFDM in PBST on a dilution plate, and transfer 100 μ L/well to the immunoplate(s). Incubate for 30-45 minutes at $36 \pm 2^\circ\text{C}$ with gentle shaking (120 ± 20 RPM).
- 5 6) Wash the plates 3 times with 275 μ L/well of PBST.
- 7) Dilute horse raddish peroxidase-Streptavidin (HRP-Streptavidin) to a final dilution of 1:1000 in 1% NFDM in PBST.
- 8) Add 100 μ L/well of HRP-Streptavidin to the immunoplate(s) and incubate for 30-45 minutes at $36 \pm 2^\circ\text{C}$ with gentle shaking (120 ± 20 RPM).
- 10 9) Wash the plates 3 times with 275 μ L/well of PBST.
- 10) Combine equal volumes of pre-warmed TMP 2-Component substrate immediately before use.
- 11) Add 100 μ L/well of prepared 3,3',5,5'-tetramethylbenzidine (TMP) substrate to the immunoplate(s) and incubate in the dark for 10 to 15 minutes at $36 \pm 2^\circ\text{C}$ with gentle shaking
- 15 (120 $\pm$ 20 RPM).
- 12) Stop the reaction by addition of 100 μ L/well of 1 M H_3PO_4 .
- 13) Read the plates using a microplate reader at a wavelength of 450 nm with a reference wavelength of 540 nm.

The extracellular domain (ECD) of canine IL-31RA was tested for its ability to bind to canine IL-31 (*see*, Figure 1). The results indicate that IL-31RA ECD binds in a dose-dependent manner to biotinylated canine IL-31 with an EC_{50} of 0.55 ng/ml.

EXAMPLE 4

MONOCLONAL ANTIBODIES AGAINST CANINE IL-31 RECEPTOR *alpha*

25 Monoclonal antibodies (mAbs) against canine IL-31RA were produced by the immunization of mice multiple times with canine IL-31RA ECD. Mice were immunized *via* the Intraperitoneal route with IL-31RA ECD in GS proprietary adjuvant 3 times on days 0, 14, and 28 using 50 μ g per mouse for first immunization and 25 μ g per mouse for the subsequent boosts. On day 48 mice were immunized once more with 25 μ g of antigen and 4 days later their spleen

30 cells were fused with the myeloma SP2/0 cell line to produce hybridomas secreting antibodies. At various time points after immunization, sera were collected from mice and tested against canine IL-31RA by ELISA. The spleen cells from the mouse with highest IL-31RA ECD reactivity were fused with the myeloma SP2/0 cell line to produce hybridomas. Approximately

14 days after the fusion, supernatants from growing hybridomas were screened by flow cytometry using cells expressing the IL-31RA protein. The reactivities of hybridoma were confirmed by ELISA as follows:

Procedure for the ELISA:

- 5 1) Coat 96-well plates with IL-31RA (1 µg/mL in PBS buffer), 25 µL/well.
- 2) Incubate the plates at 4°C overnight.
- 3) Wash the plates 3 times with PBST (PBS +0.05% Tween 20)
- 4) Block the plates with blocking buffer [PBS with 5% fetal bovine serum (FBS)], 25µl/well for 30 minutes at room temperature.
- 10 5) Transfer 25 µl/well hybridoma supernatant to the 96-well plates, incubate 60 minutes at room temperature.
- 6) Wash the plates 3 times by PBST.
- 7) Add 25µl/well anti-mouse HRP, 1:4000 dilution in blocking buffer, to the plates and incubate 60 minutes at room temperature.
- 15 8) Wash the plates 5 times by PBST.
- 9) Add TMB based reagent to the plates for colorimetric reaction for 2-3 minutes.
- 10) Stop the reactions with 0.16M sulfuric acid.
- 11) Read the plates by plate reader.

As shown in Figures 2A-2B, the selected mouse mAbs were tested for their reactivity to canine IL-31RA. The results indicate that the selected mouse mAbs bind to canine IL-31RA in a dose-dependent manner. Ten (10) mouse monoclonal antibodies were obtained that have strong binding reactivity to canine IL-31RA, as shown in Figures 2A-2B.

The amino acid sequences of the heavy and light chain variable regions of these ten mouse monoclonal antibody are provided below.

25 100H8VH [SEQ ID NO: 52]

EVQLQQSGPELVKPGASVKIPCKASGYTFTDYNMDWVKQSHGKSLEWIGHINPNNGGILYNQKFK
GKATLTVDRSSNTAYMELRSLTSEDVAVYFCARWAPLVRQPYWYFDVWGTGTTVTVSS

100H8VL [SEQ ID NO: 53]

30 DIKMTQSPSSI FASLGERVTITCKASQDINSYLNWFQQKPGKSPKTLIYRADRLVDGVPSRFSGS
GSGQDYSLTISSLEYEDMGIYYCLQYDEFPLTFGAGTKLELN

222E7VH [SEQ ID NO: 54]

EVQLQQSGPELVKPGSSVKIPCKASGYTFTDYNMDWVKQSHGKSLEWIGHINPNIGGTLYNQKFK
GKATLSVDKSSSTAYMELRSLTSED TAVYYCARWAQLQRQPYWYFDVWGP GTTVTVSS

222E7VL [SEQ ID NO: 55]

5 DIKMTQSPSSMYASLGERTITCKASQDINSYLNWFQQKPGKSPKTLIYRVNRLVDGVPSRFSGS
GSGQDYSLTISSLEYEDMGIYYCLQYDEFPLTFGAGTKLELK

55B3VH [SEQ ID NO: 56]

EVQLQQSGPELVKPGASVKIPCKASGYTFTDYNMDWVKQSHGKSLEWIGHINPNTGGTIYNQKFK
10 GKATLTVDKSSSTAYMDLRSLTSED TAVYYCARWAQLLRQPYWYFDVWGT GTTVTVSS

55B3VL [SEQ ID NO: 57]

DIKMTQSPSSMYASLGERTITCKASQDINSYFNWVQQKPGKSPKTLIFRANRLVDGVPSRFSGS
GSGQDYSLTINSLEYEDMGIYYCLQYDEFPLTFGAGTKLELK
15

74H10VH [SEQ ID NO: 58]

EVQLQQSGPELVKPGASMKIPCKTSGYTFTDYNMDWVKQSHGKSLEWIGHINPNNGGTLYNQKFK
DKATLTVDRSSNTAYMELRSLTSED TAVYYCARWAPLLRQPYWYFDVWGT GTTVTVSS

20 74H10VL [SEQ ID NO: 59]

DIKMTQSPSSMYASLGERTITCKASQDINSYLNWFQQKPGKSPKTLIYRADRLVDGVPSRFSGS
GSGQDYSLTISSLEYEDMGIYYCLQYDEFPLTFGDGTKLELN

209G5VH [SEQ ID NO: 60]

25 EVQLQQSVAELVRPGASVKLSCTASGFNIKNTYMHVVKERPEQGLEWIGRIDPANGNSKYAPKFQ
GKATITTDTSNTAYLQLSSLTSED TAIYYCARYYYVSSHFDVWGT GTTVTVSS

209G5VL [SEQ ID NO: 61]

SIVMTQTPKFLLVSAGDRVTITCKASQSVTNDVTWYQQKPGQSPKLLIYYASNRYT
30 GVPDRFTGSGYGTDFTFITSTVQAEDLAVYFCQQDYSSPFTFGSGTKLEIK

51F8VH [SEQ ID NO: 62]

EVQLQQSVAELVRPGASVKLSCTASGFNIKNTFIHWVKQRPEQGLEWIGRIDPANGNTKYAPKFQ
GRATLTADTSSNTAYLQLSSLTSEDTAIYYCARYYYVSSYFDVWGTGTTVTVSS

51F8VL [SEQ ID NO: 63]

5 SIVMTQTPKFLLVSAGDRVTTITCKASQSVTNDVTWYQQKPGQSPKLLIFASNRYTGVPDRFTGS
GYGTDFTFTISTVQAEDLAVYFCQQDYSSPWTFGGGTKLEIK

65G9VH [SEQ ID NO: 64]

EFQLQQSGPELVKPGASVKISCKASGYSFTDYGMNWLKQINGKSLEWIAIINPNYGTASSNPCKFK
10 DKATLTVDHSSSTAYMQLSSLTSEDSAVYYCARAFDGYFYWYFDVWGTGTTVTVSS

65G9VL [SEQ ID NO: 65]

DVVMQTPLSLPVSLGDQASISCRSSQSLIHTNGNTYLHWYLQKPGQSPKLLIYKVSNRFSGVPD
RFSGSGSGTDFTLKISRLEAEDLGVIYFCSQSTHVPLTFGAGTKLELR
15

85C10VH [SEQ ID NO: 66]

EFQLQQSGPELVKPGASVKISCKASGYSFTDYSINWVKQSNQKSLEWIGVINPNYGTSSHNQKFK
GKATMTVDQSSSTAYMQLNSLTSEDSAVYYCARALDDYYFYWYFDVWGIGTTVTVSS

20 85C10VL [SEQ ID NO: 67]

DVVMQTPLSLPVSLGDQASISCRSSQSLVHTNGNTYLHWYLQKPGQSPKLLIYKVSNRFSGVPD
RFSGSGSGTDFTLKISRVEAEDLGVYFCSQTHVPLTFGAGTKLELK

218D9VH [SEQ ID NO: 68]

25 QVTLKESGPGILQPSQTLSTCSFSGFSLSTFGRGVGWIRQPSGKGLDWLTHIWDDDKYYNPAL
KSRLTISKDTSKNQVFLKIANVDTADTATYYCARIAGGLRRAPYAMDSWGQGTSTVTVSS

218D9VL [SEQ ID NO: 69]

DIQMTQSPASLSVSVGETVTITCRASENIYSSLAWYQQKQKSPQLLVYAATNLADGVPSRFSGS
30 GSGTQYSLKINSLQSEDSGNYYCQHFRDTPPTFGGGGTKLEIK

224G3VH [SEQ ID NO: 70]

EVQLVESGGDLVKPGGSLKLSCAASGFTFSSYGMSWVRQTPDKRLEWVATISNGGSYTYYPDSVK
GRFTISRDNKNTLYMQMSSLKSEDTAMYYCARNEPPDYWGQGTSTVTVSS

224G3VL [SEQ ID NO: 71]

5 QIVLTQSPAISASLGERVTMTCTASSSVGSSYLHWYQQKPGSSPKLWIYDTSNLASGVPVRFSG
SGSGTSYSLTISMEAEADAATYYCHQYHRSPYTFGGGTKLEIK

Table 2 below, provides the association rate constant (k_a), dissociation rate constant (k_d), and dissociation constant (K_D), as analyzed by Octet Kinetics (*see also*, Example 9 below).

10 These constants reflect the binding affinity of the individual monoclonal antibodies for canine IL-31 RA.

TABLE 2

BINDING AFFINITY OF SELECTED ANTI-IL-31RA ANTIBODIES

mAb	K_D (M)	k_a (1/Ms)	k_d (1/s)
100H8	1.52E-10	7.88E+05	1.20E-04
51F8	2.07E-10	6.84E+05	1.41E-04
55B3	2.72E-10	5.68E+05	1.54E-04
65G9	4.51E-10	7.87E+05	3.55E-04
74H10	1.96E-10	4.63E+05	9.05E-05
209G5	1.31E-10	3.70E+05	4.87E-05
85C10	8.51E-10	4.27E+05	3.63E-04
218D9	6.98E-11	6.64E+05	4.63E-05
222E7	1.74E-10	1.62E+06	2.82E-04
224G3	9.64E-13	1.12E+06	1.08E-06

15 The results show that the selected mAbs have low nanomolar to sub-picomolar binding affinities ranging from about 0.85 nM to about 1 pM.

The sets of the six CDRs for each of the ten monoclonal antibodies described above are provided below in Table 3. In addition, the canonical structures for each of these CDRs are provided in Table 4 below.

20

TABLE 3

AMINO ACID SEQUENCES OF THE MOUSE CDRS

	Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:
	100H8		74H10	
HCDR1	DYNMD	3	DYNMD	3
HCDR2	HINPNNGGILYNQKFKG	10	HINPNNGGTLYNQKFKD	11
HCDR3	WAPLVRQPYWY	20	WAPLLRQPYWYFDV	21
LCDR1	KASQDINSYLN	30	KASQDINSYLN	30
LCDR2	RADRLVD	40	RADRLVD	40
LCDR3	LQYDEFPLT	45	LQYDEFPLT	45
	222E7		55B3	
HCDR1	DYNMD	3	DYNMD	3
HCDR2	HINPNIGGTLYNQKFKG	12	HINPNTGGTIYNQKFKG	13
HCDR3	WAQLQRQPYWYFDV	22	WAQLLRQPYWYFDV	23
LCDR1	KASQDINSYLN	30	KASQDINSYFN	31
LCDR2	RVNRLVD	38	RANRLVD	39
LCDR3	LQYDEFPLT	45	LQYDEFPLT	45
	51F8		209G5	
HCDR1	NTFIH	5	NTYMH	6
HCDR2	RIDPANGNTKYAPKFQG	15	RIDPANGNSKYAPKFQG	16
HCDR3	YYYVSSY	25	YYYVSSHFDV	26
LCDR1	KASQSVTNDVT	32	KASQSVTNDVT	32
LCDR2	SASNRYT	41	YASNRYT	42
LCDR3	QQDYSSPWT	47	QQDYSSPFT	48
	65G9		218D9	
HCDR1	DYGMN	7	TFGRGVG	8
HCDR2	IINPNYGTASSNPKEFD	17	HIWWDDDKYYPALKS	18
HCDR3	AFDGYFYWYFDV	27	IAGGLRRAPYAMDS	28
LCDR1	RSSQSLIHTNGNTYLH	34	RASENIYSSLA	35
LCDR2	KVSNRFS	37	AATNLAD	43
LCDR3	SQSTHVPLT	50	QHFRDTPPT	51
	224G3		85C10	
HCDR1	SYGMS	9	DYSIN	4
HCDR2	TISNGGSYTYPPDSVKG	19	VINPNYGTSSHNQKFKG	14

HCDR3	NEPPDY	29	ALDDYYFYWY	24
LCDR1	TASSSVGSSYLH	36	RSSQSLVHTNGNTYLH	33
LCDR2	DTSNLAS	44	KVSNRFS	37
LCDR3	HQYHRSPYT	49	SQTTHVPLT	46

TABLE 4

CANONICAL STRUCTURE OF THE MOUSE CDRs

	L1	L2	L3	H1	H2	H3
100H8	L1-2	L2-1	L3-1	H1-1	H2-2	H3-11
85C10	L1-4	L2-1	L3-1	H1-1	H2-2	H3-10
51F8	L1-2	L2-1	L3-1	H1-1	H2-2	H3-7
222E7	L1-2	L2-1	L3-1	H1-1	H2-2	H3-14
55B3	L1-2	L2-1	L3-1	H1-1	H2-2	H3-14
209G5	L1-2	L2-1	L3-1	H1-1	H2-2	H3-10
65G9	L1-4	L2-1	L3-1	H1-1	H2-2	H3-14
218D9	L1-2	L2-1	L3-1	H1-1	H2-4	H3-14
224G3	L1-2	L2-1	L3-1	H1-1	H2-4	H3-6
74H10	L1-2	L2-1	L3-1	H1-1	H2-2	H3-14

- 5 Of these 10 sets of CDRs in Table 3 above, a particular group of four anti-canine IL-31RA antibodies that bind IL-31RA were identified as comprising a striking amino acid sequence similarity.

HEAVY CHAIN:

	<u>AB</u>	<u>HCDR1</u>	<u>SEQ ID</u>	<u>HCDR2</u>	<u>SEQ ID</u>
10	100H8	DYNMD	NO: 3	HINPNNGGILYNQKFKG	NO: 10
	74H10	DYNMD	NO: 3	HINPNNGG T LYNQKFKD	NO: 11
	222E7	DYNMD	NO: 3	HINPN I GG T LYNQKFKG	NO: 12
	55B3	DYNMD	NO: 3	HINPN T GG T IYNQKFKG	NO: 13
15	<u>AB</u>	<u>HCDR3</u>	<u>SEQ ID</u>		
	100H8	WAPLVRQPYWY	NO: 20		
	74H10	WAPL L RQPYWY FDV	NO: 21		

222E7 WAQLQRQPYWYFDV NO: 22
 55B3 WAQLLRQPYWYFDV NO: 23

LIGHT CHAIN:

	<u>AB</u>	<u>LCDR1</u>	<u>SEQ ID</u>	<u>LCDR2</u>	<u>SEQ ID</u>
5	100H8	KASQDINSYLN	NO: 30	RADRLVD	NO: 40
	74H10	KASQDINSYLN	NO: 30	RADRLVD	NO: 40
	222E7	KASQDINSYLN	NO: 30	RVNRLVD	NO: 38
	55B3	KASQSVTNDVT	NO: 31	RANRLVD	NO: 39

	<u>AB</u>	<u>LCDR3</u>	<u>SEQ ID</u>
10	100H8	LQYDEFPLT	NO: 45
	74H10	LQYDEFPLT	NO: 45
	222E7	LQYDEFPLT	NO: 45
15	55B3	LQYDEFPLT	NO: 45

Indeed, all of the HCDR1's in this group of four antibodies have the identical amino acid sequence (SEQ ID NO: 3). Whereas the four HCDR2's differ, they do not differ much. Notably, the HCDR2 of 100H8 differs from the three other HCDR2's by having an isoleucine residue at the ninth position rather than a threonine residue. 74H10 further differs from 100H8 by possessing an aspartic acid residue at its C-terminus rather than a glycine residue. Like 100H8, the other three HCDR2's have a glycine residue at their C-terminus. Unlike 100H8, the other three HCDR's have three additional amino acid residues (FDV) at their C-terminus. Both 74H10 and 55B3 have a leucine at their fourth position, rather than a valine residue like 100H8, whereas 222E7 has a glutamine residue. Finally, whereas both 100H8 and 74H10 have a proline residue at the third position, both 222E7 and 55B3 have a glutamine residue at the third position.

Three of the four LCDR1's in this group of four antibodies have the identical amino acid sequence (SEQ ID NO: 30), but whereas the LCDR1 of 55B3 shares their first four amino acid residues, its LCDR1 differs from the other three LCDR1s in its remaining seven amino acid residues. The LCDR2 of 100H8 and 74H10 have identical amino acid sequences (SEQ ID NO: 40), but the LCDR2 of 55B3 and 222E7 both differ from the other two LCDR2s by having an asparagine residue at position three instead of the aspartic acid residue of 100H8 and 74H10. In addition, the LCDR2 of 222E7 has a valine residue at position two rather than an alanine

residue found in the LCDR2 of the three other antibodies. Notably, all four LCDR3's in this group of four antibodies have the identical amino acid sequence (SEQ ID NO: 45).

The remaining six (6) antibodies to canine IL-31RA detailed above, *i.e.*, 65G9, 85C10, 224G3, 51F8, 209G5, and 218D9, can be further broken down into two pairs of antibodies that have noticeable identity in their respective CDR amino acid sequences, and two antibodies that are relative outliers. Accordingly, there is appreciable amino acid sequence identity between the sets of 6CDRs of antibody 65G9 with that of antibody 85C10 (*see*, Table 3). Consistently, antibodies 65G9 and 85C10 both bind two linear sequences in the C-terminal region of IL-31RA-ECD, one of which is the same (SEQ ID NO: 106; *see*, Table 6 and Figure 6D) and the other one has substantial overlap (compare SEQ ID NO: 107 with SEQ ID NO: 108; *see*, Table 6). Notably, one of the outliers, antibody 244G3, binds to an epitope comprising a single linear sequence in the C-terminal region of IL-31RA-ECD (*see*, Figure 6E), which is contained with the second linear sequence of antibody 85C10 (compare SEQ ID NO: 109 with SEQ ID NO: 108; *see*, Table 6).

The second group contains antibodies 51F8 and 209G5, which also have appreciable amino acid sequence identity between their respective sets of 6CDRs (*see*, Table 3). Consistently, antibodies 51F8 and 209G5 both bind two linear sequences in the N-terminal region of IL-31RA-ECD, one of which is the same (SEQ ID NO: 98; *see*, Table 6 and Figure 6B), whereas the second linear sequence of the IL-31RA-ECD that antibody 209G5 binds (SEQ ID NO: 101) is within the amino sequence of the second linear sequence of IL-31RA-ECD that antibody 51F8 binds (SEQ ID NO: 100; *see*, Table 6).

The other outlier, antibody 218D9 binds to two linear amino acid sequences located in the middle portion of the amino acid sequence of IL-31RA-ECD (SEQ ID NOs: 104 and 105; *see*, Figure 6C and Table 6). This antibody proved to be both a strong binder of IL-31RA and a good blocker of the binding of IL-31RA with IL-31.

EXAMPLE 5

BLOCKING ACTIVITY OF ANTI-IL-31 RECEPTOR *alpha* ANTIBODIES

The ability of anti-canine IL-31RA hybridoma supernatants to block the binding of IL-31 to IL-31RA were evaluated in the blocking ELISA described below.

Protocol

- 1) Coat 96-well half area plates with IL-31RA (1 µg/mL in PBS buffer), 25 µL/well.
- 2) Incubate the plates at 4°C overnight.

- 3) Wash the plates 3 times by PBST (PBS +0.05% Tween 20)
- 4) Block the plates with blocking buffer (PBS with 5% FBS), 25ul/well, for 30 minutes at room temperature.
- 5) Transfer 25 ul/well hybridoma supernatant to the 96-well plates, incubate 60 minutes at room temperature.
- 6) Wash the plates 3 times with PBST.
- 7) Transfer 25 µL/well of biotinylated IL31 (0.5 µg/mL in blocking buffer,) incubate 60 minutes at room temperature.
- 8) Wash the plates 3 times with PBST.
- 10 9) Add 25 µL/well Streptavidin-HRP, 1:5000 dilution in blocking buffer, to the plates and incubate 60 minutes at room temperature.
- 10) Wash the plates five times with PBST.
- 11) Add TMB based reagent to the plates for colorimetric reaction for 2-3 minutes.
- 12) Stop the reactions with 0.16 M sulfuric acid.
- 15 13) Read the plates by plate reader.

Results:

Out of the approximately 2000 hybridoma clones initially identified, approximately 300 were found to have binding affinity for IL-31RA, and about 10 of such clones also showed significant blocking of the binding of canine IL-31 to canine IL-31RA (*see*, Examples below).

20

EXAMPLE 6

FACS ASSAY FOR TESTING BLOCKING ACTIVITY OF MONOCLONAL ANTIBODIES AGAINST CANINE IL31-RA

In order to develop a cell-based assay to assess binding and blocking of canine IL-31 by anti-canine IL-31RA antibodies, the nucleotide sequences of the canine IL-31RA with c-terminal Flag tag and OSMR with c-terminal HA tag were prepared by chemical synthesis and then cloned into lentivirus vector Lenti-puro and Lenti-Hygro, respectively. The lentivirus Lenti-puro-IL31RA-Flag and Lenti-Hygro-OSMR-HA prepared from the Lenti-X 293T cells were co-transfected into CHO-k1 cells. The CHO stable cell pool co-expressing canine IL-31RA and OSMR was selected by FACS with anti-flag and anti-HA antibodies. Single cell clones were isolated from the stable pool. The developed CHO-IL-31RA/OSMR table cell line is applied to screen anti-canine IL-31RA monoclonal antibodies for blocking of IL-31 with its receptor complex IL-31RA/OSMR.

30

Materials

Cell line: CHO-IL-31RA/OSMR stable cell line

Cell growth medium: F-12K Medium with 10% FBS, 8µg/ml Puromycin and 200µg/ml hygromycin

5 Recombinant canine IL-31-His protein (0.5 µg/ml)

FACS Buffer: PBS

Isotype control: Mouse IgG (Genscript, A01007) (3 µg/ml)

Secondary antibody : Mouse anti-His tag antibody, 1µg/ml (Genscript, A01802)

Flow cytometer: BD FACSCanto

10 *Flow Cytometry Procedure*

- 1) CHO-IL-31RA/OSMR cells were grown in the growth medium in T75 flask.
- 2) Trypsinize to detach the cells, and then resuspend the cells in 5 mL fresh growth medium.
- 3) Spin down the cells at 300g for 3min, discard the supernatant and wash the cells twice with PBS.
- 15 4) Resuspend the cells in PBS as 2x10⁶ cells/mL.
- 5) The cells were plated into 96-well assay plate with 50µl/well.
- 6) Mix anti-IL-31RA antibody (20µg/mL) or isotype control with IL-31 (1µg/ml), and then transfer 50µl of the mixture into each well of the assay plate.
- 7) After incubation at 4°C for 40min, the cells were washed twice by 150µl of cold PBS.
- 20 8) Add 100µl of the secondary antibody (1µg/mL) into each well of the assay plate.
- 9) After incubation at 4°C for 40min, the cells were washed twice by 150µl of cold PBS.
- 10) Resuspend the cells in 100µl/well cold PBS and read by the Flow cytometry.

As shown in Figure 3, the FACS result indicate that nine of the ten mouse anti-canine
 25 IL-31RA mAbs can significantly block the binding of IL-31 with the IL-31RA/OSMR complex presented on the CHO-IL-31RA/OSMR cells. The lead antibodies are 51F8, 74H10, 100H8, 209G5 and 218D9.

EXAMPLE 7

30 STAT-3 ASSAY

Stat-3 is known to be activated by IL-31 in cells comprising the the heterodimeric receptor for IL-31. In order to develop an assay to assess the activation of STAT-3 by canine IL-31, the nucleotide sequences encoding IL-31RA and OSMR, respectively, were prepared by

chemical synthesis and then cloned into expression vectors pcDNA3.1. The vectors containing the IL-31RA and OSMR nucleotide sequences, respectively, were co-transfected into Ba/f3 cells and the transfected cells, denoted as “Ba/f3-OI”, were grown as a pool under antibiotic selection. The ability of canine IL-31 to induce STAT-3 activation was tested as follows.

5 *Materials:*

Cell line: Ba/f3-OI stable pool cells

Growth medium with mouse IL-3 or with canine IL-31 (cIL-31):

RPMI 1640 435 ml (ThermoFisher, 12633-020)

FBS 50 mL (SAFC cat# 12003c-500mL)

10 2-Mercaptoethanol (50 mM) 0.5 mL (Gibco 31350-010)

100X Pen Strep 5 mL (Gibco 15140-122 Lot1734040)

200mM L-Glu 10 ml (Gibco 25030-081 Lot1677185)

500 ng/mL Geneticin G418 (from Gibco or Sigma)

5 ng/mL mIL-3 or 100 ng/mL cIL-31

15 Starvation medium: the growth medium without mIL-3 and cIL-31

p-STAT3 (Tyr705) Assay Kit: PerkinElmer, ALSU-PST3-A-HV

Procedure:

Cell culture

- 1) Thaw a vial of the Ba/f3-OI cells, and grow the cells in the growth medium with mIL-3 in
- 20 37°C CO₂ shaker with 125 rpm.
- 2) Passage the cells 2 – 3 passages to have the cells with $\geq 90\%$ viability before set a cell-based assay.
- 3) To setup assay, harvest and resuspend the cells in the starvation medium to 1×10^7 viable cells/mL.
- 25 4) Dispense cells into 96-well plate, 50 μ L/well (about 5×10^5 cells/well).
- 5) Three-fold dilute cIL-31 in starvation medium in a dilution plate, and then transfer 50 μ L of each of the serial diluted cIL-31 aliquots into the cell plate.
- 6) Incubate the cell plate for 15-30 min in 37°C CO₂ shaker with 125 rpm for 1-2 hrs.

AlphaLISA assay as per manufacturer's instruction:

- 30 7) Spin down the cells, aspirate the supernatant, and add 1x lysis buffer of 50 – 100 μ L/well. Incubate at RT for 10 min with 1000rpm shaking.
- 8) Remove 30 μ L of the cell lysate into a ½ area plate or freeze and store at -80°C for future test.

SUREFIRE Assay:

- 8) Add 15 μ L /well acceptor mix to the cell lysate. Seal and agitate plate for 2 min at 1000 rpm and then incubate for 1-2 hours at RT.
- 9) Add 15 μ L/well donor mix to the cell lysate. Seal and agitate for 2 min at 1000 rpm, and
5 then incubate for 1-2 hours at RT (the plate can be stored at 4°C overnight. Incubate at room temp for 1 hr before reading the plate next day)
- 10) Read the plate on Alpha plate reader at 520 – 620 nm.

Results:

- Figure 4 shows the induction of STAT-3 phosphorylation by canine IL-31, which
10 stimulates activation of STAT-3 in Ba/f3-OI cells in a dose dependent manner. Ba/f3 cells were used as the control. Ba/f3-OI cells expressing the IL-31 receptor complex were tested for IL-31-induced STAT-3 phosphorylation. The results indicate that STAT-3 phosphorylation was induced by IL-31 in the Baf3-OI cells in a dose-dependent manner, implying that: (i) the canine IL-31 receptor complex is successfully expressed on the cell surface; (ii) that the binding of
15 canine IL-31 to the IL-31 receptor can stimulate the endogenous STAT3 phosphorylation; and (iii) then initiate its downstream signaling pathway.

EXAMPLE 8

BIOLOGICAL ACTIVITY OF ANTI-CANINE IL-31RA ANTIBODIES

- 20 The ability of the anti-canine IL-31RA mAbs to inhibit the activation of STAT-3 in Ba/f3-OI cells is assessed as follows:
- 1) Thaw a vial of the Ba/f3-OI cells, and grow the Ba/f3-OI cells in the growth medium with mIL-3 in 37°C CO₂ shaker with 125 rpm.
 - 2) Passage the cells 2 – 3 passages to have the cells with $\geq 90\%$ viability before set a cell-
25 based assay.
 - 3) To setup assay, harvest and resuspend the cells in the starvation medium to 1×10^7 viable cells/mL.
 - 4) Dispense cells into 96-well plate, 50 μ L/well (about 5×10^5 cells/well).
 - 5) Three-fold dilute the antibody in starvation medium in a row on a 96-well plate, starting
30 concentration at 200 μ g/mL. Then add 5 μ L cIL-31 in each well to get final concentration of 100 ng/mL.
 - 6) Transfer 50 μ L of the diluted antibody and cIL-31 mix into each well of the cell plate, gently mix.

7) Incubate the cell plate in 37°C CO₂ shaker with 125 rpm for 15-30 min. AlphaLISA assay as per manufacturer's instruction: (refer to Example 7)

As exemplified in Figure 5A for monoclonal antibodies 209G5, 218D9, and 85C10, in Figure 5B for monoclonal antibodies 100H8, 74H10, 51F8, and 85C10, and again in Figure 8 for various constructs of 218D9, all of the IL-31RA mAbs tested inhibit the canine IL-31 mediated STAT-3 phosphorylation in the Ba/f3-OI cells, whereas in the absence of these antibodies, there is no inhibition (labeled IL-31). From Figure 8, the IC₅₀ for the various 218D9 antibody constructs was calculated to be approximately: 2.2 nM for the chimeric antibody; 230 nM for c218D9VH3VL2; 8.3 nM for c218D9VH4VL2; 2.9 nM for c218D9VH4VL3; and 2.5 nM for c218D9VH3VL3.

EXAMPLE 9

IN VITRO BINDING OF ANTI-CANINE IL-31RA MONOCLONAL ANTIBODIES TO CANINE IL-31RA RECEPTOR

All kinetics measurements were performed by Octet HTX using SA biosensors and Data Acquisition 12.0 software. A biotin-labeled antigen (canine IL-31RA) was loaded onto the pre-hydrated SA biosensors for 120s at a concentration of 1 µg/mL. Next, the biosensors were placed into Octet Kinetics Buffer (PBS+ 0.02% Tween 20, 0.1% BSA) for the blocking phase for 120s. Then, for the association phase, antigen loaded biosensors were placed into 2-fold serial dilutions from 100 nM down to 3.13 nM of anti-IL-31RA monoclonal antibody in Octet Kinetics Buffer for 300s. The last well was buffer alone and that sensor was used for reference sensor subtraction. Finally, the biosensors were placed into Octet Kinetics Buffer for the dissociation phase for 300s. Analysis was performed using Data Analysis 12.0 software and curves were fitted using a 1:1 binding model.

Binding affinity measurement results indicate that all the tested monoclonal antibodies have low nanomolar to sub-picomolar binding affinities ranging from about 1.5 nM to about 1 pM (*see*, Table 2 above). Two of the top blocking antibodies: 51F8 and 218D9, had K_Ds of about 0.2 nM and about 0.07 nM respectively (*see*, Table 5B below).

EXAMPLE 10

CANINIZED ANTIBODIES

The DNA and protein sequence of the canine heavy and light chains are known and can be obtained by searching of the NCBI gene and protein databases. As indicated above, for canine antibodies there are four known IgG subtypes: IgG-A, IgG-B, IgG-C, and IgG-D, and two types of light chains, *i.e.*, *kappa* and *lambda*. Without being bound by any specific approach, the process of producing caninized heavy and light chains that can be mixed in different combinations to produce caninized anti-canine IL-31 receptor *alpha* mAbs involves the following scheme:

Identify the DNA sequence of VH and VL domains comprising the CDRs of desired anti-IL-31 receptor *alpha* mAbs

- i) Identify the H and L chain CDRs of desired anti-IL-31RA mAbs
- ii) Identify a suitable sequence for H and L chain of canine IgG
- iii) Identify the DNA sequence encoding the endogenous CDRs of canine IgG H and L chains of the above sequence.
- iv) Replace the DNA sequence encoding endogenous canine H and L chain CDRs with DNA sequences encoding the desired anti-IL-31RA CDRs. In addition, optionally replace some canine framework residues with selected residues from the desired anti-IL-31 receptor *alpha* mAb framework regions.
- v) Synthesize the DNA from step (v), clone it into a suitable expression plasmid, and transfect the plasmids containing desired caninized H and L chains into HEK 293 cells.
- vi) Purify expressed caninized antibody from HEK 293 supernatant.
- vii) Test purified caninized antibody for binding to canine IL-31 receptor *alpha* chain.

The application of the above outlined steps can result in a set of caninized H and L chain amino acid sequences provided below.

25 c51F8VH1-cIgGBm [SEQ ID NO: 72]

EVQLVQSGAEVKKPGASVKVSCKTSGYTFINTFIHWVRQAPGAGLDWMGQIDPANGNTKYAPKFQ
 GRVTLTADTSTSTAYMELSSLRAGDIAYYYCARYYYVSSYFDVWGQGTLVTVSSASTTAPSVFPL
 APSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRW
 PSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPKDTLLIAR
 TPEVTCVVVALDPEDPEVQISWFDGKQMQTAKTQPREEQFAGTYRVVSVLPIGHQDWLKGKQFT
 CKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDIDVEWQSNG
 QQEPESKYRTTPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGK

c51F8VH2-cIgGBm [SEQ ID NO: 73]

EVQLVQSGAEVKKPGASVKVSCTASGFNIKNTFIHWVRQAPGAGLDWIGRIDPANGNTKYAPKFQ
 GRVTLTADTSTSTAYMELSSLRAGDIAVYYCARYYYVSSYFDVWGQGTLVTVSSASTTAPSVFPL
 APSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRW
 5 PSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPKDTLLIAR
 TPEVTCVVVALDPEDPEVQISWFVDGKQMQTAKTQPREEQFAGTYRVVSVLPIGHQDWLKGKQFT
 CKVNNKALPSPIERTISKARGQAHQPSVYVLPSSREELSKNTVSLTCLIKDFFPPDIDVEWQSNG
 QQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGK

10 c51F8VH3-clgGBm [SEQ ID NO: 74]

EVQLVQSGAEVKKPGASVKVSCTASGFNIKNTFIHWVRQAPGAGLDWIGRIDPANGNTKYAPKFQ
 GRATLTADTSTNTAYMQLSSLRAGDIAVYYCARYYYVSSYFDVWGQGTLVTVSSASTTAPSVFPL
 APSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRW
 PSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPKDTLLIAR
 15 TPEVTCVVVALDPEDPEVQISWFVDGKQMQTAKTQPREEQFAGTYRVVSVLPIGHQDWLKGKQFT
 CKVNNKALPSPIERTISKARGQAHQPSVYVLPSSREELSKNTVSLTCLIKDFFPPDIDVEWQSNG
 QQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGK

c51F8VH4-cIgGBm [SEQ ID NO: 75]

20 EVQLVQSGAEVVKPGASVKVSCTASGFNIKNTFIHWVRQRPAGAGLDWIGRIDPANGNTKYAPKFQ
 GRATLTADTSTNTAYMQLSSLRAGDIAVYYCARYYYVSSYFDVWGQGTLVTVSSASTTAPSVFPL
 APSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRW
 PSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPKDTLLIAR
 TPEVTCVVVALDPEDPEVQISWFVDGKQMQTAKTQPREEQFAGTYRVVSVLPIGHQDWLKGKQFT
 25 CKVNNKALPSPIERTISKARGQAHQPSVYVLPSSREELSKNTVSLTCLIKDFFPPDIDVEWQSNG
 QQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGKR
 NDAQPAVYLFQPSPDQLHTGSASVVCLLNSFYPKDINVKKVDGVIQDTGIQESVTEQDKDSTYS
 LSSTLTMSSTEYLSHELYSCEITHKSLPSTLIKS FQRSECQRVD

30 c51F8VL1-cCK [SEQ ID NO: 76]

EIVMTQSPASLSLSQEEKVTITCKASQSVTNDVTWYQQKPGQAPKLLIYSASNRYTGVPSTRFSGS
 GSGTDFSFTISSLEPEDVAVYYCQQDYSSPWTFGQGTEIKRNDAPAVYLFQPSPDQLHTGSA

SVVCLLNSFYPKDINVKKVDGVIQDTGIIQESVTEQDKDSTYSLSSTLTMSSTEYLSHELYSCEI
THKSLPSTLIKSFORSECQRVD

c51F8VL6-cCK [SEQ ID NO: 77]

5 DIVMTQTPLSLSVSPGETASISCKASQSVTNDVTWFRQKPGQSPQLLIYSASNRYTGVPDRFSGS
GSGTDFTLRISTVEADDTGVYYCQQDYSSPWTFGQGTKLEIKRNDAPAVYLFQPSPDQLHTGSA
SVVCLLNSFYPKDINVKKVDGVIQDTGIIQESVTEQDKDSTYSLSSTLTMSSTEYLSHELYSCEI
THKSLPSTLIKSFORSECQRVD

10 c51F8VL7-cCK [SEQ ID NO: 78]

DIVMTQTPLSLSVSPGETASISCKASQSVTNDVTWFRQKPGQSPKLLIYSASNRYTGVPDRFSGS
GSGTDFTLRISTVEADDTGVYYCQQDYSSPWTFGQGTKLEIKRNDAPAVYLFQPSPDQLHTGSA
SVVCLLNSFYPKDINVKKVDGVIQDTGIIQESVTEQDKDSTYSLSSTLTMSSTEYLSHELYSCEI
THKSLPSTLIKSFORSECQRVD

15

c100H8VH4-cIgGBm [SEQ ID NO: 79]

EVQLVQSGAEVKKPGASVKVSCKTSGYTFTDYNMDWVRQAPGAGLDWMGHINPNNGGILYNQKFK
GRVTLTADTSTSTAYMELSSLRAGDIAVYYCARWAPLVRQPYWYFDVWGQGTLVTVSSASTTAPS
VFPLAPSCGSTSGSTVALACLVSIFYPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVP
20 SSRWPSETFTCNVAHPASKTKVDKVPKRENGRVPRPPDCPKCPAPEMLGGPSVFIFFPKPKDTL
LIARTPEVTCVVVALDPEDPEVQISWFVDGKQMOTAKTQPREEQFAGTYRVVSVLPIGHQDWLKG
KQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPSPREELSKNTVSLTCLIKDFPPDIDVEW
QSNQQQEPESKYRTTPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHS
PGK

25

c100H8VH7-cIgGBm [SEQ ID NO: 80]

EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNMDWVRQAPGAGLDWIGHINPNNGGILYNQKFK
GKATLTADTSTSTAYMELSSLRAGDIAVYYCARWAPLVRQPYWYFDVWGQGTLVTVSSASTTAPS
VFPLAPSCGSTSGSTVALACLVSIFYPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVP
30 SSRWPSETFTCNVAHPASKTKVDKVPKRENGRVPRPPDCPKCPAPEMLGGPSVFIFFPKPKDTL
LIARTPEVTCVVVALDPEDPEVQISWFVDGKQMOTAKTQPREEQFAGTYRVVSVLPIGHQDWLKG
KQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPSPREELSKNTVSLTCLIKDFPPDIDVEW

QSNQGQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHS
PGK

c100H8VH8-cIgGBm [SEQ ID NO: 81]

5 EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNMDWVRQAPGAGLDWIGHINPNNGGILYNQKFK
GKATLTVDNSTNTAYMELSSLRAGDIAVYFCARWAPLVRQPYWYFDVWGQGTLLTVSSASTTAPS
VFPLAPSCGSTSGSTVALACLVSQYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVP
SSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFIFFPKPKDTL
LIARTPEVTCVVVALDPEDPEVQISWFDGKQMQTAKTQPREEQFAGTYRVVSVLPIGHQDWLKG
10 KQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPSPSREELSKNTVSLTCLIKDFFPPDIDVEW
QSNQGQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHS
PGK

c100H8VL1-cCK [SEQ ID NO: 82]

15 EIVMTQSPASLSLSQEEKVTITCKASQDINSYLNWYQQKPGQAPKLLIYRADRLVDGVPSRFSGS
GSGTDFSFSTISSLEPEDVAVYYCLQYDEFPLTFGAGTKVELKRNDAPAVYLFQPSPDQLHTGSA
SVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSSTLTMSSTEYLSHELYSCEI
THKSLPSTLIKSFORSECQRVD

20 c100H8VL2-cCK [SEQ ID NO: 83]

EIVMTQSPASLSLSQEEKVTITCKASQDINSYLNWFQQKPGQAPKLLIYRADRLVDGVPSRFSGS
GSGTDFSFSTISSLEPEDVAVYYCLQYDEFPLTFGAGTKLELKRNDAPAVYLFQPSPDQLHTGSA
SVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSSTLTMSSTEYLSHELYSCEI
THKSLPSTLIKSFORSECQRVD

25

c100H8VL3-cCK [SEQ ID NO: 84]

DIKMTQSPASLSLSQEEKVTITCKASQDINSYLNWFQQKPGQAPKLLIYRADRLVDGVPSRFSGS
GSGTDFSFSTISSLEPEDVAVYYCLQYDEFPLTFGAGTKLELKRNDAPAVYLFQPSPDQLHTGSA
SVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSSTLTMSSTEYLSHELYSCEI
30 THKSLPSTLIKSFORSECQRVD

c100H8VL4-cCK [SEQ ID NO: 85]

DIVMTQTPLSLSVSPGETASISCKASQDINSYLNWFRQKPGQSPQLLIYRADRLVDGVPDRFSGS
GSGTDFTLRISTVEADDTGVYYCLQYDEFPLTFGAGTKVELKRND AQPAVYLFQPSPDQLHTGSA
SVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSTLTMSSTEYLSHELYSCEI
THKSLPSTLIKSFORSECQRVD

5

c218D9VH1-cIgGBm [SEQ ID NO: 86]

ELTLQESGPGLVKPSQTLSLTCTVVS GGSVTTTFGRGVGWIRQRPGRGLEWMGHIWWDDDKYYNPAL
KSRISITADTAKNQFSLQLSSMTTEDTAVYYCARIAGGLRRAPYAMD SWGQGT LVTVSSASTTAP
SVFPLAPSCGSTSGSTVALACLVSGYFPEPVT VSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTV
10 PSSRWPSETFTCNVAHPASKTKVDKPV PKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPKDT
LLIARTPEVTCVVVALDPEDPEVQISWFVDGKQMQTAKTQPREEQFAGTYRVVSVLP IGHQDWLK
GKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLP PSREELSKNTVSLTCLIKDFFPPDIDVE
WQSNQQEPESKYRTTPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSH
SPGK

15

c218D9VH2-cIgGBm [SEQ ID NO: 87]

ELTLQESGPGLVKPSQTLSLTCSFSGFSLSTFGRGVGWIRQRPGRGLEWMGHIWWDDDKYYNPAL
KSRISITADTAKNQFSLQLSSMTTEDTAVYYCARIAGGLRRAPYAMD SWGQGT LVTVSSASTTAP
SVFPLAPSCGSTSGSTVALACLVSGYFPEPVT VSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTV
20 PSSRWPSETFTCNVAHPASKTKVDKPV PKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPKDT
LLIARTPEVTCVVVALDPEDPEVQISWFVDGKQMQTAKTQPREEQFAGTYRVVSVLP IGHQDWLK
GKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLP PSREELSKNTVSLTCLIKDFFPPDIDVE
WQSNQQEPESKYRTTPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSH
SPGK

25

c218D9VH3-cIgGBm [SEQ ID NO: 88]

EVTLQESGPGLVKPSQTLSLTCSFSGFSLSTFGRGVGWIRQRPGRGLEWMGHIWWDDDKYYNPAL
KSRLSITKDTAKNQFSLQLSSMTTEDTAVYYCARIAGGLRRAPYAMD SWGQGT LVTVSSASTTAP
SVFPLAPSCGSTSGSTVALACLVSGYFPEPVT VSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTV
30 PSSRWPSETFTCNVAHPASKTKVDKPV PKRENGRVPRPPDCPKCPAPEMLGGPSVFI FPPKPKDT
LLIARTPEVTCVVVALDPEDPEVQISWFVDGKQMQTAKTQPREEQFAGTYRVVSVLP IGHQDWLK
GKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLP PSREELSKNTVSLTCLIKDFFPPDIDVE

WQSNQGQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSH
SPGK

c218D9VH4-cIgGBm [SEQ ID NO: 89]

5 EVTLQESGPGLVKPSQTLSTCSFSGFSLSTFGRGVGWIRQRPGRGLEWMGHIWDDDKYYNPAL
KSRLSITKDTAKNQVFLQLSSMTTEDTAVYYCARIAGGLRRAPYAMDSWGQGLTVTVSSASTTAP
SVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTV
PSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGPSVFIFFPKPKDT
LLIARTPEVTCVVVALDPEDPEVQISWFVDGKQMQTAKTQPREEQFAGTYRVVSVLPIGHQDWLK
10 GKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPSPREELSKNTVSLTCLIKDFFPPDIDVE
WQSNQGQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSH
SPGK

c218D9VL1-cCK [SEQ ID NO: 90]

15 EIVMTQSPASLSLSQEEKVTITCRASENIYSSLAWYQQKPGQAPKLLIYAATNLADGVPSRFSGS
GSGTDFSFSTISSLEPEDVAVYYCQHFRDTPPTFGQGTKLEIKRNDAPAVYLFQPSPDQLHTGSA
SVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSSTLTMSSTEYLSHELYSCEI
THKSLPSTLIKSFORSECQRVD

20 **c218D9VL2-cCK [SEQ ID NO: 91]**

DIVMTQTPLSLSVSPGETASISCRASENIYSSLAWFRQKPGQSPQLLIYAATNLADGVDPDRFSGS
GSGTDFTLRISRVEADDTGVYYCQHFRDTPPTFGQGTKLEIKRNDAPAVYLFQPSPDQLHTGSA
SVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSSTLTMSSTEYLSHELYSCEI
THKSLPSTLIKSFORSECQRVD

25

c218D9VL3-cCK [SEQ ID NO: 92]

DIVMTQTPLSLSVSPGETASISCRASENIYSSLAWFRQKPGQSPQLLVYAATNLADGVDPDRFSGS
GSGTDYTLRISRVEADDTGVYYCQHFRDTPPTFGQGTKLEIKRNDAPAVYLFQPSPDQLHTGSA
SVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDKDSTYSLSSSTLTMSSTEYLSHELYSCEI
30 THKSLPSTLIKSFORSECQRVD

c218D9VH1 [SEQ ID NO: 93]

ELTLQESGPGLVKPSQTLSTLCVVSGGSVTTFGRGVGWIRQRPGRGLEWMGHIWWDDDKYYNPAL
KSRISITADTAKNQFSLQLSSMTTEDTAVYYCARIAGGLRRAPYAMDSWGQGTLLTVTVSS

c218D9VH2 [SEQ ID NO: 94]

5 ELTLQESGPGLVKPSQTLSTLCVSGFSLSTFGRGVGWIRQRPGRGLEWMGHIWWDDDKYYNPAL
KSRISITADTAKNQFSLQLSSMTTEDTAVYYCARIAGGLRRAPYAMDSWGQGTLLTVTVSS

c218D9VH3 [SEQ ID NO: 95]

EVTLQESGPGLVKPSQTLSTLCVSGFSLSTFGRGVGWIRQRPGRGLEWMGHIWWDDDKYYNPAL
10 KSRLSITKDTAKNQFSLQLSSMTTEDTAVYYCARIAGGLRRAPYAMDSWGQGTLLTVTVSS

c218D9VH4 [SEQ ID NO: 96]

EVTLQESGPGLVKPSQTLSTLCVSGFSLSTFGRGVGWIRQRPGRGLEWMGHIWWDDDKYYNPAL
15 KSRLSITKDTAKNQVFLQLSSMTTEDTAVYYCARIAGGLRRAPYAMDSWGQGTLLTVTVSS

c218D9VL1 [SEQ ID NO: 128]

EIVMTQSPASLSLSQEEKVTITCRASENIYSSLAWYQQKPGQAPKLLIYAATNLADGVPSRFSGS
GSGTDFSFTISSLEPEDVAVYYCQHFRDTPPTFGQGTKLEIK

20 c218D9VL2 [SEQ ID NO: 129]

DIVMTQTPLSLSVSPGETASISCRASENIYSSLAWFRQKPGQSPQLLIYAATNLADGVDPDRFSGS
GSGTDFTLRISRVEADDTGVYYCQHFRDTPPTFGQGTKLEIK

c218D9VL3 [SEQ ID NO: 130]

25 DIVMTQTPLSLSVSPGETASISCRASENIYSSLAWFRQKPGQSPQLLVYAATNLADGVDPDRFSGS
GSGTDYTLRISRVEADDTGVYYCQHFRDTPPTFGQGTKLEIK

EXAMPLE 11

REACTIVITY OF CANINIZED ANTIBODIES AGAINST CANINE IL-31RA

30 The caninized antibodies were tested for reactivity with canine IL-31RA as follows:

1. Coat 200 ng/well of IL-31RA on an immunoplate and incubate the plate at 4°C overnight.
2. Wash the plate 3 times with phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBST).

3. Block the plate with 0.5% bovine serum albumin (BSA) in PBS for 45 - 60 min at room temperature.
4. Wash the plate 3 times with PBST.
5. Three - fold dilute the caninized antibody in each column or row of dilution plate starting at 0.3 μ g/mL.
6. Transfer the diluted caninized antibody into each column or row of the immunoplate, and incubate the plate for 45 - 60 min at room temperature.
7. Wash the plate 3 times with PBST.
8. Add 1 :4000 diluted horseradish peroxidase labeled anti - canine IgG Fc into each well of the plate, and then incubate the plate for 45 - 60 min at room temperature.
9. Wash the plate 3 times with PBST.
10. Add 3,3',5,5'-tetramethylbenzidine (TMB) Substrate into each well of the plate, and incubate the plate for 10 to 15 min at room temperature to develop the color.
11. Add 100 μ L 1.5 M phosphoric acid into each well to stop the reaction. Read plate at 450nm with 540 nm reference wavelength.

As depicted in Figures 7A-7E, the caninized antibodies were tested for their reactivity to canine IL-31RA. The results indicate that the caninized antibodies have similar binding affinity as their corresponding parental antibodies (represented by their chimeric antibodies).

Table 5A below provides the relative binding affinities of the different caninized antibodies (EC50), relative to their corresponding mouse-canine chimera antibody (*see*, Figures 7A-7E). Although these are just relative numbers, caninized antibody 218D9 stands out as an antibody that has essentially the same binding affinity as its parental chimeric murine antibody.

The term “Chimeric” before the antibody number signifies that the antibody is a murine-canine chimeric antibody, *e.g.*, Chimeric 218D9 or Chimeric 51F8. In addition, an “m” before the antibody number followed by a “Chim” signifies that the antibody is a murine-canine chimeric antibody, *e.g.*, m224G3 Chim. The lower case “c” before the antibody number signifies that it is a caninized antibody, *e.g.*, c218D9VH4VL2.

TABLE 5A

RELATIVE BINDING AFFINITIES OF THE CANINIZED ANTIBODIES (EC50)

ANTIBODY	EC50
Chimeric 100H8	2.021
c100H8VH5L4	2.561
c100H8VH7L4	0.581

Chimeric 85C10	2.583
c85C10VH3L2	1.910
c85C10VH1L2	6.002
Chimeric 224G3	0.383
c224G3VH2L2	1.078
c224G3VH2L3	0.699
Chimeric 51F8	3.417
c51F8VH3L6	16.94
c51F8VH3L7	25.02
c51F8VH4L6	8.306
c51F8VH4L7	11.33
Chimeric 218D9	3.153
c218D9VH3L2	4.289
c218D9VH3L3	3.216
c218D9VH4L2	4.096
c218D9VH4L3	3.580

Table 5B below shows the binding constants of the caninized antibodies of 51F8 and 218D9. The results again indicate that caninized 218D9 antibodies have essentially the same binding affinity as their parental chimeric murine antibody, whereas caninized 51F8 antibodies have slightly weaker binding affinity than their parental chimeric murine antibody.

TABLE 5B

BINDING CONSTANTS OF THE CANINIZED 51F8 AND 218D9 ANTIBODIES

ANTIBODY	KD (M)	ka (1/Ms)	kdis (1/s)
chimeric 51F8	2.07E-10	6.84E+05	1.41E-04
c51F8VH3VL6	2.67E-09	4.28E+05	1.14E-03
c51F8VH3VL7	3.99E-09	4.01E+05	1.60E-03
c51F8VH4VL6	1.01E-09	4.45E+05	4.48E-04
c51F8VH4VL7	1.23E-09	4.16E+05	5.12E-04
chimeric 218D9	6.98E-11	6.64E+05	4.63E-05
c218D9VH3VL2	5.28E-10	6.41E+05	3.38E-04
c218D9VH3VL3	4.16E-11	6.61E+05	2.75E-05
c218D9VH4VL2	3.05E-10	5.41E+05	1.65E-04
c218D9VH4VL3	4.47E-11	6.01E+05	2.69E-05

MAPPING OF CANINE IL-31 RECEPTOR *alpha* EPITOPES USING MASS SPECTROSCOPY

A method based on chemical crosslinking and mass spectrometry detection was employed to identify epitopes recognized by anti-canine IL-31 receptor *alpha* mAbs [CovalX Instrument Incorporated, located at 999 Broadway, Suite 305, Saugus, MA 01906-4510]. The application of this technology to epitope mapping of canine IL-31 receptor *alpha* chain resulted in identification of epitopes recognized by the mAbs listed in Table 6 below. The results from the epitope mapping of canine IL-31 receptor *alpha* with the antibodies disclosed herein indicate that the mAbs recognize specific peptide epitopes that are present within the extracellular domain of canine IL-31 receptor *alpha* (*see*, Table 6 below). The results from the epitope mapping of canine IL-31RA with the eight antibodies included in Table 6, indicate that the mAbs recognize specific peptide epitopes that are present within the extracellular domain of canine IL-31RA. Notably, seven epitopes, which are distributed from N-terminus to C-terminus of IL-31RA-ECD were identified bound by the eight antibodies. Antibodies 100H8, 51F8, 209G5, and 55B3 share epitopes towards the N-terminus, while antibodies 65G9, 85C10 and 224G3 share epitopes towards the C-terminus of the IL-31RA-ECD. Antibody 218D9 has two unique epitopes located at middle portion of IL-31RA-ECD. As indicated by the functional results, all the eight monoclonal antibodies block IL-31 mediated STAT-3 phosphorylation, meaning that the seven epitopes are important in the interaction of canine IL-31RA with its ligand. This exhibits the complexity of the interaction of the canine IL-31 and canine IL-31 receptor complex. Five of the eight monoclonal antibodies tested were caninized, and the positions of their respective epitopes and their identified binding amino acid residues of the IL-31RA ECD are both presented in Figures 6A-6E and included in Table 6 below [*see also*, Figures 6A-6E which provides the epitopes on canine IL-31RA for the antibodies: 100H8, 51F8, 218D9, 85C10, and 224G3, respectively, and the position of binding residues of the amino acid sequence of SEQ ID NO: 2 of the respective epitopes on the cIL-31R ECD antigen.]

TABLE 6
IL-31RA EPITOPES RECOGNIZED BY anti-IL-31RA MONOCLONAL

mAb	SEQ ID NO:	EPITOPE SEQUENCE	¹ RESIDUES IDENTIFIED AS BINDING
100H8	97	YWNLD AIMKIEPPEIFSVK	Y ₉₄ , K ₁₀₂ , and K ₁₁₂

¹ Residue position on the cIL-31R ECD antigen with the amino acid sequence of SEQ ID NO: 2.

	103	RCAPAESMFWSGWSQEKVGT	R ₁₈₃ , S ₁₉₃ , and T ₂₀₂
51F8	98	KIEPPEIFSVKSVLGIKR	K ₁₀₂ , S ₁₁₀ , K ₁₁₂ , K ₁₁₈ , and R ₁₁₉
	100	TEYVMTLRCAPAES	T ₁₇₆ , Y ₁₇₈ , and S ₁₈₉
209G5	98	KIEPPEIFSVKSVLGIKR	
	101	YVMTLR	
55B3	99	RPVLAPHSSTLKY	
	102	RCAPAESM	
218D9	104	RRPVQLMWKKAT	R ₂₂₅ and T ₂₃₆
	105	RIPALNEKT	R ₂₉₈ , K ₃₀₅ , and T ₃₀₆
65G9	106	RNGFIKNYTIIFY	
	107	RTSYSLQVMASSTNAGGT	
85C10	106	RNGFIKNYTIIFY	R ₄₃₀ , K ₄₃₅ , T ₄₃₈ , and Y ₄₄₁
	108	SLQVMASSTNAGGTNGT	S ₄₇₂ , T ₄₇₉ , and T ₄₈₇
224G3	109	STNAGGTNGT	S ₄₇₈ and T ₄₈₇

TABLE 7

SEQUENCES IN FIGURES 6A-6E

FIGURE	SEQ ID NO:	SEQUENCE
6A	119	SDITYWNLDAIMKIEPPEIFSVKSVLGIKRM
	120	MTLRCAPAESMFWSGWSQEKVGTTEEEAPYG
6B	121	IMKIEPPEIFSVKSVLGIKRM
	122	TELQAFTEYVMTLRCAPAESM
6C	123	AMVDGRRPVQLMWKKATGAPV
	124	GESPVATLRIPALNEKTFQCI
6D	125	RNGFIKNYTIIFYQAEDGKEFS
	126 [#]	SYSLQVMASSTNAGGTNGTKIN
6E	126	SYSLQVMASSTNAGGTNGTKIN

ST.25 SEQUENCE LISTING FROM PRIORITY FILING **SEQUENCE LISTING**

5 <110> Intervet Inc.
 Intervet International BV
 Morsey, Mohamad
 Zhang, Yuanzheng

10 <120> Caninized Antibodies To Canine Interleukin-31 Receptor Alpha II

 <130> 25365-US-PSP

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 ctgcagatca agtggatcag acccgtgctg gccccccaca gcagcaccct gaagtacacc 420

40 ctgagattca gaaccatcaa cagcgcctac tggatggagg tgaacttcac caaggaggac 480

 atcgacagag acgagacctc caacctgacc gagctgcagg ccttcaccga gtacgtgatg 540

 accctgagat gcgcccccg cgcagagcatg ttctggagcg gctggagcca ggagaaggtg 600

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	ttccccgacg tggacagcga gcccagcagc ttcagctggg agagcgtgag ccaggccaga	1080
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	cccgtgctga gagacagagt gggccagccc tacagcacc aggcctacgt gcaggagggc	1200
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20	Trp Glu Ser Val Ser Gln Ala Arg Asn Trp Thr Ile Gln Lys Asp Glu		
	355	360	365
25	Leu Lys Pro Leu Trp Cys Tyr Asn Ile Ser Val Tyr Pro Val Leu Arg		
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Gly His Ile Asn Pro Asn Asn Gly Gly Ile Leu Tyr Asn Gln Lys Phe
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Phe Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser Arg Phe Ser Gly
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 20 Ala Arg Trp Ala Pro Leu Leu Arg Gln Pro Tyr Trp Tyr Phe Asp Val
 100 105 110
 25 Trp Gly Thr Gly Thr Thr Val Thr Val Ser Ser
 115 120
 30 <210> 59
 <211> 107
 <212> PRT
 <213> Mus musculus
 <400> 59
 35 Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr Ala Ser Leu Gly
 1 5 10 15
 40 Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Ser Tyr
 20 25 30
 45 Leu Asn Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro Lys Thr Leu Ile
 35 40 45
 50 Tyr Arg Ala Asp Arg Leu Val Asp Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 50 Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser Ser Leu Glu Tyr
 65 70 75 80

Glu Asp Met Gly Ile Tyr Tyr Cys Leu Gln Tyr Asp Glu Phe Pro Leu
 85 90 95

5 Thr Phe Gly Asp Gly Thr Lys Leu Glu Leu Asn
 100 105

10 <210> 60
 <211> 119
 <212> PRT
 <213> Mus musculus

15 <400> 60

Glu Val Gln Leu Gln Gln Ser Val Ala Glu Leu Val Arg Pro Gly Ala
 1 5 10 15

20 Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asn Thr
 20 25 30

25 Tyr Met His Trp Val Lys Glu Arg Pro Glu Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Arg Ile Asp Pro Ala Asn Gly Asn Ser Lys Tyr Ala Pro Lys Phe
 50 55 60

30 Gln Gly Lys Ala Thr Ile Thr Thr Asp Thr Ser Ser Asn Thr Ala Tyr
 65 70 75 80

35 Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Ile Tyr Tyr Cys
 85 90 95

40 Ala Arg Tyr Tyr Tyr Val Ser Ser His Phe Asp Val Trp Gly Thr Gly
 100 105 110

45 Thr Thr Val Thr Val Ser Ser
 115

50 <210> 61
 <211> 107
 <212> PRT
 <213> Mus musculus

<400> 61

Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly
 1 5 10 15

5 Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Thr Asn Asp
 20 25 30

10 Val Thr Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
 35 40 45

15 Tyr Tyr Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
 50 55 60

20 Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Thr Val Gln Ala
 65 70 75 80

25 Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Ser Ser Pro Phe
 85 90 95

30 Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
 100 105

35 <210> 62
 <211> 119
 <212> PRT
 <213> Mus musculus
 <400> 62

40 Glu Val Gln Leu Gln Gln Ser Val Ala Glu Leu Val Arg Pro Gly Ala
 1 5 10 15

45 Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asn Thr
 20 25 30

50 Phe Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
 35 40 45

55 Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Ala Pro Lys Phe
 50 55 60

60 Gln Gly Arg Ala Thr Leu Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
 65 70 75 80

Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Ile Tyr Tyr Cys
 85 90 95

5

Ala Arg Tyr Tyr Tyr Val Ser Ser Tyr Phe Asp Val Trp Gly Thr Gly
 100 105 110

10

Thr Thr Val Thr Val Ser Ser
 115

15

<210> 63
 <211> 107
 <212> PRT
 <213> Mus musculus

20

Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly
 1 5 10 15

25

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Thr Asn Asp
 20 25 30

30

Val Thr Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
 35 40 45

35

Phe Ser Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
 50 55 60

40

Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Thr Val Gln Ala
 65 70 75 80

45

Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Ser Ser Pro Trp
 85 90 95

50

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105

<210> 64
 <211> 122
 <212> PRT
 <213> Mus musculus

<400> 64

1 5 10 15
 Glu Phe Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 5
 20 25 30
 Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr
 10
 35 40 45
 Gly Met Asn Trp Leu Lys Gln Ile Asn Gly Lys Ser Leu Glu Trp Ile
 15
 50 55 60
 Ala Ile Ile Asn Pro Asn Tyr Gly Thr Ala Ser Ser Asn Pro Lys Phe
 20
 65 70 75 80
 Lys Asp Lys Ala Thr Leu Thr Val Asp His Ser Ser Ser Thr Ala Tyr
 25
 85 90 95
 Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 30
 100 105 110
 Ala Arg Ala Phe Asp Gly Tyr Tyr Phe Tyr Trp Tyr Phe Asp Val Trp
 35
 115 120
 Gly Thr Gly Thr Thr Val Thr Val Ser Ser
 40
 <210> 65
 <211> 112
 <212> PRT
 <213> Mus musculus
 45
 <400> 65
 50
 1 5 10 15
 Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
 55
 20 25 30
 Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Ile His Thr
 60
 35 40 45
 Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 65
 50 55 60
 Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro

5 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80

10 Ser Arg Leu Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser
 85 90 95

15 Thr His Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Arg
 100 105 110

20 <210> 66
 <211> 122
 <212> PRT
 <213> Mus musculus

25 Glu Phe Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1 5 10 15

30 Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Asp Tyr
 20 25 30

35 Ser Ile Asn Trp Val Lys Gln Ser Asn Gly Lys Ser Leu Glu Trp Ile
 35 40 45

40 Gly Val Ile Asn Pro Asn Tyr Gly Thr Ser Ser His Asn Gln Lys Phe
 50 55 60

45 Lys Gly Lys Ala Thr Met Thr Val Asp Gln Ser Ser Ser Thr Ala Tyr
 65 70 75 80

50 Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95

55 Ala Arg Ala Leu Asp Asp Tyr Tyr Phe Tyr Trp Tyr Phe Asp Val Trp
 100 105 110

60 Gly Ile Gly Thr Thr Val Thr Val Ser Ser
 115 120

65 <210> 67

<211> 112

<212> PRT

<213> Mus musculus

5 <400> 67

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
 1 5 10 15

10 Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Thr
 20 25 30

15 Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

20 Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

25 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Thr
 85 90 95

30 Thr His Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
 100 105 110

35 <210> 68

<211> 124

<212> PRT

<213> Mus musculus

40 <400> 68

Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln Pro Ser Gln
 1 5 10 15

45 Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Phe
 20 25 30

50 Gly Arg Gly Val Gly Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Asp
 35 40 45

Trp Leu Thr His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ala

50 55 60
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
 5 65 70 75 80
 Phe Leu Lys Ile Ala Asn Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr
 10 85 90 95
 Cys Ala Arg Ile Ala Gly Gly Leu Arg Arg Ala Pro Tyr Ala Met Asp
 15 100 105 110
 Ser Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
 115 120
 20 <210> 69
 <211> 107
 <212> PRT
 <213> Mus musculus
 25 <400> 69
 Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Val Ser Val Gly
 1 5 10 15
 30 Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Ser Ser
 20 25 30
 35 Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Val
 35 40 45
 40 Tyr Ala Ala Thr Asn Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 45 Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Ser Leu Gln Ser
 65 70 75 80
 Glu Asp Ser Gly Asn Tyr Tyr Cys Gln His Phe Arg Asp Thr Pro Pro
 85 90 95
 50 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105

<210> 70
 <211> 115
 <212> PRT
 <213> Mus musculus

5

<400> 70

Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly
 1 5 10 15

10

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

15

Gly Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Trp Val
 35 40 45

20

Ala Thr Ile Ser Asn Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val
 50 55 60

25

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80

Met Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95

30

Ala Arg Asn Glu Pro Pro Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
 100 105 110

35

Val Ser Ser
 115

40

<210> 71
 <211> 108
 <212> PRT
 <213> Mus musculus

45

<400> 71

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Leu Gly
 1 5 10 15

50

Glu Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val Gly Ser Ser
 20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Trp
 35 40 45

5 Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser
 50 55 60

10 Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu
 65 70 75 80

15 Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser Pro
 85 90 95

Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105

20 <210> 72
 <211> 454
 <212> PRT
 <213> Artificial Sequence

25 <220>
 <223> caninized mouse

30 <400> 72

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

35 Ser Val Lys Val Ser Cys Lys Thr Ser Gly Tyr Thr Phe Ile Asn Thr
 20 25 30

40 Phe Ile His Trp Val Arg Gln Ala Pro Gly Ala Gly Leu Asp Trp Met
 35 40 45

45 Gly Gln Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Ala Pro Lys Phe
 50 55 60

Gln Gly Arg Val Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
 65 70 75 80

50 Met Glu Leu Ser Ser Leu Arg Ala Gly Asp Ile Ala Val Tyr Tyr Cys
 85 90 95

	Ala Arg Tyr Tyr Tyr Val Ser Ser Tyr Phe Asp Val Trp Gly Gln Gly
	100 105 110
5	Thr Leu Val Thr Val Ser Ser Ala Ser Thr Thr Ala Pro Ser Val Phe
	115 120 125
10	Pro Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly Ser Thr Val Ala Leu
	130 135 140
15	Ala Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
	145 150 155 160
20	Asn Ser Gly Ser Leu Thr Ser Gly Val His Thr Phe Pro Ser Val Leu
	165 170 175
25	Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met Val Thr Val Pro Ser
	180 185 190
30	Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val Ala His Pro Ala
	195 200 205
35	Ser Lys Thr Lys Val Asp Lys Pro Val Pro Lys Arg Glu Asn Gly Arg
	210 215 220
40	Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala Pro Glu Met Leu
	225 230 235 240
45	Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Thr Leu
	245 250 255
50	Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Ala Leu Asp
	260 265 270
55	Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys Gln
	275 280 285
60	Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln Phe Ala Gly Thr
	290 295 300
65	Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu Lys
	305 310 315 320

Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala Leu Pro Ser Pro
 325 330 335
 5
 Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala His Gln Pro Ser
 340 345 350
 10
 Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser Lys Asn Thr Val
 355 360 365
 15
 Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Asp Ile Asp Val
 370 375 380
 20
 Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser Lys Tyr Arg Thr
 385 390 395 400
 25
 Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser Lys
 405 410 415
 30
 Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile Cys
 420 425 430
 35
 Ala Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Glu Ser Leu
 435 440 445
 40
 Ser His Ser Pro Gly Lys
 450
 <210> 73
 <211> 454
 <212> PRT
 <213> Artificial Sequence
 45
 <220>
 <223> caninized mouse
 <400> 73
 50
 Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Val Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asn Thr
 20 25 30

5 Phe Ile His Trp Val Arg Gln Ala Pro Gly Ala Gly Leu Asp Trp Ile
 35 40 45

Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Ala Pro Lys Phe
 50 55 60

10 Gln Gly Arg Val Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
 65 70 75 80

15 Met Glu Leu Ser Ser Leu Arg Ala Gly Asp Ile Ala Val Tyr Tyr Cys
 85 90 95

20 Ala Arg Tyr Tyr Tyr Val Ser Ser Tyr Phe Asp Val Trp Gly Gln Gly
 100 105 110

25 Thr Leu Val Thr Val Ser Ser Ala Ser Thr Thr Ala Pro Ser Val Phe
 115 120 125

Pro Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly Ser Thr Val Ala Leu
 130 135 140

30 Ala Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145 150 155 160

35 Asn Ser Gly Ser Leu Thr Ser Gly Val His Thr Phe Pro Ser Val Leu
 165 170 175

40 Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met Val Thr Val Pro Ser
 180 185 190

Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val Ala His Pro Ala
 195 200 205

45 Ser Lys Thr Lys Val Asp Lys Pro Val Pro Lys Arg Glu Asn Gly Arg
 210 215 220

50 Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala Pro Glu Met Leu
 225 230 235 240

Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255

5 Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Ala Leu Asp
 260 265 270

10 Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys Gln
 275 280 285

15 Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln Phe Ala Gly Thr
 290 295 300

Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu Lys
 305 310 315 320

20 Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala Leu Pro Ser Pro
 325 330 335

25 Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala His Gln Pro Ser
 340 345 350

30 Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser Lys Asn Thr Val
 355 360 365

35 Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Asp Ile Asp Val
 370 375 380

Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser Lys Tyr Arg Thr
 385 390 395 400

40 Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser Lys
 405 410 415

45 Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile Cys
 420 425 430

50 Ala Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Glu Ser Leu
 435 440 445

Ser His Ser Pro Gly Lys
 450

5 <210> 74
 <211> 454
 <212> PRT
 <213> Artificial Sequence

 10 <220>
 <223> caninized mouse

 <400> 74

 15 Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

 Ser Val Lys Val Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asn Thr
 20 25 30

 20 Phe Ile His Trp Val Arg Gln Ala Pro Gly Ala Gly Leu Asp Trp Ile
 35 40 45

 25 Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Ala Pro Lys Phe
 50 55 60

 30 Gln Gly Arg Ala Thr Leu Thr Ala Asp Thr Ser Thr Asn Thr Ala Tyr
 65 70 75 80

 Met Gln Leu Ser Ser Leu Arg Ala Gly Asp Ile Ala Val Tyr Tyr Cys
 85 90 95

 35 Ala Arg Tyr Tyr Tyr Val Ser Ser Tyr Phe Asp Val Trp Gly Gln Gly
 100 105 110

 40 Thr Leu Val Thr Val Ser Ser Ala Ser Thr Thr Ala Pro Ser Val Phe
 115 120 125

 45 Pro Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly Ser Thr Val Ala Leu
 130 135 140

 50 Ala Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145 150 155 160

 Asn Ser Gly Ser Leu Thr Ser Gly Val His Thr Phe Pro Ser Val Leu
 165 170 175

5 Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met Val Thr Val Pro Ser
 180 185 190

10 Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val Ala His Pro Ala
 195 200 205

15 Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala Pro Glu Met Leu
 225 230 235 240

20 Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255

25 Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Ala Leu Asp
 260 265 270

30 Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys Gln
 275 280 285

35 Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu Lys
 305 310 315 320

40 Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala Leu Pro Ser Pro
 325 330 335

45 Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala His Gln Pro Ser
 340 345 350

50 Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser Lys Asn Thr Val
 355 360 365

Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Asp Ile Asp Val
 370 375 380

Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser Lys Tyr Arg Thr
 385 390 395 400

5 Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser Lys
 405 410 415

10 Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile Cys
 420 425 430

15 Ala Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Glu Ser Leu
 435 440 445

20 Ser His Ser Pro Gly Lys
 450

25 <210> 75
 <211> 564
 <212> PRT
 <213> Artificial Sequence

30 <220>
 <223> caninized mouse
 <400> 75

35 Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Val Lys Pro Gly Ala
 1 5 10 15

40 Ser Val Lys Val Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asn Thr
 20 25 30

45 Phe Ile His Trp Val Arg Gln Arg Pro Gly Ala Gly Leu Asp Trp Ile
 35 40 45

50 Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Ala Pro Lys Phe
 50 55 60

55 Gln Gly Arg Ala Thr Leu Thr Ala Asp Thr Ser Thr Asn Thr Ala Tyr
 65 70 75 80

60 Met Gln Leu Ser Ser Leu Arg Ala Gly Asp Ile Ala Val Tyr Tyr Cys
 85 90 95

	Ala Arg Tyr Tyr Tyr Val Ser Ser Tyr Phe Asp Val Trp Gly Thr Gly
	100 105 110
5	Thr Leu Val Thr Val Ser Ser Ala Ser Thr Thr Ala Pro Ser Val Phe
	115 120 125
10	Pro Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly Ser Thr Val Ala Leu
	130 135 140
15	Ala Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
	145 150 155 160
20	Asn Ser Gly Ser Leu Thr Ser Gly Val His Thr Phe Pro Ser Val Leu
	165 170 175
25	Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met Val Thr Val Pro Ser
	180 185 190
30	Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val Ala His Pro Ala
	195 200 205
35	Ser Lys Thr Lys Val Asp Lys Pro Val Pro Lys Arg Glu Asn Gly Arg
	210 215 220
40	Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala Pro Glu Met Leu
	225 230 235 240
45	Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Thr Leu
	245 250 255
50	Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Ala Leu Asp
	260 265 270
55	Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys Gln
	275 280 285
60	Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln Phe Ala Gly Thr
	290 295 300
65	Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu Lys
	305 310 315 320

Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala Leu Pro Ser Pro
 325 330 335
 5

Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala His Gln Pro Ser
 340 345 350

10

Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser Lys Asn Thr Val
 355 360 365

15

Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Asp Ile Asp Val
 370 375 380

20

Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser Lys Tyr Arg Thr
 385 390 395 400

25

Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser Lys
 405 410 415

30

Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile Cys
 420 425 430

35

Ala Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Glu Ser Leu
 435 440 445

40

Ser His Ser Pro Gly Lys Arg Asn Asp Ala Gln Pro Ala Val Tyr Leu
 450 455 460

45

Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly Ser Ala Ser Val Val
 465 470 475 480

50

Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys
 485 490 495

Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln Glu Ser Val Thr Glu
 500 505 510

Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Met Ser
 515 520 525

Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser Cys Glu Ile Thr His
 530 535 540

5 Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe Gln Arg Ser Glu Cys
 545 550 555 560

10 Gln Arg Val Asp

15 <210> 76
 <211> 217
 <212> PRT
 <213> Artificial Sequence

20 <220>
 <223> caninized mouse

25 Glu Ile Val Met Thr Gln Ser Pro Ala Ser Leu Ser Leu Ser Gln Glu
 1 5 10 15

30 Glu Lys Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Thr Asn Asp
 20 25 30

35 Val Thr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Lys Leu Leu Ile
 35 40 45

40 Tyr Ser Ala Ser Asn Arg Tyr Thr Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

45 Ser Gly Ser Gly Thr Asp Phe Ser Phe Thr Ile Ser Ser Leu Glu Pro
 65 70 75 80

50 Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asp Tyr Ser Ser Pro Trp
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Asn Asp Ala Gln
 100 105 110

Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
 115 120 125

Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
 130 135 140

5 Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
 145 150 155 160

10 Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
 165 170 175

15 Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
 180 185 190

Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe
 195 200 205

20 Gln Arg Ser Glu Cys Gln Arg Val Asp
 210 215

25 <210> 77
 <211> 217
 <212> PRT
 <213> Artificial Sequence

30 <220>
 <223> caninized mouse
 <400> 77

35 Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Ser Pro Gly
 1 5 10 15

40 Glu Thr Ala Ser Ile Ser Cys Lys Ala Ser Gln Ser Val Thr Asn Asp
 20 25 30

45 Val Thr Trp Phe Arg Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile
 35 40 45

Tyr Ser Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Ser Gly
 50 55 60

50 Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile Ser Thr Val Glu Ala
 65 70 75 80

Asp Asp Thr Gly Val Tyr Tyr Cys Gln Gln Asp Tyr Ser Ser Pro Trp
 85 90 95

5 Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Asn Asp Ala Gln
 100 105 110

10 Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
 115 120 125

15 Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
 130 135 140

Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
 145 150 155 160

20 Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
 165 170 175

25 Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
 180 185 190

30 Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe
 195 200 205

35 Gln Arg Ser Glu Cys Gln Arg Val Asp
 210 215

40 <210> 78
 <211> 217
 <212> PRT
 <213> Artificial Sequence

45 <220>
 <223> caninized mouse

50 <400> 78

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Ser Pro Gly
 1 5 10 15

Glu Thr Ala Ser Ile Ser Cys Lys Ala Ser Gln Ser Val Thr Asn Asp
 20 25 30

Val Thr Trp Phe Arg Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
 35 40 45

5 Tyr Ser Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Ser Gly
 50 55 60

10 Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile Ser Thr Val Glu Ala
 65 70 75 80

15 Asp Asp Thr Gly Val Tyr Tyr Cys Gln Gln Asp Tyr Ser Ser Pro Trp
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Asn Asp Ala Gln
 100 105 110

20 Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
 115 120 125

25 Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
 130 135 140

30 Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
 145 150 155 160

35 Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
 165 170 175

Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
 180 185 190

40 Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe
 195 200 205

45 Gln Arg Ser Glu Cys Gln Arg Val Asp
 210 215

50 <210> 79
 <211> 458
 <212> PRT
 <213> Artificial Sequence
 <220>

<223> caninized mouse

<400> 79

5	Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
	1 5 10 15
10	Ser Val Lys Val Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Asp Tyr
	20 25 30
15	Asn Met Asp Trp Val Arg Gln Ala Pro Gly Ala Gly Leu Asp Trp Met
	35 40 45
20	Gly His Ile Asn Pro Asn Asn Gly Gly Ile Leu Tyr Asn Gln Lys Phe
	50 55 60
25	Lys Gly Arg Val Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
	65 70 75 80
30	Met Glu Leu Ser Ser Leu Arg Ala Gly Asp Ile Ala Val Tyr Tyr Cys
	85 90 95
35	Ala Arg Trp Ala Pro Leu Val Arg Gln Pro Tyr Trp Tyr Phe Asp Val
	100 105 110
40	Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Thr Ala
	115 120 125
45	Pro Ser Val Phe Pro Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly Ser
	130 135 140
50	Thr Val Ala Leu Ala Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro Val
	145 150 155 160
55	Thr Val Ser Trp Asn Ser Gly Ser Leu Thr Ser Gly Val His Thr Phe
	165 170 175
60	Pro Ser Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met Val
	180 185 190
65	Thr Val Pro Ser Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val
	195 200 205

5 Ala His Pro Ala Ser Lys Thr Lys Val Asp Lys Pro Val Pro Lys Arg
 210 215 220

10 Glu Asn Gly Arg Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala
 225 230 235 240

15 Pro Glu Met Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro
 245 250 255

20 Lys Asp Thr Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val
 260 265 270

25 Val Ala Leu Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val
 275 280 285

30 Asp Gly Lys Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln
 290 295 300

35 Phe Ala Gly Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln
 305 310 315 320

40 Asp Trp Leu Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala
 325 330 335

45 Leu Pro Ser Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala
 340 345 350

50 His Gln Pro Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser
 355 360 365

55 Lys Asn Thr Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro
 370 375 380

60 Asp Ile Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser
 385 390 395 400

65 Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe
 405 410 415

Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp
 420 425 430

5 Thr Phe Ile Cys Ala Val Met His Glu Ala Leu His Asn His Tyr Thr
 435 440 445

10 Gln Glu Ser Leu Ser His Ser Pro Gly Lys
 450 455

15 <210> 80
 <211> 458
 <212> PRT
 <213> Artificial Sequence

20 <220>
 <223> caninized mouse
 <400> 80

25 Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

30 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
 20 25 30

35 Asn Met Asp Trp Val Arg Gln Ala Pro Gly Ala Gly Leu Asp Trp Ile
 35 40 45

40 Gly His Ile Asn Pro Asn Asn Gly Gly Ile Leu Tyr Asn Gln Lys Phe
 50 55 60

45 Lys Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
 65 70 75 80

50 Met Glu Leu Ser Ser Leu Arg Ala Gly Asp Ile Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Trp Ala Pro Leu Val Arg Gln Pro Tyr Trp Tyr Phe Asp Val
 100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Thr Ala
 115 120 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly Ser
 130 135 140

5 Thr Val Ala Leu Ala Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro Val
 145 150 155 160

10 Thr Val Ser Trp Asn Ser Gly Ser Leu Thr Ser Gly Val His Thr Phe
 165 170 175

15 Pro Ser Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met Val
 180 185 190

20 Thr Val Pro Ser Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val
 195 200 205

25 Ala His Pro Ala Ser Lys Thr Lys Val Asp Lys Pro Val Pro Lys Arg
 210 215 220

30 Glu Asn Gly Arg Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala
 225 230 235 240

35 Pro Glu Met Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro
 245 250 255

40 Lys Asp Thr Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val
 260 265 270

45 Val Ala Leu Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val
 275 280 285

50 Asp Gly Lys Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln
 290 295 300

55 Phe Ala Gly Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln
 305 310 315 320

60 Asp Trp Leu Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala
 325 330 335

65 Leu Pro Ser Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala
 340 345 350

5 His Gln Pro Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser
 355 360 365

10 Lys Asn Thr Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro
 370 375 380

15 Asp Ile Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser
 385 390 395 400

20 Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe
 405 410 415

25 Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp
 420 425 430

30 Thr Phe Ile Cys Ala Val Met His Glu Ala Leu His Asn His Tyr Thr
 435 440 445

35 Gln Glu Ser Leu Ser His Ser Pro Gly Lys
 450 455

40 <210> 81
 <211> 458
 <212> PRT
 <213> Artificial Sequence

45 <220>
 <223> caninized mouse

50 <400> 81

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

45 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
 20 25 30

50 Asn Met Asp Trp Val Arg Gln Ala Pro Gly Ala Gly Leu Asp Trp Ile
 35 40 45

Gly His Ile Asn Pro Asn Asn Gly Gly Ile Leu Tyr Asn Gln Lys Phe
 50 55 60

5 Lys Gly Lys Ala Thr Leu Thr Val Asp Arg Ser Thr Asn Thr Ala Tyr
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ala Gly Asp Ile Ala Val Tyr Phe Cys
 85 90 95

10 Ala Arg Trp Ala Pro Leu Val Arg Gln Pro Tyr Trp Tyr Phe Asp Val
 100 105 110

15 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Thr Ala
 115 120 125

20 Pro Ser Val Phe Pro Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly Ser
 130 135 140

25 Thr Val Ala Leu Ala Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro Val
 145 150 155 160

Thr Val Ser Trp Asn Ser Gly Ser Leu Thr Ser Gly Val His Thr Phe
 165 170 175

30 Pro Ser Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met Val
 180 185 190

35 Thr Val Pro Ser Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn Val
 195 200 205

40 Ala His Pro Ala Ser Lys Thr Lys Val Asp Lys Pro Val Pro Lys Arg
 210 215 220

45 Glu Asn Gly Arg Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro Ala
 225 230 235 240

Pro Glu Met Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro
 245 250 255

50 Lys Asp Thr Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val
 260 265 270

Val Ala Leu Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val
 275 280 285

5 Asp Gly Lys Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln
 290 295 300

10 Phe Ala Gly Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln
 305 310 315 320

15 Asp Trp Leu Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala
 325 330 335

20 Leu Pro Ser Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala
 340 345 350

His Gln Pro Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser
 355 360 365

25 Lys Asn Thr Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro
 370 375 380

30 Asp Ile Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser
 385 390 395 400

35 Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe
 405 410 415

40 Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp
 420 425 430

Thr Phe Ile Cys Ala Val Met His Glu Ala Leu His Asn His Tyr Thr
 435 440 445

45 Gln Glu Ser Leu Ser His Ser Pro Gly Lys
 450 455

50 <210> 82
 <211> 217
 <212> PRT
 <213> Artificial Sequence
 <220>

<223> caninized mouse

<400> 82

5 Glu Ile Val Met Thr Gln Ser Pro Ala Ser Leu Ser Leu Ser Gln Glu
 1 5 10 15

10 Glu Lys Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Ser Tyr
 20 25 30

15 Leu Asn Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Lys Leu Leu Ile
 35 40 45

20 Tyr Arg Ala Asp Arg Leu Val Asp Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

25 Ser Gly Ser Gly Thr Asp Phe Ser Phe Thr Ile Ser Ser Leu Glu Pro
 65 70 75 80

30 Glu Asp Val Ala Val Tyr Tyr Cys Leu Gln Tyr Asp Glu Phe Pro Leu
 85 90 95

35 Thr Phe Gly Ala Gly Thr Lys Val Glu Leu Lys Arg Asn Asp Ala Gln
 100 105 110

40 Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
 115 120 125

45 Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
 130 135 140

50 Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
 145 150 155 160

55 Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
 165 170 175

60 Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
 180 185 190

65 Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe
 195 200 205

5 Gln Arg Ser Glu Cys Gln Arg Val Asp
 210 215

10 <210> 83
 <211> 217
 <212> PRT
 <213> Artificial Sequence

15 <220>
 <223> caninized mouse

20 Glu Ile Val Met Thr Gln Ser Pro Ala Ser Leu Ser Leu Ser Gln Glu
 1 5 10 15

25 Glu Lys Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Ser Tyr
 20 25 30

30 Leu Asn Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Lys Leu Leu Ile
 35 40 45

35 Tyr Arg Ala Asp Arg Leu Val Asp Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

40 Ser Gly Ser Gly Thr Asp Phe Ser Phe Thr Ile Ser Ser Leu Glu Pro
 65 70 75 80

45 Glu Asp Val Ala Val Tyr Tyr Cys Leu Gln Tyr Asp Glu Phe Pro Leu
 85 90 95

50 Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Asn Asp Ala Gln
 100 105 110

55 Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
 115 120 125

60 Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
 130 135 140

65 Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
 145 150 155 160

5 Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
 165 170 175

10 Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
 180 185 190

15 Gln Arg Ser Glu Cys Gln Arg Val Asp
 210 215

20 <210> 84
 <211> 217
 <212> PRT
 <213> Artificial Sequence

25 <220>
 <223> caninized mouse

30 <400> 84
 Asp Ile Lys Met Thr Gln Ser Pro Ala Ser Leu Ser Leu Ser Gln Glu
 1 5 10 15

35 Glu Lys Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Ser Tyr
 20 25 30

40 Leu Asn Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Lys Leu Leu Ile
 35 40 45

45 Tyr Arg Ala Asp Arg Leu Val Asp Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

50 Ser Gly Ser Gly Thr Asp Phe Ser Phe Thr Ile Ser Ser Leu Glu Pro
 65 70 75 80

55 Glu Asp Val Ala Val Tyr Tyr Cys Leu Gln Tyr Asp Glu Phe Pro Leu
 85 90 95

60 Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Asn Asp Ala Gln
 100 105 110

5 Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
 115 120 125

10 Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
 130 135 140

15 Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
 145 150 155 160

20 Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
 165 170 175

25 Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
 180 185 190

30 Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe
 195 200 205

35 Gln Arg Ser Glu Cys Gln Arg Val Asp
 210 215

40 <210> 85
 <211> 217
 <212> PRT
 <213> Artificial Sequence

45 <220>
 <223> caninized mouse

50 <400> 85

55 Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Ser Pro Gly
 1 5 10 15

60 Glu Thr Ala Ser Ile Ser Cys Lys Ala Ser Gln Asp Ile Asn Ser Tyr
 20 25 30

65 Leu Asn Trp Phe Arg Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile
 35 40 45

70 Tyr Arg Ala Asp Arg Leu Val Asp Gly Val Pro Asp Arg Phe Ser Gly
 50 55 60

5 Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile Ser Thr Val Glu Ala
 65 70 75 80

Asp Asp Thr Gly Val Tyr Tyr Cys Leu Gln Tyr Asp Glu Phe Pro Leu
 85 90 95

10 Thr Phe Gly Ala Gly Thr Lys Val Glu Leu Lys Arg Asn Asp Ala Gln
 100 105 110

15 Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
 115 120 125

20 Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
 130 135 140

25 Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
 145 150 155 160

Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
 165 170 175

30 Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
 180 185 190

35 Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe
 195 200 205

40 Gln Arg Ser Glu Cys Gln Arg Val Asp
 210 215

45 <210> 86
 <211> 459
 <212> PRT
 <213> Artificial Sequence

50 <220>
 <223> caninized mouse

<400> 86

Glu Leu Thr Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
 1 5 10 15

5 Thr Leu Ser Leu Thr Cys Val Val Ser Gly Gly Ser Val Thr Thr Phe
 20 25 30

Gly Arg Gly Val Gly Trp Ile Arg Gln Arg Pro Gly Arg Gly Leu Glu
 35 40 45

10 Trp Met Gly His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ala
 50 55 60

15 Leu Lys Ser Arg Ile Ser Ile Thr Ala Asp Thr Ala Lys Asn Gln Phe
 65 70 75 80

20 Ser Leu Gln Leu Ser Ser Met Thr Thr Glu Asp Thr Ala Val Tyr Tyr
 85 90 95

25 Cys Ala Arg Ile Ala Gly Gly Leu Arg Arg Ala Pro Tyr Ala Met Asp
 100 105 110

Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Thr
 115 120 125

30 Ala Pro Ser Val Phe Pro Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly
 130 135 140

35 Ser Thr Val Ala Leu Ala Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro
 145 150 155 160

40 Val Thr Val Ser Trp Asn Ser Gly Ser Leu Thr Ser Gly Val His Thr
 165 170 175

Phe Pro Ser Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met
 180 185 190

45 Val Thr Val Pro Ser Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn
 195 200 205

50 Val Ala His Pro Ala Ser Lys Thr Lys Val Asp Lys Pro Val Pro Lys
 210 215 220

Arg Glu Asn Gly Arg Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro
 225 230 235 240

5 Ala Pro Glu Met Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys
 245 250 255

10 Pro Lys Asp Thr Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val
 260 265 270

15 Val Val Ala Leu Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe
 275 280 285

20 Val Asp Gly Lys Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu
 290 295 300

25 Gln Phe Ala Gly Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His
 305 310 315 320

30 Gln Asp Trp Leu Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys
 325 330 335

35 Ala Leu Pro Ser Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln
 340 345 350

40 Ala His Gln Pro Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu
 355 360 365

45 Ser Lys Asn Thr Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro
 370 375 380

50 Pro Asp Ile Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu
 385 390 395 400

55 Ser Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr
 405 410 415

60 Phe Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly
 420 425 430

65 Asp Thr Phe Ile Cys Ala Val Met His Glu Ala Leu His Asn His Tyr
 435 440 445

Thr Gln Glu Ser Leu Ser His Ser Pro Gly Lys
 450 455
 5
 <210> 87
 <211> 459
 <212> PRT
 10 <213> Artificial Sequence
 <220>
 <223> caninized mouse
 15 <400> 87
 Glu Leu Thr Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
 1 5 10 15
 20 Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Phe
 20 25 30
 25 Gly Arg Gly Val Gly Trp Ile Arg Gln Arg Pro Gly Arg Gly Leu Glu
 35 40 45
 30 Trp Met Gly His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ala
 50 55 60
 Leu Lys Ser Arg Ile Ser Ile Thr Ala Asp Thr Ala Lys Asn Gln Phe
 65 70 75 80
 35 Ser Leu Gln Leu Ser Ser Met Thr Thr Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 40 Cys Ala Arg Ile Ala Gly Gly Leu Arg Arg Ala Pro Tyr Ala Met Asp
 100 105 110
 45 Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Thr
 115 120 125
 Ala Pro Ser Val Phe Pro Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly
 50 130 135 140
 Ser Thr Val Ala Leu Ala Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro
 145 150 155 160

5 Val Thr Val Ser Trp Asn Ser Gly Ser Leu Thr Ser Gly Val His Thr
 165 170 175

Phe Pro Ser Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met
 180 185 190

10 Val Thr Val Pro Ser Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn
 195 200 205

15 Val Ala His Pro Ala Ser Lys Thr Lys Val Asp Lys Pro Val Pro Lys
 210 215 220

20 Arg Glu Asn Gly Arg Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro
 225 230 235 240

25 Ala Pro Glu Met Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys
 245 250 255

Pro Lys Asp Thr Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val
 260 265 270

30 Val Val Ala Leu Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe
 275 280 285

35 Val Asp Gly Lys Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu
 290 295 300

40 Gln Phe Ala Gly Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His
 305 310 315 320

Gln Asp Trp Leu Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys
 325 330 335

45 Ala Leu Pro Ser Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln
 340 345 350

50 Ala His Gln Pro Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu
 355 360 365

Ser Lys Asn Thr Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro
 370 375 380

5 Pro Asp Ile Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu
 385 390 395 400

10 Ser Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr
 405 410 415

15 Phe Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly
 420 425 430

Asp Thr Phe Ile Cys Ala Val Met His Glu Ala Leu His Asn His Tyr
 435 440 445

20 Thr Gln Glu Ser Leu Ser His Ser Pro Gly Lys
 450 455

25 <210> 88
 <211> 459
 <212> PRT
 <213> Artificial Sequence

30 <220>
 <223> caninized mouse
 <400> 88

35 Glu Val Thr Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
 1 5 10 15

40 Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Phe
 20 25 30

45 Gly Arg Gly Val Gly Trp Ile Arg Gln Arg Pro Gly Arg Gly Leu Glu
 35 40 45

Trp Met Gly His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ala
 50 55 60

50 Leu Lys Ser Arg Leu Ser Ile Thr Lys Asp Thr Ala Lys Asn Gln Phe
 65 70 75 80

	Ser Leu Gln Leu Ser Ser Met Thr Thr Glu Asp Thr Ala Val Tyr Tyr
	85 90 95
5	Cys Ala Arg Ile Ala Gly Gly Leu Arg Arg Ala Pro Tyr Ala Met Asp
	100 105 110
10	Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Thr
	115 120 125
15	Ala Pro Ser Val Phe Pro Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly
	130 135 140
20	Ser Thr Val Ala Leu Ala Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro
	145 150 155 160
25	Val Thr Val Ser Trp Asn Ser Gly Ser Leu Thr Ser Gly Val His Thr
	165 170 175
30	Phe Pro Ser Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met
	180 185 190
35	Val Thr Val Pro Ser Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn
	195 200 205
40	Val Ala His Pro Ala Ser Lys Thr Lys Val Asp Lys Pro Val Pro Lys
	210 215 220
45	Arg Glu Asn Gly Arg Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro
	225 230 235 240
50	Ala Pro Glu Met Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys
	245 250 255
55	Pro Lys Asp Thr Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val
	260 265 270
60	Val Val Ala Leu Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe
	275 280 285
65	Val Asp Gly Lys Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu
	290 295 300

5 Gln Phe Ala Gly Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His
 305 310 315 320

10 Gln Asp Trp Leu Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys
 325 330 335

15 Ala Leu Pro Ser Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln
 340 345 350

20 Ser Lys Asn Thr Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro
 370 375 380

25 Pro Asp Ile Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu
 385 390 395 400

30 Ser Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr
 405 410 415

35 Phe Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly
 420 425 430

40 Thr Gln Glu Ser Leu Ser His Ser Pro Gly Lys
 450 455

45 <210> 89
 <211> 459
 <212> PRT
 <213> Artificial Sequence

50 <220>
 <223> caninized mouse

<400> 89

Glu Val Thr Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
 1 5 10 15

5 Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Phe
 20 25 30

Gly Arg Gly Val Gly Trp Ile Arg Gln Arg Pro Gly Arg Gly Leu Glu
 35 40 45

10 Trp Met Gly His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ala
 50 55 60

15 Leu Lys Ser Arg Leu Ser Ile Thr Lys Asp Thr Ala Lys Asn Gln Val
 65 70 75 80

20 Phe Leu Gln Leu Ser Ser Met Thr Thr Glu Asp Thr Ala Val Tyr Tyr
 85 90 95

25 Cys Ala Arg Ile Ala Gly Gly Leu Arg Arg Ala Pro Tyr Ala Met Asp
 100 105 110

Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Thr
 115 120 125

30 Ala Pro Ser Val Phe Pro Leu Ala Pro Ser Cys Gly Ser Thr Ser Gly
 130 135 140

35 Ser Thr Val Ala Leu Ala Cys Leu Val Ser Gly Tyr Phe Pro Glu Pro
 145 150 155 160

40 Val Thr Val Ser Trp Asn Ser Gly Ser Leu Thr Ser Gly Val His Thr
 165 170 175

45 Phe Pro Ser Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Met
 180 185 190

Val Thr Val Pro Ser Ser Arg Trp Pro Ser Glu Thr Phe Thr Cys Asn
 195 200 205

50 Val Ala His Pro Ala Ser Lys Thr Lys Val Asp Lys Pro Val Pro Lys
 210 215 220

Arg Glu Asn Gly Arg Val Pro Arg Pro Pro Asp Cys Pro Lys Cys Pro
 225 230 235 240

5 Ala Pro Glu Met Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys
 245 250 255

10 Pro Lys Asp Thr Leu Leu Ile Ala Arg Thr Pro Glu Val Thr Cys Val
 260 265 270

15 Val Val Ala Leu Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe
 275 280 285

20 Val Asp Gly Lys Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu
 290 295 300

25 Gln Phe Ala Gly Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His
 305 310 315 320

30 Gln Asp Trp Leu Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys
 325 330 335

35 Ala Leu Pro Ser Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln
 340 345 350

40 Ala His Gln Pro Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu
 355 360 365

45 Ser Lys Asn Thr Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro
 370 375 380

50 Pro Asp Ile Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu
 385 390 395 400

55 Ser Lys Tyr Arg Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr
 405 410 415

60 Phe Leu Tyr Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly
 420 425 430

65 Asp Thr Phe Ile Cys Ala Val Met His Glu Ala Leu His Asn His Tyr
 435 440 445

Thr Gln Glu Ser Leu Ser His Ser Pro Gly Lys
 450 455
 5
 <210> 90
 <211> 217
 <212> PRT
 10 <213> Artificial Sequence
 <220>
 <223> caninized mouse
 15 <400> 90
 Glu Ile Val Met Thr Gln Ser Pro Ala Ser Leu Ser Leu Ser Gln Glu
 1 5 10 15
 20
 Glu Lys Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Ser Ser
 20 25 30
 25
 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Lys Leu Leu Ile
 35 40 45
 30
 Tyr Ala Ala Thr Asn Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 35
 Ser Gly Ser Gly Thr Asp Phe Ser Phe Thr Ile Ser Ser Leu Glu Pro
 65 70 75 80
 40
 Glu Asp Val Ala Val Tyr Tyr Cys Gln His Phe Arg Asp Thr Pro Pro
 85 90 95
 45
 Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Asn Asp Ala Gln
 100 105 110
 50
 Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
 115 120 125
 50
 Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
 130 135 140
 Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
 145 150 155 160

5 Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
 165 170 175

10 Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
 180 185 190

15 Gln Arg Ser Glu Cys Gln Arg Val Asp
 210 215

20 <210> 91
 <211> 217
 <212> PRT
 <213> Artificial Sequence

25 <220>
 <223> caninized mouse

30 <400> 91

35 Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Ser Pro Gly
 1 5 10 15

40 Glu Thr Ala Ser Ile Ser Cys Arg Ala Ser Glu Asn Ile Tyr Ser Ser
 20 25 30

45 Leu Ala Trp Phe Arg Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile
 35 40 45

50 Tyr Ala Ala Thr Asn Leu Ala Asp Gly Val Pro Asp Arg Phe Ser Gly
 50 55 60

55 Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile Ser Arg Val Glu Ala
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60 Asp Asp Thr Gly Val Tyr Tyr Cys Gln His Phe Arg Asp Thr Pro Pro
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65 Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Asn Asp Ala Gln
 100 105 110

5 Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
 115 120 125

10 Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
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15 Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
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20 Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
 165 170 175

25 Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
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65 Leu Ala Trp Phe Arg Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Val
 35 40 45

70 Tyr Ala Ala Thr Asn Leu Ala Asp Gly Val Pro Asp Arg Phe Ser Gly
 50 55 60

5 Ser Gly Ser Gly Thr Asp Tyr Thr Leu Arg Ile Ser Arg Val Glu Ala
 65 70 75 80

Asp Asp Thr Gly Val Tyr Tyr Cys Gln His Phe Arg Asp Thr Pro Pro
 85 90 95

10 Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Asn Asp Ala Gln
 100 105 110

15 Pro Ala Val Tyr Leu Phe Gln Pro Ser Pro Asp Gln Leu His Thr Gly
 115 120 125

20 Ser Ala Ser Val Val Cys Leu Leu Asn Ser Phe Tyr Pro Lys Asp Ile
 130 135 140

25 Asn Val Lys Trp Lys Val Asp Gly Val Ile Gln Asp Thr Gly Ile Gln
 145 150 155 160

Glu Ser Val Thr Glu Gln Asp Lys Asp Ser Thr Tyr Ser Leu Ser Ser
 165 170 175

30 Thr Leu Thr Met Ser Ser Thr Glu Tyr Leu Ser His Glu Leu Tyr Ser
 180 185 190

35 Cys Glu Ile Thr His Lys Ser Leu Pro Ser Thr Leu Ile Lys Ser Phe
 195 200 205

40 Gln Arg Ser Glu Cys Gln Arg Val Asp
 210 215

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 20 25 30

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 35 40 45

10 Trp Met Gly His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ala
 50 55 60

15 Leu Lys Ser Arg Ile Ser Ile Thr Ala Asp Thr Ala Lys Asn Gln Phe
 65 70 75 80

20 Ser Leu Gln Leu Ser Ser Met Thr Thr Glu Asp Thr Ala Val Tyr Tyr
 85 90 95

25 Cys Ala Arg Ile Ala Gly Gly Leu Arg Arg Ala Pro Tyr Ala Met Asp
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 35 40 45

Trp Met Gly His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ala
 50 55 60

5 Leu Lys Ser Arg Ile Ser Ile Thr Ala Asp Thr Ala Lys Asn Gln Phe
 65 70 75 80

10 Ser Leu Gln Leu Ser Ser Met Thr Thr Glu Asp Thr Ala Val Tyr Tyr
 85 90 95

15 Cys Ala Arg Ile Ala Gly Gly Leu Arg Arg Ala Pro Tyr Ala Met Asp
 100 105 110

20 Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
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 20 25 30

45 Gly Arg Gly Val Gly Trp Ile Arg Gln Arg Pro Gly Arg Gly Leu Glu
 35 40 45

50 Trp Met Gly His Ile Trp Trp Asp Asp Asp Lys Tyr Tyr Asn Pro Ala
 50 55 60

55 Leu Lys Ser Arg Leu Ser Ile Thr Lys Asp Thr Ala Lys Asn Gln Phe
 65 70 75 80

60 Ser Leu Gln Leu Ser Ser Met Thr Thr Glu Asp Thr Ala Val Tyr Tyr
 85 90 95

65 Cys Ala Arg Ile Ala Gly Gly Leu Arg Arg Ala Pro Tyr Ala Met Asp
 100 105 110

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 35 40 45
 30 Trp Met Gly His Ile Trp Trp Asp Asp Lys Tyr Tyr Asn Pro Ala
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 Leu Lys Ser Arg Leu Ser Ile Thr Lys Asp Thr Ala Lys Asn Gln Val
 65 70 75 80
 35 Phe Leu Gln Leu Ser Ser Met Thr Thr Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
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 100 105 110
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Ser Val Lys

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Lys Arg

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15 Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln Phe Asn Gly
 50 55 60

20 Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu
 65 70 75 80

25 Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala Leu Pro Ser
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30 Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala His Gln Pro
 100 105 110

35 Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser Lys Asn Thr
 115 120 125

40 Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Asp Ile Asp
 130 135 140

45 Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser Lys Tyr Arg
 145 150 155 160

50 Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser
 165 170 175

55 Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile
 180 185 190

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Asp Pro Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys
 35 40 45

25

Gln Met Gln Thr Ala Lys Thr Gln Pro Arg Glu Glu Gln Phe Ala Gly
 50 55 60

Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu
 65 70 75 80

30

Lys Gly Lys Gln Phe Thr Cys Lys Val Asn Asn Lys Ala Leu Pro Ser
 85 90 95

35

Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala His Gln Pro
 100 105 110

40

Ser Val Tyr Val Leu Pro Pro Ser Arg Glu Glu Leu Ser Lys Asn Thr
 115 120 125

45

Val Ser Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Asp Ile Asp
 130 135 140

Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Ser Lys Tyr Arg
 145 150 155 160

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Thr Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser
 165 170 175

Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile
 180 185 190

5 Cys Ala Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Glu Ser
 195 200 205

10 Leu Ser His Ser Pro Gly
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<400> 116

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 20 25 30

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Gly Arg Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys
 35 40 45

40

Glu Val His Thr Ala Lys Thr Gln Ser Arg Glu Gln Gln Phe Asn Gly
 50 55 60

45

Thr Tyr Arg Val Val Ser Val Leu Pro Ile Glu His Gln Asp Trp Leu
 65 70 75 80

50 Thr Gly Lys Glu Phe Lys Cys Arg Val Asn His Ile Asp Leu Pro Ser
 85 90 95

Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Arg Ala His Lys Pro
 100 105 110

5 Ser Val Tyr Val Leu Pro Pro Ser Pro Lys Glu Leu Ser Ser Ser Asp
 115 120 125

10 Thr Val Ser Ile Thr Cys Leu Ile Lys Asp Phe Tyr Pro Pro Asp Ile
 130 135 140

15 Asp Val Glu Trp Gln Ser Asn Gly Gln Gln Glu Pro Glu Arg Lys His
 145 150 155 160

20 Arg Met Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr
 165 170 175

25 Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Gln Gly Asp Pro Phe
 180 185 190

30 Thr Cys Ala Val Met His Glu Thr Leu Gln Asn His Tyr Thr Asp Leu
 195 200 205

35 Ser Leu Ser His Ser Pro Gly Lys
 210 215

40 <210> 117
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50 Leu Val Thr Ala Arg Thr Pro Thr Val Thr Cys Val Val Val Asp Leu
 20 25 30

55 Asp Pro Glu Asn Pro Glu Val Gln Ile Ser Trp Phe Val Asp Ser Lys
 35 40 45

60 Gln Val Gln Thr Ala Asn Thr Gln Pro Arg Glu Glu Gln Ser Asn Gly
 50 55 60

Thr Tyr Arg Val Val Ser Val Leu Pro Ile Gly His Gln Asp Trp Leu

	65	70	75	80	
5	Ser Gly Lys Gln Phe Lys Cys Lys Val Asn Asn Lys Ala Leu Pro Ser	85	90	95	
10	Pro Ile Glu Glu Ile Ile Ser Lys Thr Pro Gly Gln Ala His Gln Pro	100	105	110	
15	Asn Val Tyr Val Leu Pro Pro Ser Arg Asp Glu Met Ser Lys Asn Thr	115	120	125	
20	Val Thr Leu Thr Cys Leu Val Lys Asp Phe Phe Pro Pro Glu Ile Asp	130	135	140	
25	Met Thr Pro Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr Ser	165	170	175	
30	Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Arg Gly Asp Thr Phe Ile	180	185	190	
35	Cys Ala Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Ile Ser	195	200	205	
40	Leu Ser His Ser Pro Gly Lys	210	215		
45	<210> 118 <211> 216 <212> PRT <213> Canis familiaris				
50	Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Ile	1	5	10	15
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Gly Arg Glu Asp Pro Glu Val Gln Ile Ser Trp Phe Val Asp Gly Lys
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5 Glu Val His Thr Ala Lys Thr Gln Pro Arg Glu Gln Gln Phe Asn Ser
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10 Thr Tyr Arg Val Val Ser Val Leu Pro Ile Glu His Gln Asp Trp Leu
 65 70 75 80

15 Thr Gly Lys Glu Phe Lys Cys Arg Val Asn His Ile Gly Leu Pro Ser
 85 90 95

20 Pro Ile Glu Arg Thr Ile Ser Lys Ala Arg Gly Gln Ala His Gln Pro
 100 105 110

25 Ser Val Tyr Val Leu Pro Pro Ser Pro Lys Glu Leu Ser Ser Ser Asp
 115 120 125

30 Thr Val Thr Leu Thr Cys Leu Ile Lys Asp Phe Phe Pro Pro Glu Ile
 130 135 140

35 Asp Val Glu Trp Gln Ser Asn Gly Gln Pro Glu Pro Glu Ser Lys Tyr
 145 150 155 160

40 His Thr Thr Ala Pro Gln Leu Asp Glu Asp Gly Ser Tyr Phe Leu Tyr
 165 170 175

45 Ser Lys Leu Ser Val Asp Lys Ser Arg Trp Gln Gln Gly Asp Thr Phe
 180 185 190

50 Thr Cys Ala Val Met His Glu Ala Leu Gln Asn His Tyr Thr Asp Leu
 195 200 205

55 Ser Leu Ser His Ser Pro Gly Lys
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<210> 119
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Ser Gln Glu Lys Val Gly Thr Thr Glu Glu Glu Ala Pro Tyr Gly
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<210> 121
 <211> 21
 <212> PRT
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Ile Met Lys Ile Glu Pro Pro Glu Ile Phe Ser Val Lys Ser Val Leu
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Gly Ile Lys Arg Met
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Pro Ala Glu Ser Met
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 Thr Gly Ala Pro Val
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 Thr Phe Gln Cys Ile
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 Arg Asn Gly Phe Ile Lys Asn Tyr Thr Ile Phe Tyr Gln Ala Glu Asp
 1 5 10 15
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 Gly Lys Glu Phe Ser
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 <210> 126
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 <212> PRT
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 Ser Tyr Ser Leu Gln Val Met Ala Ser Thr Asn Ala Gly Gly Thr Asn
 1 5 10 15

Gly Thr Lys Ile Asn
20

5

WHAT IS CLAIMED IS:

1. A mammalian antibody or antigen binding fragment thereof that binds canine interleukin-31 receptor *alpha* (canine IL-31RA) comprising a heavy chain that comprises a set of
5 three heavy chain complementary determining regions (HCDRs) each comprising an amino acid sequence: a CDR heavy 1 (HCDR1), a CDR heavy 2 (HCDR2), and a CDR heavy 3 (HCDR3); wherein
- (i) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 3;
 - (ii) the HCDR2 comprises the amino acid sequence selected from the group
10 consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13;
 - (iii) the HCDR3 comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, and SEQ ID NO: 23.
2. The mammalian antibody or antigen binding fragment thereof of Claim 1,
15 that when bound to canine IL-31RA the antibody binds to an epitope comprised by an amino acid sequence selected from the group consisting of SEQ ID NO: 97, SEQ ID NO: 103, SEQ ID NO: 99, SEQ ID NO: 102, or any combination thereof.
3. The mammalian antibody or antigen binding fragment thereof of Claim 2, that
20 when bound to canine IL-31RA the antibody binds to an epitope comprised by an amino acid sequence of SEQ ID NO: 97 and SEQ ID NO: 103.
4. The mammalian antibody or antigen binding fragment thereof of Claim 2, that
when bound to canine IL-31RA the antibody binds to an epitope comprised by an amino acid
25 sequence of SEQ ID NO: 98 and SEQ ID NO: 102.
5. The mammalian antibody or antigen binding fragment thereof of Claim 1 or 3, wherein
- (i) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 3;
 - (ii) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 10; and
30
 - (iii) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 20.

6. The mammalian antibody or antigen binding fragment thereof of any one of Claims 1-4, wherein

- (i) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 3;
- (ii) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 11; and
- (iii) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 21.

7. The mammalian antibody or antigen binding fragment thereof of any one of Claims 1-4, wherein

- (i) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 3;
- (ii) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 12; and
- (iii) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 22.

8. The mammalian antibody or antigen binding fragment thereof of Claim 1 or 4, wherein

- (i) the HCDR1 comprises the amino acid sequence of SEQ ID NO: 3;
- (ii) the HCDR2 comprises the amino acid sequence of SEQ ID NO: 13; and
- (iii) the HCDR3 comprises the amino acid sequence of SEQ ID NO: 23.

9. The mammalian antibody or antigen binding fragment thereof of any one of Claims 1-8, further comprising a light chain that comprises a set of three light chain complementary determining regions (LCDRs) each comprising an amino acid sequence: a CDR light 1 (LCDR1), a CDR light 2 (LCDR2), and a CDR light 3 (LCDR3); wherein

(iv) the LCDR1 comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 30 and SEQ ID NO: 31;

(v) the LCDR2 comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40; and

(vi) the LCDR3 comprises the amino acid sequence of SEQ ID NO: 45.

10. The mammalian antibody or antigen binding fragment thereof of Claim 5 or 6, further comprising a set of three light chain complementary determining regions (LCDRs): a CDR light 1 (LCDR1), a CDR light 2 (LCDR2), and a CDR light 3 (LCDR3); wherein

(iv) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 30;

(v) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 40; and

(vi) the LCDR3 comprises the amino acid sequence of SEQ ID NO: 45.

11. The mammalian antibody or antigen binding fragment thereof of Claim 7, further comprising a set of three light chain complementary determining regions (LCDRs): a CDR light
5 1 (LCDR1), a CDR light 2 (LCDR2), and a CDR light 3 (LCDR3); wherein

(iv) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 30;

(v) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 38; and

(vi) the LCDR3 comprises the amino acid sequence of SEQ ID NO: 45.

10 12. The mammalian antibody or antigen binding fragment thereof of Claim 8, further comprising a set of three light chain complementary determining regions (LCDRs): a CDR light
1 (LCDR1), a CDR light 2 (LCDR2), and a CDR light 3 (LCDR3); wherein

(iv) the LCDR1 comprises the amino acid sequence of SEQ ID NO: 31;

(v) the LCDR2 comprises the amino acid sequence of SEQ ID NO: 39; and

15 (vi) the LCDR3 comprises the amino acid sequence of SEQ ID NO: 45.

13. The mammalian antibody or antigen binding fragment thereof of any one of Claims 1 to 12, wherein the antibody and antigen binding fragment thereof bind canine IL-31RA and block the binding of canine IL-31RA to canine interleukin-31.

20 14. The mammalian antibody or antigen binding fragment thereof of any one of Claims 1-13, wherein the mammalian antibody or antigen binding fragment thereof is a caninized antibody or a caninized antigen binding fragment thereof.

25 15. The caninized antibody or antigen binding fragment thereof of Claim 14, that comprises a hinge region that comprises an amino acid sequence that comprises at least 90%, 95%, or 100% identity with the amino acid sequence selected from the group consisting of SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, and SEQ ID NO: 115.

30 16. The caninized antibody or antigen binding fragment thereof of Claim 14 or 15, that comprises a canine fragment crystallizable region (cFc region); wherein the cFc region comprises an amino acid sequence that comprises at least 90%, 95%, 98%, 99%, or 100%

identity with the amino acid sequence selected from the group consisting of SEQ ID NO: 110, SEQ ID NO: 116, SEQ ID NO: 117, and SEQ ID NO: 118.

17. The caninized antibody or antigen binding fragment thereof of Claim 14 or 15, that comprises a canine fragment crystallizable region (cFc region); wherein the cFc region comprises an amino acid sequence that comprises at least 90%, 95%, 98%, 99%, or 100% identity with the amino acid sequence SEQ ID NO: 111, wherein both the aspartic acid residue (D) at position 31 of SEQ ID NO: 110 and the asparagine residue (N) at position 63 of SEQ ID NO: 110, remain substituted by an alanine residue (A).

18. A caninized antibody or antigen binding fragment thereof, wherein the caninized IL-31RA antibody comprises a light chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, and SEQ ID NO: 85; and a heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 79, SEQ ID NO: 80, and SEQ ID NO: 81.

19. The caninized antibody or antigen binding fragment thereof of Claim 18, that when bound to canine IL-31RA the antibody binds to an epitope comprised by an amino acid sequence of SEQ ID NO: 97 and SEQ ID NO: 103.

20. The caninized antibody or antigen binding fragment thereof of Claim 18 or 19, wherein the caninized IL-31RA antibody comprises:

a light chain comprising the amino acid sequence of SEQ ID NO: 84 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 80; or

a light chain comprising the amino acid sequence of SEQ ID NO: 84 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 81; or

a light chain comprising the amino acid sequence of SEQ ID NO: 85 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 80; or

a light chain comprising the amino acid sequence of SEQ ID NO: 85 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 81.

21. A nucleic acid that encodes the heavy chain of the caninized antibody or antigen binding fragment thereof of any one of Claims 14-20.

22. A nucleic acid that encodes the light chain of the caninized antibody or antigen binding fragment thereof of any one of Claims 14-20.

23. A pair of nucleic acids, wherein one of the pair of nucleic acids comprises a nucleotide sequence that encodes the light chain of a specific caninized antibody of any one of the antibodies of Claims 14-20 and the other of the pair of nucleic acids comprises a nucleotide sequence that encodes the heavy chain of said specific caninized antibody.

24. An expression vector comprising the pair of nucleic acids of Claim 23 or the nucleic acid of Claim 21 or 22.

25. A pair of expression vectors, wherein one of the pair of expression vectors comprises a nucleic acid comprising a nucleotide sequence that encodes the light chain of a specific caninized antibody of any one of the antibodies of Claims 14-20 and the other of the pair of expression vectors comprises a nucleic acid comprising a nucleotide sequence that encodes the heavy chain of said specific caninized antibody.

26. A host cell comprising the expression vector of Claim 24 or the pair of expression vectors of Claim 25.

27. A pharmaceutical composition comprising the caninized antibody or antigen binding fragment thereof of Claims 14-20, and a pharmaceutically acceptable carrier or diluent.

28. A method of aiding in blocking pruritus associated with atopic dermatitis in an animal subject, comprising administering to the animal subject a therapeutically effective amount of the pharmaceutical composition of Claim 27.

29. A pharmaceutical composition for use in aiding in blocking pruritus associated with atopic dermatitis in a canine comprising the caninized antibody or antigen binding fragment thereof of Claims 14-20, and a pharmaceutically acceptable carrier or diluent.

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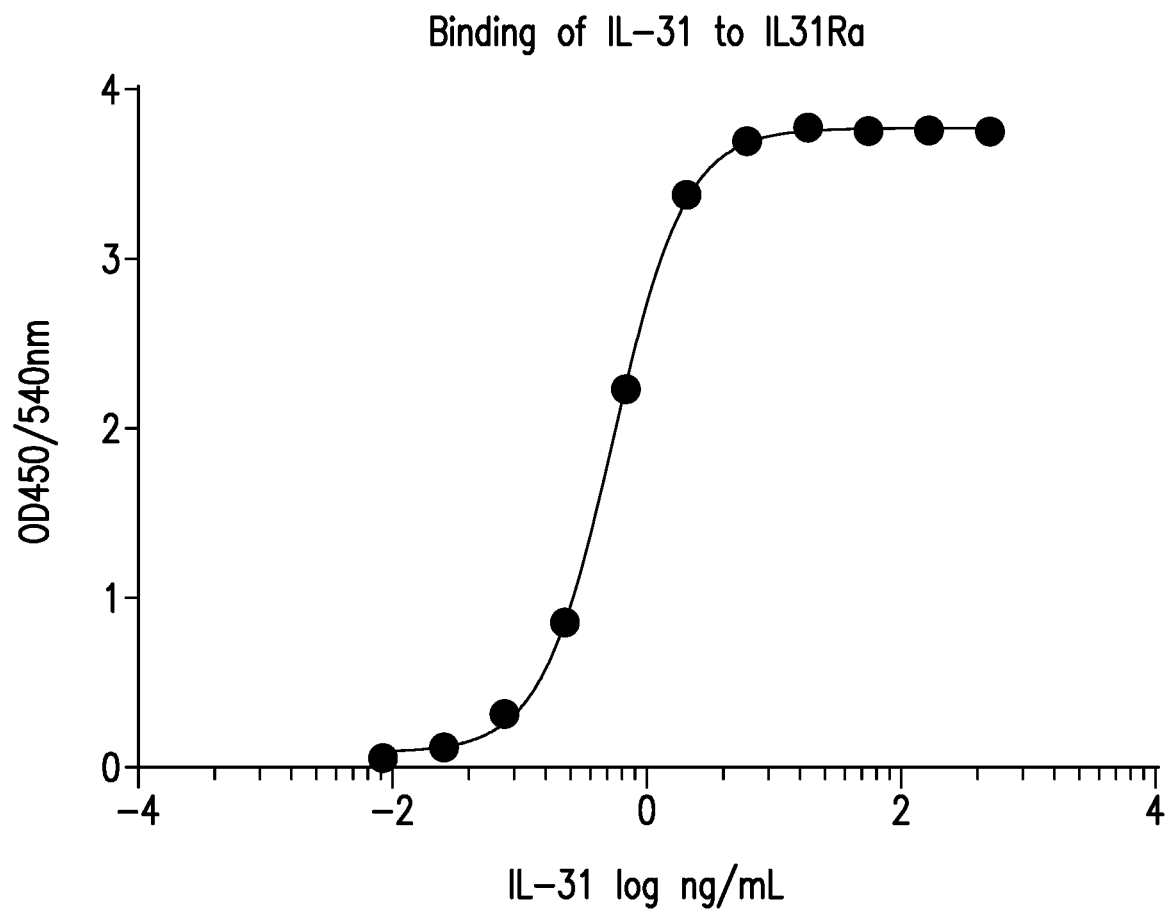


FIG.1

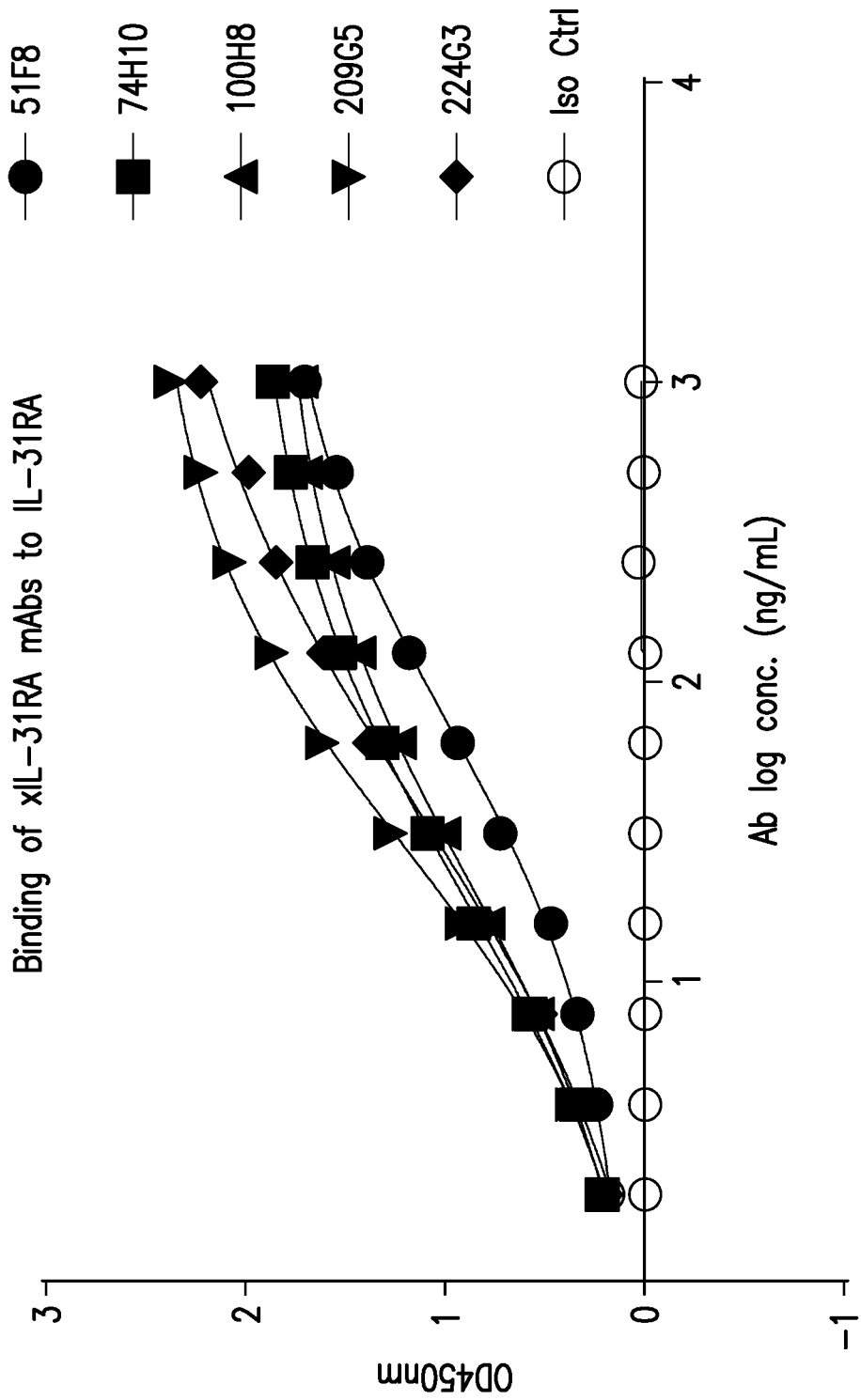


FIG.2A

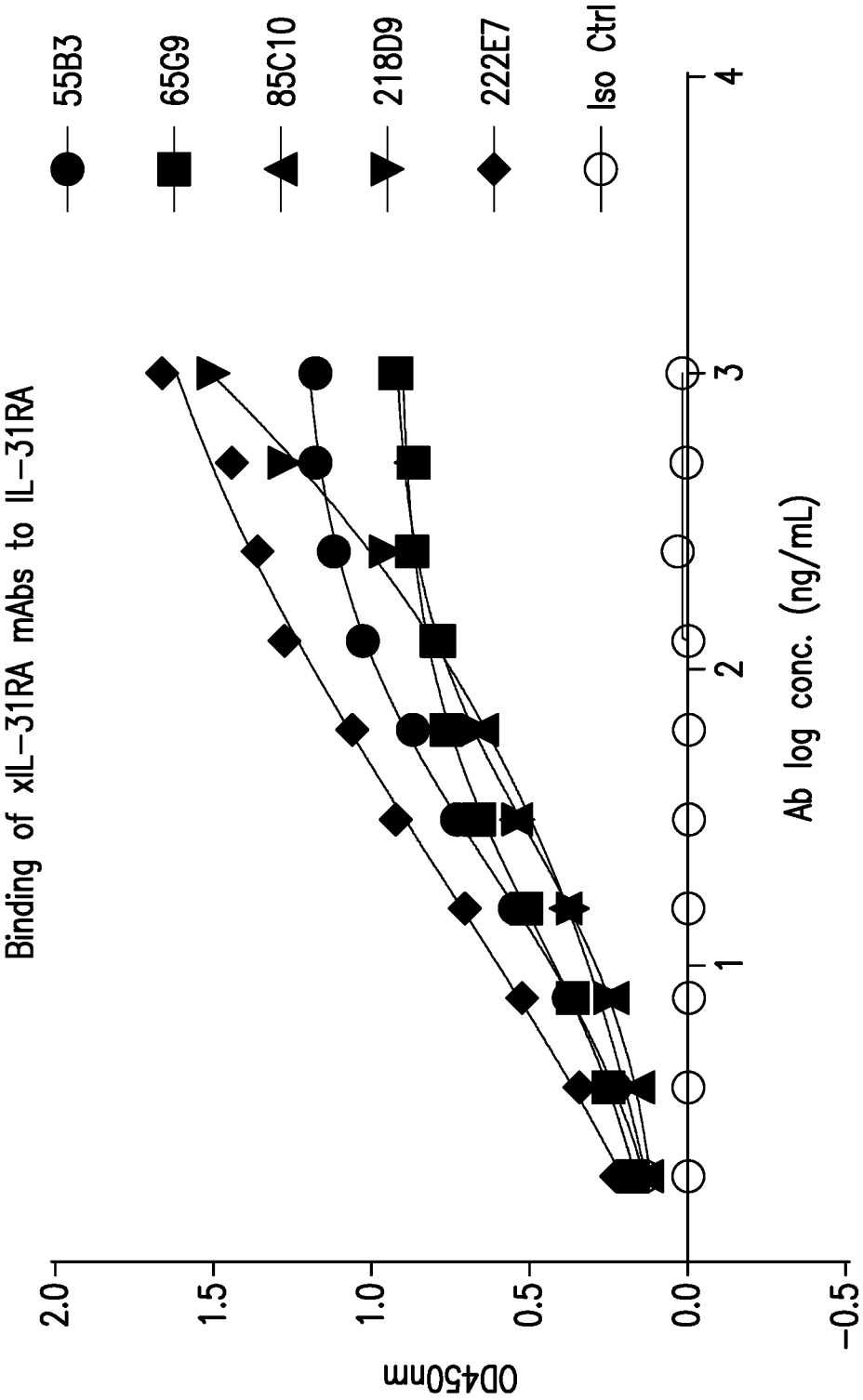


FIG.2B

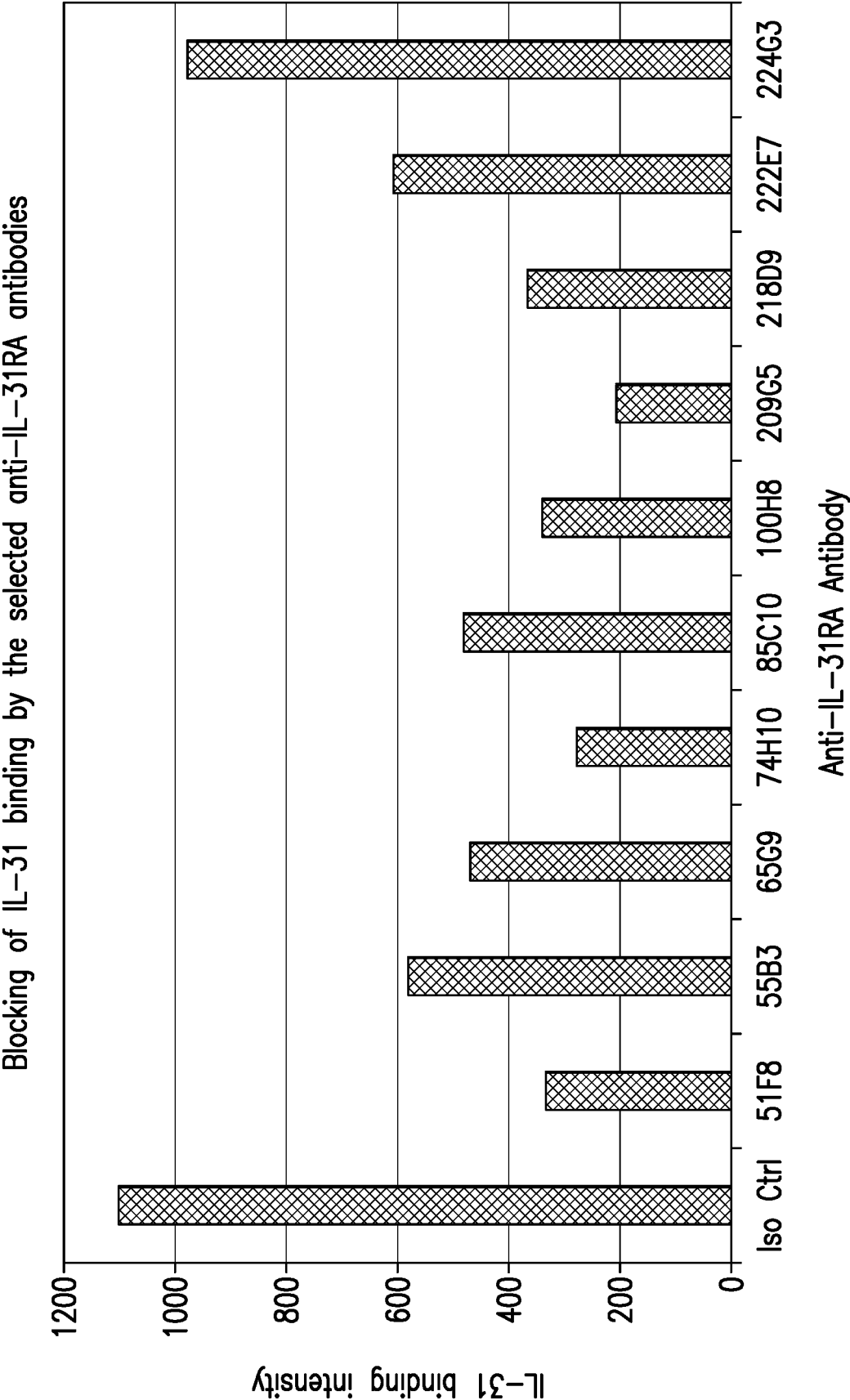
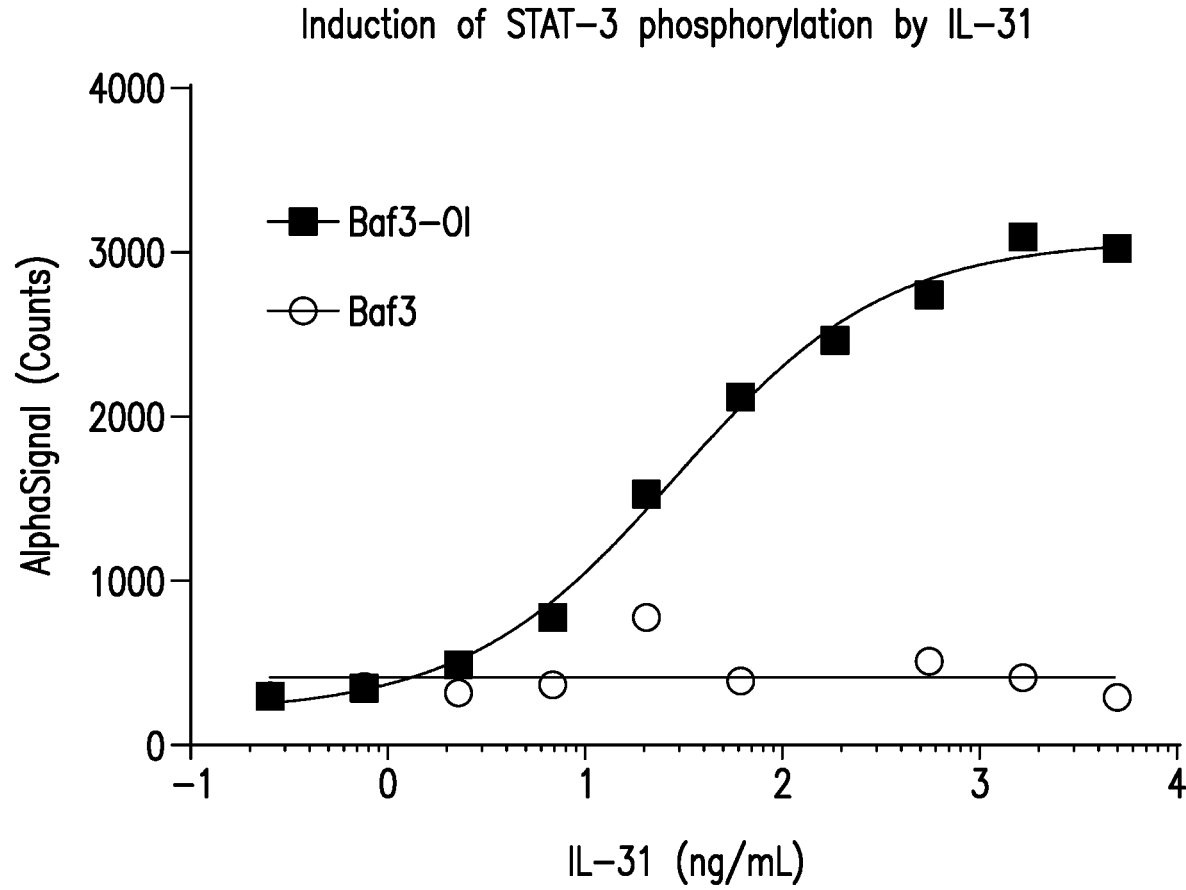


FIG.3



	Baf3	Baf3-OI
EC50	~ 0.000	29.16

FIG.4

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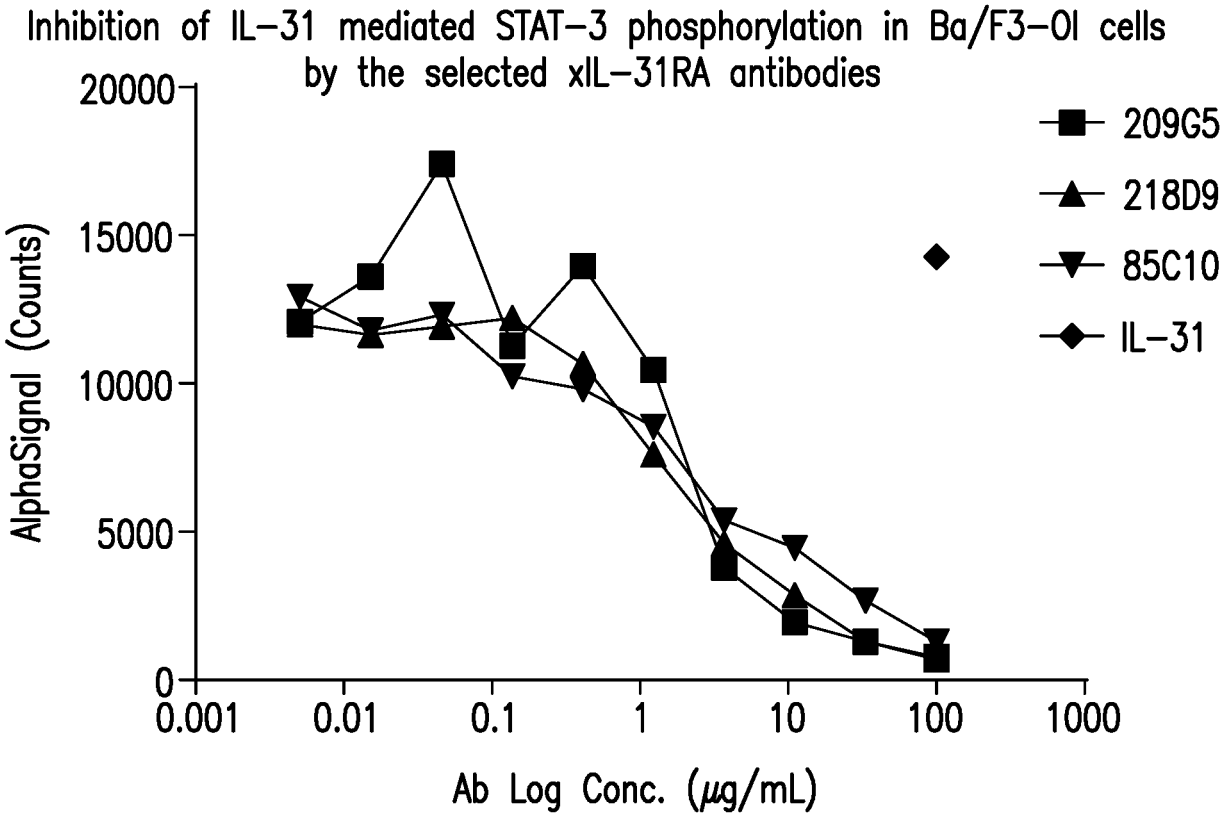


FIG.5A

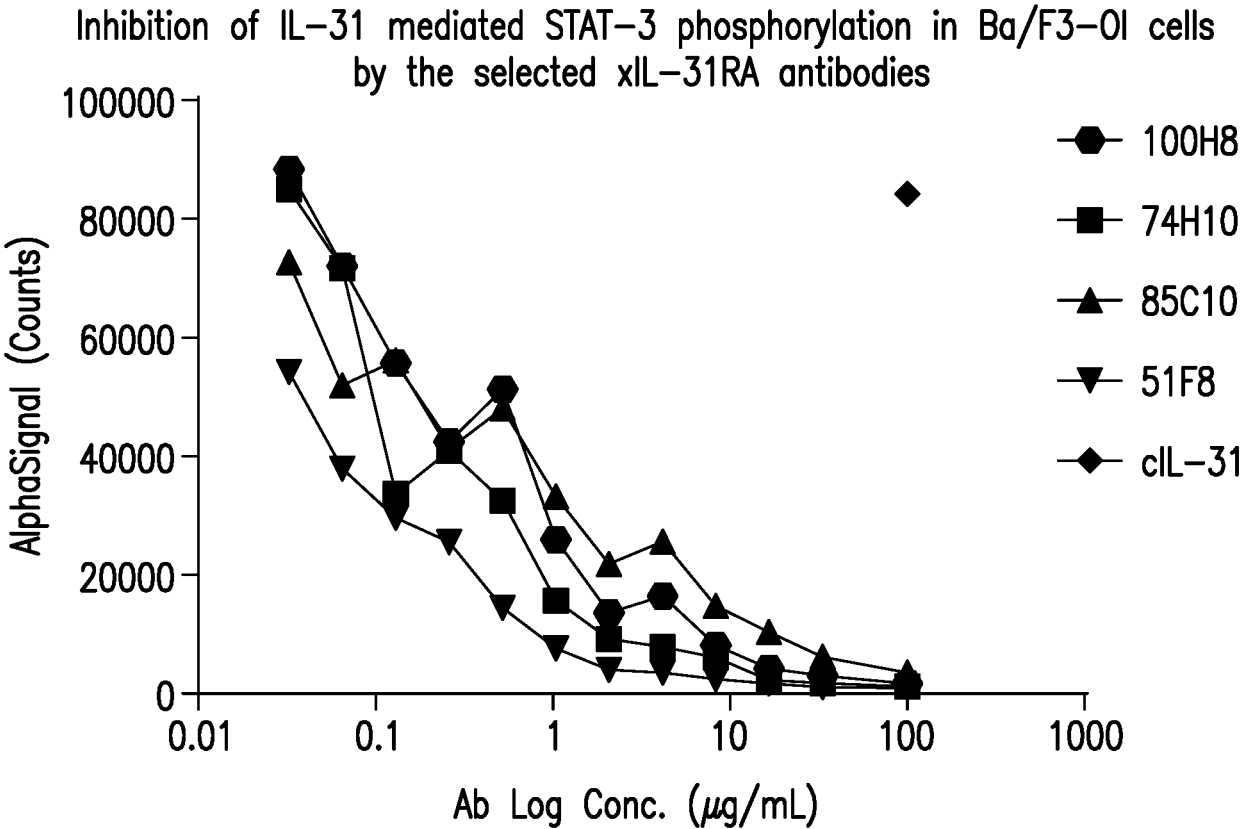


FIG.5B

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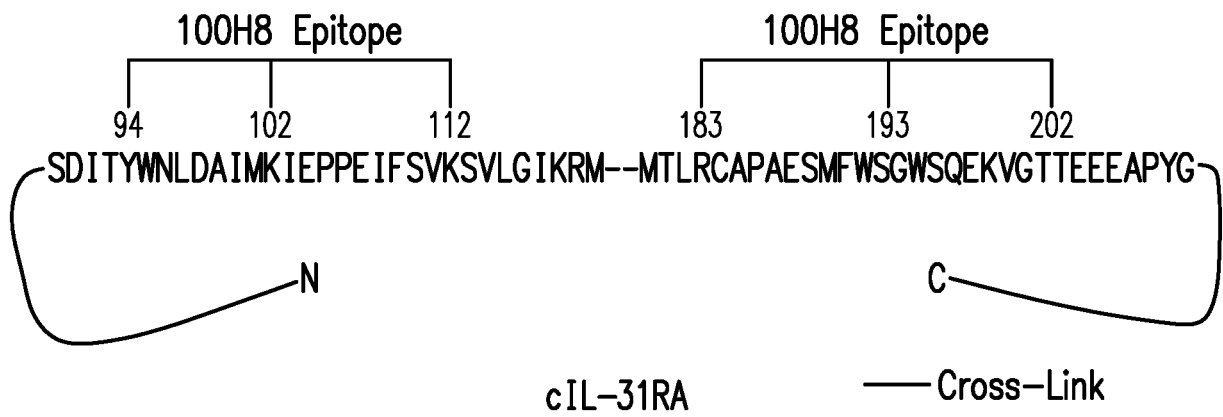


FIG.6A

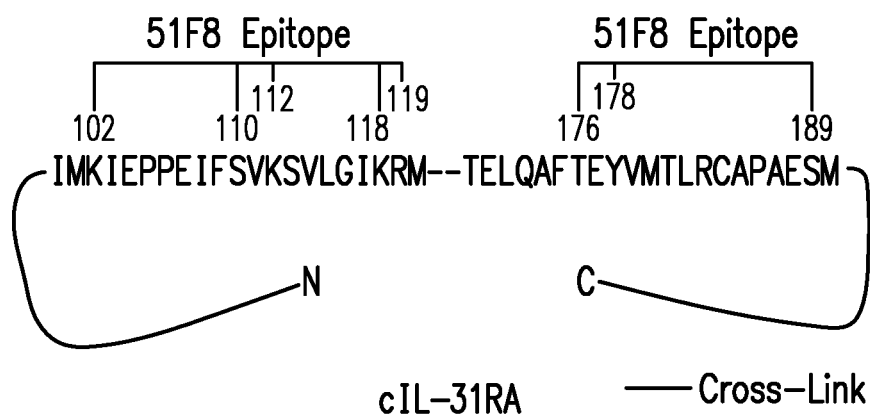


FIG.6B

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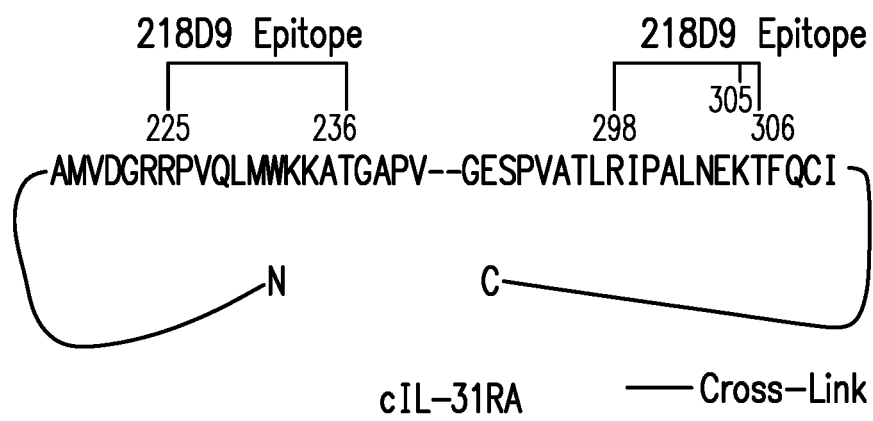


FIG.6C

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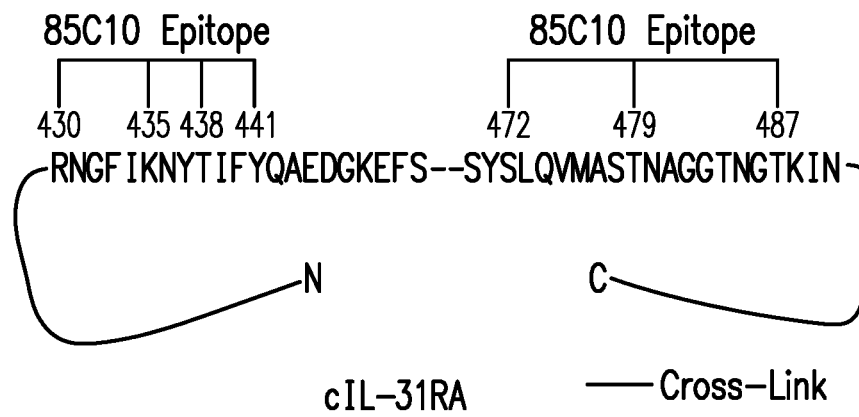


FIG.6D

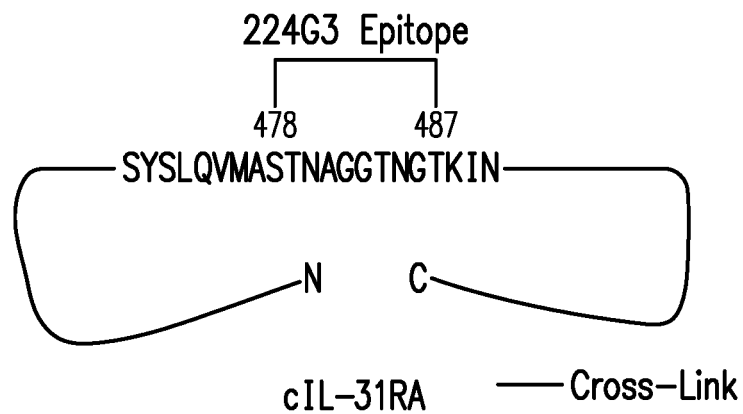


FIG.6E

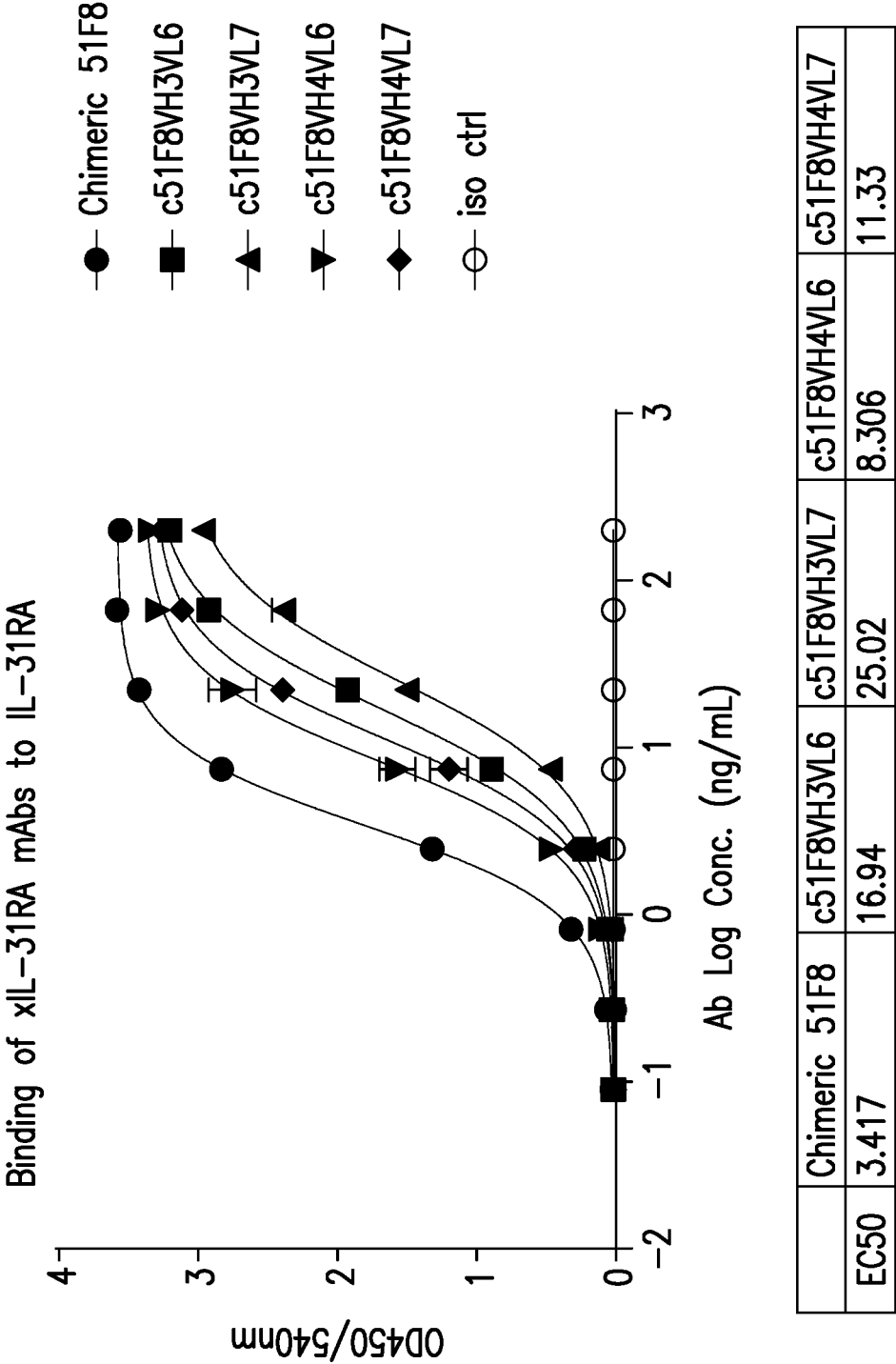


FIG.7A

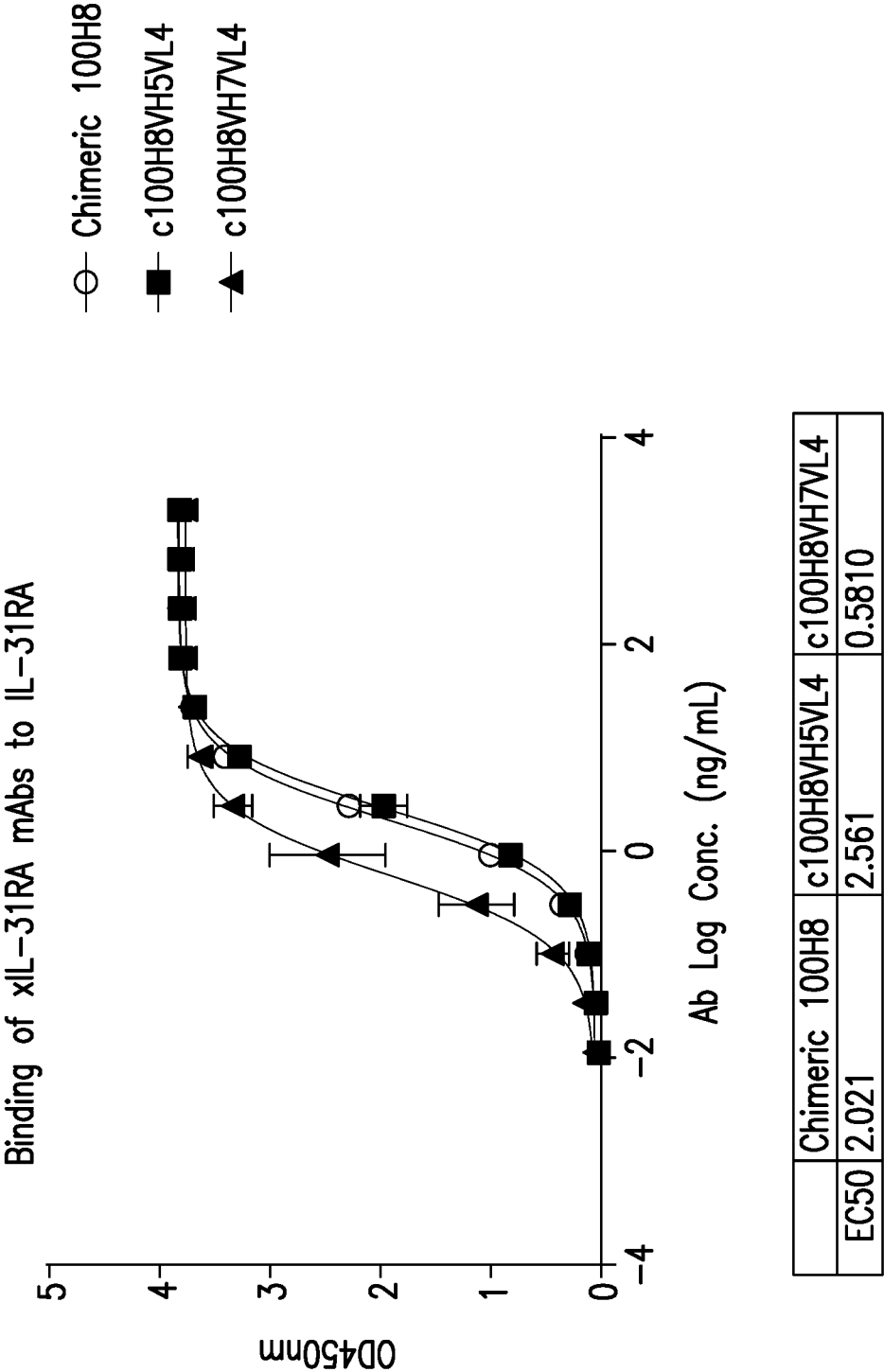


FIG.7B

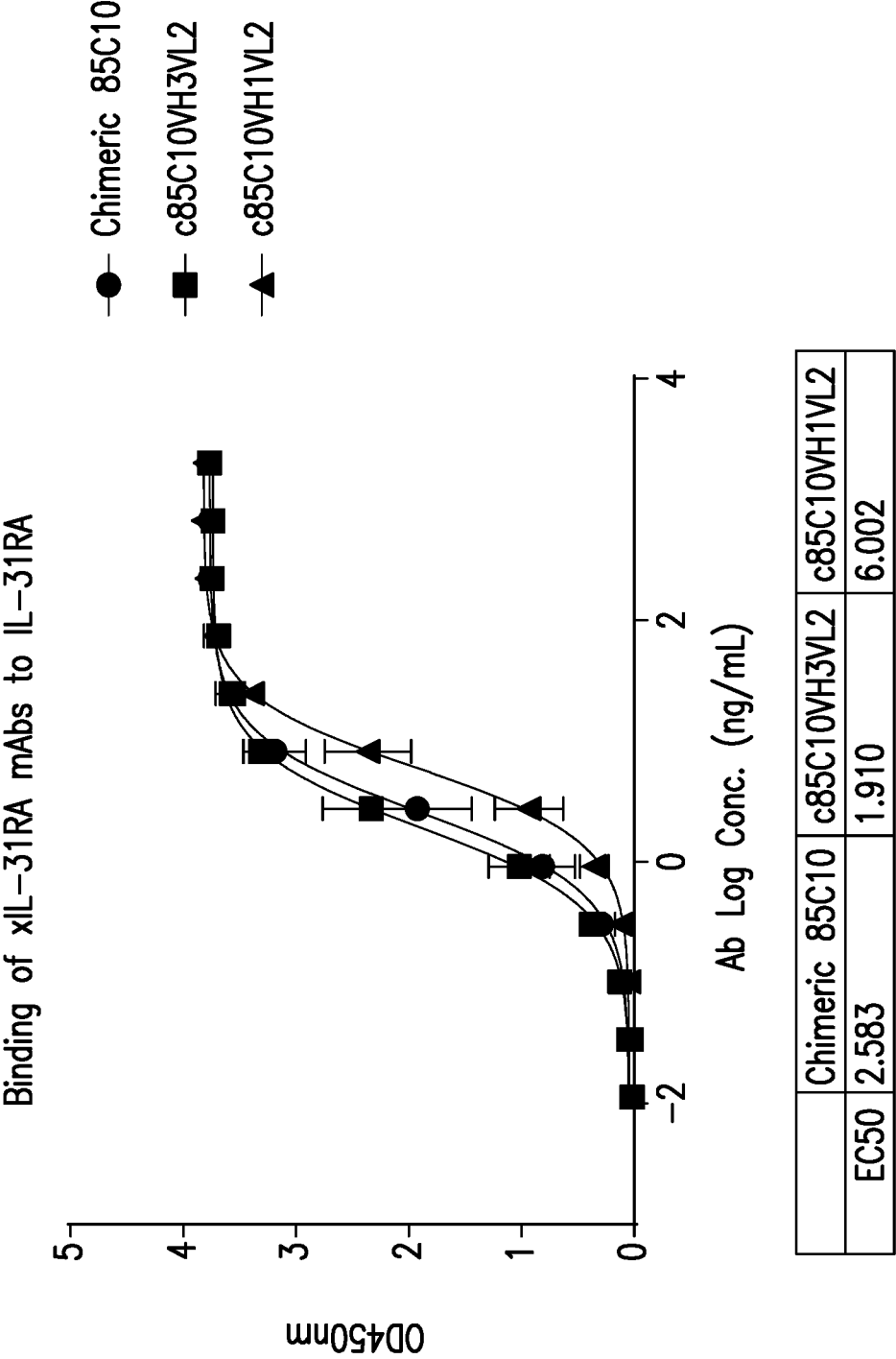


FIG.7C

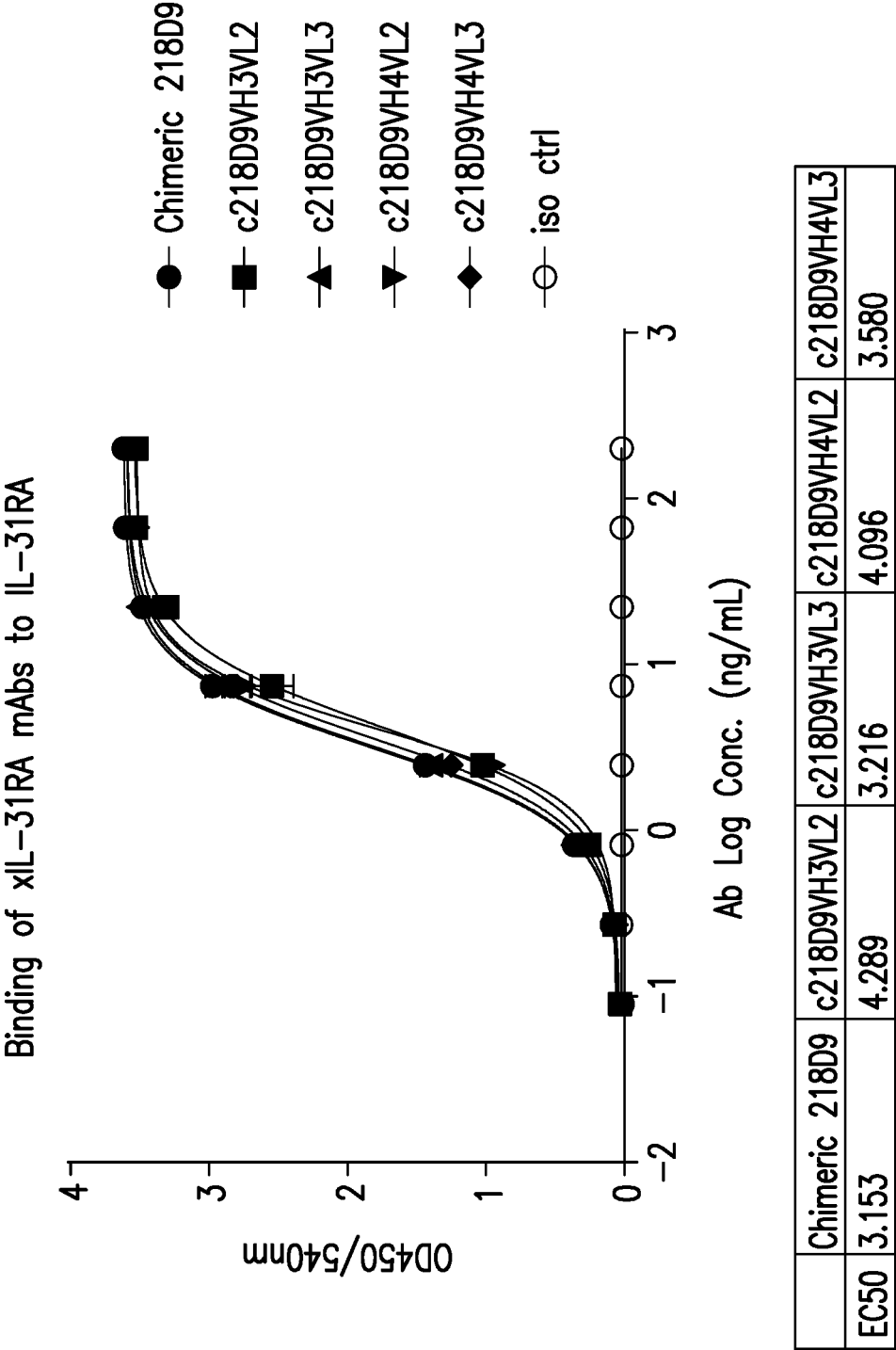


FIG. 7D

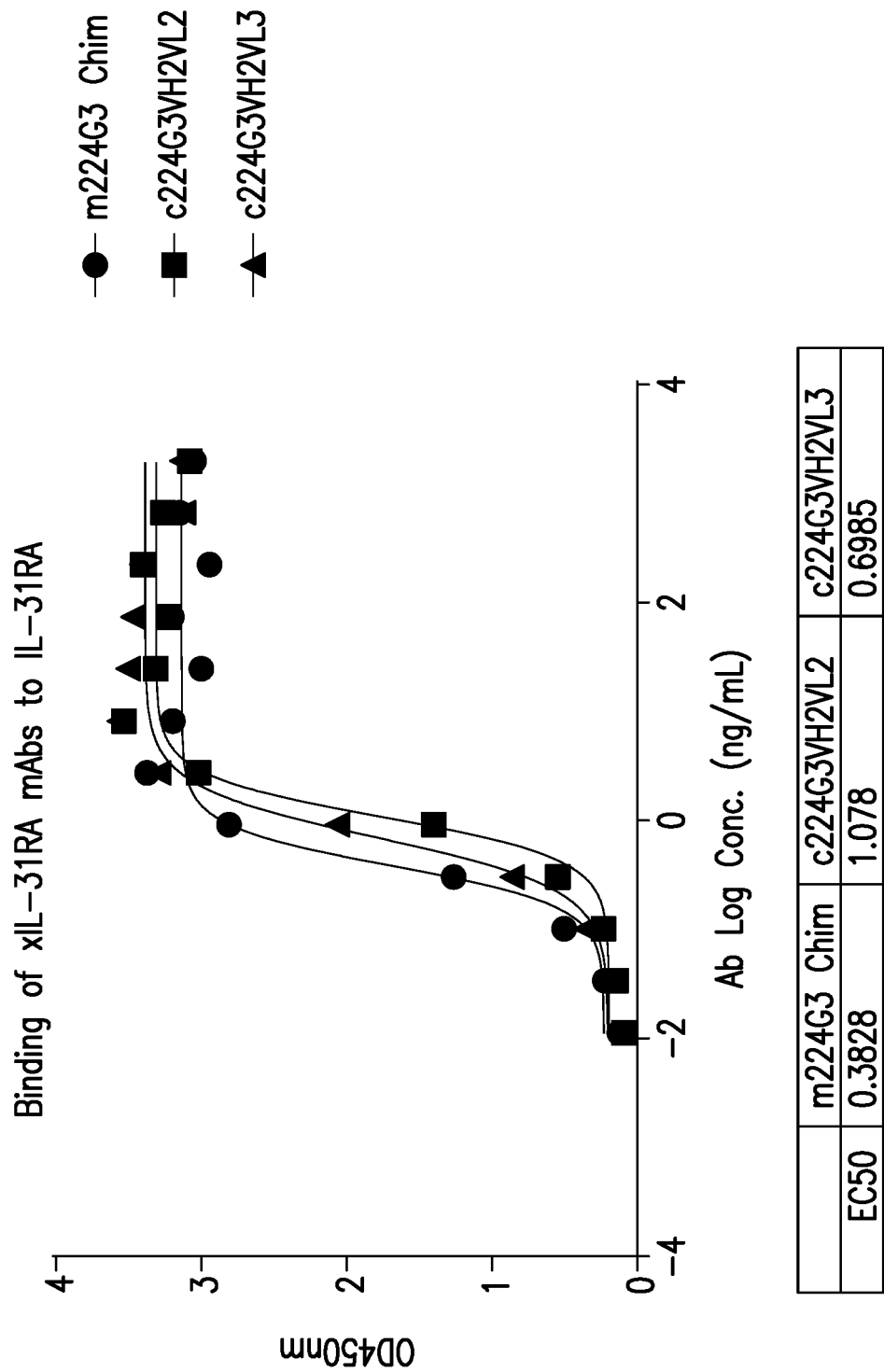
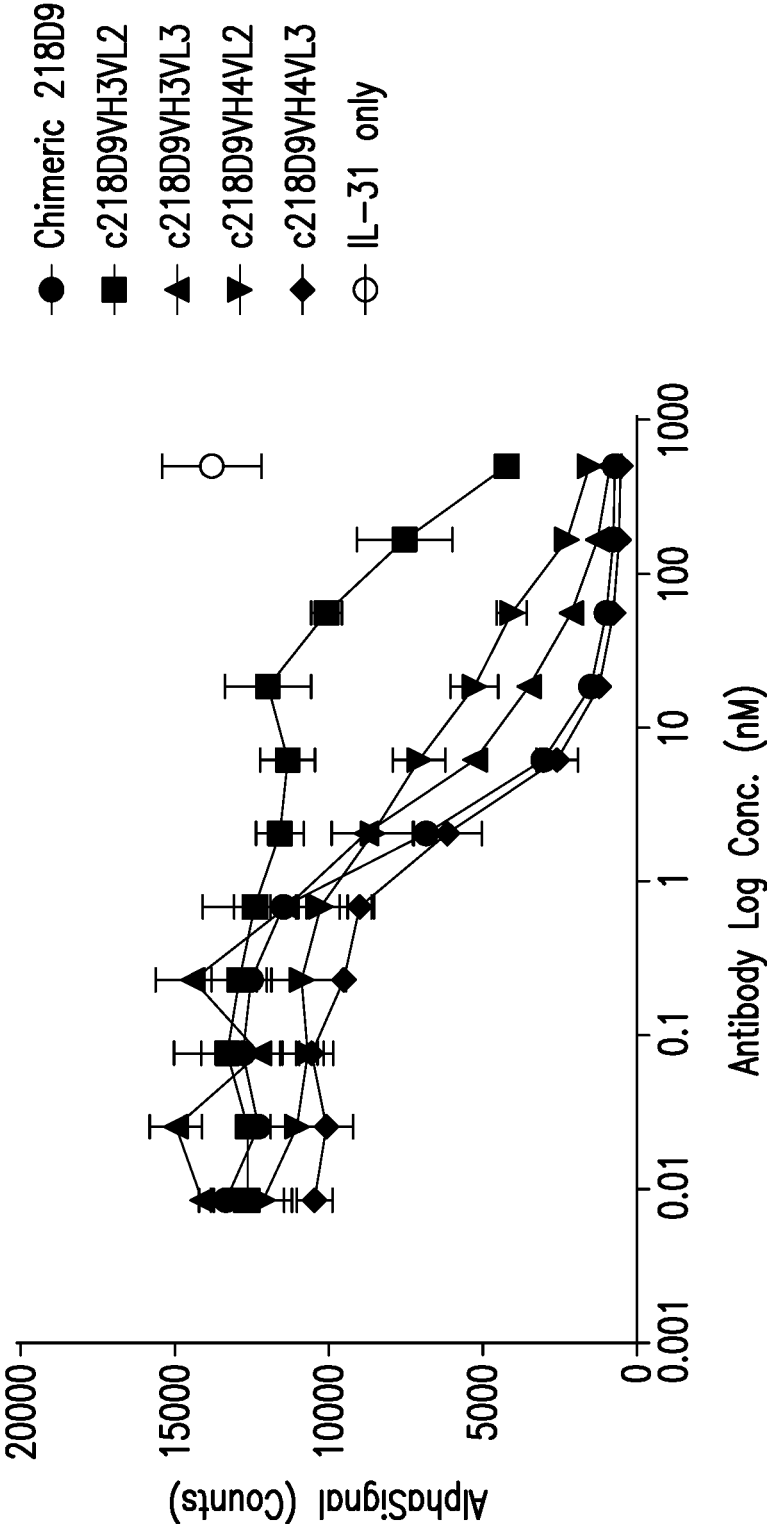


FIG.7E

Inhibition of IL-31 mediated STAT-3 phosphorylation in Ba/F3-01 cells by caninized 218D9 antibodies



	Chimeric 218D9	c218D9VH3VL2	c218D9VH3VL3	c218D9VH4VL2	c218D9VH4VL3
IC50	2.237	226.2	2.972	8.265	2.498

FIG.8