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An agency of Industry Canada

CA 2961987 A1 2016/04/07

(21) 2 961 987

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2015/09/29
(87) Date publication PCT/PCT Publication Date: 2016/04/07
(85) Entrée phase nationale/National Entry: 2017/03/21
(86) N° demande PCT/PCT Application No.: EP 2015/072324
(87) N° publication PCT/PCT Publication No.: 2016/050721
(30) Priorités/Priorities: 2014/09/30 (US62/057,541); 2015/06/08 (US62/172,511)

(51) Cl.Int./Int.Cl. *C07K 14/705* (2006.01), *A61K 39/00* (2006.01), *C07K 16/28* (2006.01), *C07K 16/46* (2006.01), *C07K 19/00* (2006.01), *C12N 15/12* (2006.01), *C12N 15/13* (2006.01), *C12N 15/62* (2006.01)

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(54) Titre : ANTICORPS PD-L1 DE LIAISON A PD-L1 CANIN
(54) Title: PD-L1 ANTIBODIES BINDING CANINE PD-L1

Figure 2

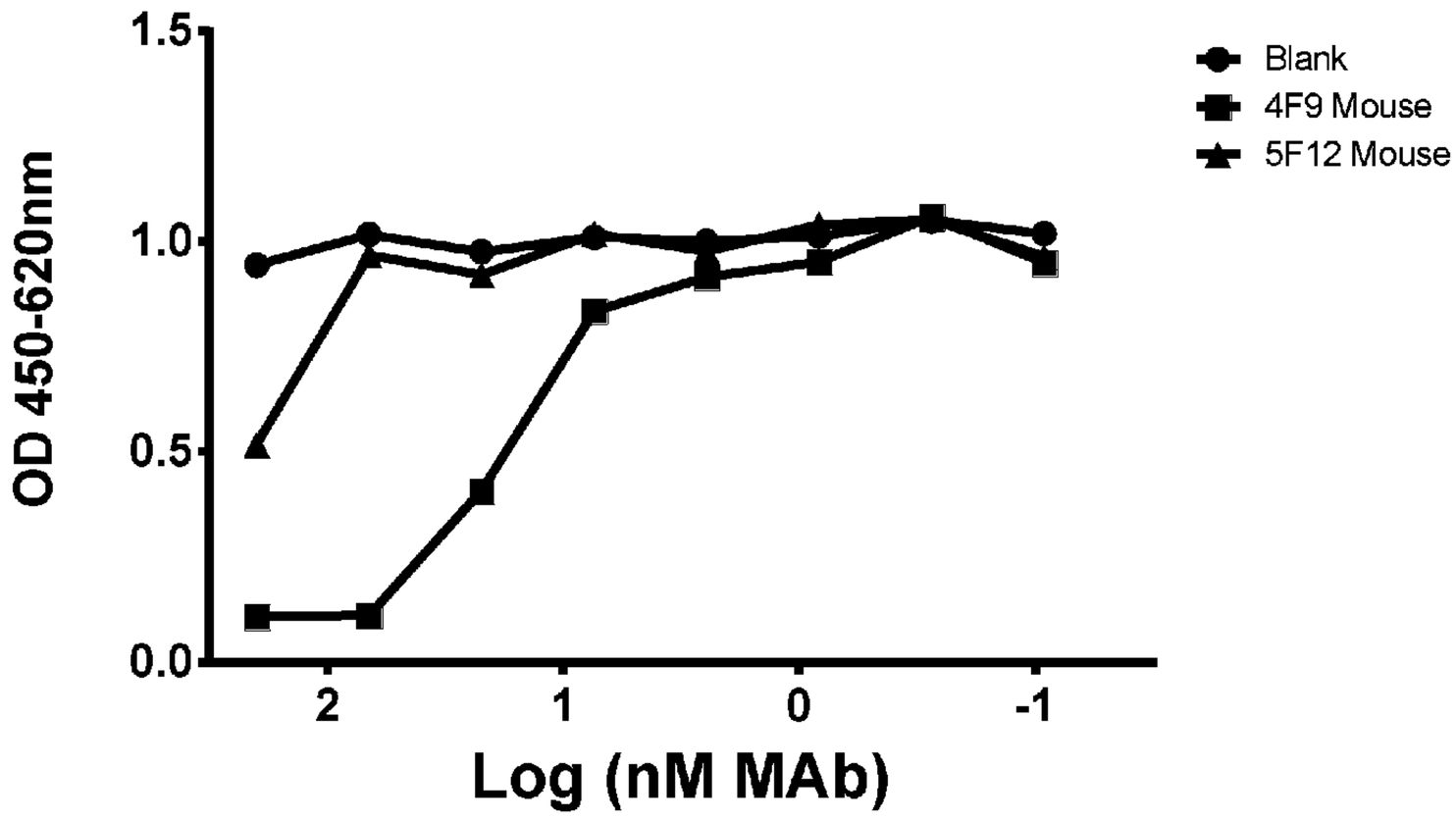

Figure 2 shows ligand blockade with mouse anti-canine PDL-1 mAbs. Two mAbs designated 4F9 and 5F12 were tested for their ability to inhibit binding of PDL-1 to PD-1 expressed on CHO cells. Both mAbs blocked the binding of PDL-1 to PD-1, although mAb 4F9 is a stronger inhibitor than 5F12.

(57) Abrégé/Abstract:
The present disclosure describes antibodies including caninized antibodies against canine PD-L1 with specific properties. The document relates to epitopes of canine PD-L1 that bind to these antibodies, as well as to anti-canine PD-L1 antibodies that bind these epitopes, and to the use of the caninized anti- canine PD-L1 antibodies in the treatment of cancer in dogs.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 April 2016 (07.04.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/050721 A1

(51) International Patent Classification:
C07K 16/28 (2006.01) *A61K 39/00* (2006.01)

(21) International Application Number:
PCT/EP2015/072324

(22) International Filing Date:
29 September 2015 (29.09.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/057,541 30 September 2014 (30.09.2014) US
62/172,511 8 June 2015 (08.06.2015) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH,

[Continued on next page]

(54) Title: PD-L1 ANTIBODIES BINDING CANINE PD-L1

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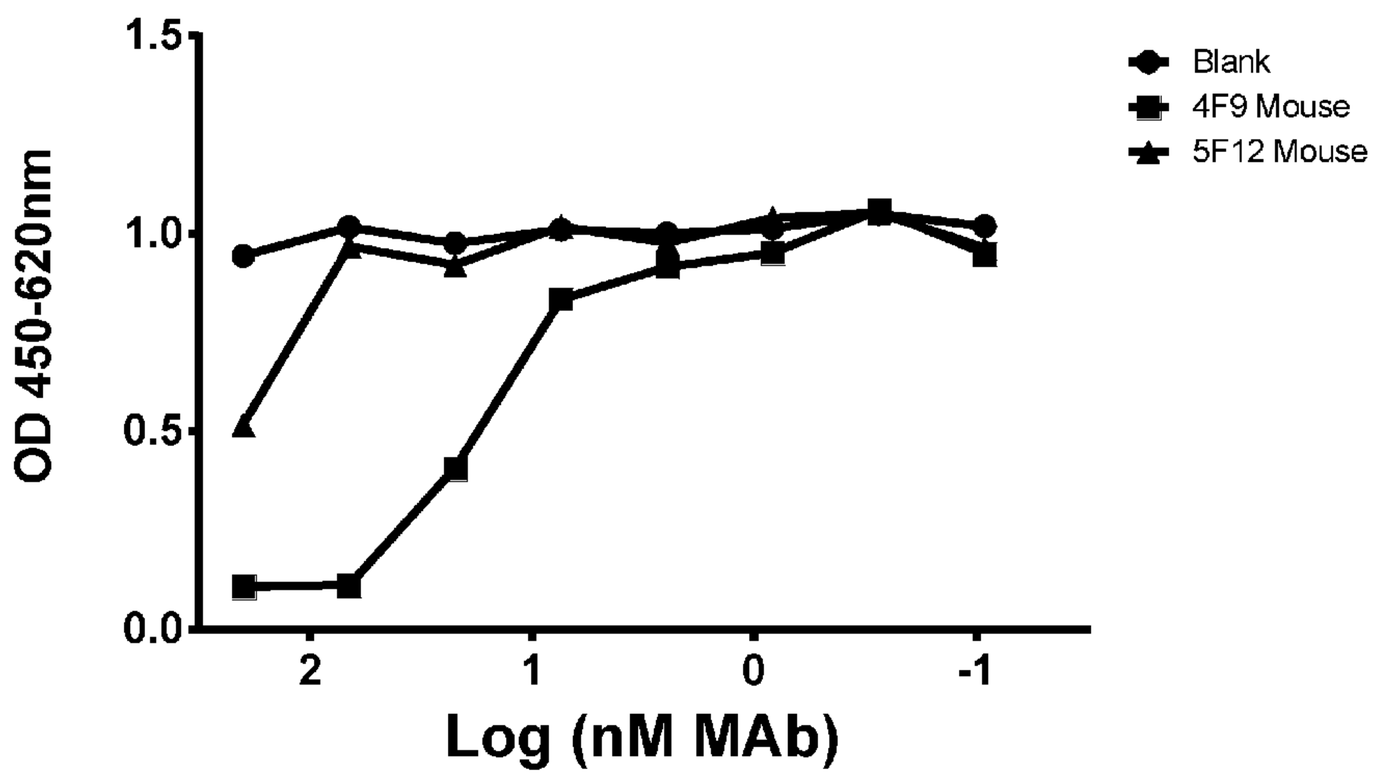
Figure 2



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WO 2016/050721 A1

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

PD-L1 ANTIBODIES BINDING CANINE PD-L1

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application Serial No. 62/057,541, filed on September 30, 2014, and U.S. Provisional Application Serial No. 62/172,511, filed on June 8, 2015, the contents of both of which are hereby incoporated by reference in their entireties.

FIELD OF THE INVENTION

The present invention relates to anti-canine PD-L1 antibodies with specific properties. The present invention also relates to caninized antibodies against canine PD-L1 that have specific sequences and a high binding affinity for canine PD-L1. The present invention further relates to epitopes of canine PD-L1 that bind to these antibodies, as well as to anti-canine PD-L1 antibodies that bind these epitopes. The invention further relates to use of the antibodies of the present invention in the treatment of dogs, including cancer treatment.

BACKGROUND OF THE INVENTION

An immunoinhibitory receptor that is primarily expressed on activated T and B cells, Programmed Cell Death Receptor 1, also referred to as Programmed Death Receptor 1 (PD-1), is a member of the immunoglobulin superfamily related to CD28 and cytotoxic T-lymphocyte associated protein-4 (CTLA-4). PD-1 and like family members are type I transmembrane glycoproteins containing an extracellular Ig Variable-type (V-type) domain that binds its ligands and a cytoplasmic tail that binds signaling molecules. The cytoplasmic tail of PD-1 contains two tyrosine-based signaling motifs, an ITIM (immunoreceptor tyrosine-based inhibition motif) and an ITSM (immunoreceptor tyrosine-based switch motif).

PD-1 attenuates T-cell responses when bound to Programmed Cell Death Ligand 1, also referred to as Programmed Death Ligand 1 (PD-L1), and/or Programmed Cell Death Ligand 2, also referred to as Programmed Death Ligand 2 (PD-L2). The binding of either

of these ligands to PD-1 negatively regulates antigen receptor signaling. Blocking the binding of PD-L1 to PD-1 enhances tumor-specific $CD8^+$ T-cell immunity, while aiding the clearance of tumor cells by the immune system. The three-dimensional structure of murine PD-1, as well as the co-crystal structure of mouse PD-1 with human PD-L1 have been reported [Zhang *et al.*, *Immunity* 20: 337-347 (2004); Lin *et al.*, *Proc. Natl. Acad. Sci. USA* 105: 3011-3016 (2008)].

PD-L1 and PD-L2 are type I transmembrane ligands that contain both IgV- and IgC-like domains in the extracellular region along with short cytoplasmic regions with no known signaling motifs. Both PD-L1 and PD-L2 are either constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as various tumor types. PD-L1 is not only expressed on B, T, myeloid and dendritic cells (DCs), but also on peripheral cells, such as microvascular endothelial cells and non-lymphoid organs *e.g.*, heart or lung. In contrast, PD-L2 is only found on macrophages and DCs. The expression pattern of PD-1 ligands suggests that PD-1 plays a role in maintaining peripheral tolerance and may further serve to regulate self-reactive T- and B-cell responses in the periphery.

In any case, it is now abundantly clear that PD-1 and PD-L1 play critical roles in at least certain human cancers, presumably by mediating immune evasion. Accordingly, PD-L1 has been shown to be expressed on a number of mouse and human tumors and is inducible by IFN- γ in the majority of PD-L1 negative tumor cell lines [Iwai *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 99: 12293-12297 (2002); Strome *et al.*, *Cancer Res.*, 63: 6501-6505 (2003)]. Furthermore, the expression of PD-1 on tumor infiltrating lymphocytes and/or PD-L1 on tumor cells has been identified in a number of primary human tumor biopsies. Such tumor tissues include cancers of the lung, liver, ovary, cervix, skin, colon, glioma, bladder, breast, kidney, esophagus, stomach, oral squamous cell, urothelial cell, and pancreas, as well as tumors of the head and neck [Brown *et al.*, *J. Immunol.* 170: 1257-1266 (2003); Dong *et al.*, *Nat. Med.* 8: 793-800 (2002); Wintterle *et al.*, *Cancer Res.* 63: 7462-7467 (2003); Strome *et al.*, *Cancer Res.*, 63: 6501-6505 (2003); Thompson *et al.*, *Cancer Res.* 66: 3381-5 (2006); Thompson *et al.*, *Clin. Cancer Res.* 13: 1757-1761 (2007); Nomi *et al.*, *Clin.Cancer Res.* 13: 2151-2157. (2007)]. More strikingly, PD-

ligand expression on tumor cells has been correlated to poor prognosis of human cancer patients across multiple tumor types [reviewed in Okazaki and Honjo, *Int. Immunol.* 19: 813-824 (2007)].

Moreover, Nomi *et al.* [*Clin. Cancer Res.* 13: 2151-2157 (2007)] demonstrated the therapeutic efficacy of blocking the binding of PD-L1 to PD-1 in a murine model of aggressive pancreatic cancer through administering either PD-1 or PD-L1 directed antibody. These antibodies effectively promoted tumor reactive $CD8^+$ T cell infiltration into the tumor resulting in the up-regulation of anti-tumor effectors including IFN-γ, granzyme B, and perforin. Similarly, the use of antibodies to block the binding of PD-L1 and PD-1 significantly inhibited tumor growth in a model of mouse squamous cell carcinoma [Tsushima *et al.*, *Oral Oncol.* 42: 268-274 (2006)].

In other studies, transfection of a murine mastocytoma line with PD-L1 led to decreased lysis of the tumor cells when co-cultured with a tumor-specific CTL clone. Lysis was restored when anti-PD-L1 monoclonal antibody was added [Iwai *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 99: 12293-12297 (2002)]. *In vivo*, blocking the PD1/PD-L1 interaction was shown to increase the efficacy of adoptive T cell transfer therapy in a mouse tumor model [Strome *et al.*, *Cancer Res.* 63: 6501-6505 (2003)]. Further evidence for the role of PD-1 and PD-L1 in cancer treatment comes from experiments performed with PD-1 knockout mice in which PD-L1 expressing myeloma cells grew only in wild-type animals (resulting in tumor growth and associated animal death), but not in PD-1 deficient mice [Iwai Y. et al., *Proc. Natl. Acad. Sci. U.S.A.* 99: 12293-12297 (2002)]. More recently, humanized murine monoclonal antibodies against human PD-1 have shown initial success in cancer therapy in humans [*see e.g.*, US 8,354,509 B2, US 8,008,449 B2, and US 7,595,048 B2].

Anti-PD-L1 antibodies may also be useful in chronic viral infection. Memory $CD8^+$ T cells generated after an acute viral infection are highly functional and constitute an important component of protective immunity. In contrast, chronic infections are often characterized by varying degrees of functional impairment (exhaustion) of virus-specific T-cell responses, and this defect is a principal reason for the inability of the host to eliminate the persisting pathogen. Although functional effector T cells are initially

generated during the early stages of infection, they gradually lose function during the course of a chronic infection. Barber *et al.* [*Nature* 439: 682-687 (2006)] showed that mice infected with a laboratory strain of LCMV developed chronic infection resulted in high levels of virus in the blood and other tissues. These mice initially developed a robust T cell response, but eventually succumbed to the infection upon T cell exhaustion. Barber *et al.* found that the decline in number and function of the effector T cells in chronically infected mice could be reversed by injecting an antibody that blocked the interaction between PD-1 and PD-L1.

Canine antibodies (also referred to as immunoglobulin G or IgG) are large tetrameric proteins of about 150 Kd. Each IgG protein is composed of two identical light chains of about 25 Kd each, and two identical heavy chains of about 50 Kd each. There are four known IgG heavy chain subclasses of canine IgG and they are referred to as IgGA, IgGB, IgGC, and IgGD. There are two types of light chains; *kappa* and *lambda* chains. Each of the *kappa* or *lambda* light chains is composed of one variable domain (VL) and one constant domain (CL). 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". In humans, IgG exists in one of four subclasses referred to as IgG1, IgG2, IgG3, and IgG4. The subclass of IgG is determined largely by the sequence of the hinge region, which differs among the four subclasses of IgG. The two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to one of the light chains also through a disulfide bond.

Digestion of IgG antibodies with the enzyme papain breaks the antibody molecule in the hinge region and results in the formation of three fragments. Two of these fragments are identical and each consists of the light chain held together with the VH and CH1 domains of the heavy chain. These fragments are called the "Fab" fragments and they contain the antigen binding sites of the antibody. The third fragment that results from digestion with papain is called the "Fc" and it contains the remainder of the two heavy chains held together by disulfide bonds. The Fc thus contains a dimer consisting of the CH2 and CH3 domain of each of the two heavy chains. While the Fab enables the antibody to bind to its

cognate epitope, the Fc enables the antibody to mediate immune effector functions such as antibody dependent cellular cytotoxicity (ADCC), antibody-dependent phagocytosis (ADCP) and complement dependent cytotoxicity (CDC).

It is well known in the art that IgG antibodies mediate effector functions such as ADCC and ADCP through binding of their Fc portion to a family of proteins known as Fc_γ receptors, whereas CDC is mediated through the binding of the Fc to the first component of complement, C1q. It is also well known in the art that different IgG sub-classes differ in their capacity to mediate these effector functions. For example, human IgG1 displays strong ADCC and CDC, whereas IgG4 displays a weak to no ADCC and CDC. In addition, methods for identification of which IgG sub-classes display or lack effector functions are well known in the art.

Approaches that rely on use of monoclonal antibodies for therapeutic purposes require the design of fit-for-purpose antibodies or antibody fragments to achieve the desired therapeutic response. For example, some therapeutic approaches for cancer require the therapeutic antibodies to have enhanced effector functions, while others require the effector functions to be significantly reduced or eliminated altogether. Enhancement or elimination of effector functions may be achieved through introduction of one or more amino acid mutations (substitutions) in the Fc portion of the antibody so as to enhance or reduce binding to Fc_γ receptors and the first component of complement. There are numerous reports in the prior art describing amino acid substitutions that may be introduced into an antibody molecule in order to modulate its effector functions. For example, Shields *et al.*, [*J. of Biol. Chem.*, 276 (9): 6591-6604 (2001)] disclosed that an asparagine to alanine (N297A) substitution, which result in a non-glycosylated antibody, significantly reduced antibody binding to several Fc_γ receptors. Additionally, Shields *et al.*, disclosed that an aspartic acid-to-alanine (D265A) substitution also significantly reduced binding of the antibody to Fc_γ receptors. Each of the N297A and D265A substitutions were also shown to significantly impair CDC. There are other similar reports identifying potential substitutions to reduce or eliminate effector function in antibodies [*e.g.*, Sazinsky *et al., Proc.Nat.Acad.Sci.*,105:20167-20172 (2008), Alegre *et*

al., Transplantation, 57:1537-1543 (1994), Hutchins *et al.*, *Proc.Nat.Acad.Sci.* 92:11980-11984 (1994), McEarchem *et al., Blood*, 109:1185-1192 (2007)].

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 antibodies and antigen binding fragments thereof (including isolated antibodies and isolated antigen binding fragments thereof) that bind canine Programmed Death Ligand 1 (canine PD-L1) with specificity. In particular embodiments the antibodies and the fragments thereof are mammalian antibodies. In more particular embodiments the mammalian antibodies are murine (*i.e.*, mouse) antibodies. In a related aspect of the present invention the isolated antibodies are caninized antibodies. In specific embodiments the caninized antibodies are caninized mammalian (*e.g.*, mouse) anti-canine PD-L1 antibodies. The antibodies and antigen binding fragments thereof of the present invention bind canine PD-L1 and can block the binding of canine PD-L1 to canine Programmed Death Receptor 1 (PD-1). The present invention further provides the use of such antibodies or antigen binding fragments thereof in the treatment of disease, *e.g.,* the treatment of cancer in canines.

In particular embodiments the antibodies or antigen binding fragments thereof that bind canine PD-L1 with specificity comprise three light chain complementary determining regions (CDRs): CDR light 1 (CDRL1), CDR light 2 (CDRL2), and CDR light 3 (CDRL3); and three heavy chain CDRs: CDR heavy 1 (CDRH1), CDR heavy 2 (CDRH2) and CDR heavy 3 (CDRH3).

In certain embodiments the CDRH1 comprises the amino acid sequence of SEQ ID NO: 13. In other embodiments the CDRH1 comprises a conservatively modified variant of SEQ ID NO: 13. In yet other embodiments the CDRH1 comprises a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 13 that comprises the canonical structure class of 1. In still other embodiments the CDRH1 comprises an amino acid sequence of

SEQ ID NO: 19. In yet other embodiments the CDRH1 comprises a conservatively modified variant of SEQ ID NO: 19. In still other embodiments the CDRH1 comprises a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 19 that comprises the canonical structure class of 1.

In certain embodiments the CDRH2 comprises the amino acid sequence of SEQ ID NO: 14. In other embodiments the CDRH2 comprises a conservatively modified variant of SEQ ID NO: 14. In still other embodiments the CDRH2 comprises a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 14 that comprises the canonical structure class of 3B. In yet other embodiments the CDRH2 comprises the amino acid sequence of SEQ ID NO: 20. In still other embodiments the CDRH2 comprises a conservatively modified variant of SEQ ID NO: 20. In yet other embodiments the CDRH2 comprises a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 20 that comprises the canonical structure class of 3B.

In certain embodiments the CDRH3 comprises the amino acid sequence of SEQ ID NO: 15. In other embodiments the CDRH3 comprises a conservatively modified variant of SEQ ID NO: 15. In still other embodiments the CDRH3 comprises a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 15 that comprises the canonical structure class of 10. In yet other embodiments the CDRH3 comprises the amino acid sequence of SEQ ID NO: 21. In still other embodiments the CDRH3 comprises a conservatively modified variant of SEQ ID NO: 21. In yet other embodiments the CDRH3 comprises a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 21 that comprises the canonical structure class of 8.

In certain embodiments the CDRL1 comprises the amino acid sequence of SEQ ID NO: 16. In other embodiments the CDRL1 comprises a conservatively modified variant of SEQ ID NO: 16. In still other embodiments the CDRL1 comprises a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 16 that comprises the canonical structure class of 2. In yet other embodiments the amino acid sequence of the CDRL1 comprises SEQ ID NO: 22. In still other embodiments the CDRL1 comprises a conservatively modified variant of SEQ ID NO: 22. In yet other embodiments the CDRL1 comprises a

variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 22 that comprises the canonical structure class of 3.

In certain embodiments the CDRL2 comprises the amino acid sequence of SEQ ID NO: 17. In other embodiments the CDRL2 comprises a conservatively modified variant of SEQ ID NO: 17. In still other embodiments the CDRL2 comprises a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 17 that comprises the canonical structure class of 1. In yet other embodiments the CDRL2 comprises SEQ ID NO: 23. In still other embodiments the CDRL2 comprises a conservatively modified variant of SEQ ID NO: 23. In yet other embodiments the CDRL2 comprises a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 23 that comprises the canonical structure class of 1.

In certain embodiments the CDRL3 comprises the amino acid sequence of SEQ ID NO: 18. In other embodiments the CDRL3 comprises a conservatively modified variant of SEQ ID NO: 18. In still other embodiments the CDRL3 comprises a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 18 that comprises the canonical structure class of 1. In yet other embodiments the CDRL3 comprises the amino acid sequence of SEQ ID NO: 24. In still other embodiments the CDRL3 comprises a conservatively modified variant of SEQ ID NO: 24. In still other embodiments the CDRL3 comprises a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 24 that comprises the canonical structure class of 1.

The present invention further provides combinations of two or more of the CDRs of the present invention (or variants thereof) in a given antibody, *e.g.*, a CDRH3 that comprises the amino acid sequence of SEQ ID NO: 15 and a CDRL3 that comprises the amino acid sequence of SEQ ID NO: 18.

In specific embodiments the antibodies and antigen binding fragments thereof that bind canine PD-L1 comprise a CDRH1 that comprises an amino acid sequence of SEQ ID NO: 13, a conservatively modified variant of SEQ ID NO: 13, or a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 13 that comprises the canonical structure class of 1; a CDRH2 that comprises the amino acid sequence of SEQ ID NO: 14, a

conservatively modified variant of SEQ ID NO: 14, or a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 14 that comprises the canonical structure class of 3B; a CDRH3 that comprises SEQ ID NO: 15, a conservatively modified variant of SEQ ID NO: 15, or a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 15 that comprises the canonical structure class of 10; a CDRL1 that comprises an amino acid sequence of SEQ ID NO: 16, a conservatively modified variant of SEQ ID NO: 16, or a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 16 that comprises the canonical structure class of 2; a CDRL2 that comprises the amino acid sequence of SEQ ID NO: 17, a conservatively modified variant of SEQ ID NO: 17 or a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 17 that comprises the canonical structure class of 1; and a CDRL3 that comprises the amino acid sequence of SEQ ID NO: 18, a conservatively modified variant of SEQ ID NO: 18, or a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 18 that comprises the canonical structure class of 1.

In related embodiments the antibodies and antigen binding fragments thereof that bind canine PD-L1 comprise a CDRH1 that comprises an amino acid sequence of SEQ ID NO: 19, a conservatively modified variant of SEQ ID NO: 19, or a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 19 that comprises the canonical structure class of 1; a CDRH2 that comprises the amino acid sequence of SEQ ID NO: 20, a conservatively modified variant of SEQ ID NO: 20, or a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 20 that comprises the canonical structure class of 3B; a CDRH3 that comprises SEQ ID NO: 21, a conservatively modified variant of SEQ ID NO: 21, or a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 21 that comprises the canonical structure class of 8; a CDRL1 that comprises an amino acid sequence of SEQ ID NO: 22, a conservatively modified variant of SEQ ID NO: 22, or a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 22 that comprises the canonical structure class of 3; a CDRL2 that comprises the amino acid sequence of SEQ ID NO: 23, a conservatively modified variant of SEQ ID NO: 23 or a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 23 that comprises the canonical structure class of 1; and a CDRL3 that comprises the amino acid sequence of SEQ ID NO: 24, a conservatively modified variant of SEQ ID NO: 24, or a variant (*e.g.*, a function-conservative variant) of SEQ ID NO: 24 that comprises the canonical structure class of 1.

Accordingly, in a particular aspect of the present invention, the present invention further provides caninized anti-canine PD-L1 antibodies. In certain embodiments the caninized anti-canine PD-L1 antibodies are caninized mammalian (*e.g.*, murine) anti-canine PD-L1 antibodies. In specific embodiments, the caninized anti-canine PD-L1 antibodies (*e.g.*, caninized mammalian anti-canine PD-L1 antibodies, such as caninized murine anti-canine PD-L1 antibodies) comprise a cFc that has been genetically modified to augment, decrease, or eliminate one or more effector functions. In particular embodiments of this type, the genetically modified cFc decreases or eliminates one or more effector functions. In other particular embodiments the genetically modified cFc 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.

The present invention further provides canine frames and/or full length heavy chains that comprise the genetically modified cFc regions. Accordingly, the present invention provides full length heavy chains of antibodies in which the full length heavy chains comprise the genetically modified cFc regions of the present invention and the CDRs of the present invention. Such full length heavy chains can also be combined with corresponding canine light (*kappa* or *lambda)* chains to form a complete antibody. In particular embodiments of this type, the resulting antibody binds to canine PD-L1.

In certain embodiments, the genetically modified cFc region comprises the amino acid sequence of SEQ ID NO: 66 (or SEQ ID NO: 68) in which one to seven of the following amino acid residues are replaced by another amino acid residue at the indicated positions:

P4, D31, N63, G64, T65, A93, or P95. The amino acid substituting for P4, D31, N63, G64, T65, A93, and/or P95 are individually selected from one of the other 19 standard naturally occurring amino acids, as listed in Table 1 below. The present invention further provides variants of the genetically modified cFc regions that comprise an amino acid sequence that is 90%, 95%, 98%, or 99% identical to the amino acid sequence of such genetically modified cFc regions and retain at least 50%, 75%, 90%, 95%, or more of the augmentation, decrease, or elimination of the ADCC and/or the CDC as the genetically modified cFc regions comprising the amino acid sequence of SEQ ID NO: 66 (or SEQ ID NO: 68) in which one or more of the following amino acid residues were replaced: *i.e.*, at P4, D31, N63, G64, T65, A93, or P95.

In other embodiments two to five of the following amino acid residues are replaced by another amino acid residue at the indicated positions: P4, D31, N63, G64, T65, A93, or P95. In particular embodiments of this type, the genetically modified cFc region comprises the amino acid sequence of SEQ ID NO: 66 or SEQ ID NO: 68 with the following substitutions: P4A, D31A, N63A, A93G, and P95A. In related embodiments, the genetically modified cFc region comprises the amino acid sequence of SEQ ID NO: 66 or SEQ ID NO: 68 with the following substitutions: P4A, D31A, N63A, and P95A. In other embodiments, the genetically modified cFc region comprises the amino acid sequence of SEQ ID NO: 66 or SEQ ID NO: 68 with substitutions at D31 and N63. In particular embodiments of this type, the aspartic acid residue at position 31 is replaced with a glutamic acid residue, an asparagine residue, or an alanine residue, whereas the asparagine residue at position 63 is replaced with a glutamine residue, a histidine residue, or an alanine residue. In a more particular embodiment of this type, the genetically modified cFc region comprises the amino acid sequence of SEQ ID NO: 66 or SEQ ID NO: 68 with the following substitutions: D31A and N63A. In particular embodiments, the genetically modified cFc region is encoded by the nucleotide sequence of SEQ ID NO: 65 or SEQ ID NO: 67 comprising nucleotide changes that correspond to the amino acid sequences that they encode.

In another embodiments, the genetically modified cFc region comprises the amino acid sequence of SEQ ID NO: 66 or SEQ ID NO: 68 with the substitution at A93. In a

particular embodiment of this type, the substitution is A93G. In a related embodiment the the substitution is A93S. As described in U.S. provisional application no. 62/030,812, filed on July 30, 2014, hereby incorporated by reference in its entirety, the substitution of A93G leads to an enhancement in complement C1q binding, which is indicative of increasing CDC activity.

In related embodiments the genetically modified cFc region further comprises a hinge region that comprises the amino acid sequence of SEQ ID NO: 45. In other embodiments the genetically modified Fc region further comprises a hinge region that comprises the amino acid sequence of SEQ ID NO: 46. In still other embodiments the genetically modified Fc region further comprises a hinge region that comprises the amino acid sequence of SEQ ID NO: 47. In yet other embodiments the genetically modified Fc region further comprises a genetically modified hinge region that comprises the amino acid sequence of SEQ ID NO: 48.

In alternative embodiments, the present invention provides a canine IgGD Fc region with a genetically modified hinge region from a canine IgGD antibody, a hinge region from a canine IgGA antibody, a hinge region from a canine IgGB antibody, or a hinge region from a canine IgGC antibody. Moreover, the present invention provides full length heavy chains of antibodies in which the full length heavy chains comprise the canine IgGD Fc region of the present invention with a genetically modified hinge region from a canine IgGD antibody, a hinge region from a canine IgGA antibody, a hinge region from a canine IgGB antibody, or a hinge region from a canine IgGC antibody. Such full length heavy chains also can be combined with corresponding canine light (*kappa* or *lambda*) chains to form a complete antibody.

Accordingly, the present invention provides a canine IgGD Fc region that further comprises a genetically modified hinge region from a canine IgGD antibody. In particular embodiments of this type the canine IgGD Fc region and genetically modified hinge region comprise the amino acid sequence of SEQ ID NO: 6 or an amino acid sequence that is 90%, 95%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 6, which comprises a proline residue at position 10 (P10). In a more particular

embodiment the canine IgGD Fc region and genetically modified hinge region is encoded by the nucleotide sequence of SEQ ID NO: 5. In other embodiments, the canine IgGD Fc region further comprises a hinge region from a canine IgGA antibody. In particular embodiments of this type the canine IgGD Fc region and hinge region comprise the amino acid sequence of SEQ ID NO: 8 or an amino acid sequence that is 90%, 95%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 8. In a more particular embodiment the canine IgGD Fc region and hinge region is encoded by the nucleotide sequence of SEQ ID NO: 7. In still other embodiments, the canine IgGD Fc region further comprises a hinge region from a canine IgGB antibody. In particular embodiments of this type the canine IgGD Fc region and hinge region comprise the amino acid sequence of SEQ ID NO: 10 or an amino acid sequence that is 90%, 95%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 10. In a more particular embodiment the canine IgGD Fc region and hinge region is encoded by the nucleotide sequence of SEQ ID NO: 9. In yet other embodiments, the canine IgGD Fc region further comprises a hinge region from a canine IgGC antibody. In particular embodiments of this type the canine IgGD cFc region and hinge region comprise the amino acid sequence of SEQ ID NO: 12 or an amino acid sequence that is 90%, 95%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 12. In a more particular embodiment the canine IgGD cFc region and hinge region is encoded by the nucleotide sequence of SEQ ID NO: 11. The present invention further provides caninized antibodies that comprise these canine IgGD Fc regions and hinge regions. In a particular embodiment the caninized antibody or antigen binding fragment thereof binds canine Programmed Death Receptor 1 (canine PD-1) with specificity.

The present invention therefore provides caninized anti-canine PD-L1 antibodies with specificity and/or that have a high binding affinity for canine PD-L1. In particular embodiments, the caninized anti-canine PD-L1 antibodies also have the ability to block the binding of canine PD-L1 to canine PD-1. Such caninized antibodies or antigen binding fragments thereof that specifically bind canine PD-L1 can comprise a canine IgG heavy chain of the present invention and a canine *kappa* or *lambda* light chain. In particular embodiments the caninized anti-canine PD-L1 antibodies are caninized murine

anti-canine PD-L1 antibodies. The present invention also relates to use of such caninized antibodies in the treatment of disease such as cancer and/or those due to infections.

In particular embodiments the caninized anti-canine PD-L1 antibody comprises a genetically modified cFc region of the present invention. In alternative embodiments the caninized anti-canine PD-L1 antibody comprises the canine IgGD Fc region with a genetically modified hinge region from a canine IgGD antibody, a hinge region from a canine IgGA antibody, a hinge region from a canine IgGB antibody, or a hinge region from a canine IgGC antibody. The present invention further provides such caninized anti-canine PD-L1 antibodies comprising the canine frames of the present invention in combination with CDRs obtained from mouse anti-canine PD-L1 antibodies, *i.e.*, three light chain CDRs: CDR light 1 (CDRL1), CDR light 2 (CDRL2), and CDR light 3 (CDRL3) and three heavy chain CDRs CDR heavy 1 (CDRH1), CDR heavy 2 (CDRH2) and CDR heavy 3 (CDRH3).

In particular embodiments,the caninized murine anti-canine PD-L1 antibodies comprise the genetically modified cFc region of IgGB or IgGC of the present invention or alternatively, the canine IgGD Fc region, together with a genetically modified hinge region from a canine IgGD antibody, a hinge region from a canine IgGA antibody, a hinge region from a canine IgGB antibody, or a hinge region from a canine IgGC antibody in combination with CDRs obtained from mouse anti-canine PD-L1 antibodies. Moreover, the present invention not only provides caninized mouse anti-canine PD-L1 antibodies with specific CDRs as detailed herein, but further provides caninized mouse anti-canine PD-L1 antibodies comprising conservatively modified variants of those CDRs as well as variants that comprise (*e.g.*, share) the same canonical structure.

Accordingly in particular embodiments the caninized anti-canine PD-L1 antibody further comprises complementary determining regions (CDRs) in which the CDRs have canonical structures of: H1-1, H2-3B, and H3-10, respectively for CDR1, CDR2, and CDR3 of the heavy chain. In even more particular embodiments, the CDRs for the corresponding light chains have canonical structures of: L1-2, L2-1, and L3-1, respectively for CDR1, CDR2, and CDR3 of the light chain. In other embodiments the

caninized anti-canine PD-L1 antibody further comprises complementary determining regions (CDRs) in which the CDRs have canonical structures of: H1-1, H2-3B, and H3-8, respectively for CDR1, CDR2, and CDR3 of the heavy chain. In even more particular embodiments of this type, the CDRs for the corresponding light chains have canonical structures of: L1-3, L2-1, and L3-1, respectively for CDR1, CDR2, and CDR3 of the light chain.

In more particular embodiments, the caninized antibody of the present invention or antigen binding fragment thereof comprises one or more of the heavy chain complementary determining region 1 (VH CDR1) with an amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 19. In another embodiment, the heavy chain complementary determining region 2 (VH CDR2) comprises an amino acid sequence of SEQ ID NO: 14 or SEQ ID NO: 20. In still another embodiment the heavy chain complementary determining region 3 (VH CDR3) comprises an amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 21. In a particular embodiment of this type, the caninized antibody or antigen binding fragment comprises both a VH CDR1 comprising an amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 19 and a VH CDR2 comprising an amino acid sequence of SEQ ID NO: 14 or SEQ ID NO: 20.

In another such embodiment, the caninized antibody or antigen binding fragment comprises both a VH CDR1 comprising an amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 19 and a VH CDR3 comprising an amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 21. In yet another such embodiment, the caninized antibody or antigen binding fragment comprises both a VH CDR2 comprising an amino acid sequence of SEQ ID NO: 14 or SEQ ID NO: 20 and a VH CDR3 comprising an amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 21. In still another such embodiment, the caninized antibody or antigen binding fragment comprises a VH CDR1 comprising an amino acid sequence of SEQ ID NO: 13 or SEQ ID NO: 19, a VH CDR2 comprising an amino acid sequence of SEQ ID NO: 14 or SEQ ID NO: 20, and a VH CDR3 comprising an amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 21.

In particular embodiments, the caninized antibody or antigen binding fragment also comprises a light chain complementary determining region 1 (VL CDR1) comprising an amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 22. In related embodiments the light chain complementary determining region 2 (VL CDR2) comprises an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 23. In still another embodiment the light chain complementary determining region 3 (VL CDR3) comprises an amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 24. In a particular embodiment of this type, the caninized antibody or antigen binding fragment comprises both a VL CDR1 comprising an amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 22 and a VL CDR2 comprising an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 23.
In another such embodiment, the caninized antibody or antigen binding fragment comprises both a VL CDR1 comprising an amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 22 and a VL CDR3 comprising an amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 24. In yet another such embodiment, the caninized antibody or antigen binding fragment comprises both a VL CDR2 comprising an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 23 and a VL CDR3 comprising an amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 24. In still another such embodiment, the caninized antibody or antigen binding fragment comprises a VL CDR1 comprising an amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 22, a VL CDR2 comprising an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 23, and a VL CDR3 comprising an amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 24.

The present invention further provides caninized antibodies that comprise the amino acid sequence of SEQ ID NO: 26 or that is 90%, 95%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 26 and SEQ ID NO: 28 or that is 90%, 95%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 28 or antigen binding fragments of these caninized antibodies. The present invention also provides caninized antibodies that comprise the amino acid sequence of SEQ ID NO: 30 or that is 90%, 95%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 30 and SEQ ID NO: 32 or that is 90%, 95%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 32 or antigen binding fragments of these caninized antibodies.

In particular embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 26 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 26) that comprises (i) P, A, G, or S at position 242, (ii) A, G, or S at position 269, (iii) A, G, or S at position 301, (iv) G, P, or A at position 302, (v) T, A, G, or S at position 303, (vi) A, G, or S at position 331, and (vii) P, A, G, or S at position 333. In other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 28 or 30 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 28 or 30) that comprises (i) P, A, G, or S at position 240, (ii) A, G, or S at position 267, (iii) A, G, or S at position 299, (iv) G, P, or A at position 300, (v) T, A, G, or S at position 301, (vi) A, G, or S at position 329, and (vii) P, A, G, or S at position 331. In yet other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 32 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 32) that comprises (i) P, A, G, or S at position 238, (ii) A, G, or S at position 265, (iii) A, G, or S at position 297, (iv) G, P, or A at position 298, (v) T, A, G, or S at position 299, (vi) A, G, or S at position 327, and (vii) P, A, G, or S at position 329.

In still other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 26 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 26) that comprises (i) P, A, G, or S at position 242, (ii) A at position 269, (iii) A at position 301, (iv) G, P, or A at position 302, (v) T, A, G, or S at position 303, (vi) A, G, or S at position 331, and (vii) P, A, G, or S at position 333. In other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 28 or 30 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 28 or 30) that comprises (i) P, A, G, or S at position 240, (ii) A at position 267, (iii) A at position 299, (iv) G, P, or A at position 300, (v) T, A, G, or S at position 301, (vi) A, G, or S at position 329, and (vii) P, A, G, or S at position 331. In yet other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 32 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 32) that comprises (i) P, A, G, or S at position 238, (ii) A at position 265, (iii) A at position 297, (iv) G, P, or A at position 298, (v) T, A, G, or S at position 299, (vi) A, G, or S at position 327, and (vii) P, A, G, or S at position 329.

In still other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 26 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 26) that comprises (i) A at position 242, (ii) A at position 269, (iii) A at position 301, (iv) P at position 302, (v) A at position 303, (vi) G, at position 331, and (vii) A, at position 333. In other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 28 or 30 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 28 or 30) that comprises (i) A at position 240, (ii) A at position 267, (iii) A at position 299, (iv) P at position 300, (v) A at position 301, (vi) G at position 329, and (vii) A at position 331. In yet other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 32 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 32) that comprises (i) A at position 238, (ii) A at position 265, (iii) A at position 297, (iv) P at position 298, (v) A at position 299, (vi) G at position 327, and (vii) A at position 329.

In yet other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 26 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 26) that comprises (i) P at position 242, (ii) A, G, or S at position 269, (iii) A, G, or S at position 301, (iv) G at position 302, (v) T at position 303, (vi) A at position 331, and (vii) P at position 333. In other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 28 or 30 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 28 or 30) that comprises (i) P at position 240, (ii) A, G, or S at position 267, (iii) A, G, or S at position 299, (iv) G at position 300, (v) T at position 301, (vi) A at position 329, and (vii) P at position 331. In yet other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 32 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 32) that comprises (i) P at position 238, (ii) A, G, or S at position 265, (iii) A, G, or S at position 297, (iv) G at position 298, (v) T at position 299, (vi) A at position 327, and (vii) P at position 329.

In still other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 26 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 26) that comprises (i) P at position 242, (ii) A at position 269, (iii) A at position 301, (iv) G at position 302, (v) T at position 303, (vi) A at position 331, and (vii) P at position 333. In other embodiments, the heavy chain of an antibody comprises the amino acid sequence of

SEQ ID NO: 28 or 30 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 28 or 30) that comprises (i) P at position 240, (ii) A at position 267, (iii) A at position 299, (iv) G at position 300, (v) T at position 301, (vi) A at position 329, and (vii) P at position 331. In yet other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 32 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 32) that comprises (i) P at position 238, (ii) A at position 265, (iii) A at position 297, (iv) G at position 298, (v) T at position 299, (vi) A at position 327, and (vii) P at position 329.

In other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 26 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 26) that comprises (i) P, A, G, or S at position 242, (ii) A, G, or S at position 269, (iii) A, G, or S at position 301, (iv) G at position 302, (v) T at position 303, (vi) A, G, or S at position 331, and (vii) P, A, G, or S at position 333. In other such embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 28 or 30 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 28 or 30) that comprises (i) P, A, G, or S at position 240, (ii) A, G, or S at position 267, (iii) A, G, or S at position 299, (iv) G at position 300, (v) T at position 301, (vi) A, G, or S at position 329, and (vii) P, A, G, or S at position 331. In yet other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 32 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 32) that comprises (i) P, A, G, or S at position 238, (ii) A, G, or S at position 265, (iii) A, G, or S at position 297, (iv) G at position 298, (v) T at position 299, (vi) A, G, or S at position 327, and (vii) P, A, G, or S at position 329.

In still other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 26 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 26) that comprises (i) P, A, G, or S at position 242, (ii) A at position 269, (iii) A at position 301, (iv) G at position 302, (v) T at position 303, (vi) A, G, or S at position 331, and (vii) P, A, G, or S at position 333. In yet other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 28 or 30 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 28 or 30) that comprises (i) P, A, G, or S at position 240, (ii) A at position 267, (iii) A at position 299, (iv) G at position 300, (v) T at position 301, (vi) A, G, or S at position 329, and (vii) P, A, G, or S at position 331. In other such

embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 32 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 32) that comprises (i) P, A, G, or S at position 238, (ii) A at position 265, (iii) A at position 297, (iv) G at position 298, (v) T at position 299, (vi) A, G, or S at position 327, and (vii) P, A, G, or S at position 329.

In still other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 26 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 26) that comprises (i) A at position 242, (ii) A at position 269, (iii) A at position 301, (iv) G at position 302, (v) T at position 303, (vi) G at position 331, and (vii) A at position 333. In yet other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 28 or 30 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 28 or 30) that comprises (i) A at position 240, (ii) A at position 267, (iii) A at position 299, (iv) G at position 300, (v) T at position 301, (vi) G at position 329, and (vii) A at position 331. In other such embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 32 (or 90%, 95%, 98%, or 99% identical to SEQ ID NO: 32) that comprises (i) A at position 238, (ii) A at position 265, (iii) A at position 297, (iv) G at position 298, (v) T at position 299, (vi) G at position 327, and (vii) A at position 329. In addition, the present invention provides caninized antibody or antigen binding fragment thereof that further comprise a canine light chain that comprises the amino acid sequence of SEQ ID NO: 38 or SEQ ID NO: 44.

Accordingly, the present invention further provides a caninized antibody or antigen binding fragment thereof that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 38. In a related embodiment, the caninized antibody or antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 36 and a light chain comprising the amino acid sequence of SEQ ID NO: 38. In another embodiment, the caninized antibody or antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 44. In a related embodiment, the caninized antibody or antigen binding fragment thereof comprises a

heavy chain comprising the amino acid sequence of SEQ ID NO: 42 and a light chain comprising the amino acid sequence of SEQ ID NO: 44.

In yet another embodiment, the caninized antibody or antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 44. In a related embodiment, the caninized antibody or antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 36 and a light chain comprising the amino acid sequence of SEQ ID NO: 44. In still another embodiment, the caninized antibody or antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 40 and a light chain comprising the amino acid sequence of SEQ ID NO: 38. In a related embodiment, the caninized antibody or antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 42 and a light chain comprising the amino acid sequence of SEQ ID NO: 38.

The present invention further provides a caninized antibody or antigen binding fragment thereof that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 26 and a light chain comprising the amino acid sequence of SEQ ID NO: 38. In a related embodiment, the caninized antibody or antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 28 and a light chain comprising the amino acid sequence of SEQ ID NO: 38. In another embodiment, the caninized antibody or antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 30 and a light chain comprising the amino acid sequence of SEQ ID NO: 44. In a related embodiment, the caninized antibody or antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 32 and a light chain comprising the amino acid sequence of SEQ ID NO: 44.

The present invention further provides a caninized antibody or antigen binding fragment thereof that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 26 and a light chain comprising the amino acid sequence of SEQ ID NO: 44. In a

related embodiment, the caninized antibody or antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 28 and a light chain comprising the amino acid sequence of SEQ ID NO: 44. In another embodiment, the caninized antibody or antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 30 and a light chain comprising the amino acid sequence of SEQ ID NO: 38. In a related embodiment, the caninized antibody or antigen binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 32 and a light chain comprising the amino acid sequence of SEQ ID NO: 38.

The present invention further provides nucleic acids that encode any of the amino acid sequences of the present invention including the CDRs, cFc regions, the cFc regions with the hinge regions, and the heavy chains, and the light chains of the caninized antibodies of the present invention. The present invention further provides expression vectors that comprise one or more of the nucleic acids of the present invention. The present invention further provides host cells that comprise one or more expression vectors of the present invention and methods for expressing the CDRs, and/or cFc regions, and/or the cFc regions with the hinge regions, and/or the heavy chains, and/or the light chains of the caninized antibodies of the present invention using such host cells. The present invention also provides host cells that have been genetically engineered to express the CDRs, and/or cFc regions, and/or the cFc regions with the hinge regions, and/or the heavy chains, and/or the light chains of the caninized antibodies of the present invention in the absence of such vectors. In particular embodiments, these nucleic acids, expression vectors, polypeptides, or host cells of the invention are useful in methods of making an antibody.

In particular embodiments, the antibody is a recombinant antibody or a recombinant antigen binding fragment thereof. In related embodiments, the variable heavy chain domain and variable light chain domain are connected by a flexible linker to form a single-chain antibody.

In certain embodiments the present invention provides an antibody or antigen binding fragment thereof (*e.g.*, an isolated antibody or isolated antigen binding fragment thereof)

that binds canine PD-L1 with specificity, and which when it is bound to canine PD-L1, the antibody or antigen binding fragment thereof binds to at least one amino acid residue within the amino acid sequence of SEQ ID NO: 82. In related embodiments, the antibody or antigen binding fragment thereof that binds canine PD-L1 with specificity, also binds to at least one amino acid residue within the amino acid sequence of SEQ ID NO: 83. In still other embodiments, the antibody or antigen binding fragment thereof that binds canine PD-L1 with specificity, also binds to at least one amino acid residue within the amino acid sequence of SEQ ID NO: 82 and to at least one amino acid residue within the amino acid sequence of SEQ ID NO: 83. In particular embodiments, these antibodies and/or antigen binding fragments thereof block the binding of canine PD-L1 to canine PD-1. In related embodiments, the antibody is a monoclonal antibody. In particular embodiments of this type the antibody is a monoclonal murine anti-canine PD-L1 antibody. In more particular embodiments, the monoclonal antibody is a caninized antibody. In still more particular embodiments, the monoclonal antibody is a caninized murine anti-canine PD-L1 antibody.

In certain embodiments, the antibody or antigen binding fragment thereof binds to 2 to 5 amino acid residues of SEQ ID NO: 82. In other embodiments, the antibody or antigen binding fragment thereof binds to 6 to 12 amino acid residues of SEQ ID NO: 82. In yet other embodiments, the isolated antibody or antigen binding fragment thereof binds to 13 to 20 amino acid residues of SEQ ID NO: 82. In related embodiments, the antibody or antigen binding fragment thereof binds to 2 to 5 amino acid residues of SEQ ID NO: 83. In other embodiments, the antibody or antigen binding fragment thereof binds to 6 to 11 amino acid residues of SEQ ID NO: 83.

In still other embodiments monoclonal antibodies or antigen binding fragments thereof are provided that cross-compete for binding with canine PD-L1 with one or more of the anti-canine PD-L1 antibodies of the present invention. In particular embodiments, the cross-competing antibodies and antigen binding fragments thereof bind canine PD-L1 and block the binding of canine PD-L1 to canine PD-1. In a more particular embodiments the monoclonal antibodies or antigen binding fragments thereof cross-compete with 4F9 (or an antibody with the 6 CDRs of 4F9) for binding canine PD-L1. In other more

particular embodiments the monoclonal antibodies or antigen binding fragments thereof cross-compete with 5F12 (or an antibody with the 6 CDRs of 5F12) for binding canine PD-L1. In yet other embodiments the monoclonal antibodies or antigen binding fragments thereof cross-compete with both 4F9 and with 5F12 for binding canine PD-L1.

In particular embodiments a monoclonal antibody of the present invention is a murine antibody. In other embodiments the monoclonal antibody is a caninized antibody. In more particular embodiments a monoclonal antibody of the present invention is a caninized murine antibody.

Furthermore, the present invention provides antibodies (*e.g.*, caninized antibodies) to canine PD-L1 that comprise the CDRs of the present invention or variants of the CDRs, which have the corresponding canonical structures provided herein, and/or that bind to the amino acid sequence of SEQ ID NO: 82 and/or 83 of PD-L1. In particular embodiments of this type, the dissociation constant (Kd) for caninized antibody-canine PD-L1 binding is 1 X 10^{-5} to 1 X 10^{-12} M. In more particular embodiments the caninized antibodies to canine PD-L1 comprise variants of the CDRs of the present invention that have the corresponding canonical structures provided herein and bind to the amino acid sequence of SEQ ID NO: 82 and/or 83 of PD-L1. The present invention therefore includes caninized antibodies and antigen binding fragments thereof that bind canine PD-L1 with specificity, and when they are bound to canine PD-L1, the antibody binds to at least one amino acid residue within the amino acid sequence of SEQ ID NO: 82 and/or 83 of PD-L1. In particular embodiments of this type, the antibodies and antigen binding fragments thereof bind canine PD-L1 and block the binding of canine PD-L1 to canine PD-1. Accordingly, in particular embodiments when bound to canine PD-L1, the caninized antibody (including the antibodies with one or more variant CDR, *e.g.*, a variant such as, but not limited to a conservatively modified variant and/or a variant that comprises a defined canonical structure class) binds to at least one amino acid residue within an epitope of PD-L1, *e.g.*, the amino acid sequence of SEQ ID NO: 82 and/or SEQ ID NO: 83.

The present invention further provides caninized antibodies or antigen binding fragments thereof that bind to canine PD-L1 with a dissociation constant (Kd) that is lower than 1 X 10^{-12} M (*e.g.*, 1 X 10^{-13} M, or even lower). In other embodiments the caninized antibodies or antigen binding fragments thereof bind to canine PD-L1 with a dissociation constant of 1 X 10^{-5} M to 1 X 10^{-12} M. In more particular embodiments the caninized antibodies or antigen binding fragments thereof bind to canine PD-L1 with a dissociation constant of 1 X 10^{-7} M to 1 X 10^{-11} M. In still more particular embodiments the caninized antibodies or antigen binding fragments thereof bind to canine PD-L1 with a dissociation constant of 1 X 10^{-8} M to 1 X 10^{-11} M. In yet more particular embodiments the caninized antibodies or antigen binding fragments thereof bind to canine PD-L1 with a dissociation constant of 1 X 10^{-8} M to 1 X 10^{-10} M.

The present invention also provides caninized antibodies or antigen binding fragments thereof that bind to canine PD-L1 with an on rate (k_{on}) that is greater than 1 X 10^{7} $M^{-1}s^{-1}$. In other embodiments the caninized antibodies or antigen binding fragments thereof bind to canine PD-L1 with an on rate of 1 X 10^{2} $M^{-1}s^{-1}$ to 1 X 10^{7} $M^{-1}s^{-1}$. In more particular embodiments the caninized antibodies or antigen binding fragments thereof bind to canine PD-L1 with an on rate of 1 X 10^{3} $M^{-1}s^{-1}$ to 1 X $10^{6}M^{-1}s^{-1}$. In still more particular embodiments the caninized antibodies or antigen binding fragments thereof bind to canine PD-L1 with an on rate of 1 X 10^{3} $M^{-1}s^{-1}$ to 1 X $10^{5}M^{-1}s^{-1}$. In yet more particular embodiments the caninized antibodies or antigen binding fragments thereof bind to canine PD-L1 with an on rate of 1 X 10^{4} $M^{-1}s^{-1}$ to 1 X $10^{5}M^{-1}s^{-1}$.

The present invention also provides caninized antibodies or antigen binding fragments thereof that bind to canine PD-L1 with an off rate (k_{off}) slower than 1 X $10^{-7}s^{-1}$. In particular embodiments the caninized antibodies or antigen binding fragments thereof bind to canine PD-L1 with an off rate of 1 X 10^{-3} s^{-1} to 1 X 10^{-8} s^{-1}. In more particular embodiments the caninized antibodies or antigen binding fragments thereof bind to canine PD-L1 with an off rate of 1 X 10^{-4} s^{-1} to 1 X 10^{-7} s^{-1}. In still more particular embodiments the caninized antibodies or antigen binding fragments thereof bind to canine PD-L1 with an off rate of 1 X 10^{-5} s^{-1} to 1 X 10^{-7} s^{-1}.

In related embodiments, the antibodies (*e.g.*, caninized antibodies) or antigen binding fragments thereof stimulate antigen-specific memory responses to a tumor or pathogen. In particular embodiments, the antibodies (*e.g.*, caninized antibodies) or antigen binding fragments thereof stimulate an antibody response *in vivo*. In other particular embodiments, the antibodies (*e.g.*, caninized antibodies) or antigen binding fragments thereof stimulate an immune response in an animal subject. In more specific embodiments the animal subject is a canine. In a related embodiment, the animal subject is a feline.

Accordingly, any of the antibodies (*e.g.*, caninized antibodies) of the present invention can exhibit one, two, three, four, five, or all these properties, *i.e.*, the aforesaid dissociation constants with canine PD-L1, the aforesaid on rates for binding with canine PD-L1, the aforesaid off rates for dissociating from the caninized antibody-canine PD-L1 binding complex, stimulating an antigen-specific memory responses to a tumor or pathogen, stimulating an antibody response *in vivo*, and/or stimulating an immune response in an animal subject.

In more particular embodiments the antibodies and/or antigen binding fragments thereof of the present invention bind canine PD-L1 and also block the binding of canine PD-L1 to PD-1. In even more particular embodiments the caninized antibodies and antigen binding fragments thereof of the present invention bind canine PD-L1 and block the binding of canine PD-L1 to PD-1.

As indicated above, the antibodies (and antigen binding fragments thereof) of the present invention, including certain aforesaid antibodies (and antigen binding fragments thereof), can be monoclonal antibodies (and antigen binding fragments thereof), mammalian antibodies (and antigen binding fragments thereof), *e.g.*, murine (mouse) antibodies (and antigen binding fragments thereof), caninized antibodies (and antigen binding fragments thereof) including caninized murine antibodies (and antigen binding fragments thereof). In certain embodiments the antibodies (and antigen binding fragments thereof) are isolated.

In particular embodiments, the antibody is a recombinant antibody or an antigen binding fragment thereof. In related embodiments, the variable heavy chain domain and variable light chain domain are connected by a flexible linker to form a single-chain antibody.

In particular embodiments, the antibody or antigen binding fragment is a Fab fragment. In other embodiments, the antibody or antigen binding fragment is a Fab' fragment. In other embodiments, the antibody or antigen binding fragment is a $(Fab')_2$ fragment. In still other embodiments, the antibody or antigen binding fragment is a diabody. In particular embodiments, the antibody or antigen binding fragment is a domain antibody. In particular embodiments, the antibody or antigen binding fragment is a camelized single domain antibody.

In particular embodiments, a caninized murine anti-canine PD-L1 antibody or antigen binding fragment increases the immune response of the animal subject (*e.g.*, canine or feline) being treated.

The present invention further provides nucleic acids (including isolated nucleic acids) that encode any one of the antibodies and portions thereof (including CDRs) of the present invention. In certain embodiments, the present invention provides nucleic acids (including isolated nucleic acids) that encode any one of the light chains of the caninized antibodies or portions thereof of the present invention. Similarly, the present invention provides nucleic acids (including isolated nucleic acids) that encode any one of the heavy chains of the caninized antibody or portions thereof of the present invention. The present invention further provides expression vectors that comprise one or more of the nucleic acids (including isolated nucleic acids) of the present invention. Accordingly, the present invention provides nucleic acids that encode the caninized murine anti-canine PD-L1 antibodies or portions thereof of the present invention. In related embodiments such antibodies or antigen binding fragments can be used for the preparation of a medicament to treat cancer in a canine and/or feline subject. Alternatively, or in conjunction, the present invention provides for the use of any of the antibodies or antibody fragments of the present invention for diagnostic use. In yet additional embodiments, a kit is provided comprising any of the caninized antibodies or antigen binding fragments disclosed herein.

In specific embodiments an expression vector is provided comprising an isolated nucleic acid encoding any of the caninized murine anti-canine PD-L1 antibodies or antigen binding fragments of the invention. The present invention further provides host cells that comprise one or more expression vectors of the present invention. In particular embodiments, these nucleic acids, expression vectors or polypeptides of the invention are useful in methods of making an antibody.

The present invention further provides antigenic peptides (including isolated antigenic peptides) that consist of 80 or fewer amino acid residues that comprise the amino acid sequence of SEQ ID NO: 82 and/or SEQ ID NO: 83. In related embodiments, the antigenic peptides (including isolated peptides) consist of 60 or fewer amino acid residues that comprise the amino acid sequence of SEQ ID NO: 82 and/or SEQ ID NO: 83. In related embodiments, the antigenic peptides (including isolated peptides) consist of 11 to 45 amino acid residues that comprise the amino acid sequence of SEQ ID NO: 82 and/or SEQ ID NO: 83. In yet other embodiments the antigenic peptides consist of 5 to 20 amino acid residues from the amino acid sequence of SEQ ID NO: 83. In still other embodiments the antigenic peptides consist of 5 to 11 amino acid residues from the amino acid sequence of SEQ ID NO: 83.

The present invention further provides antigenic peptides (including isolated peptides) that consist of 80 or fewer amino acid residues that comprise an amino acid sequence that is 80%, 85%, 90%, 95% or 100% identical with the amino acid sequence of SEQ ID NO: 82 and/or SEQ ID NO: 83 and binds to an isolated mammalian antibody or antigen binding fragment thereof of the present invention. In related embodiments, the antigenic peptides (including isolated antigenic peptides) consist of 60 or fewer amino acid residues that comprise an amino acid sequence that is 80%, 85%, 90%, 95% or 100% identical with the amino acid sequence of SEQ ID NO: 82 and/or SEQ ID NO: 83 and binds to an isolated mammalian antibody or antigen binding fragment thereof. In other embodiments the peptides consist of 5 to 20 amino acid residues from an amino acid sequence that is 80%, 85%, 90%, 95% or 100% identical with the amino acid sequence of SEQ ID NO: 82 and binds to an isolated mammalian antibody or antigen binding fragment thereof. In other embodiments the peptides consist of 5 to 11 amino acid residues from an amino

acid sequence that is 80%, 85%, 90%, 95% or 100% identical with the amino acid sequence of SEQ ID NO: 83 and binds to an isolated mammalian antibody or antigen binding fragment thereof. In particular embodiments the mammalian antibody is 4E9. In other embodiments the mammalian antibody is 5F12.

The present invention further provides fusion proteins that comprise any of the aforesaid antigenic peptides. In a particular embodiment, the fusion protein comprises such an antigenic peptide and an Fc region of a non-canine mammalian IgG antibody. In a more particular embodiment the fusion protein comprises an Fc region of a non-canine mammalian IgG antibody. In certain embodiments the non-canine mammalian IgG antibody is a murine IgG. In alternative embodiments the non-canine mammalian IgG antibody is a human IgG. In other embodiments the non-canine mammalian IgG antibody is an equine IgG. In still other embodiments the non-canine mammalian IgG antibody is a porcine IgG. In yet other embodiments the non-canine mammalian IgG antibody is a bovine IgG.

In particular embodiments the non-canine mammalian IgG antibody is an IgG1. In other embodiments the non-canine mammalian IgG antibody is an IgG2a. In still other embodiments the non-canine mammalian IgG antibody is an IgG3. In yet other embodiments the non-canine mammalian IgG antibody is an IgG4.

In other embodiments the fusion protein comprises any of the aforesaid antigenic peptides and maltose-binding protein. In yet other embodiments, the fusion protein comprises any of the aforesaid antigenic peptides and *beta*-galactosidase. In still other embodiments the fusion protein comprises any of the aforesaid antigenic peptides and glutathione S-transferase. In yet other embodiments, the fusion protein comprises any of the aforesaid antigenic peptides and thioredoxin. In still other embodiments the fusion protein comprises any of the aforesaid antigenic peptides and Gro EL. In yet other embodiments the fusion protein comprises any of the aforesaid antigenic peptides and NusA.

The present invention further provides nucleic acids (including isolated nucleic acids) that encode the antigenic peptides and the corresponding fusion proteins of the present invention. The present invention also provides expression vectors that comprise these nucleic acids and host cells that comprise one or more expression vectors of the present invention.

In addition, the present invention includes pharmaceutical compositions comprising anti-canine PD-L1 antibodies or antigen binding fragments thereof of the present invention, antigenic peptides (including isolated antigenic peptides) from canine PD-L1, fusion proteins comprising the antigenic peptides from canine PD-L1 of the present invention, nucleic acids (including isolated nucleic acids) encoding the antigenic fragments and/or fusion proteins of the present invention, the expression vectors comprising such nucleic acids, or any combination thereof, and a pharmaceutically acceptable carrier or diluent.

In particular embodiments such pharmaceutical compositions further comprise an anti-canine PD-1 antibody or antigen binding fragment thereof. In more particular embodiments the anti-canine PD-1 antibody is a caninized murine anti-canine PD-1 antibody or a antigen binding fragment of the caninized murine anti-canine PD-1 antibody. In related embodiments, such pharmaceutical compositions further comprise an anti-canine CTLA-4 antibody or an antigen binding fragment thereof. In particular embodiments the anti-canine CTLA-4 antibody is a caninized murine anti-canine CTLA-4 antibody or an antigen binding fragment of a caninized murine anti-canine CTLA-4 antibody.

Accordingly, the present invention provides pharmaceutical compositions that comprise one, two, three, or more of the following: an anti-canine PD-L1 antibody, an anti-canine PD-1 antibody, an anti-canine CTLA-4 antibody, an antigen binding fragment of an anti-canine PD-L1 antibody, an antigen binding fragment of an anti-canine PD-1 antibody, or an antigen binding fragment of an anti-canine CTLA-4 antibody. In particular embodiments, such anti-canine protein (*i.e.*, anti-canine PD-L1, PD-1, or CTLA-4) antibodies or the antigen binding fragments thereof are murine anti-canine protein antibodies. In other such anti-canine protein antibodies or the antigen binding fragments

thereof are caninized anti-canine protein antibodies. In more particular embodiments the anti-canine protein antibodies or the antigen binding fragments thereof are caninized murine anti-canine protein antibodies.

In addition, the present invention provides methods of increasing the activity of an immune cell, comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition of the present invention. In certain embodiments the method is used for the treatment of cancer. In other embodiments, the method is used in the treatment of an infection or infectious disease. In still other embodiments, a caninized antibody of the present invention or antigen binding fragment thereof is used as a vaccine adjuvant. In particular embodiments a pharmaceutical composition comprising a caninized murine anti-canine PD-L1 antibody or antigen binding fragment thereof can be administered before, after or concurrently with a caninized murine anti-canine PD-1 antibody or antigen binding fragment thereof and/or a caninized murine anti-canine CTLA-4 antibody or antigen binding fragment thereof.

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 ELISA results for reactivity of two mouse anti-canine PD-L1 mAbs against canine PD-L1, as a function of OD 650/490 versus the log mAb (nM). Both mAbs, designated 4F9 and 5F12, demonstrate strong and dose-dependent binding to canine PD-L1.

Figure 2 shows ligand blockade with mouse anti-canine PD-L1 mAbs. Two mAbs designated 4F9 and 5F12 were tested for their ability to inhibit binding of PD-L1 to PD-1 expressed on CHO cells. Both mAbs blocked the binding of PD-L1 to PD-1, although mAb 4F9 is a stronger inhibitor than 5F12.

DETAILED DESCRIPTION

Abbreviations

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

ADCC	Antibody-dependent cellular cytotoxicity
CDC	Complement-dependent cyotoxicity
CDR	Complementarity determining region in the immunoglobulin variable regions, defined for human antibodies using the Kabat numbering system
CHO	Chinese hamster ovary
EC50	concentration resulting in 50% efficacy or binding
ELISA	Enzyme-linked immunosorbant assay
FR	Antibody framework region: the immunoglobulin variable regions excluding the CDR regions.
HRP	Horseradish peroxidase
IFN	interferon
IC50	concentration resulting in 50% inhibition
IgG	Immunoglobulin G
Kabat	An immunoglobulin alignment and numbering system for human antibodies pioneered by Elvin A. Kabat [*Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)]
mAb	Monoclonal antibody (also Mab or MAb)
MES	2-(N-morpholino)ethanesulfonic acid
MOA	Mechanism of action
NHS	Normal human serum
PCR	Polymerase chain reaction
PK	Pharmacokinetics
SEB	Staphylococcus Enterotoxin B
TT	Tetanus toxoid

V region	The segment of human IgG chains which is variable in sequence between different antibodies. It extends to Kabat residue 109 in the light chain and 113 in the heavy chain.
VH	Immunoglobulin heavy chain variable region
VK	Immunoglobulin *kappa* 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.

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.

"Activation" as it applies to cells or to receptors refers to the activation or treatment of a cell or receptor with a ligand, unless indicated otherwise by the context or explicitly. "Ligand" encompasses natural and synthetic ligands, e.g., cytokines, cytokine variants, analogues, muteins, and binding compounds derived from antibodies. "Ligand" also encompasses small molecules, e.g., peptide mimetics of cytokines and peptide mimetics of antibodies. "Activation" can refer to cell activation as regulated by internal mechanisms as well as by external or environmental factors.

"Activity" of a molecule may describe or refer to the binding of the molecule to a ligand or to a receptor, to catalytic activity; to the ability to stimulate gene expression or cell signaling, differentiation, or maturation; to antigenic activity, to the modulation of activities of other molecules, and the like. "Activity" of a molecule may also refer to activity in modulating or maintaining cell-to-cell interactions, e.g., adhesion, or activity in maintaining a structure of a cell, *e.g.*, cell membranes or cytoskeleton. "Activity" can also mean specific activity, *e.g.*, [catalytic activity]/[mg protein], or [immunological

activity]/[mg protein], concentration in a biological compartment, or the like. "Activity" may refer to modulation of components of the innate or the adaptive immune systems.

"Administration" and "treatment," as it applies to an animal, *e.g.*, a canine experimental subject, cell, tissue, organ, or biological fluid, refers to contact of an exogenous pharmaceutical, therapeutic, 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. "Treat" or "treating" means to administer a therapeutic agent, such as a composition containing an antibody or antigen binding fragment of the present invention, internally or externally to a veterinary subject (*e.g.*, canine or feline) having one or more disease symptom, or being suspected of having a disease, for which the agent has therapeutic activity. Accordingly, "administration" and "treatment" also includes *in vitro* and *ex vivo* treatments, *e.g.*, of a cell, by a reagent, diagnostic, binding compound, or by another cell.

Typically, the agent is administered in an amount effective to alleviate and/or ameliorate one or more disease symptom 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 symptom (also referred to as the "therapeutically effective amount") may vary according to factors such as the disease state, age, and weight of the subject (*e.g.*, canine), and the ability of the pharmaceutical composition to elicit a desired response in the subject. Whether a disease symptom has been alleviated or ameliorated can be assessed by any clinical measurement typically used by veteranarians or other skilled healthcare providers to assess the severity or progression status of 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 symptom(s) in every subject, it should alleviate the target disease 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 chi^2-test, the U-test according to Mann and Whitney, the Kruskal-Wallis test (H-test), Jonckheere-Terpstra-test and the Wilcoxon-test.

The term "immune response" refers to the action of, for example, lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the mammalian body (*e.g.*, canine body) of cancerous cells, cells or tissues infected with pathogens, or invading pathogens.

The term "subject" includes any organism, preferably an animal, more preferably a mammal (*e.g.*, canine, feline, or human) and most preferably a canine.

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, such as any member of the subfamilies *Felinae*, *e.g.*, cats, lions, tigers, pumas, jaguars, leopards, snow leopards, panthers, North American mountain lions, cheetahs, lynx, bobcats, caracals or any cross breeds thereof. Cats also include domestic cats, pure-bred and/or mongrel companion cats, show cats, laboratory cats, cloned cats, and wild or feral cats.

Canine PD-1 has been found to comprise the amino acid sequence of SEQ ID NO: 50 [U.S. provisional application no. 61/918,946, filed on December 20, 2013, the contents of which are hereby incorporated herein in their entireties]. In a specific embodiment canine PD-1 is encoded by a nucleic acid that comprises the nucleotide sequence of SEQ ID NO: 49.

Canine PD-L1 has been found to comprise the amino acid sequence of SEQ ID NO: 56 [U.S. provisional application no. 61/918,946, filed on December 20, 2013, *supra*]. In a specific embodiment canine PD-L1 is encoded by a nucleotide sequence comprising SEQ ID NO: 55.

As used herein, a "substitution of an amino acid residue" with another amino acid residue in an amino acid sequence of, for example an antibody, 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. For example, one such substitution (replacement) is denoted as P4A of an Fc region of an IgGB or IgGC amino acid sequence, in which case, the proline residue at amino acid position 4 of the amino acid sequence of the Fc region of an IgGB or the Fc region of an IgGC has been substituted for (replaced) by an alanine residue.

Accordingly, 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.

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 a particular embodiment, 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 are discussed below.

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).

Caninized Anti-Canine Antigen Antibodies

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 a canine antigen, *e.g.*, canine PD-L1) if it binds to polypeptides comprising that portion of the amino acid sequence of the canine antigen, *e.g.*, canine PD-L1, but does not bind to other canine proteins lacking that portion of the sequence of the canine antigen, *e.g.*, canine PD-L1. For example, an antibody that specifically binds to a polypeptide comprising

canine PD-L1 may bind to a FLAG®-tagged form of canine PD-L1, but will not bind to other FLAG®-tagged canine proteins with specificity. 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, the term "antibody" refers to any form of antibody that exhibits the desired biological activity. 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, multispecific 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, 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 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.

A "Fab fragment" is comprised of one light chain and the C_H1 and variable regions of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule. A "Fab fragment" can be the product of papain cleavage of an antibody.

A "fragment crystallizable" ("Fc") region contains two heavy chain fragments (*i.e.*, two identical polypeptides) comprising the C_H2 and C_H3 domains of an antibody. The two

heavy chain fragments are held together by two or more disulfide bonds and by hydrophobic interactions of the C_H3 domains. 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.* [*Vet. Immunol. Immunopathol.* 80: 259-270 (2001)].

A "Fab' fragment" contains one light chain and a portion or fragment of one heavy chain that contains the V_H domain and the C_H1 domain and also the region between the C_H1 and C_H2 domains, such that an interchain disulfide bond can be formed between the two heavy chains of two Fab' fragments to form a $F(ab')_2$ molecule.

A "$F(ab')_2$ fragment" contains two light chains and two heavy chains containing a portion of the constant region between the C_H1 and C_H2 domains, such that an interchain disulfide bond is formed between the two heavy chains. A $F(ab')_2$ fragment thus is composed of two Fab' fragments that are held together by a disulfide bond between the two heavy chains. An "$F(ab')_2$ fragment" can be the product of pepsin cleavage of an antibody.

The "Fv region" comprises the variable regions from both the heavy and light chains, but lacks the constant regions.

The term "single-chain Fv" or "scFv" antibody refers to antibody fragments comprising the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. [*See*, Pluckthun, THE PHARMACOLOGY OF MONOCLONAL ANTIBODIES, vol. 113 Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); WO 88/01649; and U.S. 4,946,778 and U.S. 5,260,203.]

As used herein, the term “canonical structure” refers to the local conformation that can be adopted by each of the hypervariable regions of the heavy and light chain of an antibody within the framework that they reside. For each hypervariable region, there are a small number of canonical structures (generally denoted by simple integers such as 1 or 2 etc.),

which can be predicted with great accuracy from the amino acid sequences of the corresponding hypervariable region (particularly within the context of the amino acid sequence of its framework, as provided below for the corresponding caninized murine anti-canine PD-L1 variable domains). These canonical structures can be determinative regarding whether a modification of the amino acid sequence of a given CDR will result in the retention or loss of the ability to bind to its antigen binding partner [*See*, Chothia and Lesk, *Canonical Structures for the hypervariable regions of immunoglobulins, J. Mol. Biol.* 196:901-917(1987); Chothia *et al.*, *Conformation of immunoglobulin hypervaribale regions*, *Nature,* 34:877-883(1989); and Al-Lazikani *et al.*, *Standard Conformations for the canonical structures of immunoglobulins*, *J. Mol. Biol.* 273:927-948 (1997)].

A "domain antibody" is an immunologically functional immunoglobulin fragment containing only the variable region of a heavy chain or the variable region of a light chain. In some instances, two or more V_H regions are covalently joined with a peptide linker to create a bivalent domain antibody. The two V_H regions of a bivalent domain antibody may target the same or different antigens.

A "bivalent antibody" comprises two antigen binding sites. In some instances, the two binding sites have the same antigen specificities. However, bivalent antibodies may be bispecific (see below).

In certain embodiments, monoclonal antibodies herein also include camelized single domain antibodies. [*See, e.g.,* Muyldermans *et al.*, *Trends Biochem. Sci.* 26:230 (2001); Reichmann *et al., J. Immunol. Methods* 231:25 (1999); WO 94/04678; WO 94/25591; U.S. 6,005,079]. In one embodiment, the present invention provides single domain antibodies comprising two V_H domains with modifications such that single domain antibodies are formed.

As used herein, the term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H-V_L or V_L-V_H). By

using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. [*See*, EP 0 404 097 B1; WO 93/11161; and Holliger *et al., Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993)]. For a review of engineered antibody variants generally *see* [Holliger and Hudson *Nat. Biotechnol.* 23:1126-1136 (2005)].

Typically, an antibody or antigen binding fragment of the invention retains at least 10% of its canine PD-L1 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, (*e.g.*, PD-L1) binding affinity as the parental antibody. It is also intended that a caninized 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, 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.*,

canine, so that the resulting chimeric antibody will be less likely to elicit an adverse immune response in a canine subject, 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.*, murine) antibodies. In general, the caninized antibody will comprise substantially all of at least one, and 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 murine anti-canine PD-L1 CDRs as exemplified below), and all or substantially all of the canine frame.

The term "fully canine antibody" refers to an antibody that comprises canine immunoglobulin protein sequences only. A fully canine antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell, or in a hybridoma derived from a mouse cell. Similarly, "mouse antibody" refers to an antibody that comprises mouse immunoglobulin sequences only. Alternatively, a fully canine antibody may contain rat carbohydrate chains if produced in a rat, in a rat cell, or in a hybridoma derived from a rat cell. Similarly, "rat antibody" refers to an antibody that comprises rat immunoglobulin sequences only.

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 flanked 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 for human antibodies 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.* CDRL1, CDRL2 and CDRL3 in the light chain variable domain and CDRH1, CDRH2 and CDRH3 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), defininig the CDR regions of a human 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.

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. In both chains, the amino acid sequences of the native canine CDRs are replaced with the corresponding foreign CDRs (*e.g.*, those from a mouse antibody). 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 canine antibody, and/or to modify the Fc function, as exemplified below.

As used herein, an “anti-canine PD-L1 antibody” refers to an antibody that was raised against canine PD-L1 (*e.g.*, in a mammal such as a mouse or rabbit) and that specifically binds to canine PD-L1. An antibody that "specifically binds to canine PD-L1," or an antibody that "specifically binds to a polypeptide comprising the amino acid sequence of SEQ ID NO: 56", is an antibody that exhibits preferential binding to canine PD-L1 as compared to other antigens, *e.g.*, binds canine PD-L1 “with specificity”. The binding does not require absolute binding specificity. An anti-canine PD-L1 antibody is considered "specific" for canine PD-L1 if its binding is determinative of the presence of

canine PD-L1 in a sample, or if it is capable of altering the activity of canine PD-L1 without unduly interfering with the activity of other molecules in a canine sample, *e.g.,* without producing undesired results such as false positives in a diagnostic context or side effects in a therapeutic context. The degree of specificity necessary for an anti-canine PD-L1 antibody may depend on the intended use of the antibody, and at any rate is defined by its suitability for use for an intended purpose. The antibody, or binding compound derived from the antigen-binding site of an antibody, of the contemplated method binds to its antigen, or a variant or mutein thereof, with an affinity that is at least two-fold greater, preferably at least ten-times greater, more preferably at least 20-times greater, and most preferably at least 100-times greater than the affinity with any other antigen.

Accordingly the present invention provides caninized anti-canine PD-L1 antibodies or antigen binding fragments thereof (including in isolated form) that bind canine PD-L1 (*e.g.*, with specificity) and uses of such antibodies or fragments thereof. In specific embodiments murine anti-canine PD-L1 CDRs from murine anti-canine PD-L1 antibodies are provided that have been shown to both bind canine PD-L1 and to block the binding of canine PD-L1 to its receptor, *e.g.*, canine PD-1. These CDRs can be inserted into a modified canine frame of the present invention to make a caninized murine anti-canine PD-L1 antibody, as exemplified herein.

More specifically, a "caninized murine anti-PD-L1 antibody" of the present invention refers to an antibody that comprises the three heavy chain CDRs and the three light chain CDRs from a murine anti-canine PD-L1 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 augment, reduce, or eliminate antibody effector functions, to increase its binding to the canine antigen, *e.g.*, canine PD-L1, and/or increase its ability to block the binding of the canine antigen, *e.g.*, canine PD-L1, to its natural binding partner, (*e.g.*, canine PD-1 in the case where the antigen is canine PD-L1).

"Homology" 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 monomer subunit, *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 homologous positions shared by the two sequences divided by the total number of positions compared ×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.

"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 phrase "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to use promoters, polyadenylation signals, and enhancers.

A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that

participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that not all progeny will have precisely identical DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

As used herein, "germline sequence" refers to a sequence of unrearranged immunoglobulin DNA sequences. Any suitable source of unrearranged immunoglobulin sequences may be used. Human germline sequences may be obtained, for example, from JOINSOLVER® germline databases on the website for the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the United States National Institutes of Health. Mouse germline sequences may be obtained, for example, as described in Giudicelli *et al.* [*Nucleic Acids Res.* 33:D256-D261 (2005)].

Properties of Caninized Antibodies

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 IgGA, IgGB, IgGC and IgGD. The DNA 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 DNA 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 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. 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.*

The development of a therapeutic monoclonal antibody is a complex process that entails coordination of a complex set of activities to generate the desired antibody. These include optimization of the antibody specificity, affinity, functional activity, expression level in engineered cell lines, long-term stability, elimination or enhancement of effector functions and development of commercially viable manufacturing and purification methods. Considering the objectives of the present invention and aside from the capacity to activate cells of the immune systems, a caninized or canine monoclonal antibody against canine PD-L1 optimally has three additional attributes:

1. lack of effector functions such as antibody-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC),
2. relatively long half-life *in vivo*; and
3. 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 subclasses satisfy all these criteria. For example, IgGB can be purified using protein A, but has a high level of ADCC activity. IgGC also has considerable ADCC activity. On the other hand, IgGA binds weakly to protein A, but displays undesirable ADCC activity. Moreover, neither IgGC nor IgGD can be purified on protein A columns, although IgGD display no ADCC activity. Additionally IgGC has short serum half-life as it does not bind to the canine FcRn receptor. The present invention overcomes this difficulty by providing modified canine IgG antibodies specific to canine antigens, *e.g.*, canine PD-L1; such antibodies lack

effector functions such as ADCC and CDC, display relatively long half-life, and can be easily of purified using industry standard protein A chromatography.

Heretofore, genetically modified canine IgGs that lacked both ADCC and CDC effector functions and in addition, could be purified by protein A chromatography had not been previously described. As disclosed herein, a single substitution at a position in canine IgG that is analogous to that of human and mouse IgG, such as N297A or D265A, does not completely eliminate both ADCC and CDC effector functions in the corresponding canine antibody. For example, while each of the substitutions N297 and D265 in human or murine antibodies results in abrogation of binding to Fc_{γ} receptor and C1q, neither substitution alone completely abrogated the binding of canine antibodies to C1q. Instead, as further disclosed below, in order to eliminate both ADCC and CDC in canine antibodies of IgGB or IgGC sub-classes, it proved necessary to make a double substitution in the Fc of the canine antibody combining both an asparagine-to-alanine and an aspartic acid-to-alanine substitution. Moreover, completely unexpectedly, one substitution that had been shown to reduce effector functions in human antibodies actually resulted in an increase in binding of corresponding canine IgG to $Fc_{\gamma}R$ and C1q.

In order to generate variants of canine IgGB and IgGC that lack effector functions, modified canine IgGB or modified canine IgGC heavy chains can be generated. A total of seven amino acid residues which are present in both of these canine fragment crystallizable regions (cFcs) were identified for such possible substitution. These seven amino acid residues are: P4, D31, N63, G64, T65, A93, and P95 for both the amino acid sequence of SEQ ID NO: 66 for the Fc of canine IgGB; and the amino acid sequence of SEQ ID NO: 68 for the Fc of canine IgGC. Accordingly, the amino acid sequence of SEQ ID NO: 2 differs from that of SEQ ID NO: 66 by having the amino acid residues at positions: 4, 31, 63, 64, 65, 93, and 95, which are proline (P), aspartic acid (D), asparagine (N), glycine (G), threonine (T), alanine (A), and proline (P), respectively, in the amino acid sequence of SEQ ID NO: 66 as “X” (or “Xaa” in the three letter code) for all seven positions, signifying that these seven amino acid positions can be any of the twenty natural amino acids (*see* list in column 1 of Table 1 below). Similarly, the amino acid sequence of SEQ ID NO: 4 differs from that of SEQ ID NO: 68 by having the amino

acid residues at positions 4, 31, 63, 64, 65, 93, and 95 are listed as "X" (or "Xaa" in the three letter code) for all seven positions, signifying that these seven amino acid positions can be any of the twenty natural amino acids. The amino acid sequence of SEQ ID NO: 2 is encoded by the nucleotide sequence of SEQ ID NO: 1, whereas the amino acid sequence of SEQ ID NO: 4 is encoded by the nucleotide sequence of SEQ ID NO: 3.

In one embodiment, the cFc comprises the amino acid sequence of SEQ ID NO: 66 with the following substitutions P4(A, G, or S), D31(A, G, or S) N63(A, G, or S), G64(A or P), T65(A, G, or S), A93(G or S), and P95(A, G, or S); in which P4 (A G, or S) signifies that the proline residue at position 4 is replaced by either an alanine, glycine, or serine residue, and similarly G64(P or A) signifies that the glycine residue at position 64 is replaced by either a proline or an alanine residue, etc.). In a particular embodiment, the cFc comprises the amino acid sequence of SEQ ID NO: 66 with the following substitutions: P4A, D31A, N63A, G64P, T65A, A93G, and P95A,

In a related embodiment, the cFc comprises the amino acid sequence of SEQ ID NO: 4, which contains 7 amino acids designated as Xaa,with the following amino acid residues: A4, A31, A63, G64, T65, G93, and A95, *i.e.*, the amino acid sequence of SEQ ID NO: 68 with the following five (5) amino acid residue changes: P4A, D31A, N63A, A93G, and P95A and the remaining two amino acid residues of the seven, G64 and T65, being retained from the amino acid sequence of SEQ ID NO: 68.

The amino acid sequences of SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, and SEQ ID NO: 32 all contain "X" (or "Xaa" in the three letter code) at seven amino acid positions, signifying that these seven amino acid positions can be any of the twenty natural amino acids listed in column 1 of Table 1 below. Notably SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, and SEQ ID NO: 32 comprise either the amino acid sequence of SEQ ID NO: 2 or that of SEQ ID NO: 4 within their respective sequences. Specific examples of the amino acid residues at one or more of these seven positions of the amino acid sequences are delineated above and below, and are therefore included within the genus of the individual amino acid sequences of SEQ ID NO: 2, SEQ ID

NO: 4, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, and SEQ ID NO: 32, as well as within the caninized antibodies that comprise these sequences.

Table 10 provided below, specifically correlates the seven amino acid positions that can be replaced, as disclosed herein, of the cIgGB Fc (SEQ ID NO: 66 and SEQ ID NO: 2) and the cIgGC Fc (SEQ ID NO: 68 and SEQ ID NO: 4) with that of the full length canine heavy chains that comprises these cFc amino acid sequences, *i.e.*, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, and SEQ ID NO: 32. Accordingly, the actual position in the full length sequence IgGB or IgGC can be readily coordinated with that of the cFc that it comprises through the use of Table 10 below.

In particular embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 26 comprising (i) P, A, G, or S at position 242, (ii) D, A, G, or S at position 269, (iii) N, A, G, or S at position 301, (iv) G, P, or A at position 302, (v) T, A, G, or S at position 303, (vi) A, G, or S at position 331, and (vii) P, A, G, or S at position 333. In other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 28 or 30 comprising (i) P, A, G, or S at position 240, (ii) D, A, G, or S at position 267, (iii) N, A, G, or S at position 299, (iv) G, P, or A at position 300, (v) T, A, G, or S at position 301, (vi) A, G, or S at position 329, and (vii) P, A, G, or S at position 331. In other embodiments, the heavy chain of an antibody comprises the amino acid sequence of SEQ ID NO: 31 comprising (i) P, A, G, or S at position 238, (ii) D, A, G, or S at position 265, (iii) N, A, G, or S at position 297, (iv) G, P, or A at position 298, (v) T, A, G, or S at position 299, (vi) A, G, or S at position 327, and (vii) P, A, G, or S at position 329.

The present invention also provides modified canine IgGDs which comprise a hinge region from either IgGA, IgGB, or IgGC in place of its natural IgGD hinge region. Alternatively, the IgGD hinge region can be genetically modified by replacing a serine residue with a proline residue as shown in Table 5. Such modifications can lead to a canine IgGD lacking fab arm exchange. The modified canine IgGDs 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 IgGD can be modified so that it encodes the modified IgGDs. The modified nucleic acid sequences are then cloned into expression plasmids for protein expression. The nucleic acids encoding the canine IgGD Fcs with the substitute hinge region are exemplified by nucleotide sequences of SEQ ID NOs: 7, 9, and 11 which encode the amino acid sequences of SEQ ID NOs: 8, 10, and 12. A nucleic acid encoding a canine IgGD Fc with a modified IgGD hinge region comprises the nucleotide sequence of SEQ ID NO: 5 which encodes the amino acid sequence of SEQ ID NO: 6.

The present invention further provides full length canine heavy chains that can be matched with corresponding light chains to make a caninized antibody. Accordingly, the present invention further provides caninized murine anti-canine antigen antibodies (including isolated caninized murine anti-canine PD-L1 antibodies) and methods of use of the antibodies or antigen binding fragments thereof in the treatment of disease *e.g.*, the treatment of cancer in canines.

Moreover, the present invention provides caninized murine anti-canine PD-L1 antibodies or antigen binding fragments that bind to canine PD-L1 and block the binding of canine PD-1 to canine PD-L1. In certain embodiments the caninized murine anti-canine PD-L1 antibodies comprise a modified canine IgGB Fc, modified canine IgGC Fc, or a modified canine IgGD lacking fab arm exchange as described herein.

The antibody or antigen binding fragment thereof that binds the canine antigen, *e.g.*, canine PD-L1, can comprise one, two, three, four, five, or six of the complementarity determining regions (CDRs) of the murine anti-canine antibody as described herein. The one, two, three, four, five, or six CDRs may be independently selected from the CDR sequences of those provided below. In a further embodiment, the antibody or antigen-binding fragment thereof that binds canine PD-L1 comprises a canine antibody *kappa* light chain comprising a murine light chain CDR-1, CDR-2 and/or CDR-3 and a canine antibody heavy chain IgG comprising a murine heavy chain CDR-1, CDR-2 and/or CDR-3. Accordingly, the present invention further provides full length canine heavy chains then can be matched *e.g.*, with the corresponding light chains to make a caninized

antibody [*see* Table 2 below, in which the sequences of two sets of CDRs of murine anti-canine PD-L1, *e.g.*, 4F9 and 5F12 are provided].

In other embodiments, the invention provides antibodies or antigen binding fragments thereof that bind PD-L1 with specificity and have canine antibody *kappa* light chains comprising one to six different CDRs comprising at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity with the amino acid sequences of SEQ ID NOs: 16, 17, 18, 22, 23, and/or 24 and canine antibody heavy chain IgG comprising one to six different CDRs comprising at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity with the amino acid sequences of SEQ ID NOs: 13, 14, 15, 19, 20, and/or 21, while still exhibiting the desired binding and functional properties. In another embodiment the antibody or antigen binding fragment of the present invention comprises a canine frame comprising of a combination of IgG heavy chain sequence with a *kappa* light chain having one or more of the above-mentioned CDR amino acid sequences with 0, 1, 2, 3, 4, or 5 conservative or non-conservative amino acid substitutions, while still exhibiting the desired binding and functional properties.

"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 I directly below.

TABLE 1
EXEMPLARY CONSERVATIVE AMINO ACID SUBSTITUTIONS

Original residue	Conservative substitution

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
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
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 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 I above.

Nucleic Acids

The present invention further comprises the nucleic acids encoding the immunoglobulin chains of caninized murine anti-canine PD-L1 antibodies and antigen binding fragments thereof disclosed herein (see 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 CDRs and/or canine cFc's and/or antibodies 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.

This present invention also provides expression vectors comprising the nucleic acids (including isolated nucleic acids) of the invention, wherein the nucleic acid is operably linked to control sequences that are recognized by a host cell when the host cell is transfected with the vector. Also provided are host cells comprising an expression vector of the present invention and methods for producing the antibody or antigen binding fragment thereof disclosed herein comprising culturing a host cell harboring an expression vector encoding the antibody or antigen binding fragment in culture medium, and isolating the antigen or antigen binding fragment thereof from the host cell or culture medium.

A caninized murine anti-canine PD-L1 antibody for example, 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 general, glycoproteins produced in a particular cell line or transgenic animal will have a glycosylation pattern that is characteristic for glycoproteins produced in the cell line or transgenic animal. Therefore, the particular glycosylation pattern of an antibody will depend on the particular cell line or transgenic animal used to produce the antibody. However, all antibodies encoded by the nucleic acid molecules provided herein, or comprising the amino acid sequences provided herein, comprise the instant invention, independent of the glycosylation pattern that the antibodies may have. Similarly, in particular embodiments, antibodies with a glycosylation pattern comprising only non-fucosylated *N*-glycans may be advantageous, because these antibodies have been shown to typically exhibit more potent efficacy than their fucosylated counterparts both *in vitro* and *in vivo* [*See* for example, Shinkawa *et al.*, *J. Biol. Chem.* 278: 3466-3473 (2003); U.S. Patent Nos. 6,946,292 and 7,214,775].

The present invention further includes antibody fragments of the caninized murine anti-canine PD-L1 antibodies disclosed herein. The antibody fragments include $F(ab)_2$ fragments, which may be produced by enzymatic cleavage of an IgG by, for example,

pepsin. Fab fragments may be produced by, for example, reduction of $F(ab)_2$ with dithiothreitol or mercaptoethylamine. A Fab fragment is a V_L-C_L chain appended to a V_H-C_{H1} chain by a disulfide bridge. A $F(ab)_2$ fragment is two Fab fragments which, in turn, are appended by two disulfide bridges. The Fab portion of an $F(ab)_2$ molecule includes a portion of the F_c region between which disulfide bridges are located. An F_V fragment is a V_L or V_H region.

In one embodiment, the antibody or antigen binding fragment comprises a heavy chain constant region, *e.g.*, a canine constant region, such as IgGA, IgGB, IgGC and IgGD canine heavy chain constant region or a variant thereof. In another embodiment, 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 IgGB and the canine light chain constant region can be from *kappa*.

Antibody Engineering

Caninized murine anti-canine PD-L1 antibodies of the present invention can be engineered to include modifications in the canine frame of a parental (*i.e.*, canine) monoclonal antibody, *e.g.* to improve the properties of the antibody, as detailed below.

Cross-blocking Antibodies and Epitope Binding

Cross-blocking antibodies and antigen-binding fragments thereof that cross-compete with 4F9 and/or 5F12, including caninized cross-blocking antibodies and antigen-binding fragments thereof, are part of the present invention. In addition, antibodies and antigen-binding fragments thereof that bind to the same epitope as any of the anti-canine PD-L1 antibodies or fragments thereof of the present invention also form part of the present invention. Cross-blocking antibodies and antigen-binding fragments can be identified based on their ability to cross-compete with 4F9 and/or 5F12 in standard binding assays (*e.g.*, BIACore®, ELISA, or flow cytometry). For example, standard ELISA assays can be used in which a recombinant canine PD-L1 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, for example, 4F9 and/or 5F12 to canine PD-L1 demonstrates that the test antibody can compete with 4F9 and/or 5F12 for binding to canine PD-L1 and thus, may, in some cases, bind to the same epitope on canine PD-L1 as 4F9 and/or 5F12. In more particular embodiments, cross-blocking antibodies and antigen-binding fragments thereof that cross-compete with 4F9 and/or 5F12 and also bind to the same epitope on canine PD-L1 as 4F9 and/or 5F12 are also part of the present invention.

Peptide and Fusion ProteinVaccines

Peptides comprising epitopes (or portions thereof) recognized by anti-canine PD-L1 mAbs and fusion proteins comprising such peptides may be used as vaccines to elicit antibodies that block the binding of PD-L1 to PD-1 and result in T cell activation and enhancement of the immune response. Such vaccines may be useful as therapeutic vaccines for diseases such as cancer or to act as enhancers of the immune response to other vaccines. In order to use these peptides as vaccines, one or more of these peptides may be coupled chemically or through the techniques of recombinant DNA technology to a carrier protein in order to enhance the immunogenicity of these peptides and elicit peptide-specific antibodies. Techniques for coupling peptides to carrier proteins are known to those skilled in the art. Peptide (and corresponding fusion protein) vaccines may be used to vaccinate animals by *e.g.*, intramuscular (IM), subcutaneous (S/C), oral, spray or *in ovo* routes (see below too). Such vaccines may be used as subunit proteins expressed from bacterial, viral, yeast or baculovirus virus systems. Alternatively such peptide (or fusion protein) vaccines may be delivered following administration of a variety of viral or bacterial vectors that express such peptide or fusion proteins as can be practiced by methods known to those skilled in the art. The peptide or fusion protein vaccines may be administered in doses from 1-1000 μg and may optionally contain an adjuvant and an acceptable pharmaceutical carrier

Pharmaceutical Compositions and Administration

To prepare pharmaceutical or sterile compositions of a caninized murine anti-canine PD-L1 antibody or antigen binding fragment thereof it 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, anti-PD-L1 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 LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index (LD_{50}/ ED_{50}). 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 ED_{50} 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 caninized murine anti-canine PD-L1 antibody or antigen binding fragment thereof can be administered by an invasive route such as by injection. In further embodiments of the invention, a murine anti-canine PD-L1 antibody or antigen binding fragment thereof, or pharmaceutical composition thereof, is administered intravenously, subcutaneously, intramuscularly, intraarterially, intratumorally, 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.

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.

Alternately, one may administer a caninized murine anti-canine PD-L1 antibody in a local rather than systemic manner, for example, via injection of the antibody directly into an arthritic joint or pathogen-induced lesion characterized by immunopathology, often in a depot or sustained release formulation. Furthermore, one may administer the antibody in a targeted drug delivery system, for example, in a liposome coated with a tissue-specific antibody, targeting, for example, arthritic joint or pathogen-induced lesion characterized by immunopathology. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

The administration regimen depends on several factors, including the serum or tissue turnover rate of the therapeutic antibody, the level of symptoms, the immunogenicity of the therapeutic antibody, and the accessibility of the target cells in the biological matrix. Preferably, the administration regimen delivers sufficient therapeutic antibody to effect improvement in the target disease state, while simultaneously minimizing undesired side effects. Accordingly, the amount of biologic delivered depends in part on the particular therapeutic antibody 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 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. 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 veteranarian, *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 until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, *e.g.*, the inflammation or level of inflammatory cytokines produced.

Antibodies or antigen binding fragments thereof disclosed 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, 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 a caninized murine anti-canine PD-L1 antibody in the subject's serum, such as 0.1, 0.3, 1, 3, 10, 30, 100, 300 μg/ml or more. In other embodiments, a caninized murine anti-canine PD-L1 antibody 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 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 with a disorder, disease 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 a caninized murine anti-canine PD-L1 antibody or antigen binding fragment thereof of the present invention that, when administered alone or in combination with an additional therapeutic agent to a cell, tissue, or subject, 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 dose further refers to that amount of the binding compound 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 an individual active ingredient administered alone, a therapeutically effective dose refers to that ingredient alone. 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 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 disease severity.

Other Combination Therapies

As previously described, a caninized murine anti-canine PD-L1 antibody or antigen binding fragment thereof may be coadministered with one or other more therapeutic agents (such as a chemotherapeutic agent). The antibody may be linked to the agent (as an immunocomplex) or can be administered separately from the agent. In the latter case (separate administration), the antibody can be administered before, after or concurrently with the agent or can be co-administered with other known therapies.

In particular combination therapies, a caninized murine anti-canine PD-L1 antibody or antigen binding fragment thereof may be coadministered with a caninized murine anti-canine PD-1 antibody or antigen binding fragment thereof [*e.g.*, as disclosed in U.S. Provisional Application Serial No. 61/918,946, filed on December 20, 2013, and U.S. Provisional Application Serial No. 62/030,812, filed on July 30, 2014, Provisional Application Serial No. 62/092,496, filed December 16, 2014, PCT/EP2014/078655 (WO2015091911), and PCTEP2014/078653 (WO2015/091910), the contents of which are all hereby incorporated by reference in their entireties] and/or a caninized murine anti-canine cytotoxic T-lymphocyte associated protein-4 (CTLA-4) antibody, or antigen binding fragment thereof. Accordingly, any combination of these three caninized murine anti-canine protein (*i.e.*, anti-canine PD-L1, PD-1, or CTLA-4) antibodies or antigen binding fragments thereof can be co-administered. For example, the caninized murine anti-canine PD-L1 antibody or antigen binding fragment thereof can be administered before, after, or concurrently with a caninized murine anti-canine PD-1 antibody or antigen binding fragment thereof and/or with a caninized murine CTLA-4 antibody or antigen binding fragment thereof. In addition, the combination of the caninized murine anti-canine PD-L1 antibody or antigen binding fragment thereof with the caninized murine anti-canine PD-1 antibody or antigen binding fragment thereof, and/or the

caninized murine CTLA-4 antibody or antigen binding fragment thereof also can be co-administered with other known therapies, *i.e.*, before, after, or concurrently.

Kits

Further provided are kits comprising one or more components that include, but are not limited to, an antibody or antigen binding fragment, as discussed herein, which specifically binds PD-L1 (*e.g.*, a caninized murine anti-canine PD-L1 antibody or antigen binding fragment thereof) in association with one or more additional components including, but not limited to a caninized murine anti-canine PD-1 antibody or antigen binding fragment thereof, and/or a caninized murine anti-canine CTLA-4 antibody or antigen binding fragment thereof, a pharmaceutically acceptable carrier and/or a chemotherapeutic agent, as discussed herein. In alternative embodiments, the kit can comprise one or more peptide comprising an epitope or portion thereof (or a fusion protein comprising the epitope or portion thereof) recognized by anti-canine PD-L1 mAbs as discussed above. The binding composition and/or the chemotherapeutic agent or the peptide comprising an epitope or portion thereof (or a fusion protein comprising the epitope or portion thereof) can be formulated as a pure composition or in combination with a pharmaceutically acceptable carrier, in a pharmaceutical composition.

In one embodiment, the kit includes a binding composition of the present invention, *e.g.*, a caninized murine anti-canine PD-L1 (or antigen binding fragment thereof) or a pharmaceutical composition thereof in one container (*e.g.*, in a sterile glass or plastic vial) and/or a pharmaceutical composition thereof and/or a chemotherapeutic agent in another container (*e.g.*, in a sterile glass or plastic vial) and/or a caninized murine anti-canine PD-1(or antigen binding fragment thereof) and/or a caninized murine anti-canine CTLA-4 (or antigen binding fragment thereof) or a pharmaceutical composition thereof in one or more other containers (*e.g.*, in a sterile glass or plastic vial).

If the kit includes a pharmaceutical composition for parenteral administration to a subject, the kit can also include a device for performing such administration. For example, the kit can include one or more hypodermic needles or other injection devices as discussed above. The kit can also include a package insert including information concerning the

pharmaceutical compositions and dosage forms in the kit. Generally, such information aids pet owners and veteranarians in using the enclosed pharmaceutical compositions and dosage forms effectively and safely. For example, the following information regarding a combination of the invention may be supplied in the insert: pharmacokinetics, pharmacodynamics, clinical studies, efficacy parameters, indications and usage, contraindications, warnings, precautions, adverse reactions, overdosage, proper dosage and administration, how supplied, proper storage conditions, references, manufacturer/distributor information and patent information.

As a matter of convenience, an antibody or specific binding agent disclosed herein can be provided in a kit, *i.e.*, a packaged combination of reagents in predetermined amounts with instructions for performing the diagnostic or detection assay. Where the antibody is labeled with an enzyme, the kit will include substrates and cofactors required by the enzyme (*e.g.*, a substrate precursor which provides the detectable chromophore or fluorophore). In addition, other additives may be included such as stabilizers, buffers (*e.g.*, a block buffer or lysis buffer) and the like. The relative amounts of the various reagents may be varied widely to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. Particularly, the reagents may be provided as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

EXAMPLES

EXAMPLE 1

CANINE PD-1 AND PD-L1

Canine PD-1 and PD-L1 Sequences:

U.S. provisional application nos. 61/918,946, filed on December 20, 2013 and 62/030,812, filed on July 30, 2014, hereby incorporated by reference in their entireties, provides: the full length nucleotide sequence for canine PD-1 (cPD-1) of SEQ ID NO: 49 [SEQ ID NO: 69 includes the signal sequence]; the corresponding translated amino acid sequence of SEQ ID NO: 50 [SEQ ID NO: 70 includes the signal sequence]; the nucleotide sequence encoding the extra-cellular domain (ECD) of canine PD-1, SEQ ID NO: 51; the amino acid sequence of the ECD of canine PD-1, SEQ ID NO: 52; the

nucleotide sequence of canine PD-1 ECD plus a GT linker and the Fc part of human IgG1 Fc gene, SEQ ID NO: 53; and the amino acid sequence of the canine PD-1 ECD plus a GT linker and the Fc part of human IgG1 Fc gene, SEQ ID NO: 54 [SEQ ID NO: 81 includes the signal sequence].

U.S. provisional application nos. 61/918,946, filed on December 20, 2013 and 62/030,812, filed on July 30, 2014, hereby incorporated by reference in their entireties further provide: the full length nucleotide sequence for canine PD-L1 (cPD-L1) of SEQ ID NO: 55 [SEQ ID NO: 71 includes the signal sequence]; the corresponding translated amino acid sequence of SEQ ID NO: 56 [SEQ ID NO: 72 includes the signal sequence]; the nucleotide sequence encoding the extra-cellular domain (ECD) of canine PD-L1, SEQ ID NO: 57; the amino acid sequence of the ECD of canine PD-L1, SEQ ID NO: 58; the nucleotide sequence of canine PD-L1 ECD plus a GT linker and the Fc part of human IgG1 Fc gene, of SEQ ID NO: 59; and the amino acid sequence of the PD-L1 ECD plus a GT linker and the Fc part of human IgG1 Fc gene, SEQ ID NO: 60.

Identification and Cloning of Canine PD-1:
A nucleic acid encoding a full length canine PD-1 (cPD-1) was identified through a search of the NCBI gene bank data bases (accession number XM_543338.4). The translated amino acid sequence (accession number XP_543338.3) corresponded to a putative canine PD-1 protein which was further identified through searching the gene bank (NCBI) protein databases and aligning the identified amino acid sequence with murine, feline, and human PD-1 amino acid sequences. The DNA sequence corresponding to the full length canine PD-1 gene that was codon optimized for CHO cells was synthesized and cloned into a plasmid designated p96793. Comparison of DNA and protein sequences of predicted canine PD-1 with known PD-1 DNA and protein sequences led to the identification of the DNA sequences encoding the extra-cellular domain (ECD) of canine PD-1 and the amino acid sequence of the ECD of canine PD-1.

A DNA sequence encoding the ECD of canine PD-1 in addition to a GT linker and 8 histidine residues was synthesized and cloned into a plasmid designated LPD2726. A nucleic acid sequence corresponding to the canine PD-1 ECD plus a GT linker and the Fc part of human IgG1 Fc gene was chemically synthesized and cloned into a plasmid

designated LPD2727. Canine PD-1 ECD and the Fc part of human IgG1 Fc comprises the amino acid sequence of SEQ ID NO: 81(including the signal sequence).

Identification and Cloning of Canine PD-L1:
A nucleic acid encoding a full length canine PD-L1 was identified through a search of the NCBI gene bank data bases (accession number XM_541302.4). The translated amino acid sequence (accession number XP-541302.4) corresponding to the putative canine PD-L1 protein was identified by searching the gene bank (NCBI) protein databases and alignment of the identified sequence with known PD-L1 mouse and human sequences.

Comparison of DNA encoding canine PD-L1 with known PD-L1 sequences identified the DNA sequence corresponding to the ECD domain of canine PD-L1 (which was codon optimized for CHO cells). The predicted amino acid sequence of the ECD of canine PD-L1 is SEQ ID NO: 58. DNA encoding PD-L1 ECD plus GT linker and 8 histidine residues was synthesized and cloned into a plasmid designated LPD2695.

A DNA sequence encoding the amino acid sequence of canine PD-L1 ECD plus GT linker and the Fc part of human IgG1 Fc was chemically synthesized and cloned into a plasmid designated LPD2697. Canine PD-L1 ECD plus GT linker and the Fc part of human IgG1 comprises the amino acid sequence of SEQ ID NO:60.

Expression of PD-1 and PD-L1 proteins:
Expression plasmids encoding the PD-1ECD-HIS, PD-1ECD-Fc, PD-L1 ECD-HIS, and PD-L1ECD-Fc proteins were transfected into HEK 293 cells and the proteins were purified from the supernatant of transfected cells using Protein A for Fc fusion proteins or Nickel (Ni^{2+}) column chromatography for HIS-tagged proteins. Purified proteins were used for: ELISA or binding assays as detailed below. Expressed proteins were analyzed by SDS-PAGE gels.

Full length canine PD-1 DNA sequence: signal sequence **underlined and in bold**
Nucleotide sequence **SEQ ID NO: 49**, is without the signal sequence.
atggggagccggcgggggccctggccgctcgtctgggccgtgctgcagctgggctggtggccagg atggctcctagactcccctgacaggccctggagcccgctcaccttctccccggcgcagctcacgg

tgcaggagggagagaacgccacgttcacctgcagcctggccgacatccccgacagcttcgtgctc
aactggtaccgcctgagcccccgcaaccagacggacaagctggccgccttccaggaggaccgcat
cgagccgggccgggacaggcgcttccgcgtcatgcggctgcccaacgggcgggacttccacatga
gcatcgtcgctgcgcgcctcaacgacagcggcatctacctgtgcggggccatctacctgccccc
aacacacagatcaacgagagtccccgcgcagagctctccgtgacggagagaaccctggagccccc
cacacagagccccagccccccacccagactcagcggccagttgcaggggctggtcatcggcgtca
cgagcgtgctggtgggtgtcctgctactgctgctgctgacctgggtcctggccgctgtcttcccc
agggccacccgaggtgcctgtgtgtgcgggagcgaggacgagcctctgaaggagggccccgatgc
agcgcccgtcttcaccctggactacggggagctggacttccagtggcgagagaagacgccggagc
ccccggcgccctgtgccccggagcagaccgagtatgccaccatcgtcttcccgggcaggccggcg
tccccgggccgcagggcctcggccagcagcctgcagggagcccagcctccgagccccgaggacgg
acccggcctgtggcccctctga

Full length canine PD-1 Amino acid sequence: signal sequence **underlined and in bold** Amino acid sequence **SEQ ID NO: 50** is without the signal sequence.

MGSRRGPWPLVWAVLQLGWWPGWLLDSPDRPWSPLTFSPAQLTVQEGENATFTCSLADIPDSFVL
NWYRLSPRNQTDKLAAFQEDRIEPGRDRRFRVMRLPNGRDFHMSIVAARLNDSGIYLCGAIYLPP
NTQINESPRAELSVTERTLEPPTQSPSPPPRLSGQLQGLVIGVTSVLVGVLLLLLLTWVLAAVFP
RATRGACVCGSEDEPLKEGPDAAPVFTLDYGELDFQWREKTPEPPAPCAPEQTEYATIVFPGRPA
SPGRRASASSLQGAQPPSPEDGPGLWPL

Canine PD-1 extracellular domain DNA sequence: **SEQ ID NO: 51** (Codon optimized for expression in CHO cells)

ctggattcccccgacagaccctggagccctctcaccttctcccctgcccagctgaccgtccagga
aggcgagaatgccaccttcacctgcagcctcgccgacatccccgacagcttcgtgctgaactggt
acagactgagccccaggaaccagaccgacaagctggccgctttccaggaggacaggatcgaaccc
ggcagggacaggaggtttagggtcatgaggctgcccaacggcagggacttccacatgtccatcgt
ggccgccagactgaacgactccggcatctacctgtgcggcgctatctacctgcccccaacaccc
agatcaacgagagccccagggccgaactgagcgtgacagagagaaccctggaacctcccacccag
agcccttcccctcctcctagactgagcggacagctgcagggcctggtg

Canine PD-1 extracellular domain: **SEQ ID NO: 52**

LDSPDRPWSPLTFSPAQLTVQEGENATFTCSLADIPDSFVLNWYRLSPRNQTDKLAAFQEDRIEP
GRDRRFRVMRLPNGRDFHMSIVAARLNDSGIYLCGAIYLPPNTQINESPRAELSVTERTLEPPTQ
SPSPPPRLSGQLQGLV

Canine PD-1 extracellular domain – human IgG1 Fc DNA sequence: **SEQ ID NO: 53** (Codon optimized for expression in HEK-293 cells)

ctggattcccccgacagaccctggagccctctcaccttctcccctgcccagctgaccgtccagga
aggcgagaatgccaccttcacctgcagcctcgccgacatccccgacagcttcgtgctgaactggt
acagactgagccccaggaaccagaccgacaagctggccgctttccaggaggacaggatcgaaccc
ggcagggacaggaggtttagggtcatgaggctgcccaacggcagggacttccacatgtccatcgt
ggccgccagactgaacgactccggcatctacctgtgcggcgctatctacctgcccccaacaccc
agatcaacgagagccccagggccgaactgagcgtgacagagagaaccctggaacctcccacccag
agcccttcccctcctcctagactgagcggacagctgcagggcctggtgggtaccgacaaaactca
cacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttcccccaa
aacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagc
cacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagac
aaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcacc
aggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatc
gagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatc

ccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcg
acatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtg
ctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagca
ggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcc
tctccctgtctccgggtaaatga

Canine PD-1 extracellular domain – human IgG1 Fc fusion protein: signal sequence **underlined and in bold:** amino acid **SEQ ID NO: 54** is without the signal sequence.

MNFLLSWVHWSLALLLYLHHAKWSQALDSPDRPWSPLTFSPAQLTVQEGENATFTCSLADIPDSF
VLNWYRLSPRNQTDKLAAFQEDRIEPGRDRRFRVMRLPNGRDFHMSIVAARLNDSGIYLCGAIYL
PPNTQINESPRAELSVTERTLEPPTQSPSPPPRLSGQLQGLVGTDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK

Full length canine PD-L1 DNA sequence: signal sequence **underlined and in bold**Nucleotide sequence **SEQ ID NO: 55** is without the signal sequence.

atgagaatgtttagtgtctttacattcatggcctactgccatttgctaaaagcatttacgatcac
agtttctaaggacctgtatgtggtagagtatggtggcaatgtgacaatggaatgcaaattcccgg
tggaaaaacagttaaacttgtttgcactaatcgtctactgggaaatggaggataaaaaaattata
caatttgtgaatggaaaggaagacctgaaagttcagcacagcagctacagccagagggctcagct
attgaaggaccagctcttcttggggaaggctgcgcttcagatcacagatgtgagattgcaggatg
caggggtttactgctgcttgatcggctatggcggtgctgactacaagcggattactttgaaagtt
catgccccgtaccgcaacatcagccaaagaatttctgtggatcctgtcacctctgaacatgaact
aatgtgtcaggctgagggttaccctgaggctgaagtcatctggacaagcagtgaccaccgagtcc
tgagtggcaaaaccaccatcactaattccaatagggaagagaagcttttcaatgtgaccagcacg
ctgaacatcaatgcaacagctaatgagattttctactgcacttttcaaagatcaggtcctgagga
aaacaatactgccgagttggtcatcccagaacgactgcccgttccagcaagtgagaggactcatt
tcatgattctgggacctttcctgttgcttcttggtgtagtcctggcagtcactttctgtctaaaa
aaacatgggagaatgatggatgtggaaaaatgttgcacccgagataggaactcaaagaaacgaaa
tgatatacaatttgaagagacataa

Full length canine PD-L1: signal sequence **underlined and in bold**Amino acid sequence **SEQ ID NO: 56** is without the signal sequence.

MRMFSVFTFMAYCHLLKAFTITVSKDLYVVEYGGNVTMECKFPVEKQLNLFALIVYWEMEDKKII
QFVNGKEDLKVQHSSYSQRAQLLKDQLFLGKAALQITDVRLQDAGVYCCLIGYGGADYKRITLKV
HAPYRNISQRISVDPVTSEHELMCQAEGYPEAEVIWTSSDHRVLSGKTTITNSNREEKLFNVTST
LNINATANEIFYCTFQRSGPEENNTAELVIPERLPVPASERTHFMILGPFLLLLGVVLAVTFCLK
KHGRMMDVEKCCTRDRNSKKRNDIQFEET

Canine PD-L1 extracellular domain DNA sequence: **SEQ ID NO: 57** (Codon optimized for expression in CHO cells)

tttaccatcaccgtgtccaaggacctgtacgtggtcgagtacggcggcaatgtgaccatggagtg
caagttccccgtggagaagcagctgaacctgttcgccctcatcgtgtactgggagatggaggaca
agaagatcatccagttcgtgaacggcaaggaggacctgaaggtgcagcactccagctactcccag
agagcccagctgctgaaggaccagctgttcctgggcaaggccgccctgcagatcaccgacgtgag
actgcaggacgccggcgtgtattgctgcctgatcggctacggaggcgccgactacaagaggatca
ccctgaaggtgcatgcaccctacaggaacatcagccagaggatcagcgtcgatcccgtgaccagc
gagcacgagctgatgtgccaagccgagggctatcccgaggccgaagtgatctggaccagcagcga

ccacagggtcctgagcggcaagaccaccatcaccaacagcaacagggaggagaagctgttcaacg
tgaccagcaccctcaacatcaacgccaccgccaacgagatcttctactgcaccttccagaggagc
ggccccgaagagaacaacaccgccgagctggtgatccccgagagactgcctgtgcctgccagcga
gaggacccac

Canine PD-L1 extracellular domain protein: **SEQ ID NO: 58**

FTITVSKDLYVVEYGGNVTMECKFPVEKQLNLFALIVYWEMEDKKIIQFVNGKEDLKVQHSSYSQ
RAQLLKDQLFLGKAALQITDVRLQDAGVYCCLIGYGGADYKRITLKVHAPYRNISQRISVDPVTS
EHELMCQAEGYPEAEVIWTSSDHRVLSGKTTITNSNREEKLFNVTSTLNINATANEIFYCTFQRS
GPEENNTAELVIPERLPVPASERTH

Canine PD-L1 extracellular domain – human IgG1 Fc DNA sequence: **SEQ ID NO: 59**
(Codon optimized for expression in HEK-293 cells)

tttaccatcaccgtgtccaaggacctgtacgtggtcgagtacggcggcaatgtgaccatggagtg
caagttccccgtggagaagcagctgaacctgttcgccctcatcgtgtactgggagatggaggaca
agaagatcatccagttcgtgaacggcaaggaggacctgaaggtgcagcactccagctactcccag
agagcccagctgctgaaggaccagctgttcctgggcaaggccgccctgcagatcaccgacgtgag
actgcaggacgccggcgtgtattgctgcctgatcggctacggaggcgccgactacaagaggatca
ccctgaaggtgcatgcaccctacaggaacatcagccagaggatcagcgtcgatcccgtgaccagc
gagcacgagctgatgtgccaagccgagggctatcccgaggccgaagtgatctggaccagcagcga
ccacagggtcctgagcggcaagaccaccatcaccaacagcaacagggaggagaagctgttcaacg
tgaccagcaccctcaacatcaacgccaccgccaacgagatcttctactgcaccttccagaggagc
ggccccgaagagaacaacaccgccgagctggtgatccccgagagactgcctgtgcctgccagcga
gaggacccacggtaccgacaaaactcacacatgcccaccgtgcccagcacctgaactcctggggg
gaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgag
gtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtgga
cggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtg
tggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtc
tccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgaga
accacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacct
gcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggag
aacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagct
caccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctc
tgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga

Canine PD-L1 extracellular domain – human IgG1 Fc fusion protein:
SEQ ID NO: 60

FTITVSKDLYVVEYGGNVTMECKFPVEKQLNLFALIVYWEMEDKKIIQFVNGKEDLKVQHSSYSQ
RAQLLKDQLFLGKAALQITDVRLQDAGVYCCLIGYGGADYKRITLKVHAPYRNISQRISVDPVTS
EHELMCQAEGYPEAEVIWTSSDHRVLSGKTTITNSNREEKLFNVTSTLNINATANEIFYCTFQRS
GPEENNTAELVIPERLPVPASERTHGTDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

EXAMPLE 2

MURINE ANTI-CANINE PD-L1 ANTIBODIES

Generation of anti-Canine PD-L1 monoclonal antibodies:

A total of three Balb/c mice were immunized multiple times (with 10 μg each time) over a 17 day period. The immunizing antigen was the canine PD-L1 ECD-Fc fusion protein. Following immunization, serum was collected from each mouse and tested for reactivity with canine PD-L1 ECD-HIS tagged protein. The spleen cells of the mouse with the highest serum anti-PD-L1 ECD-HIS titer were fused to the myeloma P3X63Ag8.653 cell line. Approximately 2 weeks following fusion, supernatant from putative hybridoma cells were tested by ELISA for their reactivity to the PD-L1 ECD-HIS tagged protein. Hybridomas producing strong positive signals in the ELISA were subcloned by limiting dilution and tested again for reactivity to canine PD-L1 ECD-HIS tagged protein.

Confirmation of monoclonal antibodies reactivity against canine PD-L1:
The reactivity of antibodies secreted by hybridomas to ECD of canine PD-L1 was confirmed by ELISA. Hybridoma cells were cultured using CELLine bioreactors (Integra-biosciences) for 10-30 days. Cells were initially maintained in DMEM supplemented with 4 mM L-glutamine and 10% Ultra Low IgG fetal bovine serum (FBS) from Gibco. Hybridoma cells were seeded in CELLine bioreactor cell chambers at a cell density of approximately $2x10^6$ cells/mL in 15 mL of the same medium with the FBS concentration increased to 20%. The outer chamber was filled with 1 L of nutrient medium (DMEM with 4mM L-glutamine and 2% standard FBS). Hybridoma cells in the cell chamber were expanded to approximately $2.5x10^7$ cells/mL over 3-7 days. Then, 10 mL of cell suspension was harvested from the cell chamber and replaced with fresh media to allow for re-expansion of cells and subsequent harvests. This procedure was repeated as necessary to obtain adequate amounts of mAb from each hybridoma clone. Harvested cell suspensions were centrifuged and the supernatants were filtered through 0.2 micron filter membranes. For antibody purification, each clone's supernatant was purified using a Protein G Sepharose 4 Fast flow 5 mL column (GE Healthcare) by gravity flow. After washing with Tris-EDTA (TE) buffer pH 8.0, bound antibodies were eluted using 0.1 M glycine buffer, pH 2.7, followed by pH neutralization using 1 M Tris, pH 8.0. Antibodies were concentrated and buffer exchanged into phosphate-buffered saline (PBS) using Centriprep YM-10,10 kDa NMWL centrifugal filter units (Millipore). Antibody concentrations were quantified using spectrophotometry.
Purified anti-canine PD-L1 mAbs were tested for reactivity with canine PD-L1-hFc fusion protein by ELISA as follows: Canine PD-L1-hFc fusion protein is diluted to 10μg/mL in

coating buffer (Carbonate/Bicarbonate pH 9.0) and dispensed at 100 µl/well in 96-well flat bottomed ELISA plates (NUNC). The plates are incubated at 4°C overnight. The plates are then washed three times with phosphate buffered saline containing 0.05% Tween-20 (PBST). Next, 200 µl of blocking buffer (5% skim milk in PBST) is added to each well and the plates are incubated at 37°C for 60 minutes. The plates are then washed three times with PBST. Next, 100 µl of test mAbs diluted in blocking buffer is added to the first wells of the appropriate columns. Test mAbs are then diluted two-fold to the appropriate plate position. Following incubation of the plates at 37°C for 60 minutes, the plates are washed three times with PBST. Next, 100 µl per well of a 1:2,000 dilution of a horseradish peroxidase conjugated goat anti-mouse IgG (KPL) is added to the plates, which are then incubated at 37°C for 60 minutes. Then the plates are washed three times with PBST, and 100 µl/well of 3,3',5,5' tetramethyl benzidine, (TMB) substrate (from KPL) is added to the plates. The color reaction is allowed to develop for 5-20 minutes at 37°C prior to measuring absorbance at 650nm.

CHO cells expressing canine PD-1 protein:

The full length canine PD-1 gene was cloned into plasmid p96793. In this plasmid the expression of the PD-1 protein is driven by an hCMV promoter. CHO DXB11 cells (dhfr-) were maintained in MEM-*alpha* (Gibco) supplemented with 10% fetal bovine serum. Transfection of CHO cells with plasmid p96793 was carried out in 75 cm^2 flasks containing approximately $6x10^6$ cells by liposome-mediated gene delivery using Lipofectamine (Invitrogen). After 48 hours, cells were passaged into MEM-*alpha* medium without nucleosides, supplemented with 10% FBS and 400µg/mL hygromycin B (selective medium). Limited-dilution cloning was performed on the pool of dhfr+, hygromycin resistant cells. Clones were assessed for expression of canine PD-1 by immunofluorescence assay. Briefly, cell monolayers were fixed in 96 well plates with 80% acetone. Fixed and dried cell monolayers were then incubated for 1 hour with a polyclonal goat anti-human PD-1 antibody (R&D Systems). Plates were washed with PBS, and then incubated for 1 hour with a fluorescein-labeled rabbit anti-goat IgG antibody (KPL). Plates were washed with PBS. Clones exhibiting fluorescence were expanded and cell stocks were established.

Ligand blockade by mouse anti-PD-L1 mAbs:

A cell-based ELISA (CELISA) assay based on the CHO cell line expressing canine PD-1 was used to demonstrate the ability of mouse anti-canine PD-L1 antibodies to block the interaction between (*e.g.*, block the binding of) canine PD-L1 and its receptor canine PD-1. Ligand blockade was confirmed using this assay in conjunction with canine PD-L1/h Fc protein as follows:

1. Seed cPD-1 CHO cells in 96-well plates and grow the cells to 95-100% confluent.

   General guidelines for plating CHO cells,

   on day -3: 1x10^3 c/well (1x10^{5}c/mL)

   -2: 2x10^4 c/well (2x10^{5}c/mL)

   -1: 4x10^4 c/well (4x10^{5}c/mL)

2. 3-fold dilute anti-cPDL1 mAbs in CHO media, starting at 30 µg/mL, 100 µL/well. Add cPD-L1-hFc to 4 µg/ml in CHO media, 100 µL/well, co-incubate in a dilution plate at 37°C, 5% CO_2 with shaking for 60min.
3. Aspirate cell culture media from the cell coated plates, wash the plates 3 x PBS + 0.05% Tween20 and 1x CHO media.
4. Add the co-incubated cPD-L1 mAbs/PD-L1 Fc from the dilution plate to the cell coated plate. 100 uL/well. Incubate at 37°C, 5% CO_2 with shaking for 60min.
5. Wash the plates 6 x PBS + 0.05% Tween 20 (using manual wash protocol).
6. Add Anti-Human Fc-HRP (Calbiochem) (1:2500) in CHO media, 100ul/well, incubate 30-60min at 37°C/5% CO_2.
7. Wash the plates 5x PBS + 0.05% Tween20 (using manual wash protocol).
8. Add 100 µl/well TMB mircowell substrate. Incubate at room temp for 10 minutes. Use one step substrate from Pierce.
9. Stop with 100 µl/well 1.5M Phosphoric acid.
10. Measure A450 – A620 on the ELISA reader.

Cloning and identification of DNA sequences corresponding to mouse anti-canine PD-L1 mAbs variable regions:

The DNA sequence of mouse VH and VL chains and the DNA sequences encoding their CDRs are identified following isolation of mRNA from each hybridoma using standard molecular biology methods. The sequences of the variable regions of heavy and light chains of the two antibodies exemplified herein are provided in Table 12 below. The SEQ ID NOs. of predicted amino acid sequences of the CDRs from these hybridomas are listed in Table 2 below:

TABLE 2

AMINO ACID SEQUENCES OF THE CDRs

4F9

		SEQ ID NO.
VH CDR1	SYAMS	13
VH CDR2	TISDGGSYTHYPDNLMG	14
VH CDR3	ESYDGYYVAN	15
VL CDR1	RASQSISNNLH	16
VL CDR2	YASQSIS	17
VL CDR3	QQSNSWPQT	18

5F12

VH CDR1	DYYMN	19
VH CDR2	WIFPGSGATYYNERFMG	20
VH CDR3	SDWDVGDF	21
VL CDR1	RSSRSLLHTNGITYLS	22
VL CDR2	QMSNLAS	23
VL CDR3	AQTLGLPRT	24

Canonical structures (classes) for VH chain CDRs

mAbs: 4F9:	CDR1: H1-1;	CDR2: H2-3B;	CDR3: H3-10
mAb: 5F12:	CDR1: H1-1;	CDR2: H2-3B;	CDR3: H3-8

Canonical structures (classes) for VL chain CDRs

mAbs: 4F9:	CDR1: L1-2;	CDR2: L2-1;	CDR3: L3-1
mAbs: 5F12:	CDR1: L1-3;	CDR2: L2-1;	CDR3: L3-1

EXAMPLE 3

CANINIZATION AND CHARACTERIZATION OF CANINIZED ANTIBODIES

In order to produce caninized antibodies it was necessary to identify the DNA sequence encoding the heavy and light chains of canine IgG. The nucleotide and amino acid sequences of the canine heavy chain can be obtained from the NCBI gene and protein databases. There are four known IgG subclasses of canine IgG: IgGA, IgGB, IgGC, and IgGD and two types of light chains: *kappa* and *lambda*. Table 7 lists the amino acid and nucleotide SEQ ID NOs of the unmodified canine Fc fragments.

Without being bound by any specific approach, the process of producing variants of anti-PD-L1 monoclonal antibodies with various contents of canine and mouse sequences involved the general following scheme:

i) Determine the nucleotide sequence of VH and VL chains of mouse mAbs;

ii) Identify the H and L chain CDRs of mouse mAbs;

iii) Identify a suitable H and L chain of canine IgG;

iv) Determine the nucleotide sequence of canine IgG H and L chains;

v) Replace the nucleotide sequence encoding endogenous canine H and L chain CDRs with nucleotide sequences encoding the respective mouse CDRs. Also, optionally replace some canine framework residues with selected residues from the mouse framework regions;

vi) Synthesize the nucleotide from step (v) and insert it into a suitable expression plasmid; Transfect plasmids into appropriate cells, *e.g.*, HEK 293 cells;

vii) Purify the expressed antibody from HEK 293 supernatent; and

viii) Test purified antibody for binding to canine PD-L1.

EXAMPLE 4

GENETICALLY MODIFIED CANINE IgGs

In order to generate variants of canine IgG that lack effector functions, a number of mutant canine IgGB heavy chains were generated [*see*, U.S. provisional application no. 62/030,812, filed on July 30, 2014, hereby incorporated by reference in its entirety]. These variants may include one 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) were cloned into expression

plasmids and transfected into HEK 293 cells along with a plasmid containing the gene encoding a light chain. Intact antibodies expressed and purified from HEK 293 cells were evaluated for binding to $Fc_{\gamma}RI$ and C1q to assess their potential for mediation of immune effector functions. Table 3 lists examples of the plasmids encoding the genetically modified caninized heavy chains, the caninized heavy chains; and the genetic modifications in these heavy chains. The variant heavy chains were used for assessment of effector function in the genetically modified mAbs. All of the heavy chains comprised the CDRs from murine anti-canine PD-1 antibodies. [*See*, U.S. provisional application no. 62/030,812, *supra*]

TABLE 3

Plasmid	**Heavy chain**	**Modification**	**AA position in native Fc**
YZZ1057/ Mut-1	can2H9VH4	D31 to A	D31
YZZ1058/ Mut-2	can2H9VH4	N63 to A	N63
YZZ1062	can2H9VH4	D31 to A + N63 to A	D31 and N63
YZZ1059	can2H9VH4	P4 to A	P4
YZZ1060	can2H9VH4	A93 to G	A93
YZZ1061	can2H9VH4	P95 to A	P95
YZZ1068	can2H9VH4	D31 to A, N63 to A, P4 to A, A93 to G, and P95 to A	D31, N63, P4, A93, P95

TABLE 4
MODIFIED cFc or NATIVE cFc WITH HINGE SEQUENCES

#	**N.**	**A.**	**Modified Fcs**
1*	√		Modified Fc –cIgGB
2*		√	Modified Fc –cIgGB
3*	√		Modified Fc –cIgGC
4*		√	Modified Fc –cIgGC
5#	√		cIgGD Fc with S of cIgGD hinge to P
6#		√	cIgGD Fc with S of cIgGD hinge to P
7	√		cIgGD Fc with A hinge
8		√	cIgGD Fc with A hinge
9	√		cIgGD Fc with B hinge

10		√	cIgGD Fc with B hinge
11	√		cIgGD Fc with C hinge
12		√	cIgGD Fc with C hinge

*The substitutions are at P4, D31, N63, G64, T65, A93, and P95 of amino acid sequences SEQ ID NOs: 2 and 4; or at the nucleotides that encode those amino acids for nucleotide sequences SEQ ID NOs: 1 and 3. # Single amino acid substitution as shown in Table 5 below in hinge region of IgGD.

TABLE 5
HINGE REGION SEQUENCES

#	A.A.	Hinge	Sequence
45	√	IgGA	FNECRCTDTPPCPVPEP
46	√	IgGB	PKRENGRVPRPPDCPKCPAPEM
47	√	IgGC	AKECECKCNCNNCPCPGCGL
48	√	IgGD#	PKESTCKCI**P**PCPVPES

Single amino acid substitution of a serine to a proline as in bold and underlined.

TABLE 6
CANINE PD-1/PD-L1 SEQUENCES

#	N.	A.	PD-1	Description	#	N.	A.	PD-L1	Description
49	√		√	Full Length	55	√		√	Full Length
50		√	√	Full Length	56		√	√	Full Length
51	√		√	ECD	57	√		√	ECD
52		√	√	ECD	58		√	√	ECD
53	√		√	cECD-hIgG1	59	√		√	cECD-hIgG1
54		√	√	cECD-hIgG1	60		√	√	cECD-hIgG1
69	√		√	+ signal seq.	71	√		√	+ signal seq.
70		√	√	+ signal seq.	72		√	√	+ signal seq.
81		√	√	+ signal seq.					

TABLE 7
NATIVE cFc SEQUENCES

#	N.	A.		#	N.	A.	
61	√		Fc-cIgGA	65	√		Fc-cIgGB
62		√	Fc-cIgGA	66		√	Fc-cIgGB
63	√		Fc-cIgGD	67	√		Fc-cIgGC
64		√	Fc-cIgGD	68		√	Fc-cIgGC

TABLE 8
CDR AMINO ACID SEQUENCES

#	A.A.	CDR
13	√	VH CDR1 4F9
14	√	VH CDR2 4F9
15	√	VH CDR3 4F9
16	√	VL CDR1 4F9
17	√	VL CDR2 4F9
18	√	VL CDR3 4F9
19	√	VH CDR1 5F12
20	√	VH CDR2 5F12
21	√	VH CDR3 5F12
22	√	VL CDR1 5F12
23	√	VL CDR2 5F12
24	√	VL CDR3 5F12

TABLE 9
INDIVIDUAL SUBSTITUTED CANINIZED HEAVY CHAINS

#	N.	A.	
25	√		4F9- VH3-CH1-hinge-FC -cIgGB Fc
26		√	4F9- VH3-CH1-hinge-FC -cIgGB Fc
27	√		4F9- VH3-CH1-hinge-FC -cIgGC Fc
28		√	4F9- VH3-CH1-hinge-FC -cIgGC Fc
29	√		5F12- VH3-CH1-hinge-FC -cIgGB Fc
30		√	5F12- VH3-CH1-hinge-FC -cIgGB Fc
31	√		5F12- VH3-CH1-hinge-FC -cIgGC Fc
32		√	5F12- VH3-CH1-hinge-FC -cIgGC Fc

The potential specific substitutions are at P4, D31, N63, G64,T65, A93, and P95

TABLE 10
CORRELATION OF AMINO ACID RESIDUE POSITIONS [#]

66/68	P4	D31	N63	G64	T65	A93	P95
2/4	4	31	63	64	65	93	95
26	242	269	301	302	303	331	333
28	240	267	299	300	301	329	331
30	240	267	299	300	301	329	331
32	238	265	297	298	299	327	329

[#] The correlation of amino acid residue positions of native and substituted cFcs with that of the corresponding substituted canine heavy chains. First Column lists SEQ ID NOs.; remaining columns list corresponding amino acid positions. For the two native amino acid sequences (SEQ ID NOs. 66 and 68), the one letter code for the natural amino acid residues are also provided.

TABLE 11
INDIVIDUAL UNSUBSTITUTED CANINIZED HEAVY AND LIGHT CHAINS

#	N.	A.	
33	√		4F9- VH3-CH1-hinge-FC -cIgGA Fc
34		√	4F9- VH3-CH1-hinge-FC -cIgGA Fc
35	√		4F9- VH3-CH1-hinge-FC -cIgGD Fc
36		√	4F9- VH3-CH1-hinge-FC -cIgGD Fc
37	√		4F9- VL3-CL-Kappa
38		√	4F9- VL3-CL-Kappa
39	√		5F12- VH3-CH1-hinge-FC -cIgGA Fc
40		√	5F12- VH3-CH1-hinge-FC -cIgGA Fc
41	√		5F12-VH3-CH1-hinge-FC-cIgGD Fc
42		√	5F12-VH3-CH1-hinge-FC -cIgGD Fc
43	√		5F12-VL3-CL-Kappa
44		√	5F12-VL3-CL-Kappa

TABLE 12
VARIABLE REGIONS OF HEAVY AND LIGHT CHAINS

#	N.	A.	Antibody	#	N.	A.	Antibody
73	√		4F9-Heavy	77	√		5F12-Heavy
74		√	4F9-Heavy	78		√	5F12-Heavy
75	√		4F9-Light	79	√		5F12-Light
76		√	4F9-Light	80		√	5F12-Light

Modified Fc

cIgGB Fc [SEQ ID NO: 2]

LGGXSVFIFPPKPKDTLLIARTPEVTCVVVXLDPEDPEVQISWFVDGKQMQTAKTQPREEQF
XXXYRVVSVLPIGHQDWLKGKQFTCKVNNKXLXSPIERTISKARGQAHQPSVYVLPPSREEL
SKNTVSLTCLIKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQ
RGDTFICAVMHEALHNHYTQESLSHSPGK

cIgGB Fc[SEQ ID NO: 1]

ctgggcggcnnnagcgtgtttatttttccgccgaaaccgaaagataccctgctgattgcgcg
caccccggaagtgacctgcgtggtggtgnnnctggatccggaagatccggaagtgcagatta
gctggtttgtggatggcaaacagatgcagaccgcgaaaacccagccgcgcgaagaacagttt
nnnnnnnnntatcgcgtggtgagcgtgctgccgattggccatcaggattggctgaaaggcaa
acagtttacctgcaaagtgaacaacaaannnctgnnnagcccgattgaacgcaccattagca
aagcgcgcggccaggcgcatcagccgagcgtgtatgtgctgccgccgagccgcgaagaactg
agcaaaaacaccgtgagcctgacctgcctgattaaagatttttttccgccggatattgatgt
ggaatggcagagcaacggccagcaggaaccggaaagcaaatatcgcaccaccccgccgcagc
tggatgaagatggcagctattttctgtatagcaaactgagcgtggataaaagccgctggcag
cgcggcgatacctttatttgcgcggtgatgcatgaagcgctgcataaccattatacccagga
aagcctgagccatagcccgggcaaa

cIgGC Fc [SEQ ID NO: 4]

LGGXSVFIFPPKPKDILVTARTPTVTCVVVXLDPENPEVQISWFVDSKQVQTANTQPREEQS
XXXYRVVSVLPIGHQDWLSGKQFKCKVNNKXLXSPIEEIISKTPGQAHQPNVYVLPPSRDEM
SKNTVTLTCLVKDFFPPEIDVEWQSNGQQEPESKYRMTPPQLDEDGSYFLYSKLSVDKSRWQ
RGDTFICAVMHEALHNHYTQISLSHSPGK

cIgGC Fc [SEQ ID NO: 3]

ctgggcggcnnnagcgtgtttatttttccgccgaaaccgaaagatattctggtgaccgcgcg
caccccgaccgtgacctgcgtggtggtgnnnctggatccggaaaacccggaagtgcagatta
gctggtttgtggatagcaaacaggtgcagaccgcgaacacccagccgcgcgaagaacagagc
nnnnnnnnntatcgcgtggtgagcgtgctgccgattggccatcaggattggctgagcggcaa
acagtttaaatgcaaagtgaacaacaaannnctgnnnagcccgattgaagaaattattagca
aaaccccgggccaggcgcatcagccgaacgtgtatgtgctgccgccgagccgcgatgaaatg
agcaaaaacaccgtgaccctgacctgcctggtgaaagatttttttccgccggaaattgatgt
ggaatggcagagcaacggccagcaggaaccggaaagcaaatatcgcatgaccccgccgcagc
tggatgaagatggcagctattttctgtatagcaaactgagcgtggataaaagccgctggcag
cgcggcgatacctttatttgcgcggtgatgcatgaagcgctgcataaccattatacccagat
tagcctgagccatagcccgggcaaa

cIgGD Fc (S-->P in D hinge) fab arm exchange [SEQ ID NO: 6]

PKESTCKCIPPCPVPESLGGPSVFIFPPKPKDILRITRTPEITCVVLDLGREDPEVQISWFV
DGKEVHTAKTQPREQQFNSTYRVVSVLPIEHQDWLTGKEFKCRVNHIGLPSPIERTISKARG
QAHQPSVYVLPPSPKELSSSDTVTLTCLIKDFFPPEIDVEWQSNGQPEPESKYHTTAPQLDE
DGSYFLYSKLSVDKSRWQQGDTFTCAVMHEALQNHYTDLSLSHSPGK

cIgGD Fc (S-->P in D hinge) fab arm exchange [SEQ ID NO: 5]

ccgaaagaaagcacctgcaaatgcattccgccgtgcccggtgccggaaagcctgggcggccc
gagcgtgtttatttttccgccgaaaccgaaagatattctgcgcattacccgcaccccggaaa
ttacctgcgtggtgctggatctgggccgcgaagatccggaagtgcagattagctggtttgtg
gatggcaaagaagtgcataccgcgaaaacccagccgcgcgaacagcagtttaacagcaccta
tcgcgtggtgagcgtgctgccgattgaacatcaggattggctgaccggcaaagaatttaaat
gccgcgtgaaccatattggcctgccgagcccgattgaacgcaccattagcaaagcgcgcggc
caggcgcatcagccgagcgtgtatgtgctgccgccgagcccgaaagaactgagcagcagcga
taccgtgaccctgacctgcctgattaaagatttttttccgccggaaattgatgtggaatggc
agagcaacggccagccggaaccggaaagcaaatatcataccaccgcgccgcagctggatgaa
gatggcagctattttctgtatagcaaactgagcgtggataaaagccgctggcagcagggcga
tacctttacctgcgcggtgatgcatgaagcgctgcagaaccattataccgatctgagcctga
gccatagcccgggcaaa

cIgGD Fc (A hinge) fab arm exchange [SEQ ID NO: 8]

FNECRCTDTPPCPVPEPLGGPSVFIFPPKPKDILRITRTPEITCVVLDLGREDPEVQISWFV
DGKEVHTAKTQPREQQFNSTYRVVSVLPIEHQDWLTGKEFKCRVNHIGLPSPIERTISKARG
QAHQPSVYVLPPSPKELSSSDTVTLTCLIKDFFPPEIDVEWQSNGQPEPESKYHTTAPQLDE
DGSYFLYSKLSVDKSRWQQGDTFTCAVMHEALQNHYTDLSLSHSPGK

cIgGD Fc (A hinge) fab arm exchange [SEQ ID NO: 7]

tttaacgaatgccgctgcaccgataccccgccgtgcccggtgccggaaccgctgggcggc
ccgagcgtgtttatttttccgccgaaaccgaaagatattctgcgcattacccgcaccccg
gaaattacctgcgtggtgctggatctgggccgcgaagatccggaagtgcagattagctgg
tttgtggatggcaaagaagtgcataccgcgaaaacccagccgcgcgaacagcagtttaac
agcacctatcgcgtggtgagcgtgctgccgattgaacatcaggattggctgaccggcaaa
gaatttaaatgccgcgtgaaccatattggcctgccgagcccgattgaacgcaccattagc
aaagcgcgcggccaggcgcatcagccgagcgtgtatgtgctgccgccgagcccgaaagaa
ctgagcagcagcgataccgtgaccctgacctgcctgattaaagatttttttccgccggaa
attgatgtggaatggcagagcaacggccagccggaaccggaaagcaaatatcataccacc
gcgccgcagctggatgaagatggcagctattttctgtatagcaaactgagcgtggataaa
agccgctggcagcagggcgatacctttacctgcgcggtgatgcatgaagcgctgcagaac
cattataccgatctgagcctgagccatagcccgggcaaa

cIgGD Fc (B hinge) fab arm exchange [SEQ ID NO: 10]

PKRENGRVPRPPDCPKCPAPEMLGGPSVFIFPPKPKDILRITRTPEITCVVLDLGREDPEVQ
ISWFVDGKEVHTAKTQPREQQFNSTYRVVSVLPIEHQDWLTGKEFKCRVNHIGLPSPIERTI
SKARGQAHQPSVYVLPPSPKELSSSDTVTLTCLIKDFFPPEIDVEWQSNGQPEPESKYHTTA
PQLDEDGSYFLYSKLSVDKSRWQQGDTFTCAVMHEALQNHYTDLSLSHSPGK

cIgGD Fc (B hinge) fab arm exchange [SEQ ID NO: 9]

ccgaaacgcgaaaacggccgcgtgccgcgcccgccggattgcccgaaatgcccggcgccg
gaaatgctgggcggcccgagcgtgtttatttttccgccgaaaccgaaagatattctgcgc
attacccgcaccccggaaattacctgcgtggtgctggatctgggccgcgaagatccggaa
gtgcagattagctggtttgtggatggcaaagaagtgcataccgcgaaaacccagccgcgc
gaacagcagtttaacagcacctatcgcgtggtgagcgtgctgccgattgaacatcaggat

tggctgaccggcaaagaatttaaatgccgcgtgaaccatattggcctgccgagcccgatt
gaacgcaccattagcaaagcgcgcggccaggcgcatcagccgagcgtgtatgtgctgccg
ccgagcccgaaagaactgagcagcagcgataccgtgaccctgacctgcctgattaaagat
ttttttccgccggaaattgatgtggaatggcagagcaacggccagccggaaccggaaagc
aaatatcataccaccgcgccgcagctggatgaagatggcagctattttctgtatagcaaa
ctgagcgtggataaaagccgctggcagcagggcgatacctttacctgcgcggtgatgcat
gaagcgctgcagaaccattataccgatctgagcctgagccatagcccgggcaaa

cIgGD Fc (C hinge) fab arm exchange [SEQ ID NO: 12]

AKECECKCNCNNCPCPGCGLLGGPSVFIFPPKPKDILRITRTPEITCVVLDLGREDPEVQIS
WFVDGKEVHTAKTQPREQQFNSTYRVVSVLPIEHQDWLTGKEFKCRVNHIGLPSPIERTISK
ARGQAHQPSVYVLPPSPKELSSSDTVTLTCLIKDFFPPEIDVEWQSNGQPEPESKYHTTAPQ
LDEDGSYFLYSKLSVDKSRWQQGDTFTCAVMHEALQNHYTDLSLSHSPGK

cIgGD Fc (C hinge) fab arm exchange [SEQ ID NO: 11]

gccaaggagtgcgagtgcaagtgcaactgcaacaactgcccctgccccggctgcggcctg
ctgggcggccccagcgtgttcatcttcccccccaagcccaaggacatcctgagaatcacc
agaacccccgagatcacctgcgtggtgctggacctgggcagagaggaccccgaggtgcag
atcagctggttcgtggacggcaaggaggtgcacaccgccaagacccagcccagagagcag
cagttcaacagcacctacagagtggtgagcgtgctgcccatcgagcaccaggactggctg
accggcaaggagttcaagtgcagagtgaaccacatcggcctgcccagccccatcgagaga
accatcagcaaggccagaggccaggcccaccagcccagcgtgtacgtgctgcccccagc
cccaaggagctgagcagcagcgacaccgtgaccctgacctgcctgatcaaggacttcttc
cccccgagatcgacgtggagtggcagagcaacggccagcccgagcccgagagcaagtac
cacaccaccgcccccagctggacgaggacggcagctacttcctgtacagcaagctgagc
gtggacaagagcagatggcagcagggcgacaccttcacctgcgccgtgatgcacgaggcc
ctgcagaaccactacaccgacctgagcctgagccacagccccggcaag

Native Fc

cIgGA Fc **[SEQ ID NO: 62]**

LGGPSVLIFPPKPKDILRITRTPEVTCVVLDLGREDPEVQISWFVDGKEVHTAKTQSREQQF
NGTYRVVSVLPIEHQDWLTGKEFKCRVNHIDLPSPIERTISKARGRAHKPSVYVLPPSPKEL
SSSDTVSITCLIKDFYPPDIDVEWQSNGQQEPERKHRMTPPQLDEDGSYFLYSKLSVDKSRW
QQGDPFTCAVMHETLQNHYTDLSLSHSPGK

cIgGA Fc[SEQ ID NO: 61]

ctgggcggccccagcgtgctgatcttcccccccaagcccaaggacatcctgagaatcacc
agaacccccgaggtgacctgcgtggtgctggacctgggcagagaggaccccgaggtgcag
atcagctggttcgtggacggcaaggaggtgcacaccgccaagacccagagcagagagcag
cagttcaacggcacctacagagtggtgagcgtgctgcccatcgagcaccaggactggctg
accggcaaggagttcaagtgcagagtgaaccacatcgacctgcccagccccatcgagaga
accatcagcaaggccagaggcagagcccacaagcccagcgtgtacgtgctgcccccagc
cccaaggagctgagcagcagcgacaccgtgagcatcacctgcctgatcaaggacttctac
ccccccgacatcgacgtggagtggcagagcaacggccagcaggagcccgagagaaagcac
agaatgaccccccccagctggacgaggacggcagctacttcctgtacagcaagctgagc
gtggacaagagcagatggcagcagggcgacccttcacctgcgccgtgatgcacgagacc
ctgcagaaccactacaccgacctgagcctgagccacagccccggcaag

cIgGD Fc [SEQ ID NO: 64]

LGGPSVFIFPPKPKDILRITRTPEITCVVLDLGREDPEVQISWFVDGKEVHTAKTQPREQQF
NSTYRVVSVLPIEHQDWLTGKEFKCRVNHIGLPSPIERTISKARGQAHQPSVYVLPPSPKEL
SSSDTVTLTCLIKDFFPPEIDVEWQSNGQPEPESKYHTTAPQLDEDGSYFLYSKLSVDKSRW
QQGDTFTCAVMHEALQNHYTDLSLSHSPGK

cIgGD Fc [SEQ ID NO: 63]

ctgggcggccccagcgtgttcatcttccccccaagcccaaggacatcctgagaatcacc
agaacccccgagatcacctgcgtggtgctggacctgggcagaggaccccgaggtgcag
atcagctggttcgtggacggcaaggaggtgcacaccgccaagacccagcccagagagcag
cagttcaacagcacctacagagtggtgagcgtgctgcccatcgagcaccaggactggctg
accggcaaggagttcaagtgcagagtgaaccacatcggcctgcccagccccatcgagaga
accatcagcaaggccagaggccaggcccaccagcccagcgtgtacgtgctgcccccagc
cccaaggagctgagcagcagcgacaccgtgaccctgacctgcctgatcaaggacttcttc
ccccccgagatcgacgtggagtggcagagcaacggccagcccgagcccgagagcaagtac
cacaccaccgcccccccagctggacgaggacggcagctacttcctgtacagcaagctgagc
gtggacaagagcagatggcagcagggcgacaccttcacctgcgccgtgatgcacgaggcc
ctgcagaaccactacaccgacctgagcctgagccacagccccggcaag

cIgGB Fc [SEQ ID NO: 66]

LGGPSVFIFPPKPKDTLLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGTYRVVSVL
PIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPDID
VEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPGK

cIgGB Fc[SEQ ID NO: 65]

ctgggcggccccagcgtgttcatcttccccccaagcccaaggacaccctgctgatcgcc
agaacccccgaggtgacctgcgtggtggtggacctggaccccgaggaccccgaggtgcag
atcagctggttcgtggacggcaagcagatgcagaccgccaagacccagcccagagaggag
cagttcaacggcacctacagagtggtgagcgtgctgcccatcggccaccaggactggctg
aagggcaagcagttcacctgcaaggtgaacaacaaggccctgcccagccccatcgagaga
accatcagcaaggccagaggccaggcccaccagcccagcgtgtacgtgctgcccccagc
agagaggagctgagcaagaacaccgtgagcctgacctgcctgatcaaggacttcttcccc
cccgacatcgacgtggagtggcagagcaacggccagcaggagcccgagagcaagtacaga
accacccccccccagctggacgaggacggcagctacttcctgtacagcaagctgagcgtg
gacaagagcagatggcagagaggcgacaccttcatctgcgccgtgatgcacgaggccctg
cacaaccactacacccaggagagcctgagccacagccccggcaag

cIgGC Fc [SEQ ID NO: 68]

LGGPSVFIFPPKPKDILVTARTPTVTCVVVDLDPENPEVQISWFVDSKQVQTANTQPREEQS
NGTYRVVSVLPIGHQDWLSGKQFKCKVNNKALPSPIEEIISKTPGQAHQPNVYVLPPSRDEM
SKNTVTLTCLVKDFFPPEIDVEWQSNGQQEPESKYRMTPPQLDEDGSYFLYSKLSVDKSRWQ
RGDTFICAVMHEALHNHYTQISLSHSPGK

cIgGC Fc [SEQ ID NO: 67]

ctgggcggccccagcgtgttcatcttccccccaagcccaaggacatcctggtgaccgcc
agaaccccccaccgtgacctgcgtggtggtggacctggaccccgagaaccccgaggtgcag
atcagctggttcgtggacagcaagcaggtgcagaccgccaacacccagcccagagaggag
cagagcaacggcacctacagagtggtgagcgtgctgcccatcggccaccaggactggctg
agcggcaagcagttcaagtgcaaggtgaacaacaaggccctgcccagccccatcgaggag
atcatcagcaagacccccggccaggcccaccagcccaacgtgtacgtgctgcccccccagc
agagacgagatgagcaagaacaccgtgaccctgacctgcctggtgaaggacttcttcccc
cccgagatcgacgtggagtggcagagcaacggccagcaggagcccgagagcaagtacaga
atgaccccccccccagctggacgaggacggcagctacttcctgtacagcaagctgagcgtg
gacaagagcagatggcagagaggcgacaccttcatctgcgccgtgatgcacgaggccctg
cacaaccactacacccagatcagcctgagccacagccccggcaag

Individual substituted heavy chains

4F9-VH3 cIgGB [SEQ ID NO: 26]

EVQLVQSGGDLVKPGGSVRLSCVASGFTFSYAMSWVRQAPGKGLQWMGTISDGGSYTHYPDN
LMGRFTFSLDTAKNTAYLQLNSLRAEDTAVYYCARESYDGYYVANWGQGTLVTVSSASTTAP
SVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSM
VTVPSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGXSVFIFP
PKPKDTLLIARTPEVTCVVVXLDPEDPEVQISWFVDGKQMQTAKTQPREEQFXXXYRVVSVL
PIGHQDWLKGKQFTCKVNNKXLXSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCL
IKDFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVM
HEALHNHYTQESLSHSPGK

4F9-VH3 cIgGB DNA [SEQ ID NO: 25]

gaggtgcagctggtgcagagcggcggcgacctggtgaagcccggcggcagcgtgagactgag
ctgcgtggccagcggcttcaccttcagctacgccatgagctgggtgagacaggcccccggca
agggcctgcagtggatgggcaccatcagcgacggcggcagctacacccactaccccgacaac
ctgatgggcagattcaccttcagcctggacaccgccaagaacaccgcctacctgcagctgaa
cagcctgagagccgaggacaccgccgtgtactactgcgccagagagagctacgacggctact
acgtggccaactggggccagggcaccctggtgaccgtgagcagcgccagcaccaccgccccc
agcgtgttcccccctggcccccagctgcggcagcaccagcggcagcaccgtggccctggcctg
cctggtgagcggctacttccccgagcccgtgaccgtgagctggaacagcggcagcctgacca
gcggcgtgcacaccttccccagcgtgctgcagagcagcggcctgtacagcctgagcagcatg
gtgaccgtgcccagcagcagatggcccagcgagaccttcacctgcaacgtggcccaccccgc
cagcaagaccaaggtggacaagcccgtgcccaagagagagaacggcagagtgcccagacccc
ccgactgccccaagtgccccgcccccgagatgctgggcggcnnnagcgtgttcatcttcccc
cccaagcccaaggacaccctgctgatcgccagaaccccccgaggtgacctgcgtggtggtgnn
nctggaccccgaggaccccgaggtgcagatcagctggttcgtggacggcaagcagatgcaga
ccgccaagacccagcccagagaggagcagttcnnnnnnnnnntacagagtggtgagcgtgctg
cccatcggccaccaggactggctgaagggcaagcagttcacctgcaaggtgaacaacaagnn
nctgnnnagccccatcgagagaaccatcagcaaggccagaggccaggcccaccagcccagcg
tgtacgtgctgcccccccagcagagaggagctgagcaagaacaccgtgagcctgacctgcctg
atcaaggacttcttcccccccgacatcgacgtggagtggcagagcaacggccagcaggagcc
cgagagcaagtacagaaccacccccccccagctggacgaggacggcagctacttcctgtaca
gcaagctgagcgtggacaagagcagatggcagagaggcgacaccttcatctgcgccgtgatg
cacgaggccctgcacaaccactacacccaggagagcctgagccacagccccggcaag

4F9-VH3 cIgGC [SEQ ID NO: 28]

EVQLVQSGGDLVKPGGSVRLSCVASGFTFSYAMSWVRQAPGKGLQWMGTISDGGSYTHYPDN
LMGRFTFSLDTAKNTAYLQLNSLRAEDTAVYYCARESYDGYYVANWGQGTLVTVSSASTTAP
SVFPLAPSCGSQSGSTVALACLVSGYIPEPVTVSWNSVSLTSGVHTFPSVLQSSGLYSLSSM
VTVPSSRWPSETFTCNVAHPATNTKVDKPVAKECECKCNCNNCPCPGCGLLGGXSVFIFPPK
PKDILVTARTPTVTCVVVXLDPENPEVQISWFVDSKQVQTANTQPREEQSXXXYRVVSVLPI
GHQDWLSGKQFKCKVNNKXLXSPIEEIISKTPGQAHQPNVYVLPPSRDEMSKNTVTLTCLVK
DFFPPEIDVEWQSNGQQEPESKYRMTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHE
ALHNHYTQISLSHSPGK

4F9-VH3 cIgGC DNA [SEQ ID NO: 27]

gaggtgcagctggtgcagagcggcggcgacctggtgaagcccggcggcagcgtgagactgag
ctgcgtggccagcggcttcaccttcagctacgccatgagctgggtgagacaggcccccggca
agggcctgcagtggatgggcaccatcagcgacggcggcagctacacccactaccccgacaac
ctgatgggcagattcaccttcagcctggacaccgccaagaacaccgcctacctgcagctgaa
cagcctgagagccgaggacaccgccgtgtactactgcgccagagagagctacgacggctact
acgtggccaactggggccagggcaccctggtgaccgtgagcagcgccagcaccaccgccccc
agcgtgttccccctggcccccagctgcggcagccagagcggcagcaccgtggccctggcctg
cctggtgagcggctacatccccgagcccgtgaccgtgagctggaacagcgtgagcctgacca
gcggcgtgcacaccttccccagcgtgctgcagagcagcggcctgtacagcctgagcagcatg
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caccaacaccaaggtggacaagcccgtggccaaggagtgcgagtgcaagtgcaactgcaaca
actgcccctgccccggctgcggcctgctgggcggcnnnagcgtgttcatcttcccccccaag
cccaaggacatcctggtgaccgccagaacccccaccgtgacctgcgtggtggtgnnnctgga
ccccgagaaccccgaggtgcagatcagctggttcgtggacagcaagcaggtgcagaccgcca
acacccagcccagagaggagcagagcnnnnnnnnntacagagtggtgagcgtgctgcccatc
ggccaccaggactggctgagcggcaagcagttcaagtgcaaggtgaacaacaagnnnctgnn
nagccccatcgaggagatcatcagcaagacccccggccaggcccaccagcccaacgtgtacg
tgctgcccccccagcagagacgagatgagcaagaacaccgtgaccctgacctgcctggtgaag
gacttcttcccccccgagatcgacgtggagtggcagagcaacggccagcaggagcccgagag
caagtacagaatgacccccccccagctggacgaggacggcagctacttcctgtacagcaagc
tgagcgtggacaagagcagatggcagagaggcgacaccttcatctgcgccgtgatgcacgag
gccctgcacaaccactacacccagatcagcctgagccacagccccggcaag

5F12-VH3 CIgGB [SEQ ID NO: 30]

EVQLVQSGGDLVKPGGSVRLSCVASFTFDYYMNWVRQAPGKGLQWIGRWIFPGSGATYYNER
FMGKATISADTAKNTAYMQLNSLRAEDTAVYYCLRSDWDVGDFWGQGTLVTVSSASTTAPSV
FPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVT
VPSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPRPPDCPKCPAPEMLGGXSVFIFPPK
PKDTLLIARTPEVTCVVVXLDPEDPEVQISWFVDGKQMQTAKTQPREEQFXXXYRVVSVLPI
GHQDWLKGKQFTCKVNNKXLXSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIK
DFFPPDIDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHE
ALHNHYTQESLSHSPGK

5F12-VH3 CIgGB DNA [SEQ ID NO: 29]

gaggtgcagctggtgcagagcggcggcgacctggtgaagcccggcggcagcgtgagactgag
ctgcgtggccagcttcaccttcgactactacatgaactgggtgagacaggcccccggcaagg
gcctgcagtggatcggcagatggatcttccccggcagcggcgccacctactacaacgagaga
ttcatgggcaaggccaccatcagcgccgacaccgccaagaacaccgcctacatgcagctgaa
cagcctgagagccgaggacaccgccgtgtactactgcctgagaagcgactgggacgtgggcg
acttctggggccagggcaccctggtgaccgtgagcagcgccagcaccaccgcccccagcgtg
ttcccccctggcccccagctgcggcagcagcgtgaccctgcctgcctgcctggt
gagcggctacttccccgagcccgtgaccgtgagctggaacagcggcagcctgaccagcggcg
tgcacaccttccccagcgtgctgcagagcagcggcctgtacagcctgagcagcatggtgacc
gtgcccagcagcagatggcccagcgagaccttcacctgcaacgtgcccaccccgccagcaa
gaccaaggtggacaagcccgtgcccaagagagagaacggcagagtgcccagacccccccgact
gccccaagtgccccgcccccgagatgctgggcggcnnnagcgtgttcatcttcccccccaag
cccaaggacaccctgctgatcgccagaacccccgaggtgacctgcgtggtggtgnnnctgga
ccccgaggaccccgaggtgcagatcagctggttcgtggacggcaagcagatgcagaccgcca
agacccagcccagagaggagcagttcnnnnnnnnntacagagtggtgagcgtgctgcccatc
ggccaccaggactggctgaagggcaagcagttcacctgcaaggtgaacaacaagnnnctgnn
nagccccatcgagagaaccatcagcaaggccagaggccaggcccaccagcccagcgtgtacg
tgctgcccccccagcagagaggagctgagcaagaacaccgtgagcctgacctgcctgatcaag
gacttcttcccccccgacatcgacgtggagtggcagagcaacggccagcaggagcccgagag
caagtacagaaccacccccccccagctggacgaggacggcagctacttcctgtacagcaagc
tgagcgtggacaagagcagatggcagagaggcgacaccttcatctgcgccgtgatgcacgag
gccctgcacaaccactacacccaggagagcctgagccacagccccggcaag

5F12-VH3-cIgGC Fc **[SEQ ID NO: 32]**

EVQLVQSGGDLVKPGGSVRLSCVASFTFDYYMNWVRQAPGKGLQWIGRWIFPGSGATYYNER
FMGKATISADTAKNTAYMQLNSLRAEDTAVYYCLRSDWDVGDFWGQGTLVTVSSASTTAPSV
FPLAPSCGSQSGSTVALACLVSGYIPEPVTVSWNSVSLTSGVHTFPSVLQSSGLYSLSSMVT
VPSSRWPSETFTCNVAHPATNTKVDKPVAKECECKCNCNNCPCPGCGLLGGXSVFIFPPKPK
DILVTARTPTVTCVVVXLDPENPEVQISWFVDSKQVQTANTQPREEQSXXXYRVVSVLPIGH
QDWLSGKQFKCKVNNKXLXSPIEEIISKTPGQAHQPNVYVLPPSRDEMSKNTVTLTCLVKDF
FPPEIDVEWQSNGQQEPESKYRMTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEAL
HNHYTQISLSHSPGK

5F12-VH3-cIgGC Fc DNA [SEQ ID NO: 31]

gaggtgcagctggtgcagagcggcggcgacctggtgaagcccggcggcagcgtgagactgag
ctgcgtggccagcttcaccttcgactactacatgaactgggtgagacaggcccccggcaagg
gcctgcagtggatcggcagatggatcttccccggcagcggcgccacctactacaacgagaga
ttcatgggcaaggccaccatcagcgccgacaccgccaagaacaccgcctacatgcagctgaa
cagcctgagagccgaggacaccgccgtgtactactgcctgagaagcgactgggacgtgggcg
acttctggggccagggcaccctggtgaccgtgagcagcgccagcaccaccgcccccagcgtg
ttcccccctggcccccagctgcggcagccagagcggcagcaccgtggccctggcctgcctggt
gagcggctacatccccgagcccgtgaccgtgagctggaacagcgtgagcctgaccagcggcg
tgcacaccttccccagcgtgctgcagagcagcggcctgtacagcctgagcagcatggtgacc
gtgcccagcagcagatggcccagcgagaccttcacctgcaacgtggcccaccccgccaccaa
caccaaggtggacaagcccgtggccaaggagtgcgagtgcaagtgcaactgcaacaactgcc
cctgccccggctgcggcctgctgggcggcnnnagcgtgttcatcttcccccccaagcccaag

gacatcctggtgaccgccagaaccccccaccgtgacctgcgtggtggtgnnnctggaccccga
gaaccccgaggtgcagatcagctggttcgtggacagcaagcaggtgcagaccgccaacaccc
agcccagagaggagcagagcnnnnnnnnntacagagtggtgagcgtgctgcccatcggccac
caggactggctgagcggcaagcagttcaagtgcaaggtgaacaacaagnnnctgnnnagccc
catcgaggagatcatcagcaagacccccggccaggcccaccagcccaacgtgtacgtgctgc
cccccagcagagacgagatgagcaagaacaccgtgaccctgacctgcctggtgaaggacttc
ttcccccccgagatcgacgtggagtggcagagcaacggccagcaggagcccgagagcaagta
cagaatgacccccccccagctggacgaggacggcagctacttcctgtacagcaagctgagcg
tggacaagagcagatggcagagaggcgacaccttcatctgcgccgtgatgcacgaggccctg
cacaaccactacacccagatcagcctgagccacagccccggcaag

Individual un-substituted caninized heavy and light chains

4F9-VH3-cIgGA Fc [SEQ ID NO: 34]

EVQLVQSGGDLVKPGGSVRLSCVASGFTFSYAMSWVRQAPGKGLQWMGTISDGGSYTHYPDN
LMGRFTFSLDTAKNTAYLQLNSLRAEDTAVYYCARESYDGYYVANWGQGTLVTVSSASTTAP
SVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLHSLSSM
VTVPSSRWPSETFTCNVVHPASNTKVDKPVFNECRCTDTPPCPVPEPLGGPSVLIFPPKPKD
ILRITRTPEVTCVVLDLGREDPEVQISWFVDGKEVHTAKTQSREQQFNGTYRVVSVLPIEHQ
DWLTGKEFKCRVNHIDLPSPIERTISKARGRAHKPSVYVLPPSPKELSSSDTVSITCLIKDF
YPPDIDVEWQSNGQQEPERKHRMTPPQLDEDGSYFLYSKLSVDKSRWQQGDPFTCAVMHETL
QNHYTDLSLSHSPGK

4F9-VH3-cIgGA Fc [SEQ ID NO: 33]

gaggtgcagctggtgcagagcggcggcgacctggtgaagcccggcggcagcgtgagactg
agctgcgtggccagcggcttcaccttcagctacgccatgagctgggtgagacaggccccc
ggcaagggcctgcagtggatgggcaccatcagcgacggcggcagctacacccactacccc
gacaacctgatgggcagattcaccttcagcctggacaccgccaagaacaccgcctacctg
cagctgaacagcctgagagccgaggacaccgccgtgtactactgcgccagagagagctac
gacggctactacgtggccaactggggccagggcaccctggtgaccgtgagcagcgccagc
accaccgcccccagcgtgttccccctggcccccagctgcggcagcaccagcggcagcacc
gtggccctggcctgcctggtgagcggctacttccccgagcccgtgaccgtgagctggaac
agcggcagcctgaccagcggcgtgcacaccttccccagcgtgctgcagagcagcggcctg
cacagcctgagcagcatggtgaccgtgcccagcagcagatggcccagcgagaccttcacc
tgcaacgtggtgcaccccgccagcaacaccaaggtggacaagcccgtgttcaacgagtgc
agatgcaccgacacccccccctgccccgtgcccgagcccctgggcggccccagcgtgctg
atcttcccccccaagcccaaggacatcctgagaatcaccagaaccccccgaggtgacctgc
gtggtgctggacctgggcagagaggaccccgaggtgcagatcagctggttcgtggacggc
aaggaggtgcacaccgccaagacccagagcagagagcagcagttcaacggcacctacaga
gtggtgagcgtgctgcccatcgagcaccaggactggctgaccggcaaggagttcaagtgc
agagtgaaccacatcgacctgcccagccccatcgagagaaccatcagcaaggccagaggc
agagcccacaagcccagcgtgtacgtgctgcccccccagccccaaggagctgagcagcagc
gacaccgtgagcatcacctgcctgatcaaggacttctacccccccgacatcgacgtggag
tggcagagcaacggccagcaggagcccgagagaaagcacagaatgacccccccccagctg
gacgaggacggcagctacttcctgtacagcaagctgagcgtggacaagagcagatggcag
cagggcgacccccttcacctgcgccgtgatgcacgagaccctgcagaaccactacaccgac
ctgagcctgagccacagccccggcaag

4F9-VH3 cIgGD Fc [SEQ ID NO: 36]

EVQLVQSGGDLVKPGGSVRLSCVASGFTFSYAMSWVRQAPGKGLQWMGTISDGGSYTHYPDN
LMGRFTFSLDTAKNTAYLQLNSLRAEDTAVYYCARESYDGYYVANWGQGTLVTVSSASTTAP
SVFPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSST
VTVPSSRWPSETFTCNVVHPASNTKVDKPVPKESTCKCISPCPVPESLGGPSVFIFPPKPKD
ILRITRTPEITCVVLDLGREDPEVQISWFVDGKEVHTAKTQPREQQFNSTYRVVSVLPIEHQ
DWLTGKEFKCRVNHIGLPSPIERTISKARGQAHQPSVYVLPPSPKELSSSDTVTLTCLIKDF
FPPEIDVEWQSNGQPEPESKYHTTAPQLDEDGSYFLYSKLSVDKSRWQQGDTFTCAVMHEAL
QNHYTDLSLSHSPGK

4F9-VH3 cIgGD Fc [SEQ ID NO: 35]

gaggtgcagctggtgcagagcggcggcgacctggtgaagcccggcggcagcgtgagactg
agctgcgtggccagcggcttcaccttcagctacgccatgagctgggtgagacaggccccc
ggcaagggcctgcagtggatgggcaccatcagcgacggcggcagctacacccactacccc
gacaacctgatgggcagattcaccttcagcctggacaccgccaagaacaccgcctacctg
cagctgaacagcctgagagccgaggacaccgccgtgtactactgcgccagagagagctac
gacggctactacgtggccaactggggccagggcaccctggtgaccgtgagcagcgccagc
accaccgccccagcgtgttccccctggcccccagctgcggcagcaccagcggcagcacc
gtggccctggcctgcctggtgagcggctacttccccgagcccgtgaccgtgagctggaac
agcggcagcctgaccagcggcgtgcacaccttccccagcgtgctgcagagcagcggcctg
tacagcctgagcagcaccgtgaccgtgcccagcagcagatggcccagcgagaccttcacc
tgcaacgtggtgcaccccgccagcaacaccaaggtggacaagcccgtgcccaaggagagc
acctgcaagtgcatcagcccctgccccgtgcccgagagcctgggcggccccagcgtgttc
atcttcccccccaagcccaaggacatcctgagaatcaccagaaccccgagatcacctgc
gtggtgctggacctgggcagaggaccccgaggtgcagatcagctggttcgtggacggc
aaggaggtgcacaccgccaagacccagcccagagagcagcagttcaacagcacctacaga
gtggtgagcgtgctgcccatcgagcaccaggactggctgaccggcaaggagttcaagtgc
agagtgaaccacatcggcctgcccagccccatcgagagaaccatcagcaaggccagaggc
caggcccaccagcccagcgtgtacgtgctgccccccagccccaaggagctgagcagcagc
gacaccgtgaccctgacctgcctgatcaaggacttcttcccccccgagatcgacgtggag
tggcagagcaacggccagcccgagcccgagagcaagtaccacaccaccgcccccccagctg
gacgaggacggcagctacttcctgtacagcaagctgagcgtggacaagagcagatggcag
cagggcgacaccttcacctgcgccgtgatgcacgaggccctgcagaaccactacaccgac
ctgagcctgagccacagccccggcaag

4F9-VL3-cL-Kappa [SEQ ID NO: 38]

DIVMTQTPLSLSVSPGEPASMSCRASQSISNNLHWYRQKPGQSPQVLVKYASQSISGVPDRF
IGSGSGTDFTLRISRVEADDLGVYYCQQSNSWPQTFGQGTKLELKRNDAQPAVYLFQPSPDQ
LHTGSASVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDSKDSTYSLSSTLTMSSTEY
LSHELYSCEITHKSLPSTLIKSFQRSECQRVD

4F9-VL3-cL-Kappa [SEQ ID NO: 37]

gacatcgtgatgacccagacccccctgagcctgagcgtgagccccggcgagcccgccagc
atgagctgcagagccagccagagcatcagcaacaacctgcactggtacagacagaagccc

ggccagagcccccaggtgctggtgaagtacgccagccagagcatcagcggcgtgcccgac
agattcatcggcagcggcagcggcaccgacttcaccctgagaatcagcagagtggaggcc
gacgacctgggcgtgtactactgccagcagagcaacagctggccccagaccttcggccag
ggcaccaagctggagctgaagagaaacgacgcccagcccgccgtgtacctgttccagccc
agccccgaccagctgcacaccggcagcgccagcgtggtgtgcctgctgaacagcttctac
cccaaggacatcaacgtgaagtggaaggtggacggcgtgatccaggacaccggcatccag
gagagcgtgaccgagcaggacagcaaggacagcacctacagcctgagcagcaccctgacc
atgagcagcaccgagtacctgagccacgagctgtacagctgcgagatcacccacaagagc
ctgcccagcaccctgatcaagagcttccagagaagcgagtgccagagagtggac

5F12-VH3-cIgGA Fc [SEQ ID NO: 40]

EVQLVQSGGDLVKPGGSVRLSCVASFTFDYYMNWVRQAPGKGLQWIGRWIFPGSGATYYNER
FMGKATISADTAKNTAYMQLNSLRAEDTAVYYCLRSDWDVGDFWGQGTLVTVSSASTTAPSV
FPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLHSLSSMVT
VPSSRWPSETFTCNVVHPASNTKVDKPVFNECRCTDTPPCPVPEPLGGPSVLIFPPKPKDIL
RITRTPEVTCVVLDLGREDPEVQISWFVDGKEVHTAKTQSREQQFNGTYRVVSVLPIEHQDW
LTGKEFKCRVNHIDLPSPIERTISKARGRAHKPSVYVLPPSPKELSSSDTVSITCLIKDFYP
PDIDVEWQSNGQQEPERKHRMTPPQLDEDGSYFLYSKLSVDKSRWQQGDPFTCAVMHETLQN
HYTDLSLSHSPGK

5F12-VH3-cIgGA Fc [SEQ ID NO: 39]

gaggtgcagctggtgcagagcggcggcgacctggtgaagcccggcggcagcgtgagactg
agctgcgtggccagcttcaccttcgactactacatgaactgggtgagacaggccccggc
aagggcctgcagtggatcggcagatggatcttccccggcagcggcgccacctactacaac
gagagattcatgggcaaggccaccatcagcgccgacaccgccaagaacaccgcctacatg
cagctgaacagcctgagagccgaggacaccgccgtgtactactgcctgagaagcgactgg
gacgtgggcgacttctggggccagggcaccctggtgaccgtgagcagcgccagcaccacc
gcccccagcgtgttccccctggcccccagctgcggcagcaccagcggcagcaccgtggcc
ctggcctgcctggtgagcggctacttccccgagcccgtgaccgtgagctggaacagcggc
agcctgaccagcggcgtgcacaccttccccagcgtgctgcagagcagcggcctgcacagc
ctgagcagcatggtgaccgtgcccagcagcagatggcccagcgagaccttcacctgcaac
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accgacacccccccctgccccgtgcccgagcccctgggcggccccagcgtgctgatcttc
ccccccaagcccaaggacatcctgagaatcaccagaaccccccgaggtgacctgcgtggtg
ctggacctgggcagagaggaccccgaggtgcagatcagctggttcgtggacggcaaggag
gtgcacaccgccaagacccagagcagagagcagcagttcaacggcacctacagagtggtg
agcgtgctgcccatcgagcaccaggactggctgaccggcaaggagttcaagtgcagagtg
aaccacatcgacctgcccagccccatcgagagaaccatcagcaaggccagaggcagagcc
cacaagcccagcgtgtacgtgctgccccccagccccaaggagctgagcagcagcgacacc
gtgagcatcacctgcctgatcaaggacttctacccccccgacatcgacgtggagtggcag
agcaacggccagcaggagcccgagagaaagcacagaatgacccccccccagctggacgag
gacggcagctacttcctgtacagcaagctgagcgtggacaagagcagatggcagcagggc
gacccccttcacctgcgccgtgatgcacgagaccctgcagaaccactacaccgacctgagc
ctgagccacagccccggcaag

5F12-VH3-cIgGD Fc [SEQ ID NO: 42]

EVQLVQSGGDLVKPGGSVRLSCVASFTFDYYMNWVRQAPGKGLQWIGRWIFPGSGATYYNER
FMGKATISADTAKNTAYMQLNSLRAEDTAVYYCLRSDWDVGDFWGQGTLVTVSSASTTAPSV
FPLAPSCGSTSGSTVALACLVSGYFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSTVT
VPSSRWPSETFTCNVVHPASNTKVDKPVPKESTCKCISPCPVPESLGGPSVFIFPPKPKDIL
RITRTPEITCVVLDLGREDPEVQISWFVDGKEVHTAKTQPREQQFNSTYRVVSVLPIEHQDW
LTGKEFKCRVNHIGLPSPIERTISKARGQAHQPSVYVLPPSPKELSSSDTVTLTCLIKDFFP
PEIDVEWQSNGQPEPESKYHTTAPQLDEDGSYFLYSKLSVDKSRWQQGDTFTCAVMHEALQN
HYTDLSLSHSPGK

5F12-VH3-cIgGD Fc [SEQ ID NO: 41]

gaggtgcagctggtgcagagcggcggcgacctggtgaagcccggcggcagcgtgagactg
agctgcgtggccagcttcaccttcgactactacatgaactgggtgagacaggcccccggc
aagggcctgcagtggatcggcagatggatcttccccggcagcggcgccacctactacaac
gagagattcatgggcaaggccaccatcagcgccgacaccgccaagaacaccgcctacatg
cagctgaacagcctgagagccgaggacaccgccgtgtactactgcctgagaagcgactgg
gacgtgggcgacttctggggccagggcaccctggtgaccgtgagcagcgccagcaccacc
gcccccagcgtgttccccctggcccccagctgcggcagcaccagcggcagcaccgtggcc
ctggcctgcctggtgagcggctacttccccgagcccgtgaccgtgagctggaacagcggc
agcctgaccagcggcgtgcacaccttccccagcgtgctgcagagcagcggcctgtacagc
ctgagcagcaccgtgaccgtgcccagcagcagatggcccagcgagaccttcacctgcaac
gtggtgcaccccgccagcaacaccaaggtggacaagcccgtgcccaaggagagcacctgc
aagtgcatcagcccctgccccgtgcccgagagcctgggcggccccagcgtgttcatcttc
cccccaagcccaaggacatcctgagaatcaccagaaccccgagatcacctgcgtggtg
ctggacctgggcagaggaccccgaggtgcagatcagctggttcgtggacggcaaggag
gtgcacaccgccaagacccagcccagagagcagcagttcaacagcacctacagagtggtg
agcgtgctgcccatcgagcaccaggactggctgaccggcaaggagttcaagtgcagagtg
aaccacatcggcctgcccagccccatcgagagaaccatcagcaaggccagaggccaggcc
caccagcccagcgtgtacgtgctgcccccccagccccaaggagctgagcagcagcgacacc
gtgaccctgacctgcctgatcaaggacttcttcccccccgagatcgacgtggagtggcag
agcaacggccagcccgagcccgagagcaagtaccacaccaccgcccccccagctggacgag
gacggcagctacttcctgtacagcaagctgagcgtggacaagagcagatggcagcagggc
gacaccttcacctgcgccgtgatgcacgaggccctgcagaaccactacaccgacctgagc
ctgagccacagccccggcaag

5F12-VL3-cLKappa [SEQ ID NO: 44]

DIVMTQTPLSLSVSLGEPASISCRSSRSLLHTNGITYLSWYRQKPGQIPQLLIYQMSNLASG
VPDRFSGSGSGTDFTLRISRVEADDAGVYYCAQTLGLPRTFGQGTKVEIKRNDAQPAVYLFQ
PSPDQLHTGSASVVCLLNSFYPKDINVKWKVDGVIQDTGIQESVTEQDSKDSTYSLSSTLTM
SSTEYLSHELYSCEITHKSLPSTLIKSFQRSECQRVD

5F12-VL3-cLKappa [SEQ ID NO: 43]

gacatcgtgatgacccagacccccctgagcctgagcgtgagcctgggcgagcccgccagc
atcagctgcagaagcagcagaagcctgctgcacaccaacggcatcacctacctgagctgg
tacagacagaagcccggccagatcccccagctgctgatctaccagatgagcaacctggcc
agcggcgtgcccgacagattcagcggcagcggcagcggcaccgacttcaccctgagaatc
agcagagtggaggccgacgacgccggcgtgtactactgcgcccagaccctgggcctgccc
agaaccttcggccagggcaccaaggtggagatcaagagaaacgacgcccagcccgccgtg

tacctgttccagcccagccccgaccagctgcacaccggcagcgccagcgtggtgtgcctg
ctgaacagcttctaccccaaggacatcaacgtgaagtggaaggtggacggcgtgatccag
gacaccggcatccaggagagcgtgaccgagcaggacagcaaggacagcacctacagcctg
agcagcaccctgaccatgagcagcaccgagtacctgagccacgagctgtacagctgcgag
atcacccacaagagcctgcccagcaccctgatcaagagcttccagagaagcgagtgccag
agagtggac

Variable regions of mouse anti-canine PDL-1:4F9

Heavy chain: DNA sequence [SEQ ID NO: 73]

gaagtgcagctggtggagtctgggggaggcttagtgaagcctggagggtccctgaaactctc
ctgtgcagcctctggattcactttcagtagctatgccatgtcttgggttcgccagactccgg
acaagagactggagtgggtcgcaaccattagtgatggtggaagttacacccactaccccgac
aatttaatgggccgattcaccatctccagagacaatgccaagaacaacctgtacctgcaaat
gagccatctgaagtctgacgacacagccatgtattactgtgcacgagagagctatgatggtt
actacgtggctaactggggccaagggactctggtcactgtctcagca

Heavy chain: Amino acid sequence [SEQ ID NO: 74]

EVQLVESGGGLVKPGGSLKLSCAASGFTFSSYAMSWVRQTPDKRLEWVATISDGGSYTHYPD
NLMGRFTISRDNAKNNLYLQMSHLKSDDTAMYYCARESYDGYYVANWGQGTLVTVSA

Light chain: DNA sequence [SEQ ID NO: 75]

gatattgtgctaactcagtctccagccaccctgtctgtgaatccaggagatagcgtcagtct
ttcctgcagggccagccaaagtattagcaacaacctacactggtatcaacaaaaatcacatg
agtctccaaggcttctcatcaagtatgcttcccagtccatctctgggatcccctccaggttc
agtggcagtggatcagggacagatttcactctcagtatcaacagtgtggagactgaagattt
tggaatgtatttctgtcaacagagtaacagctggcctcagacgttcggtggaggcaccaagc
tggaaatcaaa

Light chain: Amino acid sequence [SEQ ID NO: 76]

DIVLTQSPATLSVNPGDSVSLSCRASQSISNNLHWYQQKSHESPRLLIKYASQSISGIPSRF
SGSGSGTDFTLSINSVETEDFGMYFCQQSNSWPQTFGGGTKLEIK

Variable regions of mouse anti-canine PDL-1: Anti-PDL-1 5F12

Heavy chain: DNA sequence [SEQ ID NO: 77]

caggtccagctacagcagtctggacctgagctggtgaagcctggggcttcagtgaagatatc
ctgcaaggcttctggctacaccttcactgactactatatgaattgggtgaaacagaggcctg
gacagggacttgagtggattggatggatttttcccggaagtggtgctacttactacaatgag
aggttcatgggcaaggccacacttactgtggataaatcttccaacacagcctacatgttgtt
cagtagcctgacctctgaggactctgcggtctatttctgtttaagatctgactgggacgtcg
gggacttctggggccaaggcaccactctcacagtctcctca

Heavy chain: Amino acid sequence [SEQ ID NO: 78]

QVQLQQSGPELVKPGASVKISCKASGYTFTDYYMNWVKQRPGQGLEWIGWIFPGSGATYYNE
RFMGKATLTVDKSSNTAYMLFSSLTSEDSAVYFCLRSDWDVGDFWGQGTTLTVSS

Light chain: DNA sequence [SEQ ID NO: 79]

gatattgtgatgacgcaggctgcattctccaatccagtcactcttggaacatcagcttccat
ctcctgcaggtctagtaggagtctcctacatactaatggcatcacttatttgtcttggtttc

tgcagaagccaggccagtctcctcagctcctgatttatcagatgtccaaccttgcctcagga
gtcccagacaggttcagtagcagtgggtcaggaactgatttcacactgagaatcagtagagt
ggaggctgaggatgtgggtatttattactgtgctcaaactctaggacttcctcggacgttcg
gtggaggcaccaagctggaaatcaaa

Light chain: Amino acid sequence [SEQ ID NO: 80]

DIVMTQAAFSNPVTLGTSASISCRSSRSLLHTNGITYLSWFLQKPGQSPQLLIYQMSNLASG
VPDRFSSSGSGTDFTLRISRVEAEDVGIYYCAQTLGLPRTFGGGTKLEIK

EXAMPLE 5

EPITOPE MAPPING OF ANTI-CANINE PD-L1 ANTIBODIES

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 PD-L1 can also elucidate unique cancer 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.

Mapping of PD-L1 epitopes using Mass spectrometry:

A method based on chemical crosslinking and mass spectroscopy detection was employed to identify the epitopes recognized by anti-canine PD-L1 mAbs [CovalX® Instrument Incorporated]. The application of this technology to epitope mapping of canine PD-L1 resulted in identification of epitopes recognized by the indicated mAbs that are listed in Table 13. The results of epitope mapping of canine PD-L1 with mAb 4F9 shows that this mAb recognizes an epitope located in the extracellular domain of canine PD-L1 comprised of the amino acid sequence represented by SEQ ID NO: 82 and the amino acid sequence represented by SEQ ID NO: 83 [*see*, Table 13]. Moreover, the results of epitope mapping experiment with mAb 5F12 shows that this mAb recognizes an epitope located in the extracellular domain of canine PD-L1 comprised of the amino acid sequence represented by SEQ ID NO: 82. Notably, the location of the epitopes on canine PD-L1 identified herein are reasonably consistent with those recently reported for the corresponding human PD-L1, [Hao *et al.*, *J. Mol. Recognit.* 28:269-276 (2015)] which provides further confidence for the determination provided above.

Table 13
PD-L1 EPITOPES RECOGNIZED BY ANTI-CANINE PD-L1 MABS

ANTIBODY DESIGNATION	SEQ ID NO.	PEPTIDE SEQUENCE
4F9	82	LNLFALIVYWEMEDKKIIQF
4F9	83	KRITLKVHAPY
5F12	82	LNLFALIVYWEMEDKKIIQF

All references cited herein are incorporated by reference to the same extent as if each individual publication, database entry (*e.g.*, Genbank sequences or GeneID entries), patent application, or patent, was specifically and individually indicated to be incorporated by reference. This statement of incorporation by reference is intended by Applicants, pursuant to 37 C.F.R. §1.57(b)(1), to relate to each and every individual publication, database entry (*e.g.*, Genbank sequences or GeneID entries), patent application, or patent, each of which is clearly identified in compliance with 37 C.F.R. §1.57(b)(2), even if such citation is not immediately adjacent to a dedicated statement of incorporation by reference. The inclusion of dedicated statements of incorporation by reference, if any, within the specification does not in any way weaken this general statement of incorporation by reference. Citation of the references herein is not intended as an admission that the reference is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

We Claim:

1. An isolated antibody or antigen binding fragment thereof that binds canine Programmed Death Ligand 1 (canine PD-L1) with specificity comprising three light chain complementary determining regions (CDRs): CDR light 1 (CDRL1), CDR light 2 (CDRL2), and CDR light 3 (CDRL3); and three heavy chain CDRs: CDR heavy 1 (CDRH1), CDR heavy 2 (CDRH2) and CDR heavy 3 (CDRH3):

(a) wherein CDRH1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 13, a conservatively modified variant of SEQ ID NO: 13, a variant of SEQ ID NO: 13 that comprises the canonical structure class of 1, SEQ ID NO: 19, a conservatively modified variant of SEQ ID NO: 19, and a variant of SEQ ID NO: 19 that comprises the canonical structure class of 1;

(b) wherein CDRH2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 14, a conservatively modified variant of SEQ ID NO: 14, a variant of SEQ ID NO: 14 that comprises the canonical structure class of 3B, SEQ ID NO: 20, a conservatively modified variant of SEQ ID NO: 20, and a variant of SEQ ID NO: 20 that comprises the canonical structure class of 3B;

(c) wherein CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 15, a conservatively modified variant of SEQ ID NO: 15, a variant of SEQ ID NO: 15 that comprises the canonical structure class of 10, SEQ ID NO: 21, a conservatively modified variant of SEQ ID NO: 21, and a variant of SEQ ID NO: 21 that comprises the canonical structure class of 8;

(d) wherein CDRL1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 16, a conservatively modified variant of SEQ ID NO: 16, a variant of SEQ ID NO: 16 that comprises the canonical structure class of 2, SEQ ID NO: 22, a conservatively modified variant of SEQ ID NO: 22, and a variant of SEQ ID NO: 22 that comprises the canonical structure class of 3;

(e) wherein CDRL2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 17, a conservatively modified variant of SEQ ID NO: 17, a variant of SEQ ID NO: 17 that comprises the canonical structure class of 1, SEQ ID NO: 23, a conservatively modified variant of SEQ ID NO: 23, and a variant of SEQ ID NO: 23 that comprises the canonical structure class of 1;

(f) wherein CDRL3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 18, a conservatively modified variant of SEQ ID NO: 18, a variant of SEQ ID NO: 18 that comprises the canonical structure class of 1, SEQ ID NO: 24, a conservatively modified variant of SEQ ID NO: 24, and a variant of SEQ ID NO: 24 that comprises the canonical structure class of 1; and

wherein the antibody and antigen binding fragment thereof bind canine PD-L1 and block the binding of canine PD-L1 to canine Programmed Death 1 (PD-1).

2. The isolated antibody or antigen binding fragment of Claim 1,

(a) wherein CDRH1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 13, a conservatively modified variant of SEQ ID NO: 13, and a variant of SEQ ID NO: 13 that comprises the canonical structure class of 1;

(b) wherein CDRH2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 14, a conservatively modified variant of SEQ ID NO: 14, and a variant of SEQ ID NO: 14 that comprises the canonical structure class of 3B;

(c) wherein CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 15, a conservatively modified variant of SEQ ID NO: 15, and a variant of SEQ ID NO: 15 that comprises the canonical structure class of 10;

(d) wherein CDRL1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 16, a conservatively modified variant of SEQ ID NO: 16, and a variant of SEQ ID NO: 16 that comprises the canonical structure class of 2;

(e) wherein CDRL2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 17, a conservatively modified variant of SEQ ID NO: 17, and a variant of SEQ ID NO: 17 that comprises the canonical structure class of 1;

(f) wherein CDRL3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 18, a conservatively modified variant of SEQ ID NO: 18, and a variant of SEQ ID NO: 18 that comprises the canonical structure class of 1; and

wherein the antibody and antigen binding fragment thereof bind canine PD-L1 and block the binding of canine PD-L1 to canine Programmed Death 1 (PD-1).

3. The isolated antibody or antigen binding fragment of Claim 1,

(a) wherein CDRH1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 19, a conservatively modified variant of SEQ ID NO: 19, and a variant of SEQ ID NO: 19 that comprises the canonical structure class of 1;

(b) wherein CDRH2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 20, a conservatively modified variant of SEQ ID NO: 20, and a variant of SEQ ID NO: 20 that comprises the canonical structure class of 3B;

(c) wherein CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 21, a conservatively modified variant of SEQ ID NO: 21, and a variant of SEQ ID NO: 21 that comprises the canonical structure class of 8;

(d) wherein CDRL1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 22, a conservatively modified variant of SEQ ID NO: 22, and a variant of SEQ ID NO: 22 that comprises the canonical structure class of 3;

(e) wherein CDRL2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 23, a conservatively modified variant of SEQ ID NO: 23, and a variant of SEQ ID NO: 23 that comprises the canonical structure class of 1;

(f) wherein CDRL3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 24, a conservatively modified variant of SEQ ID NO: 24, and a variant of SEQ ID NO: 24 that comprises the canonical structure class of 1; and

wherein the antibody and antigen binding fragment thereof bind canine PD-L1 and block the binding of canine PD-L1 to canine Programmed Death 1 (PD-1).

4. The isolated antibody of Claims 1, 2, or 3 wherein the antibody is a murine antibody.

5. The isolated antibody of Claims 1, 2, 3, or 4 wherein the antibody is a caninized antibody.

6. The caninized antibody or antigen binding fragment thereof of Claim 5 that comprises a canine fragment crystallizable region (cFc region) that comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 66 and SEQ ID NO: 68; wherein one to seven amino acid residues are substituted for at the indicated positions selected from the group consisting of P4, D31, N63, G64, T65, A93, and P95.

7. The caninized antibody of Claim 6 wherein the substitutions of the one to seven amino acid residues that are substituted for are selected from the group consisting of P4A, D31A, N63A, G64A, T65A, A93G, and P95A.

8. The caninized antibody or antigen binding fragment thereof of Claim 5 that comprises a canine fragment crystallizable region (cFc region) that comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 62 and SEQ ID NO: 64.

9. The caninized antibody or antigen binding fragment thereof of Claim 6, 7, or 8, that comprises a hinge region that comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48.

10. The caninized antibody or antigen binding fragment thereof of Claim 5, that comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, and SEQ ID NO: 32; wherein the caninized antibody comprises a cFc region comprising the amino acid sequence of SEQ ID NO: 66 or SEQ ID NO: 68 in which at the indicated positions of the cFc region at least one of seven amino acid residues selected from the group consisting of P4, D31, N63, G64, T65, A93, and P95 of SEQ ID NO: 66 or SEQ ID NO: 68 is replaced by a different amino acid residue.

11. The caninized antibody or antigen binding fragment thereof of Claim 5, that comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, and SEQ ID NO: 44.

12. The caninized antibody or antigen binding fragment thereof of Claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 further comprising a canine light chain that comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 38 and SEQ ID NO: 44.

13. An isolated antibody or antigen binding fragment thereof that binds canine Programmed Death Ligand 1 (canine PD-L1) with specificity, wherein when bound to canine PD-L1, said antibody binds to at least one amino acid residue within the amino acid sequence of SEQ ID NO: 82 or SEQ ID NO: 83, or to at least one amino acid residue within the amino

acid sequence of SEQ ID NO: 82 and to at least one amino acid residue within the amino acid sequence of SEQ ID NO: 83; wherein the antibody and antigen binding fragment thereof binds canine PD-L1 and blocks the binding of canine PD-L1 to canine Programmed Death Receptor 1 (PD-1).

14. The isolated antibody or antigen binding fragment thereof of Claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12, wherein when bound to canine PD-L1, said antibody binds to at least one amino acid residue within the amino acid sequence of SEQ ID NO: 82 or SEQ ID NO: 83; wherein the antibody and antigen binding fragment thereof binds canine PD-L1 and blocks the binding of canine PD-L1 to canine PD-1.

15. The isolated antibody or antigen binding fragment thereof of Claims 13 or 14, wherein when bound to canine PD-L1 said antibody binds to at least one amino acid residue within SEQ ID NO: 82 and to at least one amino acid residue within SEQ ID NO: 83.

16. A monoclonal antibody or antigen binding fragment thereof that cross-competes for binding with canine PD-L1 with one or more antibody of Claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15;

wherein the antibody and antigen binding fragment thereof binds canine PD-L1 and blocks the binding of canine PD-L1 to canine PD-1.

17. The monoclonal antibody of Claim 13, 14, 15, or 16 that is a murine antibody, a caninized antibody, or a caninized murine antibody.

18. The antibody or antigen binding fragment thereof of Claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 11, 12, 13, 14, 15, 16, or 17 which exhibits one, two, three, four, five, six, or all of the following properties:

(i) binding to canine PD-L1 with a dissociation constant (Kd) of 1 X 10^{-5} M to 1 X 10^{-12} M;

(ii) binding to canine PD-L1 with an on rate (k_{on}) of 1 X 10^{2} $M^{-1}s^{-1}$ to 1 X 10^{7} $M^{-1}s^{-1}$;

(iii) binding to canine PD-L1 with an off rate (k_{off}) of 1 X 10^{-3} s^{-1}

to 1 X 10^{-8} s^{-1};

(iv) cross-competing for binding with canine PD-L1 with one or more antibody of Claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15

(v) stimulating antigen-specific memory responses to a tumor or pathogen;

(vi) stimulating antibody response *in vivo*; and

(vii) stimulating an immune response in an animal subject.

19. An isolated nucleic acid that encodes the light chain of the antibody of Claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18.

20. An isolated nucleic acid that encodes the heavy chain of the antibody of Claims Claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18.

21. An isolated nucleic acid of Claims 19 or 20 that encodes one or more amino acid sequences selected from the group consisting of SEQ ID NOs: 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, and 24.

22. An expression vector comprising the nucleic acid of Claim 19, 20, or 21.

23. A host cell comprising one or more expression vectors of Claim 22.

24. An isolated antigenic peptide consisting of 60 or fewer amino acid residues that comprises the amino acid sequence of SEQ ID NO: 82 or SEQ ID NO: 83.

25. An isolated antigenic peptide consisting of 60 or fewer amino acid residues that comprises an amino acid sequence that is 95% identical with SEQ ID NO: 82 or SEQ ID NO: 83 and binds to the isolated antibody or antigen binding fragment thereof of Claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18.

26. A fusion protein that comprises the antigenic peptide of Claims 24 or 25.

27. The fusion protein of Claim 26 that also comprises an Fc region of a non-canine mammalian IgG antibody.

28. The fusion protein of Claim 27 wherein the non-canine mammalian IgG antibody is from a source selected from the group consisting of murine, human, equine, porcine, and bovine.

29. The fusion protein of Claim 27 or 28, wherein the non-canine mammalian IgG antibody is selected from the group consisting of IgG1, IgG2a, IgG3, and IgG4.

30. An isolated nucleic acid that encodes the antigenic peptide of Claims 24 or 25, or the fusion protein of Claims 26, 27, 28, or 29.

31. An expression vector comprising the isolated nucleic acid of Claim 30.

32. A pharmaceutical composition comprising the antibody of Claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18, or the antigenic peptide of Claims 24 or 25, or the fusion protein of Claims 26, 27, 28, or 29 or the nucleic acid of Claims 19, 20, 21, or 31, the expression vector of Claims 22 or 31, or any combination thereof, and a pharmaceutically acceptable carrier or diluent.

33. The pharmaceutical composition of Claim 32 further comprising a caninized antibody or fragment thereof selected from the group consisting of a caninized anti-canine PD-1 antibody, an antigen binding fragment of a caninized anti-canine PD-1 antibody, a caninized anti-canine cytotoxic T-lymphocyte associated protein-4 (CTLA-4) antibody, a caninized antigen binding fragment of an anti-canine CTLA-4 antibody, or any combination thereof.

34. A method of increasing the activity of an immune cell, comprising administering to a subject in need thereof a therapeutically effective amount of the pharmaceutical composition of Claims 32 or 33.

35. The method of claim 34, wherein said method is used for:

(i) the treatment of cancer;

(ii) the treatment of an infection or infectious disease;

(iii) as a vaccine adjuvant; or

(iv) any combination thereof.

Figure 1

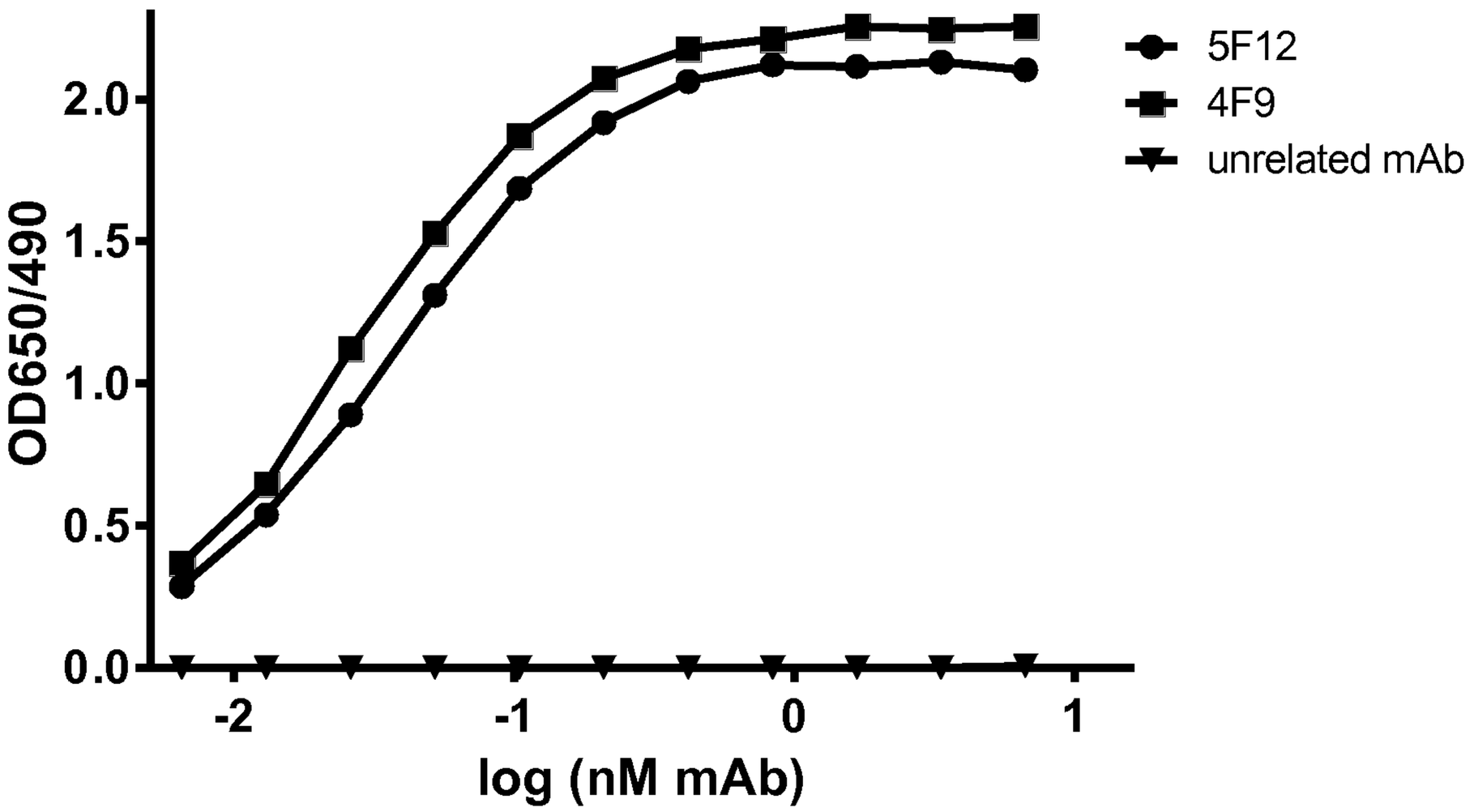


Figure 1 shows the ELISA results for reactivity of two mouse anti-canine PDL-1 mAbs against canine PDL-1, as a function of OD 650/490 versus the log mAb (nM). Both mAbs, designated 4F9 and 5F12, demonstrate strong and dose-dependent binding to canine PDL-1.

Figure 2

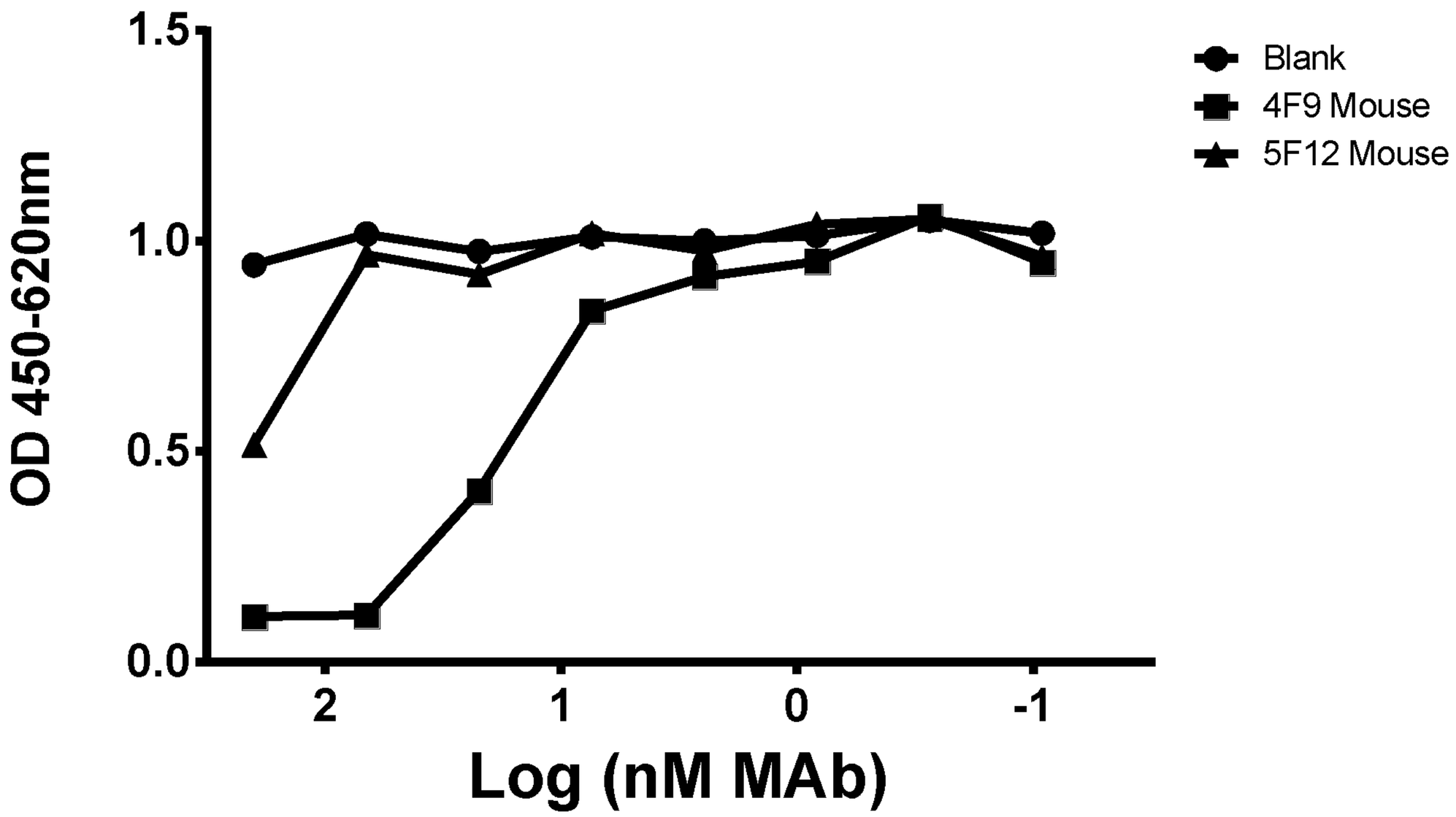
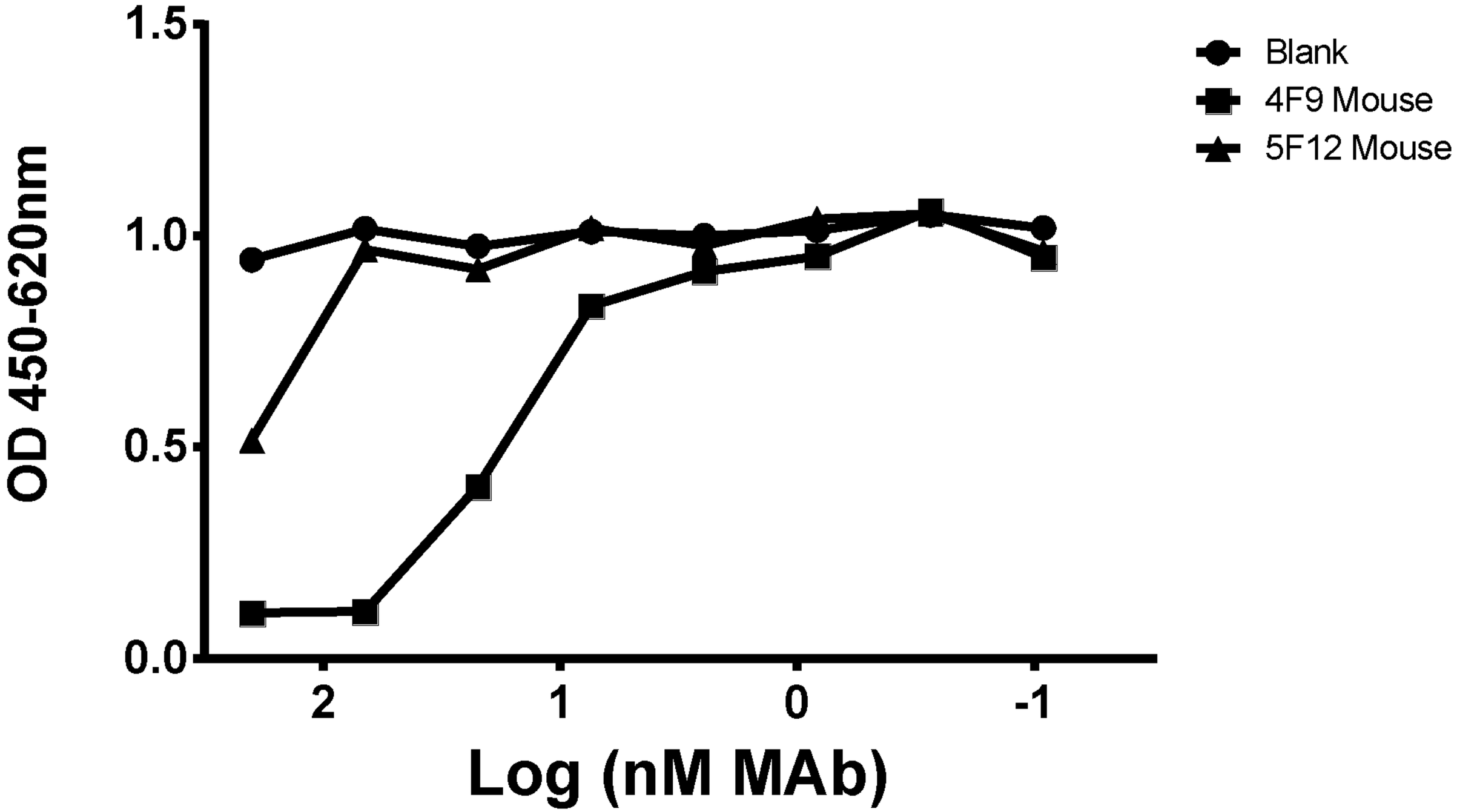


Figure 2 shows ligand blockade with mouse anti-canine PDL-1 mAbs. Two mAbs designated 4F9 and 5F12 were tested for their ability to inhibit binding of PDL-1 to PD-1 expressed on CHO cells. Both mAbs blocked the binding of PDL-1 to PD-1, although mAb 4F9 is a stronger inhibitor than 5F12.

Figure 2



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