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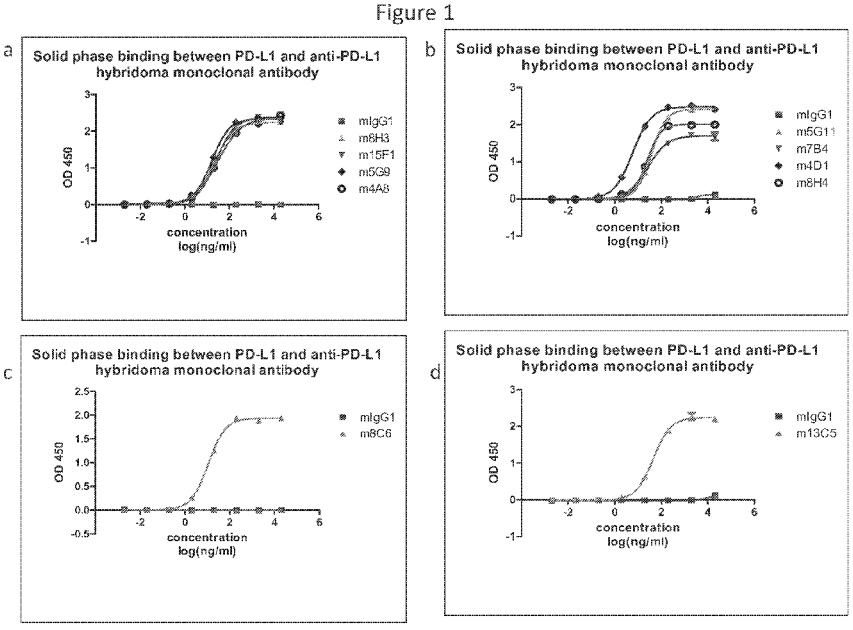
(54) **Title**: ANTI-PD-L1 ANTIBODIES

Figure 1



(57) **Abstract**: The present disclosure relates to antibodies and antigen-binding fragments thereof that bind to PD-L1, and to methods of using such antibodies and antigen-binding fragments. For example, the present invention provides humanized anti-PD-L1 antibodies and methods of use thereof.

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ANTI-PD-L1 ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to International Application No. PCT/CN2014/083715, filed August 5, 2014, which is incorporated herein by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates to antibodies and antigen-binding fragments thereof that bind to PD-L1, and to methods of using such antibodies and antigen-binding fragments.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0003] The content of the text file submitted electronically herewith is incorporated herein by reference in its entirety: A computer readable format copy of the Sequence Listing (filename: CRBI_007_01WO_SeqList_ST25.txt); date recorded: August 4, 2015; file size 153 KB).

BACKGROUND

[0004] Programmed death receptor Ligand 1 (PD-L1) is a ligand of programmed death receptor 1 (PD-1). PD-1 is primarily expressed on lymphocytes and has two ligands, PD-L1 and PD-L2. PD-L2 is not as common as PD-L1. PD-L1 is also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1) and is a 40kDa type 1 transmembrane protein which is encoded by the CD274 gene. Both PD-L1 and PD-1 belong to immunoglobulin superfamily and consist of two extracellular Ig domains, an N-terminal V domain, and a C-terminal constant domain. The binding interface of PD-L1 to programmed death 1 (PD-1) and B7-1 (CD80) is on the IgV-like domain (Lin et al. (2008) PNAS 105:3011-3016). While PD-L1 contains a conserved short intracellular tail (about 30 amino acids), PD-1 contains two cytoplasmic tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T cell stimulation, PD-1 recruits the tyrosine phosphatase SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 Zeta, PKC theta and ZAP70 that are involved in the CD3 T cell signaling cascade (Freeman et al. (2000) J Exp Med 192:1027-34; Latchman, et. al. (2001) Nat Immunol 2:261-8; Carter et al. (2002) Eur J Immunol 32:634-43).

[0005] PD-L1 is not only widely distributed on leukocytes and nonhematopoietic cells in lymphoid and nonlymphoid tissues, but also in various cancer cells. Clinical data suggest that high tumor expression of PD-L1 is associated with increased tumor aggressiveness and poorer prognosis. The formation of PD-1/PD-L1 complex transmits an inhibitory signal and negatively regulates T cell immune responses; it inhibits TCR-mediated T cell activation, cytokine production and T cell proliferation (Fife et al. (2011) Nature Immunology 10:1185-1193); induces exhaustion or anergy among cognate antigen-specific T cells (Hofmeyer et al. (2011) Journal of Biomedicine and Biotechnology 2011:1-9); promotes the differentiation of Th1 cells into Foxp3+ regulatory T cells (Armanath et al. (2011) Science TransMed 3:1-13; Francisco et al. (2009) J. Exp. Med. 206:3015-3029); and induces apoptosis of effctor T cells. Disruption of the PD-L1 gene leads to up-regulated T cell responses and the generation of self-reactive T cells (Latchman et al. (2004) PNAS 101:10691–10696). Antibody blockade of either PD-1or PD-L1 leads to increased antitumor immunity (Iwai et al. (2002) PNAS 99:12293–12297).

[0006] Thus, there is an important role for the PD-1/PD-L1 pathway in controlling immune responses. Dysfunction of PD-1/PD-L1 signaling appears to be correlated with initiation and development of diseases such as cancer and viral infection. Analysis of knockout animals has led to the understanding that PD-1/PD-L1 functions mainly in inducing and regulating peripheral tolerance. Thus, therapeutic blockade of the PD-1/PD-L1 pathway would be helpful in overcoming immune tolerance and in the treatment of cancer or infection as well as in boosting immunity during vaccination (either prophylactic or therapeutic). There is a need in the art for improved methods for blocking the PD-1/PD-L1 pathway.

SUMMARY OF THE INVENTION

[0007] In one aspect, the present invention provides antibodies and antigen-binding fragments thereof that bind to programmed death-1 ligand 1(PD-L1). In some embodiments, the antibodies and antigen-binding fragments thereof bind to human PD-L1. In some embodiments, the antibodies and antigen-binding fragments thereof bind to PD-L1 and block binding of PD-1 and/or CD80 to PD-L1. In further embodiments, the anti-PD-L1 antibodies and fragments thereof bind to PD-L1 and disrupt the PD-L1/PD-1 or PD-L1/CD80 pathway. In one embodiment, the antibody or fragment thereof is a murine antibody, a chimeric antibody, a human antibody or a humanized antibody. In one embodiment, the anti-PD-L1 antibody or

fragment thereof is a monoclonal antibody, scFv, Fab fragment, Fab' fragment, F(ab)' fragment, bispecific antibody, immunoconjugate, or a combination thereof

[0008] In one embodiment, the present invention provides an isolated antibody or fragment thereof comprising one or more CDRs selected from the group consisting of SEQ ID NOs: 81-140.

[0009] In one embodiment, the antibody or fragment thereof comprises a heavy chain CDR1 sequence having at least 80% homology, at least 81% homology, at least 82% homology, at least 83% homology, at least 84% homology, at least 85% homology, at least 86% homology, at least 87% homology, at least 88% homology, at least 89% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 81, 87, 93, 99, 105, 111, 117, 123, 129, and 135.

[0010] In one embodiment, the antibody or fragment thereof comprises a heavy chain CDR2 sequence having at least 80% homology, at least 81% homology, at least 82% homology, at least 83% homology, at least 84% homology, at least 85% homology, at least 86% homology, at least 87% homology, at least 88% homology, at least 89% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 82, 88, 94, 100, 106, 112, 118, 124, 130, and 136.

[0011] In one embodiment, the antibody or fragment thereof comprises a heavy chain CDR3 sequence having at least 80% homology, at least 81% homology, at least 82% homology, at least 83% homology, at least 84% homology, at least 85% homology, at least 86% homology, at least 87% homology, at least 88% homology, at least 89% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 83, 89, 95, 101, 107, 113, 119, 125, 131, and 137.

[0012] In one embodiment, the antibody or fragment thereof comprises a light chain CDR1 sequence having at least 80% homology, at least 81% homology, at least 82% homology, at least

83% homology, at least 84% homology, at least 85% homology, at least 86% homology, at least 87% homology, at least 88% homology, at least 89% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 84, 90, 96, 102, 108, 114, 120, 126, 132, and 138.

[0013] In one embodiment, the antibody or fragment thereof comprises a light chain CDR2 sequence having at least 80% homology, at least 81% homology, at least 82% homology, at least 83% homology, at least 84% homology, at least 85% homology, at least 86% homology, at least 87% homology, at least 88% homology, at least 89% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 85, 91, 97, 103, 109, 115, 121, 127, 133, and 139.

[0014] In one embodiment, the antibody or fragment thereof comprises a light chain CDR3 sequence having at least 80% homology, at least 81% homology, at least 82% homology, at least 83% homology, at least 84% homology, at least 85% homology, at least 86% homology, at least 87% homology, at least 88% homology, at least 89% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 86, 92, 98, 104, 110, 116, 122, 128, 134, and 140.

[0015] In one embodiment, the antibody or fragment thereof comprises a heavy chain CDR1 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 81, 87, 93, 99, 105, 111, 117, 123, 129, and 135; a heavy chain CDR2 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 82, 88, 94, 100, 106, 112, 118, 124, 130, and 136; a heavy chain CDR3 consisting of an amino acid sequences selected from the group consisting of SEQ ID NOs: 83, 89, 95, 101, 107, 113, 119, 125, 131, and 137; a light chain CDR1 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 84, 90, 96, 102, 108, 114, 120, 126, 132, and 138; a light chain CDR2 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 85, 91, 97, 103, 109, 115, 121,

127, 133, and 139 and a light chain CDR3 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 86, 92, 98, 104, 110, 116, 122, 128, 134, and 140.

[0016] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 81, 82, and 83, respectively; and a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 84, 85, and 86, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 81, 82, and 83, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 84, 85, and 86, respectively.

[0017] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 87, 88, and 89, respectively; and a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 90, 91, and 92, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 87, 88, and 89, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 90, 91, and 92, respectively.

[0018] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 93, 94, and 95, respectively; and a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 96, 97, and 98, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 93, 94, and 95, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 96, 97, and 98, respectively.

[0019] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 99, 100, and 101, respectively; and a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 102, 103, and 104, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 99, 100, and 101, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 102, 103, and 104, respectively.

[0020] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92%

homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 105, 106, and 107, respectively; and a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 108, 109, and 110, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 105, 106, and 107, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 108, 109, and 110, respectively.

[0021] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 111, 112, and 113, respectively; and a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 114, 115, and 116, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 111, 112, and 113, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 114, 115, and 116, respectively.

[0022] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 117, 118, and 119, respectively; and a light

chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 120, 121, and 122, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 117, 118, and 119, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 120, 121, and 122, respectively.

[0023] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 123, 124, and 125, respectively; and a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 126, 127, and 128, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 123, 124, and 125, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 126, 127, and 128, respectively.

[0024] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 129, 130, and 131, respectively; and a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96%

homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 132, 133, and 134, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 129, 130, and 131, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 132, 133, and 134, respectively.

[0025] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 135, 136, and 137, respectively; and a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence according to SEQ ID NOs: 138, 139, and 140, respectively. In a further embodiment, the antibody or antibody fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 135, 136, and 137, respectively, and a light chain CDR1, CDR2, and CDR3 according to SEQ ID NOs: 138, 139, and 140, respectively.

[0026] In one embodiment, the antibody or fragment thereof binds PD-L1 and comprises a heavy chain variable region comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, and 46; and a light chain variable region comprising an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48. In a further embodiment, the isolated antibody or fragment thereof binds

PD-L1 and comprises a heavy chain variable region comprising, consisting essentially of, or consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, and 46; and a light chain variable region comprising, consisting essentially of, or consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48.

[0027] In one embodiment, the invention provides anti-PD-L1 antibodies that comprise a variable heavy chain of an antibody selected from the group consisting of 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and 15F1 and a variable light chain of an antibody selected from the group consisting of 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and 15F1. Thus, in one embodiment, the invention provides an antibody or fragment thereof comprising a heavy chain variable region comprising SEQ ID NO: 2 and a light chain variable region comprising SEQ ID NO: 4; a heavy chain variable region comprising SEQ ID NO: 6 and a light chain variable region comprising SEQ ID NO: 8; a heavy chain variable region comprising SEQ ID NO: 10 and a light chain variable region comprising SEQ ID NO: 12; a heavy chain variable region comprising SEQ ID NO: 14 and a light chain variable region comprising SEQ ID NO: 16; a heavy chain variable region comprising SEQ ID NO: 18 and a light chain variable region comprising SEQ ID NO: 20; a heavy chain variable region comprising SEQ ID NO: 22 and a light chain variable region comprising SEQ ID NO: 24; a heavy chain variable region comprising SEQ ID NO: 26 and a light chain variable region comprising SEQ ID NO: 28; a heavy chain variable region comprising SEQ ID NO: 30 and a light chain variable region comprising SEQ ID NO: 32; a heavy chain variable region comprising SEQ ID NO: 34 and a light chain variable region comprising SEQ ID NO: 36; or a heavy chain variable region comprising SEQ ID NO: 38 and a light chain variable region comprising SEQ ID NO: 40.

[0028] In one embodiment, the present invention provides a chimeric anti-PD-L1 antibody, wherein the antibody comprises a heavy chain having an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 54, 58, 60, 64, and 66; and a light chain having an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93%

homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 52, 56, 62 and 68.

[0029] In one embodiment, the present invention provides a humanized anti-PD-L1 antibody, wherein the antibody comprises a heavy chain variable region having an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 42 and 46. In another embodiment, the present invention provides a humanized anti-PD-L1 antibody, wherein the antibody comprises a light chain variable region having an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 44 and 48.

[0030] In another embodiment, the present invention provides a humanized anti-PD-L1 antibody, wherein the antibody comprises a heavy chain variable region having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to SEQ ID NO: 42 and a light chain variable region having least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to SEQ ID NO: 44. In another embodiment, the present invention provides a humanized anti-PD-L1 antibody, wherein the antibody comprises a heavy chain variable region having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to SEQ ID NO: 46 and a light chain variable region having least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at

least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to SEQ ID NO: 48.

[0031] In one embodiment, the present invention provides a humanized anti-PD-L1 antibody, wherein the antibody comprises a full heavy chain having an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 70, 72, 76, and 78. In another embodiment, the present invention provides a humanized anti-PD-L1 antibody, wherein the antibody comprises a full light chain having an amino acid sequence having at least 80% homology, at least 85% homology, at least 90% homology, at least 91% homology, at least 92% homology, at least 93% homology, at least 94% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, or at least 99% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 74 and 80.

[0032] In one embodiment, the present invention provides a humanized anti-PD-L1 antibody, wherein the antibody comprises a heavy chain according to SEQ ID NO: 70 and a light chain according to SEQ ID NO: 74. In another embodiment, the present invention provides a humanized anti-PD-L1 antibody, wherein the antibody comprises a heavy chain according to SEQ ID NO: 72 and a light chain according to SEQ ID NO: 74. In another embodiment, the present invention provides a humanized anti-PD-L1 antibody, wherein the antibody comprises a heavy chain according to SEQ ID NO: 76 and a light chain according to SEQ ID NO: 80. In another embodiment, the present invention provides a humanized anti-PD-L1 antibody, wherein the antibody comprises a heavy chain according to SEQ ID NO: 78 and a light chain according to SEQ ID NO: 80.

[0033] In one embodiment, the present invention provides anti-PD-L1 antibodies or fragments thereof that bind to the same epitope on PD-L1 as any of the exemplary antibodies provided herein. In one embodiment, the antibodies or fragments thereof compete with any of the exemplary antibodies provided herein for binding to PD-L1. Binding to PD-L1 may be measured by ELISA, flow cytometry, surface plasmon resonance (SPR) assay, or any other method known in the art.

[0034] In one embodiment, the present invention provides anti-PD-L1 antibodies and fragments thereof that bind to PD-L1 with a binding affinity kD of about 10 nM to about 0.01 nM. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of from about 10 nM to about 0.05 nM. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of from about 8 nM to about 0.1 nM. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of from about 5nM to about 0.2 nM. In another embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 10 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 6nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 4nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 2 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 1nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 0.75 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 0.5 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 0.25 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 0.2 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 0.15 nM or less. In a further embodiment, the anti- PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 0.1 nM or less. In a further embodiment, the anti- PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 0.075 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 0.05 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 0.025 nM or less. In a

further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 0.02 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 0.015 nM or less. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1 with a binding affinity kD of about 0.01 nM or less. In one embodiment, the binding affinity kD of the anti-PD-L1 antibodies and fragments provided herein is measured by Biacore assay.

[0035] In one embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC50 for PD-L1 of about 1 ng/mL to about 2000 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC50 for PD-L1 of about 1 ng/mL to about 1500 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC50 for PD-L1 of about 1 ng/mL to about 1000 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC50 for PD-L1 of about 2 ng/mL to about 500 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC50 for PD-L1 of about 2 ng/mL to about 250 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC50 for PD-L1 of about 5 ng/mL to about 200 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC50 for PD-L1 of about 5 ng/mL to about 50 ng/mL. In one embodiment, the anti PD-L1 antibodies and fragments thereof provided herein have a binding EC50 for PD-L1 of about 500 ng/mL or less, about 400 ng/mL or less, about 300 ng/mL or less, about 250 ng/mL or less, about 200 ng/mL or less, about 150 ng/mL or less, about 100 ng/mL or less, about 75 ng/mL or less, about 60 ng/mL or less, about 50 ng/mL or less, about 40 ng/mL or less, or about 30 ng/mL or less. In one embodiment, the EC50 of the anti-PD-L1 antibodies and fragments provided herein is measured by ELISA or FACS.

[0036] In one embodiment, the anti PD-L1 antibodies and fragments thereof provided herein inhibit PDL1/PD-1 binding with an IC50 of about of about 1 ng/mL to about 1500 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein inhibit PDL1/PD-1 binding with an IC50 of about 2 ng/mL to about 1200 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein inhibit PDL1/PD-1 binding with an IC50 of about 5 ng/mL to about 500 ng/mL. In a further embodiment, the anti

PD-L1 antibodies and fragments thereof provided herein inhibit PDL1/PD-1 binding with an IC50 of about 5 ng/mL to about 100 ng/mL. In a further embodiment, the anti PD-L1 antibodies and fragments thereof provided herein inhibit PDL1/PD-1 binding with an IC50 of about 10 ng/mL to about 50 ng/mL. In one embodiment, the anti PD-L1 antibodies and fragments thereof provided herein inhibit PDL1/PD-1 binding with an IC50 of about 1200 ng/mL or less, about 1000 ng/mL or less, about 800 ng/mL or less, about 400 ng/mL or less, about 300 ng/mL or less, about 250 ng/mL or less, about 200 ng/mL or less, about 150 ng/mL or less, about 100 ng/mL or less, about 75 ng/mL or less, about 60 ng/mL or less, about 50 ng/mL or less, about 40 ng/mL or less, about 30 ng/mL or less, about 20 ng/mL or less, or about 10 ng/mL or less. In one embodiment, the IC50 of the anti-PD-L1 antibodies and fragments provided herein is measured by ELISA or FACS.

[0037] In one embodiment, the anti-PD-L1 antibody provided herein is a humanized antibody having a heavy chain variable region amino acid sequence according to SEQ ID NO: 42 and a light chain variable region amino acid according to SEQ ID NO: 44; or having a heavy chain variable region amino acid sequence according to SEQ ID NO: 46 and a light chain variable region amino acid sequence according to SEQ ID NO: 48; wherein the anti-PD-L1 antibody has a PD-L1 binding EC50 of about 200ng/ml or less or about 150 ng/mL or less or about 100 ng/mL or less or about 80 ng/ml or less or about 60 ng/mL or less or about 50 ng/mL or less, as measured by ELISA or FACS. In another embodiment, the anti-PD-L1 antibody provided herein is a humanized antibody having a heavy chain variable region amino acid sequence according to SEQ ID NO: 42 and a light chain variable region amino acid according to SEQ ID NO: 44; or having a heavy chain variable region amino acid sequence according to SEQ ID NO: 46 and a light chain variable region amino acid sequence according to SEQ ID NO: 48; wherein the anti-PD-L1 antibody has a PDL1/PD-1 blockage IC50 of about 1200 ng/mL or less, or about 1000 ng/mL or less, or about 800 ng/mL or less, or about 600 ng/mL or less, or about 500 ng/mL or less, or about 400 ng/mL or less, or about 300 ng/mL or less, or about 200 ng/mL or less, or about 100 ng/mL or less, or about 60 ng/mL or less, or about 30 ng/mL or less, or about 25 ng/mL or less, or about 20 ng/mL or less, or about 10 ng/mL or less, as measured by ELISA or FACS. In another embodiment, the anti-PD-L1 antibody provided herein is a humanized antibody having a heavy chain variable region amino acid sequence according to SEQ ID NO: 42 and a light chain variable region amino acid according to SEQ ID NO: 44; or having a heavy

chain variable region amino acid sequence according to SEQ ID NO: 46 and a light chain variable region amino acid sequence according to SEQ ID NO: 48; wherein the anti-PD-L1 antibody has a binding affinity kD for PD-L1 of about 10 nM or less, or about 5 nM or less, or about 2nM or less, or about 1 nM or less, or about 0.5 nM or less, or about 0.1 nM or less, or about 0.05nM or less, as measured by Biacore assay. In one embodiment, the humanized anti-PD-L1 antibody has a binding affinity kD for PD-L1 of about 2 nM. In another embodiment, the humanized anti-PD-L1 antibody has a binding affinity kD for PD-L1 of about 1 nM. In another embodiment, the humanized anti-PD-L1 antibody has a binding affinity kD for PD-L1 of about 0.5 nM. In another embodiment, the humanized anti-PD-L1 antibody has a binding affinity kD for PD-L1 of about 0.1 nM.

[0038] In one embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein bind to PD-L1, disrupting the PD-1/PD-L1 interaction and resulting in an increase in T cell activation. In a further embodiment, the antibodies and fragments thereof bind PD-L1 and result in an increase in T cell proliferation and/or cytokine production. In a yet further embodiment, the antibodies and fragments thereof bind PD-L1 and result in an increase of one or more cytokines selected from the group consisting of IL-2, IFNγ, TNF, IL-1, IL-4, IL-5, IL-6, IL-12, IL-13, IL-17, and GM-CSF. Thus, in one aspect, the present invention provides methods for modulating an immune response comprising contacting T cells and antigen presenting cells with the anti-PD-L1 antibody or fragment thereof. In one embodiment, the modulation of an immune response by the anti-PD-L1 antibodies and fragments provided herein may be measured in a mixed lymphocyte (MLR) reaction. In one embodiment, the anti-PD-L1 antibodies provided herein increase the level of cytokine production from lymphocytes in an MLR. In a further embodiment, the anti-PD-L1 antibodies increase the level of IL-2 production and/or IFNγ production in an MLR. In a yet further embodiment, the anti-PD-L1 antibodies increase the level of IL-2 production and IFNγ production in an MLR. In one embodiment, the anti-PD-L1 antibodies enhance memory T cell responses. In a further embodiment, the anti-PD-L1 antibodies enhance memory T cell responses as measured by an increase in IFNγ production from memory T cells.

[0039] In one embodiment, the anti-PD-L1 antibodies and fragments thereof provided herein inhibit regulatory T cell function. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof inhibit the suppression of effector T cells by regulatory T cells. In another embodiment, the anti-PD-L1 antibodies and fragments thereof restore the effector functions of T

cells in the presence of regulatory T cells. In a further embodiment, the anti-PD-L1 antibodies and fragments thereof restore the ability of effector T cells to proliferate and/or produce cytokines in the presence of regulatory T cells. Thus, in one embodiment, the present invention provides a method for inhibiting the suppressive effects of regulatory T cells in vitro or in a subject in need thereof.

[0040] In one aspect, an isolated antibody or fragment thereof that binds to PD-L1 is provided, wherein the antibody is produced by a hybridoma selected from the group consisting of the hybridomas herein termed 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and 15F1. Thus, the present invention also encompasses the hybridomas 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and 15F1, as well as any hybridoma producing an antibody disclosed herein. The present invention also provides isolated polynucleotides encoding the antibodies and fragments thereof provided herein. Expression vectors comprising the isolated polynucleotides, and host cells comprising such expression vectors, are also encompassed in the invention.

[0041] In one embodiment, the present invention provides anti-PD-L1 antibody immunoconjugates. Thus, the present invention provides an antibody or fragment thereof that binds to PD-L1 and that is linked or conjugated to a therapeutic agent. Therapeutic agents that may be linked or conjugated to the anti-PD-L1 antibody may include, but are not limited to, cytotoxic drugs, radioactive isotopes, immunomodulators, or antibodies.

[0042] In one aspect, the present invention provides compositions comprising one or more anti-PD-L1 antibody or fragment thereof provided herein, and a pharmaceutically acceptable carrier.

[0043] In one aspect, the present invention provides methods for modulating an immune response in a subject, the method comprising administering to the subject a therapeutically effective amount of an anti-PD-L1 antibody or fragment thereof provided herein. In one embodiment, the present invention provides methods for treating or preventing a disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an anti-PD-L1 antibody or fragment thereof provided herein.

[0044] In one embodiment, the present invention provides a method for enhancing anti-tumor responses in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an anti-PD-L1 antibody or fragment of the invention. In another embodiment, the present invention provides a method for reducing tumors or inhibiting the growth of tumor cells in a subject in need thereof, comprising administering to the subject a

therapeutically effective amount of an anti-PD-L1 antibody or fragment of the invention. In another embodiment, the present invention provides a method for treating cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an anti-PD-L1 antibody or fragment of the invention. In a further embodiment, the cancer is selected from the group consisting of lymphoma, leukemia, melanoma, glioma, breast cancer, lung cancer, colon cancer, bone cancer, ovarian cancer, bladder cancer, kidney cancer, liver cancer, stomach cancer, rectal cancer, testicular cancer, salivary cancer, thyroid cancer, thymic cancer, epithelial cancer, head or neck cancer, gastric cancer, pancreatic cancer, or a combination thereof.

[0045] In one embodiment, the present invention provides a method for treating an infectious disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an anti-PD-L1 antibody or fragment of the invention. In a further embodiment, the infectious disease is selected from the group consisting of candidiasis, candidemia, aspergillosis, streptococcal pneumonia, streptococcal skin and oropharyngeal conditions, gram negative sepsis, tuberculosis, mononucleosis, influenza, respiratory illness caused by Respiratory Syncytial Virus, malaria, schistosomiasis, and trypanosomiasis.

BRIEF DESCIRPTION OF THE FIGURES

[0046] **Figure 1a-d** is set of graphs showing the binding of the murine hybridoma anti-PD-L1 antibodies to PD-L1 over a range of antibody concentrations as measured by ELISA. Binding of hybridoma antibodies 8H3-mIgG (m8H3), 15F1-mIgG (m15F1), 5G9-mIgG (m5G9), and 4A8-mIgG (m4A8) is shown in **Figure 1a**. Binding of hybridoma antibodies 5G11-mIgG (m5G11), 7B4-mIgG (m7B4), 4D1-mIgG (m4D1), and 8H4-mIgG (m8H4) is shown in **Figure 1b**. Binding of hybridoma antibody 8C6-mIgG (m8C6) is shown in **Figure 1c**. Binding of hybridoma antibody 13C5-mIgG (m13C5) is shown in **Figure 1d**. In each of Figures 1a-1d, binding of mIgG1 is shown as a negative control.

[0047] **Figure 2a-c** is set of graphs showing the binding of chimeric anti-PD-L1 antibodies to PD-L1 over a range of concentrations as measured by ELISA. Binding of chimeric antibodies ch5G11-hIgG4 and ch5G11-hIgG1 is shown in **Figure 2a**. Binding of chimeric antibodies ch13C5-hIgG4, ch13C5-hIgG1, and ch8H4-hIgG4 is shown in **Figure 2b**. Binding of chimeric

antibody ch8C6-hIgG4 is shown in **Figure 2c**. In each of Figures 2a-2c, binding of hIgG4 is shown as a negative control.

[0048] Figure 3a-b is set of graphs showing the binding of humanized anti-PD-L1 antibodies to PD-L1 over a range of antibody concentrations as measured by ELISA. Binding of control hIgG4 and humanized antibodies hu5G11-hIgG1 and hu5G11-hIgG4 is shown in **Figure 3a**. Binding of control hIgG4 and humanized antibodies hu13C5-hIgG1 and hu13C5-hIgG4 is shown in **Figure 3b**.

[0049] . **Figure 4a-c** is a set of graphs showing the blockage of the PD-1/PD-L1 interaction by hybridoma anti-PD-L1 antibodies over a range of antibody concentrations as measured by ELISA. Blockage of PD-1/PD-L1 binding by hybridoma antibodies 13C5-mIgG (m13C5), 8C6-mIgG (m8C6), 5G9-mIgG (m5G9), and 4A8-mIgG (m4A8) as compared to control mIgG1 is shown in **Figure 4a**. Blockage of PD-1/PD-L1 binding by hybridoma antibodies 5G11-mIgG (m5G11), 7B4-mIgG (m7B4), 4D1-mIgG (m4D1), and 8H4-mIgG (m8H4) as compared to control mIgG1 is shown in **Figure 4b**. Blockage of PD-1/PD-L1 binding by hybridoma antibodies 8H3-mIgG (m8H3) and 15F1-mIgG (m15F1) as compared to control mIgG1 is shown in **Figure 4c**.

[0050] Figure 5a-c is a set of graphs showing the blockage of the PD-1/PD-L1 interaction by chimeric anti-PD-L1 antibodies over a range of antibody concentrations as measured by ELISA. Blockage of PD-1/PD-L1 binding by chimeric antibodies ch5G11 hIgG4 and ch5G11 hIgG1 as compared to control hIgG4 is shown in **Figure 5a**. Blockage of PD-1/PD-L1 binding by chimeric antibody ch8C6-hIgG4 as compared to control hIgG4 is shown in **Figure 5b**. Blockage of PD-1/PD-L1 binding by chimeric antibodies ch8H4-hIgG4, ch13C5-hIgG1, and ch13C5-hIgG4 as compared to control hIgG4 is shown in **Figure 5c**.

[0051] Figure 6a-b is a set of graphs showing the blockage of the PD-1/PD-L1 interaction by humanized anti-PD-L1 antibodies over a range of antibody concentrations as measured by ELISA. Blockage of PD-1/PD-L1 binding by control hIgG4 and humanized antibodies 5G11-hIgG1 and 5G11-hIgG4 is shown in **Figure 6a**. Blockage of PD-1/PD-L1 binding by control hIgG4 and humanized antibodies 13C5-hIgG1 and 13C5-hIgG4 is shown in **Figure 6b.**

[0052] Figure 7a and 7b show the binding of the hybridoma anti-PD-L1 antibodies to PD-L1 over a range of antibody concentrations as measured by FACS. Binding (as measured by the mean fluorescence intensity) of hybridoma antibodies 4A8, 15F1, 4D1, 13C5, 8H4, and 8H3 as

compared to control antibody mIgG1 is shown in **Figure 7a**. Binding (as measured by the mean fluorescence intensity) of hybridoma antibodies 5G11, 8C6, 5G9, or 7B4 as compared to control antibody mIgG1 is shown in **Figure 7b**.

[0053] Figure 8 shows the binding of the chimeric anti-PD-L1 antibodies to PD-L1 over a range of antibody concentrations as measured by FACS. Binding of control antibody hIgG4, and chimeric antibodies ch13C5-hIgG1, ch5G11-hIgG1, and ch5G11-hIgG4 are shown.

[0054] Figure 9 shows the binding of humanized anti-PD-L1 antibodies to PD-L1 over a range of antibody concentrations as measured by FACS. Binding of control antibody hIgG4 and humanized antibodies hu13C5-hIgG1, hu13C5-hIgG4, hu5G11-hIgG1, and hu5G11-hIgG4 are shown.

[0055] . **Figure 10a** and **10b** show the blockage of the PD-1/PD-L1 interaction by hybridoma anti-PD-L1 antibodies over a range of antibody concentrations as measured by FACS. Blockage of PD-1/PD-L1 binding by control antibody mIgG1 and hybridoma antibodies m4D1, m5G11, m13C5, m7B4, and m8H4 is shown in **Figure 10a.** Blockage of PD-1/PD-L1 binding by control antibody mIgG1 and hybridoma antibodies m4A8, m5G9, m8C6, m8H3, and m15F1 is shown in **Figure 10b.**

[0056] Figure 11 shows the blockage of the PD-1/PD-L1 interaction over a range of concentrations of control antibody hIgG4 or chimeric anti-PD-L1 antibodies ch8C6-hIgG4, ch5G11-hIgG1, ch5G11-hIgG4, ch13C5-hIgG1, ch13C5-hIgG4, or ch8H4-hIgG4, as measured by FACS.

[0057] Figure 12 shows the blockage of the PD-1/PD-L1 interaction over a range of concentrations of control antibody hIgG4 or humanized antibodies hu 13C5-hIgG1, hu13C5-hIgG4, hu5G11-hIgG1, or hu5G11-hIgG4, as measured by FACS.

[0058] Figure 13a is a graph showing IL-2 (pg/mL) production in an MLR in response to different concentrations of hybridoma anti-PD-L1 antibodies. **Figure 13b** is a graph showing IFNγ (pg/mL) production in an MLR in response to different concentrations of hybridoma anti-PD-L1 antibodies. For both **Figure 13a** and **13b**, the antibodies tested were, from left to right, control mIgG1, m8C6, m4D1, m5G11, m7B4, m8H4, m5G9, m13C5, m8H3, and m15F1. T cell only and/or DC only wells were also included as negative controls. As shown on the x-axis for both **Figure 13a** and **13b**, each antibody was tested at 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

[0059] Figure 14a is a graph showing IL-2 (pg/mL) production in an MLR in response to different concentrations of chimeric anti-PD-L1 antibodies. **Figure 14b** is a graph showing IFNγ (pg/mL) production in an MLR in response to different concentrations of chimeric anti-PD-L1 antibodies. For both **Figure 14a** and **14b**, the antibodies tested were, from left to right, control hIgG4, chimeric 8C6-hIgG4, chimeric 8H4-hIgG4, chimeric 5G11-hIgG4, and chimeric 13C5-hIgG1. As shown on the x-axis for both **Figure 14a** and **14b**, each antibody was tested at 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

[0060] Figure 15a is a graph showing IL-2 (pg/mL) production in an MLR in response to different concentrations of humanized anti-PD-L1 antibodies. **Figure 15b** is a graph showing IFNγ (pg/mL) production in an MLR in response to different concentrations of humanized anti-PD-L1 antibodies. For both **Figure 15a** and **15b**, the antibodies tested were, from left to right, control hIgG4, hu13C5-hIgG1, hu13C5-hIgG4, hu5G11-hIgG1, and hu5G11-hIgG4. As shown on the x-axis for both **Figure 15a** and **15b**, each antibody was tested at 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

[0061] Figure 16 shows the effects of chimeric (ch) or humanized (hu) anti-PD-L1 antibodies on Treg-mediated inhibition of IFNγ production (pg/mL), in an allogeneic MLR with CD4+ CD25+ Treg cells, CD4+CD25- T cells, and dendritic cells. The antibodies tested were, from left to right, control hIgG4, ch13C5-hIgG1, ch13C5-hIgG4, hu13C5-hIgG1, hu13C5-hIgG4, ch5G11-hIgG1, ch5G11-hIgG4, hu5G11-hIgG1, and hu5G11-hIgG4.

[0062] Figure 17 shows IFN-γ production (pg/mL) from T cells in response to costimulation with autologous DCs and anti-CD3 antibody, in the presence of humanized anti-PD-L1 antibody (hu13C5-hIgG1, hu13C5-hIgG4, hu5G11-hIgG1, or hu5G11-hIgG4), isotype control (hIgG4) antibody, or no antibody.

[0063] Figures 18a and 18b show the effect of humanized anti-PD-L1 antibodies on memory T cell responses recalled by tetanus toxin, as measured by IFN-γ production (pg/mL). Negative control hIgG4 or humanized antibody hu13C5-hIgG1, hu13C5-hIgG4, hu5G11-hIgG1, or hu5G11-hIgG4 were tested at the following concentrations: 20 μg/mL, 2 μg/mL, 0.2 μg/mL, 0.02 μg/mL, and 0.002 μg/mL.

DETAILED DESCRIPTION

[0064] PD1/PDL1 interactions inhibit T cell receptor signaling by recruiting the SHP1 and SHP2 phosphatases, which interfere with TCR signaling (Chemnitz et al. (2004) J. Immunol. 17:945–954). PD-L1 can not only promote tumor progression through inhibition of PD1-expressing immune effectors, but also modulate cell-mediated immunity in some infectious diseases (Mueller et al. (2010) J. Clin. Invest. 120:2508–2515). Furthermore, allogeneic effector T cell responses are susceptible to PD-1 pathway modulation in graft rejection (Lee et al. (2003) J. Immunol. 171:6929–6935). Therefore, the interaction of PD-1 with PD-L1 exerts a vital and diverse range of immunoregulatory roles in T cell activation, tolerance, and immune-mediated tissue damage. However, the interaction can be reversed by blocking the local binding of PD-1 with PD-L1 (Iwai et al. (2002) Proc. Nat'l. Acad Sci. USA 99: 12293-7; Brown et al. (2003) J. Immunol. 170:1257-66).

[0065] PD-1 has been found to have a correlation with cancer growth and development due to its role in protecting tumor cells from efficient immune destruction. Its ligand, PD-L1, has been revealed to have significant expression on a number of mouse and human tumors, which is postulated to mediate immune evasion (Iwai, Y. et al., Proc. Natl. Acad. Sci. USA.99: 12293-12297 (2002); Strome S. E. et al., Cancer Res., 63:6501-6505 (2003); Dong et al. (2002) Nat. Med. 8:787-9). In humans, expression of PD-1 (on tumor infiltrating lymphocytes) and/or PD-L1 (on tumor cells) has been found in a number of primary tumor biopsies as assessed by immunohistochemistry. Such 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 J. A.et al., J. Immunol. 170: 1257-1266 (2003); Dong H. et al., Nat.Med. 8: 793-800 (2002); Wintterle et al., Cancer Res. 63:7462-7467 (2003); Strome S. E. et al., Cancer Res., 63: 6501 -6505 (2003); Thompson R. H. et al., Cancer Res. 66: 3381-5(2006); Thompson et al., Clin. Cancer Res. 13: 1757-61(2007); Nomi T. et al., Clin. Cancer Res. 13: 2151-7. (2007)). More strikingly, PD-l ligand expression on tumor cells has been correlated to poor prognosis of cancer patients across multiple tumor types (reviewed in OkaZaki and Honjo, Int. Immunol. 19: 813-824 (2007)).

[0066] While the interaction between PD-1 and PD-L1 results in a decrease in tumor infiltrating lymphocytes, a decrease in T-cell receptor mediated proliferation, and immune evasion by the cancerous cells (Dong et al. (2003) J. Mol. Med. 81:281-7; Blank et al. (2005) Cancer Immunol.

Immunother. 54: 3 07-3 14; Konishi et al. (2004) Clin. Cancer Res. 10:5094-100), blockade of the PD-1/PD-L1 interaction was accordingly shown to enhance tumor-specific T-cell immunity and be helpful in clearance of tumor cells by the immune system. In a murine model of aggressive pancreatic cancer, for example, Nomi T., et al. (Clin. Cancer Res. 13: 2151-2157, 2007) demonstrated the therapeutic efficacy of PD-1/PD-L1 blockade. Administration of either PD-1 or PD-L1 directed antibody significantly inhibited tumor growth. Antibody blockade 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. Additionally, the authors showed that PDL1/PD-1 blockade can be effectively combined with chemotherapy to yield a synergistic effect. In another study, using a model of squamous cell carcinoma in mice, antibody blockade of PD-l or PD-Ll significantly inhibited tumor growth (Tsushima F. et al., Oral Oncol. 42:268-274 (2006)).

[0067] Furthermore, transfection of a murine mastocytoma line with PD-Ll led to decreased lysis of the tumor cells when co-cultured with a tumor-specific CTL clone. Lysis was restored when anti-PD-Ll mAb was added (Iwai Y. et al., Proc. Natl. Acad. Sci. USA. 99: 12293-12297 (2002)). In vivo, blocking the PDl/PD-Ll interaction was shown to increase the efficacy of adoptive T cell transfer therapy in a mouse tumor model (Strome S. E. et al., Cancer Res. 63:6501-6505 (2003)). Further evidence for the role of PD-1 in cancer treatment comes from experiments performed with PD-l knockout mice. PD-Ll 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. USA. 99: 12293-12297(2002)).In human studies, R. M. Wong et al. (Int. Immunol. 19:1223-1234 (2007)) showed that PD-l blockade using a fully human anti-PD-l antibody augmented the absolute numbers of tumor-specific CD8+ T cells (CTLs) in ex vivo stimulation assays using vaccine antigens and cells from vaccinated individuals. In a similar study, antibody blockade of PD-Ll resulted in enhanced cytolytic activity of tumor-associated antigen-specific cytotoxic T cells and increased cytokine production by tumor specific TH cells (Blank C. et al., Int. J. Cancer 119: 317-327 (2006)). The same authors showed that PD-Ll blockade augments tumor-specific T cell responses in vitro when used in combination with anti-CTLA-4 blockade. Overall, the PD-l/PD-Ll pathway is a target for the development of antibody therapeutics for cancer treatment. Anti-PD-Ll 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. (Barber et al., Nature 439: 682-687 (2006)) showed that mice infected with a laboratory strain of LCMV developed chronic infection resulting 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. The authors 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-l and PD-Ll.

[0068] In one aspect, the present invention provides antibodies or antigen binding fragments thereof that bind to programmed death ligand 1 (PD-L1). In one embodiment, the antibodies or fragments thereof bind to human PD-L1. In another embodiment, the antibodies or fragments thereof bind to human and to cynomolgous PD-L1. In another embodiment, the antibodies or fragments thereof block the interaction of PD-L1 with its receptor PD-1 on T cells. In one aspect, the present invention provides methods of making and using the anti-PD-L1 antibodies or fragments thereof, and compositions comprising anti-PD-L1 antibodies or fragments thereof, including pharmaceutical compositions.

[0069] As used herein, the term "antibody" refers to a binding protein having at least one antigen binding domain. The antibodies and fragments thereof of the present invention may be whole antibodies or any fragment thereof. Thus, the antibodies and fragments of the invention include monoclonal antibodies or fragments thereof and antibody variants or fragments thereof, as well as immunoconjugates. Examples of antibody fragments include Fab fragments, Fab' fragments, F(ab)' fragments, Fv fragments, isolated CDR regions, single chain Fv molecules (scFv), and other antibody fragments known in the art. Antibodies and fragments thereof may also include recombinant polypeptides, fusion proteins, and bi-specific antibodies. The anti-PD-L1 antibodies and fragments thereof disclosed herein may be of an IgG1, IgG2, IgG3, or IgG4 isotype. The term "isotype" refers to the antibody class encoded by the heavy chain constant region genes. In one embodiment, the anti-PD-L1 antibodies and fragments thereof disclosed herein are of an IgG1 or an IgG4 isotype. The PD-L1 antibodies and fragments thereof of the present invention

may be derived from any species including, but not limited to, mouse, rat, rabbit, primate, llama, and human. The PD-L1 antibodies and fragments thereof may be chimeric, humanized, or fully human antibodies. In one embodiment, the anti-PD-L1 antibodies are antibodies produced by a hybridoma cell line derived from a mouse. Thus, in one embodiment, the anti-PD-L1 antibodies are murine antibodies. In another embodiment, the anti-PD-L1 antibodies are chimeric antibodies. In a further embodiment, the chimeric antibodies are mouse-human chimeric antibodies. In another embodiment, the antibodies are humanized antibodies. In a further embodiment, the antibodies are derived from murine antibodies and are humanized.

[0070] A "chimeric antibody" is an antibody having at least a portion of the heavy chain variable region and at least a portion of the light chain variable region derived from one species; and at least a portion of a constant region derived from another species. For example, in one embodiment, a chimeric antibody may comprise murine variable regions and a human constant region.

[0071] A "humanized antibody" is an antibody containing complementarity determining regions (CDRs) that are derived from a non-human antibody; and framework regions as well as constant regions that are derived from a human antibody. For example, the anti-PD-L1 antibodies provided herein may comprise CDRs derived from one or more murine antibodies and human framework and constant regions. Thus, in one embodiment, the humanized antibody provided herein binds to the same epitope on PD-L1 as the murine antibody from which the antibody's CDRs are derived. Exemplary humanized antibodies are provided herein. Additional anti-PD-L1 antibodies comprising the heavy and light chain CDRs provided herein, or variants thereof, may be generated using any human framework sequence, and are also encompassed in the present invention. In one embodiment, framework sequences suitable for use in the present invention include those framework sequences that are structurally similar to the framework sequences provided herein. Further modifications in the framework regions may be made to improve the properties of the antibodies provided herein. Such further framework modifications may include chemical modifications; point mutations to reduce immunogenicity or remove T cell epitopes; or back mutation to the residue in the original germline sequence. In some embodiments, such modifications include those corresponding to the mutations exemplified herein, including backmutations to the germline sequence. For example, in one embodiment, one or more amino acids in the human framework regions of the VH and/or VL of the humanized antibodies

provided herein are back mutated to the corresponding amino acid in the parent murine antibody. As an example, as for VH and VL of humanized 5G11 and humanized 13C5, several sites of framework amino acid of the aforementioned template human antibody were back mutated to the corresponding amino acid sequences in mouse 5G11 and 13C5 antibodies. In one embodiment, the amino acid at positions 53 and/or 60 and/or 67 of the light chain variable region is back mutated to the corresponding amino acid found at that position in the mouse 5G11 or 13C5 light chain variable region. In another embodiment, the amino acid at positions 24 and/or 28 and/or 30 and/or 49 and/or 73 and/or 83 and/or 94 of the heavy chain variable region is back mutated to the corresponding amino acid found at that position in the mouse 5G11 or 13C5 heavy chain variable region. In one embodiment, the humanized 5G11 antibody comprises a light chain variable region wherein the amino acid at position 60 is mutated from Ser (S) to Asp (D) and the amino acid at position 67 is mutated from Ser (S) to Tyr (Y); and a heavy chain variable region wherein the amino acid at position 24 is mutated from Phe (F) to Val (V), the amino acid at position 49 is mutated from Ala (A) to Gly (G), the amino acid at position 73 is mutated from Thr (T) to Asn (N), and the amino acid at position 83 is mutated from Thr (T) to Asn (N). In one embodiment, the humanized 13C5 antibody comprises a light chain variable region wherein the amino acid at position 53 is mutated from Tyr (Y) to Lys (K); and a heavy chain variable region wherein the amino acid at position 28 is mutated from Thr (T) to Ile (I), the amino acid at position 30 is mutated from Ser (S) to Arg (R), the amino acid at position 49 is mutated from Ser (S) to Ala (A), and the amino acid at position 94 is mutated from Tyr (Y) to Asp (D). Additional or alternate back mutations may be made in the framework regions of the humanized antibodies provided herein in order to improve the properties of the antibodies. The present invention also encompasses humanized antibodies that bind to PD-L1 and comprise framework modifications corresponding to the exemplary modifications described herein with respect to any suitable framework sequence, as well as other framework modifications that otherwise improve the properties of the antibodies.

[0072] As used herein, the term "derived" when used to refer to a molecule or polypeptide relative to a reference antibody or other binding protein, means a molecule or polypeptide that is capable of binding with specificity to the same epitope as the reference antibody or other binding protein.

[0073] The antibodies and antigen-binding fragments thereof disclosed herein are specific for PD-L1. In one embodiment, the antibodies and fragments thereof are specific for human PD-L1. In one embodiment, the antibodies and fragments provided herein bind to human or primate PD-L1 but not to PD-L1 from any other mammal. In a further embodiment, the antibodies and fragments thereof do not bind to mouse PD-L1. The terms "human PD-L1," "hPD-L1", and "huPD-L1" and the like are used interchangeably herein and refer to human PD-L1 and variants or isoforms of human PD-L1. By "specific for" is meant that the antibodies and fragments thereof bind PD-L1 with greater affinity than any other target. As used herein, the term "EC50" refers to the effective concentration, 50% maximal response of the antibody. As used herein, the term "IC50" refers to the inhibitory concentration, 50% maximal response of the antibody. Both EC50 and IC50 may be measured by ELISA or FACS analysis, or any other method known in the art.

[0074] In one embodiment, the anti-PD1 antibodies and fragments or variants thereof have a binding affinity (KD) for PD-L1 in the range of about 0.001 nM to about 100 nM, about 0.002 nM to about 50 nM, about 0.005 nM to about 5 nM, about 0.01 nM to about 1 nM, or about 0.05 nM to about 0.1 nM. In one embodiment, the antibodies and fragments thereof have a binding affinity (KD) for PD-L1 of about 50 nM or less, about 25 nM or less, about 20 nM or less, about 15 nM or less, about 10 nM or less, about 8 nM or less, about 6 nM or less, about 5 nM or less, about 4 nM or less, about 3 nM or less, about 2 nM or less, about 1 nM or less, about 0.9 nM or less, about 0.8 nM or less, about 0.7 nM or less, about 0.6 nM or less, about 0.5 nM or less, about 0.4 nM or less, about 0.3 nM or less, about 0.2 nM or less, about 0.1 nM or less, about 0.09 nM or less, about 0.08 nM or less, about 0.07 nM or less, about 0.06 nM or less, about 0.05 nM or less, about 0.04 nM or less, about 0.03 nM or less, about 0.02 nM or less, about 0.01 nM or less, about 0.009 nM or less, about 0.008 nM or less, about 0.007 nM or less, about 0.006 nM or less, about 0.005 nM or less, about 0.004 nM or less, about 0.003 nM or less, about 0.002 nM or less, or about 0.001 nM or less. In one embodiment, the antibodies and fragments thereof have a binding affinity (KD) for PD-L1 of about 10 nM, about 9 nM, about 8 nM, about 7 nM, about 6 nM, about 5 nM, about 4 nM, about 3 nM, about 2 nM, about 1 nM, about 0.9 nM, about 0.8 nM, about 0.7 nM, about 0.6 nM, about 0.5 nM, about 0.4 nM, about 0.3 nM, about 0.2 nM, about 0.1 nM, about 0.09 nM, about 0.08 nM, about 0.07 nM, about 0.06 nM, about 0.05 nM, about 0.04 nM, about 0.03 nM, about 0.02 nM, about 0.01 nM, about 0.009 nM,

about 0.008 nM, about 0.007 nM, about 0.006 nM, about 0.005 nM, about 0.004 nM, about 0.003 nM, about 0.002 nM, or about 0.001 nM.

[0075] In one embodiment, the antibodies and fragments provided herein comprise a light chain and a heavy chain, each of which comprises three CDR regions. Exemplary heavy chain CDR sequences (HCDR1, HCDR2, and HCDR3) for PD-L1 antibodies of the invention are provided below in **Table 1**. Exemplary light chain CDR sequences (LCDR1, LCDR2, and LCDR3) for PD-L1 antibodies of the invention are provided below in **Table 2**. Exemplary variable regions and full length heavy and light chain sequences for PD-L1 antibodies of the invention are provided below in **Table 3**.

Table 1. Heavy Chain CDR Sequences

Name	HCDR	SEQ ID NO	Sequence
13C5	1	81	SYGMS
	2	82	SISSGGSTYYPDSVKG
	3	83	GYDSGFAY
5G9	1	87	SYGMS
	2	88	SISSGGTTYYPDSVKG
	3	89	GYDSGFAY
5G11	1	93	TYGVH
	2	94	VIWRGVTTDYNAAFMS
	3	95	LGFYAMDY
8C6	1	99	SYGVH
	2	100	VIWSGGVTDYNAAFIS
	3	101	LGFYAMDY
7B4	1	105	TYWMH
	2	106	QINPDSTTINYAPSLKD
	3	107	PGDYGYDFDC

4D1	1	111	SGYWN
	2	112	YISYSGSTYYNPSLKS
	3	113	SLLWFSTGFAY
4A8	1	117	SYGVH
	2	118	VIWSGGITDYNAAFKS
	3	119	LGFYAMDY
8H4	1	123	SYGMS
	2	124	SISSGGTTYYLGSVQG
	3	125	GYDAGFAY
8H3	1	129	SGYWT
	2	130	YISYTGSTYYNPSLKS
	3	131	QRDWLGFAY
15F1	1	135	SYGMS
	2	136	SISSGGSIYYPDSVKG
	3	137	GYDAGFAF

Table 2. Light chain CDR Sequences

Name	LCDR	SEQ ID NO	Sequence
13C5	1	84	ASQSVSTSSSSFMH
	2	85	YASNLES
	3	86	QHSWEIPYT
5G9	1	90	RASQSVSTSSSSYMH
	2	91	YASNLES
	3	92	QHSWEIPYT

5G11	1	96	KASQSVSNDVA
	2	97	YAANRYT
	3	98	QQDYTSPYT
8C6	1	102	KASQSVSNDVG
	2	103	YASNRYS
	3	104	QQDYTSPYT
7B4	1	108	RSSQIIVHSNANTYLE
	2	109	KVSNRFS
	3	110	FQGSHVPYT
4D1	1	114	SASSSVSSSYLY
	2	115	NTSNLAS
	3	116	HQWRSYPPT
4A8	1	120	SANSSVSYMH
	2	121	DTSKLAS
	3	122	QQWSSNPWT
8H4	1	126	RASQSVSTSSYSYMH
	2	127	YASNLES
	3	128	QNSWEIPYT
8H3	1	132	KSSQSLLYSSNQKNSLA
	2	133	WASNRES
	3	134	QQYYSYPLT
15F1	1	138	RASQSVSTSSYSYVH
	2	139	YASNLES
	3	140	QHSWEIPYT

Table 3. Heavy chain and light chain variable region and full length heavy and light chain amino acid sequences

Name	Region	SEQ ID NO	Sequence
13C5 murine	Heavy chain variable	2	EVKLVESGGGLVKPGGSLKLSCAASGFIFRSYGMSWVRQTPE KRLEWVASISSGGSTYYPDSVKGRFTISRDNAR NILYLQMSSLRSEDTAMYDCARGYDSGFAYWGQGTLVTVSE
13C5 murine	Light chain variable	4	DIVLTQSPASLAVSLGQRATISCRASQSVSTSSSSFMHWYQQK PGQPPKLLIKYASNLESGVPARFSGSGSGTDFT LNIHPVEEEDTATYYCQHSWEIPYTFGGGTKLEIKR
5G9 murine	Heavy chain variable	6	EVKLVESGGGLVKPGGSLKLSCAASGFTFRSYGMSWVRQTP EKRLEWVASISSGGTTYYPDSVKGRFIISRDNARNILYLQMSS LRSEDTAMYYCAKGYDSGFAYWGQGTLVIVSA
5G9 murine	Light chain variable	8	DIVLTQSPPSLAVSLGQRATISCRASQSVSTSSSSYMHWYQQK PGQPPKLLIKYASNLESGVPARFSGSGSGTDFTLNIHPVEEEDT ATYYCQHSWEIPYTFGGGTKLEIK
5G11 murine	Heavy chain variable	10	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTTYGVHWVRQSPG KGLEWLGVIWRGVTTDYNAAFMSRLTITKDNSKSQVFFKMN SLQANDTAIYYCARLGFYAMDYWGQGTSVTVSS
5G11 murine	Light chain variable	12	SIVMTQTPKFLLVSAGDRVTITCKASQSVSNDVAWYQQKPG QSPKLLIYYAANRYTGVPDRFTGSGYGTDFTFTISIVQAEDLA VYFCQQDYTSPYTFGGGTKLEIK
8C6 murine	Heavy chain variable	14	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTSYGVHWVRQSPG KGLEWLGVIWSGGVTDYNAAFISRLSISKDNSKSQVFFKMNS LQANDTAIYYCARLGFYAMDYWGQGTSVTVSS
8C6 murine	Light chain variable	16	SIVMTQTPKFLLVSAGDRVTITCKASQSVSNDVGWYQQKPG QSPKLLIYYASNRYSGVPDRFTGSGYGTDFTFTISTVQAEDLA VYFCQQDYTSPYTFGGGTKLEIK
7B4 murine	Heavy chain variable	18	EVKLFESGGGLVQPGGSLKLSCVASGFDFSTYWMHWVRQAP GQGLEWIGQINPDSTTINYAPSLKDRFIISRDNAKNTLFLQMS KVRSEDTALYYCAKPGDYGYDFDCWGQGTTLTVSS
7B4 murine	Light chain variable	20	DVLMTQTPLYLPVSLGDQASISCRSSQIIVHSNANTYLEWFLQ KPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAE DLGVYYCFQGSHVPYTFGGGTKLEIK
4D1 murine	Heavy	22	EVQLQESGPSLVKPSQTLSLTCSVTGDSITSGYWNWIRKFPGN KLEYMGYISYSGSTYYNPSLKSRISITRDTSKNQYYLQLNSVT

	chain variable		TEDTATYYCARSLLWFSTGFAYWGQGTLVTVSA
4D1 murine	Light chain variable	24	QIVLTQSPAIMSASPGEKVTLTCSASSSVSSSYLYWNQQKPGS SPKVWIYNTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAAS YFCHQWRSYPPTLGAGTKLELK
4A8 murine	Heavy chain variable	26	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTSYGVHWVRQSPG KGLEWLGVIWSGGITDYNAAFKSRLSISKDNSKSQVFFKMNS LQANDTAIYFCARLGFYAMDYWGQGTSVTVSS
4A8 murine	Light chain variable	28	QIVLTQSPAIMSASPGEKVTMTCSANSSVSYMHWYQQKSGTS PKRWIYDTSKLASGVPARFSGSGSGTSYSLTISSMGAEDAAT YYCQQWSSNPWTFGGGTKLEIK
8H4 murine	Heavy chain variable	30	EVKLVESGGGLVKPGGSLKLSCAASGFTFRSYGMSWARQIPE KRLEWVASISSGGTTYYLGSVQGRFTISRDNARNILYLQMSSL RSEDTAMYYCARGYDAGFAYWGQGTLVSVSE
8H4 murine	Light chain variable	32	DIVLTQSPASLAVSLGQRATISCRASQSVSTSSYSYMHWYQQ KPGQPPKLLIKYASNLESGVPARFSGSGSGTDFTLNIHPVEEE DTATYYCQNSWEIPYTFGGGTKLEIK
8H3 murine	Heavy chain variable	34	EVQLQESGPSLVKPSQTLSLTCSVTGDSITSGYWTWIRKFPGN KLEYMGYISYTGSTYYNPSLKSRISISRDTSKSQYYLQLNSVT TEDTATYYCARQRDWLGFAYWGQGTLVTVSA
8H3 murine	Light chain variable	36	DIVMTQTPSSLAVSLGEKVTMSCKSSQSLLYSSNQKNSLAWY QQKPGQSPKLLIYWASNRESGVPDRFTGSSSGTDFTLTISSVK AEDLAVYYCQQYYSYPLTFGAGTKLELK
15F1 murine	Heavy chain variable	38	EEKLVESGGGLVKPGGSLKLSCAASGFSFSSYGMSWVRQTPE KRLEWVASISSGGSIYYPDSVKGRFTISRDNARNILYLQMSSL RSEDTAMYYCARGYDAGFAFWGQGTLVTASA
15F1 murine	Light chain variable	40	DIVLTQSPASLAVSLGQRATISCRASQSVSTSSYSYVHWYQQ KPGQPPKLLIKYASNLESGVPARFSGSGSGTDFTLNIHPVEEE DTATYYCQHSWEIPYTFGGGTKLEIK
5G11 humanized	Heavy chain variable	42	QITLKESGPTLVKPTQTLTLTCTVSGFSLSTYGVHWIRQPPGK ALEWLGVIWRGVTTDYNAAFMSRLTITKDNSKNQVVLTMN NMDPVDTATYYCARLGFYAMDYWGQGTLVTVSS
5G11 humanized	Light chain variable	44	DIQMTQSPSSLSASVGDRVTITCKASQSVSNDVAWYQQKPGK APKLLIYYAANRYTGVPDRFSGSGYGTDFTFTISSLQPEDIAT YFCQQDYTSPYTFGQGTKLEIK

13C5 humanized	Heavy chain variable	46	EVQLVESGGGLVKPGGSLRLSCAASGFIFRSYGMSWVRQAP GKGLEWVASISSGGSTYYPDSVKGRFTISRDNAKNSLYLQMN SLRAEDTAVYDCARGYDSGFAYWGQGTLVTVSS
13C5 humanized	Light chain variable	48	DIVLTQSPASLAVSPGQRATITCRASQSVSTSSSSFMHWYQQK PGQPPKLLIKYASNLESGVPARFSGSGSGTDFTLTINPVEAND TANYYCQHSWEIPYTFGQGTKLEIK
Chimeric 8C6-IgG4 (F234A/ L235A)	Full length heavy chain (IgG4)	50	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTSYGVHWVRQSPG KGLEWLGVIWSGGVTDYNAAFISRLSISKDNSKSQVFFKMNS LQANDTAIYYCARLGFYAMDYWGQGTSVTVSSASTKGPSVF PLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV DKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKA KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF SCSVMHEALHNHYTQKSLSLSLG
Chimeric 8C6	Full length light chain	52	SIVMTQTPKFLLVSAGDRVTITCKASQSVSNDVGWYQQKPG QSPKLLIYYASNRYSGVPDRFTGSGYGTDFTFTISTVQAEDLA VYFCQQDYTSPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC
Chimeric 8H4-IgG4 (F234A/ L235A)	Full length heavy chain (IgG4)	54	EVKLVESGGGLVKPGGSLKLSCAASGFTFRSYGMSWARQIPE KRLEWVASISSGGTTYYLGSVQGRFTISRDNARNILYLQMSSL RSEDTAMYYCARGYDAGFAYWGQGTLVSVSSASTKGPSVFP LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV DKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKA KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF SCSVMHEALHNHYTQKSLSLSLG
Chimeric 8H4	Full length light chain	56	DIVLTQSPASLAVSLGQRATISCRASQSVSTSSYSYMHWYQQ KPGQPPKLLIKYASNLESGVPARFSGSGSGTDFTLNIHPVEEE DTATYYCQNSWEIPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC
Chimeric 5G11-IgG1 (D265A)	Full length heavy chain	58	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTTYGVHWVRQSPG KGLEWLGVIWRGVTTDYNAAFMSRLTITKDNSKSQVFFKMN SLQANDTAIYYCARLGFYAMDYWGQGTSVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV

	(IgG1)		HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
Chimeric 5G11-IgG4 (F234A/ L235A)	Full length heavy chain (IgG4)	60	QVQLKQSGPGLVQPSQSLSITCTVSGFSLTTYGVHWVRQSPG KGLEWLGVIWRGVTTDYNAAFMSRLTITKDNSKSQVFFKMN SLQANDTAIYYCARLGFYAMDYWGQGTSVTVSSASTKGPSV FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK VDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN VFSCSVMHEALHNHYTQKSLSLSLG
Chimeric 5G11	Full length light chain	62	SIVMTQTPKFLLVSAGDRVTITCKASQSVSNDVAWYQQKPG QSPKLLIYYAANRYTGVPDRFTGSGYGTDFTFTISIVQAEDLA VYFCQQDYTSPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC
Chimeric 13C5-IgG1 (D265A)	Full length heavy chain (IgG1)	64	EVKLVESGGGLVKPGGSLKLSCAASGFIFRSYGMSWVRQTPE KRLEWVASISSGGSTYYPDSVKGRFTISRDNARNILYLQMSSL RSEDTAMYDCARGYDSGFAYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK
Chimeric 13C5-IgG4 (F234A/ L235A)	Full length heavy chain (IgG4)	66	EVKLVESGGGLVKPGGSLKLSCAASGFIFRSYGMSWVRQTPE KRLEWVASISSGGSTYYPDSVKGRFTISRDNARNILYLQMSSL RSEDTAMYDCARGYDSGFAYWGQGTLVTVSSASTKGPSVFP LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV DKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKA KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF SCSVMHEALHNHYTQKSLSLSLG

Chimeric 13C5	Full length light chain	68	DIVLTQSPASLAVSLGQRATISCRASQSVSTSSSSFMHWYQQK PGQPPKLLIKYASNLESGVPARFSGSGSGTDFTLNIHPVEEEDT ATYYCQHSWEIPYTFGGGTKLEIKRTRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC
Humanized 5G11-IgG1 (D265A)	Full length heavy chain (IgG1)	70	QITLKESGPTLVKPTQTLTLTCTVSGFSLSTYGVHWIRQPPGK ALEWLGVIWRGVTTDYNAAFMSRLTITKDNSKNQVVLTMN NMDPVDTATYYCARLGFYAMDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK
Humanized 5G11-IgG4 (F234A/ L235A)	Full length heavy chain (IgG4)	72	QITLKESGPTLVKPTQTLTLTCTVSGFSLSTYGVHWIRQPPGK ALEWLGVIWRGVTTDYNAAFMSRLTITKDNSKNQVVLTMN NMDPVDTATYYCARLGFYAMDYWGQGTLVTVSSASTKGPS VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNT KVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN VFSCSVMHEALHNHYTQKSLSLSLG
Humanized 5G11	Full length light chain	74	DIQMTQSPSSLSASVGDRVTITCKASQSVSNDVAWYQQKPGK APKLLIYYAANRYTGVPDRFSGSGYGTDFTFTISSLQPEDIAT YFCQQDYTSPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC
Humanized 13C5-IgG1 (D265A)	Full length heavy chain (IgG1)	76	EVQLVESGGGLVKPGGSLRLSCAASGFIFRSYGMSWVRQAP GKGLEWVASISSGGSTYYPDSVKGRFTISRDNAKNSLYLQMN SLRAEDTAVYDCARGYDSGFAYWGQGTLVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVAVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK

Humanized 13C5-IgG4 (F234A/ L235A)	Full length heavy chain (IgG4)	78	EVQLVESGGGLVKPGGSLRLSCAASGFIFRSYGMSWVRQAP GKGLEWVASISSGGSTYYPDSVKGRFTISRDNAKNSLYLQMN SLRAEDTAVYDCARGYDSGFAYWGQGTLVTVSSASTKGPSV FPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTK VDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN VFSCSVMHEALHNHYTQKSLSLSLG
Humanized 13C5	Full length light chain	80	DIVLTQSPASLAVSPGQRATITCRASQSVSTSSSSFMHWYQQK PGQPPKLLIKYASNLESGVPARFSGSGSGTDFTLTINPVEAND TANYYCQHSWEIPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS FNRGEC

[0076] In one embodiment, the invention provides anti-PD-L1 antibodies that comprise the light chain CDRs and heavy chain CDRs of antibodies 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and/or 15F1. The person of skill in the art will understand that the heavy and light chain CDRs of the antibodies provided herein may be independently selected, or mixed and matched, to form an antibody or binding fragment thereof comprising any heavy chain CDR1, CDR2, and CDR3; and any light chain CDR1, CDR2, and CDR3 from the antibodies provided herein. Thus, the invention provides anti-PD-L1 antibodies that comprise a heavy chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 81, 87, 93, 99, 105, 111, 117, 123, 129, and 135; a heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 82, 88, 94, 100, 106, 112, 118, 124, 130, and 136; a heavy chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 83, 89, 95, 101, 107, 113, 119, 125, 131, and 137; a light chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 84, 90, 96, 102, 108, 114, 120, 126, 132, and 138; a light chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 85, 91, 97, 103, 109, 115, 121, 127, 133, and 139; and a light chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:86, 92, 98, 104, 110, 116, 122, 128, 134, and 140. In one embodiment, the present invention provides anti-PD-L1 antibodies comprising heavy and light chain CDR regions comprising amino acid sequences having at least 75%, at least 80%, at least at least 81%, at least

82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homology to the corresponding light or heavy chain CDR1, CDR2, or CDR3 provided herein. In one embodiment, the present invention provides anti-PD-L1 antibodies comprising heavy and light chain CDR regions comprising amino acid sequences having 1, 2, 3, 4, 5, or 6 amino acid substitutions, deletions, or insertions relative to the corresponding light or heavy chain CDR1, CDR2, or CDR3 provided herein.

[0077] In one embodiment, the invention provides anti-PD-L1 antibodies that comprise a variable heavy chain of an antibody selected from the group consisting of 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and/or 15F1 and a variable light chain of an antibody selected from the group consisting of 13C5, 5G9, 5G11, 8C6, 7B4, 4D1, 4A8, 8H4, 8H3, and/or 15F1. In one embodiment, the antibodies and fragments provided herein comprise a heavy chain variable region comprising an amino acid sequence that is at least 75%, at least 80%, at least at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homology to a heavy chain variable region selected from the group consisting of SEQ ID NOs: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, and 46. In one embodiment, the antibodies and fragments provided herein comprise a heavy chain variable region comprising an amino acid sequence according to SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, or a variant thereof, wherein the variant comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions or deletions, or a combination thereof. In a further embodiment, the amino acid substitutions are conservative substitutions.

[0078] In one embodiment, the antibodies and fragments provided herein comprise a light chain variable region comprising an amino acid sequence that is at least 75%, at least 80%, at least at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homology to a light chain variable region selected from the group consisting of SEQ ID NOs: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, or 48. In one embodiment, the antibodies and fragments provided herein comprise a light chain variable region comprising an amino acid sequence according to SEQ ID NO: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, or a variant thereof, wherein the variant comprises 1, 2, 3,

4, 5, 6, 7, 8, 9, 10, or more amino acid substitutions, insertions, or deletions, or a combination thereof. In a further embodiment, the amino acid substitutions are conservative substitutions.

[0079] The anti-PD-L1 antibodies disclosed herein having one or more amino acid substitution, insertion, deletion, or combination thereof in the CDR or variable light or heavy chain region retain the biological activity of the corresponding anti-PD-L1 antibody that does not have an amino acid substitution, insertion, or deletion. Thus, the variant anti-PD-L1 antibodies provided herein retain binding to PD-L1. Percent homology, as used herein, refers to the number of identical amino acid sequences shared by two reference sequences, divided by the total number of amino acid positions, multiplied by 100.

[0080] In some embodiments, the anti-PD-L1 antibodies provided herein comprise conservative amino acid substitutions. The person of skill in the art will recognize that a conservative amino acid substitution is a substitution of one amino acid with another amino acid that has a similar structural or chemical properties, such as, for example, a similar side chain. Exemplary conservative substitutions are described in the art, for example, in Watson *et al.*, *Molecular Biology of the Gene*, The Bengamin/Cummings Publication Company, 4th Ed. (1987).

[0081] The skilled person will understand that the variable light and variable heavy chains may be independently selected, or mixed and matched, from the antibodies provided herein. Thus, the present invention provides anti-PD-L1 antibodies comprising a heavy chain variable region having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, and 46; and a light chain variable region having at least 80% homology to an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48.

[0082] In one embodiment, the present invention provides antibodies that bind to the same epitope as any one of the exemplary antibodies disclosed herein. Thus, in one embodiment, the present invention provides antibodies that compete for binding to PD-L1 with the exemplary antibodies provided herein.

[0083] The anti-PD-L1 antibodies and fragments thereof provided herein may further comprise Fc region modifications to alter effector functions. Fc modifications may be amino acid insertions, deletions, or substitutions, or may be chemical modifications. For example, Fc region modifications may be made to increase or decrease complement binding, to increase or decrease antibody-dependent cellular cytoxicity, or to increase or decrease the half life of the antibody.

Some Fc modifications increase or decrease the affinity of the antibody for an Fcγ receptor such as FcγRI, FcγRII, FcγRIII, or FcRn. Various Fc modifications have been described in the art, for example, in Shields et al., *J Biol. Chem* 276; 6591 (2001); Tai et al. *Blood* 119; 2074 (2012); Spiekermann et al. *J Exp. Med* 196; 303 (2002); Moore et al. *mAbs* 2:2; 181 (2010); Medzihradsky *Methods in Molecular Biology* 446; 293 (2008); Mannan et al. *Drug Metabolism and Disposition* 35; 86 (2007); and Idusogie et al. *J Immunol* 164; 4178 (2000). In some embodiments, Fc region glycosylation patters are altered. In other embodiments, the Fc region is modified by pegylation (e.g., by reacting the antibody or fragment thereof with polyethylene glycol (PEG).

[0084] In one embodiment, the antibodies or fragments thereof provided herein are immunoconjugates comprising an anti-PD-L1 antibody or fragment thereof and further comprising an agent selected from the group including an additional therapeutic agent, a cytotoxic agent, an immunoadhesion molecule, and an imaging agent. In some embodiments, the imaging agent is selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin. In some embodiments, the imaging agent is a radiolabel selected from the group consisting of: ^{3}H, ^{14}C, ^{35}S, ^{62}Cu, ^{64}Cu, ^{89}Zr, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm. In some embodiments, the therapeutic agent or cytotoxic agent is selected from the group including a chemotherapeutic agent, an immunosuppressive agent, an immuno-stimulatory agent, an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, a toxin, and an apoptotic agent. In some embodiments, the binding protein is conjugated directly to the agent. In other embodiments, the binding protein is conjugated to the agent via a linker. Suitable linkers include, but are not limited to, amino acid and polypeptide linkers disclosed herein. Linkers may be cleavable or non-cleavable.

[0085] In one embodiment, the present invention provides bispecific or multispecific antibodies specific for PD-L1 and at least one other antigen or epitope. The anti-PD-L1 antibodies and fragments thereof provided herein may be tested for binding to PD-L1 using the binding assays provided herein, or any other binding assay known in the art.

[0086] Unless otherwise stated, the practice of the present invention employs conventional molecular biology, cell biology, biochemistry, and immunology techniques that are well known in the art and described, for example, in Methods in Molecular Biology, Humana Press;

Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989), Current Protocols in Immunology (J. E. Coliganet al., eds., 1991); Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Phage display: a laboratory manual (C. Barbas III et al, Cold Spring Harbor Laboratory Press, 2001); and Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999).

[0087] In one aspect the present invention provides methods for treating a subject for a disease or condition responsive to enhancing, stimulating, or eliciting an immune response. As used herein, the terms "treatment" or "treating" refers to both therapeutic treatment and prophylactic or preventive measures. Subjects in need of treatment include those subjects that already have the disease or condition, as well as those that may develop the disease or condition and in whom the object is to prevent, delay, or diminish the disease or condition. As used herein, the term "subject" denotes a mammal, such as a rodent, a feline, a canine, and a primate. Preferably, a subject according to the invention is a human.

[0088] The term "therapeutically effective amount," as used herein, refers to the amount of a compound or composition that is necessary to provide a therapeutic and/or preventative benefit to the subject.

[0089] In one aspect, the antibodies and antigen binding fragments thereof are useful in the treatment of solid or non-solid tumors. Thus, in one aspect, the present invention provides methods for treatment of cancer. "Cancer" as used herein refers to the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to carcinoma, lymphoma, blastoma, sarcoma (including liposarcoma, osteogenic sarcoma, angiosarcoma, endotheliosarcoma, leiomyosarcoma, chordoma, lymphangiosarcoma, lymphangioendotheliosarcoma, rhabdomyosarcoma, fibrosarcoma, myxosarcoma, chondrosarcoma), neuroendocrine tumors, mesothelioma, synovioma, schwanoma, meningioma, adenocarcinoma, melanoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), Hodgkin's lymphoma; non-Hodgkin's lymphomas (Burkitt's lymphoma, small lymphocytic lymphoma/chronic lymphocytic leukemia, mycosis fungoides, mantle cell lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, marginal zone lymphoma, hairy

cell leukemia and lymphoplasmacytic leukemia), tumors of lymphocyte precursor cells, including B-cell acute lymphoblastic leukemia/lymphoma, and T-cell acute lymphoblastic leukemia/lymphoma, thymoma, tumors of the mature T and NK cells, including peripheral T-cell leukemias, adult T-cell leukemia/T-cell lymphomas and large granular lymphocytic leukemia, Langerhans cell histocytosis, myeloid neoplasias such as acute myelogenous leukemias, including AML with maturation, AML without differentiation, acute promyelocytic leukemia, acute myelomonocytic leukemia, and acute monocytic leukemias, myelodysplastic syndromes, and chronic myeloproliferative disorders, including chronic myelogenous leukemia,B-cell acute lymphoblastic leukemia/lymphoma, T-cell acute lymphoblastic leukemia/lymphoma, lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, small cell lung carcinoma, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, testicular cancer, esophageal cancer, tumors of the biliary tract, Ewing's tumor, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, testicular tumor, lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemia, lymphoma, multiple myeloma, Waldenstrom's macroglobulinemia, myelodysplastic disease, heavy chain disease, neuroendocrine tumors, Schwanoma, and other carcinomas, as well as head and neck cancer.

[0090] In one embodiment, the antibodies and fragments thereof provided herein are useful in the treatment of diseases caused by infectious agents. Infectious agents include, but are not limited to, bacterial, mycological, parasitic, and viral agents. Examples of such infectious agents include the following: staphylococcus, methicillin-resistant staphylococcus aureus, Escherichia coli, streptococcaceae, neisseriaaceae, cocci, enterobacteriaceae, enterococcus, vancomycin-

resistant enterococcus, cryptococcus, histoplasmosis, aspergillus, pseudomonadaceae, vibrionaceae, campylobacter, pasteurellaceae, bordetella, francisella, brucella, legionellaceae, bacteroidaceae, gram-negativebacilli, clostridium, corynebacterium, propionibacterium, gram-positive bacilli, anthrax, actinomyces, nocardia, mycobacterium, treponema, borrelia, leptospira, mycoplasma, ureaplasma, rickettsia, chlamydiae, candida, systemic mycoses, opportunistic mycoses, protozoa, nematodes, trematodes, cestodes, adenoviruses, herpesviruses (including, for example, herpes simplex virus and Epstein Barr virus, and herpes zoster virus), poxviruses, papovaviruses, hepatitis viruses, (including, for example, hepatitis B virus and hepatitis C virus), papilloma viruses, orthomyxoviruses (including, for example, influenza A, influenza B, and influenza C), paramyxoviruses, coronaviruses, picornaviruses, reoviruses, togaviruses, flaviviruses, bunyaviridae, rhabdoviruses, rotavirus, respiratory syncitial virus, human immunodeficiency virus and retroviruses. Exemplary infectious diseases include but are not limited to candidiasis, candidemia, aspergillosis, streptococcal pneumonia, streptococcal skin and oropharyngeal conditions, gram negative sepsis, tuberculosis, mononucleosis, influenza, respiratory illness caused by Respiratory Syncytial Virus, malaria, schistosomiasis, and trypanosomiasis.

[0091] In one embodiment, the antibodies and fragments thereof provided herein are useful in the treatment of diseases mediated by T-helper type 2 (Th2) T cells, such as, for example, asthma, allergy, or graft versus host disease.

[0092] In one embodiment, the antibodies and fragments thereof provided herein are useful in for the stimulation of an immune response in a subject in need thereof. For example, in one embodiment, the anti-PD-L1 antibodies and fragments thereof may be administered in conjunction with an antigen of interest for the purpose of eliciting an immune response to said antigen. An antigen of interest may be an antigen associated with a pathogen such as a virus or bacterium. Thus, in one embodiment, the present invention provides a vaccine comprising an anti-PD-L1 antibody and an antigen, wherein the vaccine elicits an antigen-specific immune response.

[0093] In one embodiment, the anti-PD-L1 antibodies provided herein modulate regulatory T cell function. CD4+ CD25+ regulatory T cells are lymphocytes that suppress or reduce the effects of effector T cell functions. The terms “regulatory T cell” and “Treg” are used interchangeably herein. In one embodiment, the anti-PD-L1 antibodies provided herein prevent

or reverse the inhibitory effects of regulatory T cells on effector T cell cytokine production. For example, in one embodiment, the anti-PD-L1 antibodies provided herein restore the capacity for IFNγ production to effector T cells in contact with regulatory T cells.

[0094] In one embodiment, the antibodies and fragments thereof disclosed herein may be administered to the subject by at least one route selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intratympanic, intrauterine, intravesical, intravitreal, bolus, subconjunctival, vaginal, rectal, buccal, sublingual, intranasal, intratumoral, and transdermal.

[0095] In one embodiment, the antibodies and fragments thereof disclosed herein may be administered to a subject in need thereof in combination with one or more additional therapeutic agent. In one embodiment, the antibodies and fragments thereof may be administered to a subject before, during, and/or after administration to the subject of the additional therapeutic agent. In one embodiment, the additional therapeutic agent is a chemotherapeutic agent, radiotherapeutic agent, cytokine, antibody or fragment thereof, or any other additional therapeutic that is indicated for the disease to be treated. In one embodiment, the anti-PD-L1 antibody and the additional therapeutic agent exhibit therapeutic synergy when administered together, whether concurrently or sequentially. In one embodiment, the anti-PD-L1 antibody and the additional therapeutic agent are administered in separate formulations. In another embodiment, the anti-PD-L1 antibody and the additional therapeutic agent are administered in the same formulation. In one embodiment, the anti-PD-L1 antibodies and fragments provided herein enhance the immune modulating effect of the one or more additional therapeutic agent. In another embodiment, the one or more additional therapeutic agent enhances the effect of the anti-PD-L1 antibody or fragment thereof.

[0096] The present invention provides isolated antibodies and antigen binding fragments thereof, and nucleic acids encoding such antibodies and fragments, as well as compositions comprising such isolated antibodies, fragments, and nucleic acids. The term “isolated” refers to a compound of interest (e.g., an antibody or nucleic acid) that has been separated from its natural

environment. The present invention further provides pharmaceutical compositions comprising the isolated antibodies or fragments thereof, or nucleic acids encoding such antibodies or fragments, and further comprising one or more pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers include, for example, excipients, diluents, encapsulating materials, fillers, buffers, or other agents.

[0097] The use of the singular includes the plural unless specifically stated otherwise. The word "a" or "an" means "at least one" unless specifically stated otherwise. The use of "or" means "and/or" unless stated otherwise. The meaning of the phrase "at least one" is equivalent to the meaning of the phrase "one or more." Furthermore, the use of the term "including," as well as other forms, such as "includes" and "included," is not limiting. Also, terms such as "element" or "component" encompass both elements or components comprising one unit and elements or components comprising more than one unit unless specifically stated otherwise.

[0098] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims. The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of non-critical parameters that could be changed or modified to yield essentially similar results.

EXAMPLES

Example 1: Generation of hPD-L1 monoclonal antibody

Immunization of mice with hPD-L1-HisTag and hPD-L1-mFc

[0099] To generate antibodies against the human PD-L1, cDNAs encoding the open reading frame of the extracellular domain of hPD-L1 fused with a histidine tag (hPD-L1-HisTag, SEQ ID NOs:143 and 144), mouse Fc (hPD-L1-mFc, SEQ ID NOs:145 and 146), and human Fc tag (hPD-L1-hFc, SEQ ID NO:147 and 148) were obtained by PCR and subcloned into expression vector pcDNA3.1 (Invitrogen CAT#:V-790), respectively. After transient expression in freestyle 293 cells, hPD-L1-HisTag was purified with NTA column (GE healthcare), hPD-L1-mFc and hPD-L1-hFc were purified with Protein G column (GE healthcare).

[00100] BALB/cJ mice were immunized subcutaneously every 2 weeks for 6 weeks with recombinant hPD-L1-HisTag protein (100μg/mouse) or hPD-L1-mFc emulsified with an equal volume of Freund's complete/incomplete adjuvant. Three days before fusion, mice were boosted by intravenous injection of the antigen without adjuvant. Spleen cells (1×10^8) from immunized mouse were fused with SP2/0 myeloma cells (1.5×10^7) with PEG Hybri-Max (Sigma Inc., CAT#:7181). After fusion, the cells were distributed into 96-well plates at 0.1 ml per well and incubated at 37°C, 5% CO_2 incubator. On day 1, cells were fed by adding an additional 0.1 ml per well with media containing serum and HAT plus 2×methotrexate. On day 3 and day 7, 0.1 ml of media from each well was replaced with 0.1 ml of fresh HT media. The screening typically occurred between days 9-14, and culture supernatant was tested for antibody reacting with hPD-L1-hFc by ELISA.

[00101] To clone the selected hybridoma cell, limiting dilution was carried out four times. The hybridoma cells were cultured in Dulbecco's Modified Eagle's medium (GIBCO; Invitrogen Corporation, Carlsbad, Calif.) containing 10% fetal calf serum, 1% penicillin/streptomycin, 2% L-glutamine, and 1% adjusted $NaHC0_3$ solution. The selected hybridoma cells were then adapted in serum free culture medium and the antibody was purified from the supernatant using Protein-G column (GE healthcare). After washing with PBS, bound antibodies were eluted using 0.1 M Glycine pH3.0, followed by pH neutralization using 2.0 M Tris. Ultra-15 centrifugal concentrators (Amicon) were used for buffer exchanging and antibody concentrating.

Example 2: Anti-PD-L1 antibodies cDNA sequences cloning and humanization

Cloning of immunoglobulin cDNAs

[00102] Total RNA isolated from the hybridoma cell line producing hPD-L1 antibody by RNeasy Mini Kit (Qiagen, CAT#:74104) was used as the template to synthesize first-strand cDNA with SuperScript® II Reverse Transcriptase (Life Technology, CAT#:18064-14) according to the manufacturer's instructions. The cDNA product was then subjected to PCR in a 50 μl volume reaction mixture using degenerate mouse IgG primers (Kettleborough CA, et al, European Journal of Immunology 23: 206-211 (1993), Strebe N, et al, Antibody Engineering 1:3-14 (2010)). The reaction was carried out in a S1000™ Thermal Cycler (Bio-Rad, CAT#:184-2000) with 30 cycles of: 94° C, 1.5 minutes for denaturation; 50° C, 1 minutes for annealing; and

72° C, 1 minute for synthesis. At the end of the 30th cycle, the reaction mixture was incubated another 7 minutes at 72° C for extension.

[00103] The PCR mixture was subjected to electrophoresis in a 1% agarose/Tris-Borate gel containing 0.5 μg/ml ethidium bromide. DNA fragments having the expected sizes (approximately 450 bp for the heavy chain and the light chain) were excised from the gel and purified. 3 μl of purified PCR product were cloned into the pMD-18T vector (Takara, CAT#:D101A) and transformed into One Shot® TOP10 chemically competent E. coli (Invitrogen, CAT#:C4040-03). Clones were screened by colony PCR using universal M13 forward and reverse primers, and 10 positive clones from each reaction were chosen for DNA sequencing in both directions using M13 forward and M13 reverse primers.

[00104] The heavy and light variable region sequences of antibodies m4A8 (SEQ ID NOs: 25-28), m4D1 (SEQ ID NOs: 21-24), m5G9 (SEQ ID NOs: 5-8), m5G11 (SEQ ID NOs: 9-12), m8C6 (SEQ ID NOs: 13-16), m8H3 (SEQ ID NOs: 33-36), m8H4 (SEQ ID NOs: 29-32), m7B4 (SEQ ID NOs: 17-20), m13C5 (SEQ ID NOs: 1-4) and m15F1 (SEQ ID NOs: 37-40) were amplified from the corresponding hybridoma clones. These antibodies showed desired functions, such as blocking PD-L1 binding to PD-1, and enhanced T cell activation and cytokine release.

Construction and expression of chimeric 5G11 and 13C5 antibody

[00105] 8C6, 8H4, 5G11 and 13C5 chimeric light chains (SEQ ID NOs: 52, 56, 62, and 68, respectively) were constructed by linking the PCR-cloned cDNAs of mouse VL regions to human kappa chain constant region, respectively. 8C6, 8H4, 5G11 and 13C5 chimeric heavy chains (SEQ ID NOs: 50 (8C6-IgG4), 54 (8H4-IgG4), 58 (5G11-IgG1), 60 (5G11-IgG4), 64 (13C5-IgG1), and 66 (13C5-IgG4)) were constructed by linking the PCR-cloned cDNAs of mouse VH regions to human IgG1 and IgG4 constant regions. The 5′ends of the mouse cDNA sequences were modified using PCR primers designed to add a leader sequence to both light chain and heavy chain.

[00106] Freestyle 293 cells (200 mL at 10^6/mL) were transfected with 100μg of each of the chimeric heavy and light chain expression plasmids and cultured for 6 days. The chimeric antibody in the supernatant was then purified with Protein-G column (GE healthcare). Binding of the chimeric antibody with PD-L1 was measured by ELISA and Biacore, and was shown to bind to PD-L1 with comparable affinity to that of the murine parent antibody.

Antibody humanization design

[00107] 5G11 and 13C5 antibodies were humanized using CDR grafting approach (see, for example, U.S. Pat. No.5,225,539). The light chain and heavy chain variable chain sequences of the murine antibody 5G11 and 13C5 were compared to those available in the Research Collaboratory for Structural Bioinformatics (RCSB) protein databank (http://www.ncbi.nlm.nih.gov/igblast/igblast.cgi). The model of 5G11 and 13C5 were generated respectively based on the VH and VL structure with the highest sequence homology.

[00108] The template human antibodies to be grafted with the complementary determining regions (CDRs) in the VH and VL of mouse 5G11 and 13C5 antibody were selected from human antibody germlines having high sequence homology with mouse 5G11 and 13C5 antibody by searching the international immunogenetics information system website (http://www.imgt.org/3Dstructure-DB/cgi/DomainGapAlign.cgi). For 5G11, the template human VH selected was a combination of IGHV2-5*10 and IGHJ4*01, and template human VL selected was a combination of IGKV1-33*01 and IGKJ2*01. For 13C5, the template human VH selected was a combination of IGHV3-21*04 and IGHJ4*01, and template human VL selected was a combination of IGKV7-3*01 and IGKJ2*01.

[00109] CDR amino acid sequences of the aforementioned template human antibodies were substituted by the CDRs of hybridoma (mouse) 5G11 (SEQ ID NOs 93-98) and 13C5 (SEQ ID NOs 81-86) antibodies. The frameworks of the above-mentioned template human antibody VH and VL were grafted with the necessary amino acid sequences from VH and VL of mouse 5G11 and 13C5 antibody to give a functional humanized antibody. As for VH and VL of 5G11 and 13C5, several sites of framework amino acid of the aforementioned template human antibody were backmutated to the corresponding amino acid sequences in mouse 5G11 and 13C5 antibody. For the light chain variable region of humanized 5G11 antibody, the amino acid at position 60 is mutated from Ser (S) to Asp (D), and the amino acid at position 67 is mutated from Ser (S) to Tyr (Y); and for the heavy chain variable region of humanized 5G11 antibody, the amino acid at position 24 is mutated from Phe (F) to Val (V), the amino acid at position 49 is mutated from Ala (A) to Gly (G), the amino acid at position 73 is mutated from Thr (T) to Asn (N), and the amino acid at position 83 is mutated from Thr (T) to Asn (N). For the light chain variable region of humanized 13C5, the amino acid at position 53 is mutated from Tyr (Y) to Lys

(K); and for the heavy chain variable region of humanized 13C5, the amino acid at position 28 is mutated from Thr (T) to Ile (I), the amino acid at position 30 is mutated from Ser (S) to Arg (R), the amino acid at position 49 is mutated from Ser (S) to Ala (A), and the amino acid at position 94 is mutated from Tyr (Y) to Asp (D). The amino acid sequences of VH and VL of humanized 5G11 are provided as SEQ ID NOs:42 and 44, respectively; DNA sequences encoding the VH and VL of humanized 5G11 are provided as SEQ ID NOs: 41 and 43, respectively. The amino acid sequences of VH and VL of humanized 13C5 are provided as SEQ ID NOs: 46 and 48, respectively); DNA sequences encoding the VH and VL of humanized 13C5 are provided as SEQ ID NOs: 45 and 47, respectively.

[00110] The amino acid sequences of the full light chain for humanized antibodies 5G11 and 13C5 are provided as SEQ ID NOs: 74 and 80, respectively. The DNA sequences encoding the full length humanized 5G11 and 13C5 are provided as SEQ ID NOs: 73 and 79, respectively. IgG1 and IgG4 versions of the humanized 5G11 and 13C5 antibodies were produced. The IgG1 constant region carries D265A mutation (Clynes R, et al, Nature Medicine 6: 443-446 (2000)), while IgG4 constant region has F234A and L235A double mutation (Xu D, et al, Cellular Immunology 200: 16-26 (2000)). The DNA and amino acid sequences for the full length IgG1 heavy chain of humanized antibody 5G11-hIgG1 are provided as SEQ ID NOs: 69 and 70, respectively. The DNA and amino acid sequences for the full length IgG4 heavy chain of humanized antibody 5G11-hIgG4 are provided as SEQ ID NOs: 71 and 72, respectively. The DNA and amino acid sequences for the full length IgG1 heavy chain of humanized antibody 13C5-hIgG1 are provided as SEQ ID NOs: 75 and 76, respectively. The DNA and amino acid sequences for the full length IgG4 heavy chain of humanized antibody 13C5-hIgG4 are provided as SEQ ID NOs: 77 and 78, respectively.

Construction and expression of humanized 5G11 and 13C5 antibody

[00111] DNA encoding humanized 5G11 and 13C5 antibody light chain and heavy chain was synthesized and cloned to the expression vector pcDNA3.1 (Invitrogen, CAT: #V-790). Freestyle 293 cells (200 mL at 10^6/mL) were transfected with 100μg of each of the humanized heavy and light chain expression plasmids and cultured for 6 days. The humanized antibody in the supernatant was then purified with Protein-G column (GE healthcare).

[00112] The binding kinetics between PD-L1 and PD-L1 antibodies were measured by Biacore analysis, which was performed at 25°C on a Biacore3000 instrument and recorded with a data collection rate of 1 Hz. Polyclonal rabbit anti-mouse IgG (GE, BR-1008-38) was diluted with 10 mM pH 5.0 sodium acetate and immobilized onto reference and experiment flow cells of a CM5 biosensor chip to around 15000RU using an amine coupling kit (GE, BR10050). In the beginning of each cycle, diluted test antibody (1.5 μg/mL) was injected over experiment flow cell for 1 minute to be captured. PD-L1 analyte series were prepared by diluting the stocks with running buffer to 100nM followed by 2X serial dilution in the same buffer down to 0.78nM. Analytes were injected in series over the reference and experiment flow cells for 3 minutes at a flow rate of 30 μL/minute. Running buffer (PBS with 0.05% P20) was allowed to flow over for 10 minutes at a flow rate of 30 μL/minute. At the end of each cycle, the biosensor surface was regenerated with 3 minutes injection of 10 mM pH1.7 Glycine-HCl buffer at a flow rate of 10 μL/minute. For each analyte sample injection (i.e. each cycle), binding responses obtained from the experimental biosensor surface were double referenced by subtracting simultaneously recorded responses from the reference surface followed by additional subtraction of responses from a single referenced running buffer sample. The association and dissociation rate constants (ka and kd) were determined simultaneously by fitting double-referenced sensorgrams of the entire titration series to Langmuir model (1:1) using Biaevaluation 4.0 software. The dissociation constant, KD, was calculated from the determined rate constants by the relation KD = kd/ka. The binding affinity of anti-PD-L1 antibodies with human PD-L1 and cynomolgus PD-L1 (cyno-PD-L1) are summarized in **Table 4**.

Table 4. PD-L1 binding affinity of anti-PD-L1 antibodies

Selected Antibody	Antigen	KD (M)
m4A8	Human PD-L1	2.33E-9
m4D1	Human PD-L1	4.39E-9
m5G9	Human PD-L1	4.78E-9
m5G11	Human PD-L1	1.90E-10
m7B4	Human PD-L1	6.01E-9
m8H3	Human PD-L1	6.60E-9
m8H4	Human PD-L1	4.56E-9
m8C6	Human PD-L1	1.53E-9

m13C5	Human PD-L1	1.35E-9
m15F1	Human PD-L1	3.59E-9
ch5G11	Human PD-L1	2.86E-10
ch13C5	Human PD-L1	2.28E-09
hu5G11	Human PD-L1	2.25E-10
hu13C5	Human PD-L1	1.74E-09
hu5G11	Cyno- PD-L1	2.75E-10
hu13C5	Cyno- PD-L1	2.43E-09

Example 3: ELISA based binding analysis of anti-PD-L1 antibodies

[00113] ELISA binding analyses were conducted based on human PD-L1-mFc (for chimeric and humanized antibody detection) and PD-L1-hFc protein (for hybridoma antibody detection). 96-well plates (Costar, Cat No: 9018) were coated with 100 µL of 2 µg/ml PD-L1-mFc (Crownbio) in coating buffer PBS (Hyclone, Cat No:SH30256.01B) overnight at 4° C. The wells were aspirated and non-specific binding sites were blocked by adding 200 µL of blocking buffer (PBS with 1% (w/v) of bovine serum albumin (BSA, Roche, Cat No:738328)) and incubating for 1 hour at 37° C. After the plates were washed three times with wash buffer (PBS with 0.05% (v/v) Tween20 (Sigma, Cat No:P1379)), 100 µL/well of 1:10 serial dilutions of hybridoma (**Figure 1**), chimeric (**Figure 2**), or humanized (**Figure 3**) anti-PD-L1 antibodies in blocking buffer (starting from 20 µg/mL) were added and incubated at room temperature for 1 hour. The plates were washed and incubated with 100 µL/well of Goat anti-Mouse IgG (H+L) (Thermo, Cat No: 31432) in blocking buffer for 60 min. After the plates were washed, 100 µL/well of substrate solution TMB (eBioscience, Cat No: 00-4201-56) were added and the plates were incubated for 2min at room temperature. 100 µL/well of stop solution (2N H_2SO_4) was added to stop the reaction. The colorimetric signals were developed and read at 450 nm using an Auto Plate SpectraMax Plus (Supplier: Moleculer Devices; Model: MNR0643; Software: SoftMax Pro v5.4). Data were analyzed using GraphPad Prism 5 and EC50 was calculated (**Figures 1-3**; **Tables 5-7**). These data demonstrated that anti-PD-L1 antibodies (hybridoma, chimeric, and humanized) bind PD-L1, as measured by ELISA.

Table 5. ELISA based binding EC50 of anti-PD-L1 hybridoma monoclonal antibody with PD-L1

hybridoma Ab	m5G11	m7B4	m4D1	m8H4	m13C5
EC50 ng/ml	45.9	31.42	7.14	29.04	65.1
hybridoma Ab	**m8C6**	**m5G9**	**m4A8**	**m8H3**	**m15F1**
EC50 ng/ml	18.2	31.2	57.6	48.7	48.7

Table 6. ELISA based binding EC50 of anti-PD-L1 chimeric antibody with PD-L1

Chimeric Ab	ch5G11 hIgG1	ch5G11 hIgG4	ch8C6 hIgG4	ch8H4-hIgG4	ch13C5 hIgG1	ch13C5 hIgG4
EC50 ng/ml	82.1	90	76	133.6	72.1	118

Table 7. ELISA based binding EC50 of humanized anti-PD-L1 antibody with PD-L1

Humanized Ab	hu13C5-hIgG1	hu13C5-hIgG4	hu5G11-hIgG1	hu5G11-hIgG4
EC50 (ng/ml)	85.6	126.82	49.5	69.9

[00114] ELISA based ligand blockage analyses were conducted via blocking biotinylated human PD-L1-mFc's binding to human PD-1-hFc. PD-1-hFc antigen (Crownbio) was suspended in PBS buffer (2ug/ml, 100ul/well) and coated on the 96 well plate (Costar, Cat No: 9018) 4°C overnight. The wells were aspirated and non-specific binding sites were blocked by adding 200 µL of blocking buffer (PBS with 1% (w/v) of bovine serum albumin (BSA, Roche, Cat No:738328)) and incubating for 1 hour at 37° C. After the plate was washed three times with wash buffer (PBS with 0.05% (v/v) Tween20 (Sigma, Cat No:P1379)), 100 µL/well of 1:3 serial dilutions of hybridoma (**Figure 4**), chimeric (**Figure 5**), or humanized (**Figure 6**) anti-PD-L1 antibodies in blocking buffer (starting from 20 µg/mL) were added and incubated at 37°C for 1 hour. 100 µl PDL-1-mFc-biotin (0.1µg/ml) was then added to each well and incubated at 37°C for 2h. After the plate was washed 3 times, secondary antibody (Avidin HRP eBioscience cat No.:E07418-1632, 1:500, 100 µl/well) was added and incubated at 37 °C for 0.5 hour. After the plate was washed, 100 µL/well of substrate solution TMB (eBioscience, Cat No: 00-4201-56) was added and the plate was incubated for 3min at room temperature. 100 µL/well of stop solution (2N H_2SO_4) was added to stop the reaction. The colorimetric signals were developed and read at 450 nm using an Auto Plate SpectraMax Plus (Supplier: Moleculer Devices; Model:

MNR0643; Software: SoftMax Pro v5.4). Data were analyzed using GraphPad Prism 5 and IC50 was calculated (**Figurse 4-6**; **Tables 8-10**). These data demonstrated that anti-PD-L1 antibodies (hybridoma, chimeric, and humanized) can block PD-1's binding with PD-L1 on the cell surface, as measured by ELISA.

Table 8. IC50 of anti-PD-L1 hybridoma monoclonal antibody inhibiting PD-1 binding with PD-L1 on solid surface

Hybridoma Ab	m5G11	m7B4	m4D1	m8H4	m13C5	m8C6	m5G9	m4A8	m8H3	m15F1
IC50 (ng/ml)	710.2	892.0	332.2	787.8	871.7	343.7	613.2	867.8	647.4	655.3

Table 9. IC50 of anti-PD-L1 chimeric antibody inhibiting PD-1 binding with PD-L1 on solid surface

Chimeric Ab	ch5G11-hIgG1	ch5G11-hIgG4	ch8C6-hIgG4	ch8H4-hIgG4	ch13C5-hIgG1	ch13C5-hIgG4
IC50 (ng/mL)	1006	926.1	476.6	848.1	805.2	375.3

Table 10. IC50 of humanized anti-PD-L1 antibody inhibiting PD-1 binding with PD-L1 on solid surface

Humanized Ab	hu5G11-hIgG1	hu5G11-hIgG4	hu13C5-hIgG1	hu13C5-hIgG4
IC50 (ng/ml)	793.6	822.5	1202.6	1192.4

Example 4: Cell-based binding analysis of anti-PD-L1 antibodies

[00115] Cell binding analyses of anti-PD-L1 antibodies were performed based on binding to a 293T cell line stably expressing PD-L1 (PD-L1-293T). $2x10^5$ 293T-PD-L1 cells were added into each well of 96-well culture plates and incubated with the indicated antibody (20μg/ml with the dilution of 1:5) at 4^0C for 1 h. After the cells were washed three times with FACS buffer, the secondary antibody (PE Goat anti-mouse: 1:200; PE mouse anti-human: 1:10) was added to the cells at 100μl/well, and incubated at 4^0C for 40min. Cells were washed three times with FACS buffer and analyzed by FACS Array. Binding of hybridoma antibodies is shown in **Figure 7a** and **7b**. Binding of chimeric antibodies is shown in **Figure 8**. Binding of humanized antibodies is shown in **Figure 9**. The calculated EC50 for hybridoma , chimeric, and humanized antibodies are shown below in **Tables 11**, **12**, and **13**, respectively. These data demonstrated that anti-PD-

L1 antibodies (hybridoma, chimeric, and humanized) bind PD-L1, as measuerd by FACS analysis.

Table 11. EC50 of anti-PD-L1 hybridoma monoclonal antibody with the PD-L1 on cell surface

Hybridoma Ab	**m4D1**	**m4A8**	**m5G11**	**m8H4**	**m8H3**
EC50 ng/ml	36.07	67.83	35.94	43.49	50.81
Hybridoma Ab	**m8C6**	**m9G9**	**m7B4**	**m13C5**	**m15F1**
EC50 ng/ml	40.97	33.7	47.41	45.29	47.8

Table 12. EC50 of anti-PD-L1 chimeric antibody with the PD-L1 on cell surface

Chimeric Ab	**ch13C5 hIgG1**	**ch5G11 hIgG1**	**ch5G11 hIgG4**
EC50 ng/ml	75.75	58.26	89.68

Table 13. EC50 of humanized anti-PD-L1 antibody with the PD-L1 on cell surface

Humanized Ab	**hu5G11-hIgG1**	**hu5G11-hIgG4**	**hu13C5-hIgG1**	**hu13C5-hIgG4**
EC50 ng/ml	47.93	54.33	80.01	80.39

[00116] The effect of anti-PD-L1 antibody on PD-1 binding to PD-L1 on the cell surface was also investigated. Briefly, PD-L1-293T cells were suspended in FACS buffer (PBS with 3% fetal calf serum). Various concentrations of the hybridoma (**Figure 10**), chimeric (**Figure 11**), or humanized (**Figure 12**) anti-PD-L1 antibodies were added to the cell suspension and incubated at 4^0C for 60 minutes in 96 well plates. Biotin-labeled PD-L1 protein was then added to the wells and incubated at 4^0C for 60 minutes. The cells were washed 3 times with PBS and incubated with mouse anti-biotin PE (Biolegend, cat# 409004). The cell-associated fluorescence was then detected by flow cytometry analysis using FACS array. The effects of anti-PD-L1 antibodies on PD-1 binding with PD-L1-293T were measured by the mean fluorescent intensity (MFI) of staining. Inhibition of PD-1 binding by anti-PD-L1 hybridoma antibodies is shown in **Figures 10a** and **10b**. Inhibition of PD-1 binding by anti-PD-L1 chimeric antibodies is shown in **Figure 11.** Inhibition of PD-1 binding by anti-PD-L1 humanized antibodies is shown in **Figure 12**. The calculated IC50 for the hybridoma (**Table 14**), chimeric (**Table 15**), and humanized (**Table 16**)

antibodies are shown in the tables below. These data demonstrated that anti-PD-L1 antibodies (hybridoma, chimeric, and humanized) can block PD-1's binding with PD-L1 on the cell surface, as measured by FACS analysis.

Table 14. IC50 of anti-PD-L1 hybridoma monoclonal antibody inhibiting PD-1 binding with PD-L1 on cell surface

Hybridoma Ab	**mIgG1**	**m4D1**	**m5G11**	**m13C5**	**m7B4**	**m8H4**
IC50 ng/ml	NA	27.3	16.3	28.9	38.1	30. 6
Hybridoma Ab	**m4A8**	**m5G9**	**m8C6**	**m8H3**	**m15F1**	
IC50 ng/ml	29.1	49.1	8.2	33.6	21.1	

Table 15. IC50 of anti-PD-L1 chimeric antibody inhibiting PD-1 binding with PD-L1 on cell surface

Chimeric Ab	**ch5G11-hIgG1**	**ch5G11-hIgG4**	**ch8C6-hIgG4**	**ch8H4-hIgG4**	**ch13C5-hIgG1**	**ch13C5-hIgG4**
IC50 ng/ml	40.36	33.18	34.91	42.02	42.71	35.78

Table 16. IC50 of humanized anti-PD-L1 antibody inhibiting PD-1 binding with PD-L1 on cell surface

Humanized Ab	**hIgG4**	**hu13C5-hIgG1**	**hu13C5-hIgG4**	**hu5G11-hIgG1**	**hu5G11-hIgG4**
IC50 ng/ml	NA	18.5	49.9	16.5	9.6

Example 5: Effect of anti-PD-L1 antibodies on T cell activation in a mixed lymphocyte reaction

[00117] A mixed lymphocyte reaction was employed to demonstrate the effect of murine (**Figure 13a, 13b**), chimeric (**Figure 14a, 14b**), or humanized (**Figure 15a, 15b**) anti-PD-L1 antibodies in blocking the PD-L1/PD-1 pathway in lymphocyte effector cells. T cells in the assay were tested for IFN-γ and IL-2 secretion in the presence or absence of humanized anti-PD-L1 antibody.

[00118] Human $CD4^+$ T-cells were purified from human PBMC using a $CD4^+$ negative selection isolation kit (Mitenyi Biotech, cat# 130-091-155). Immature dendritic cells (DC) were derived from monocytes isolated from human PBMC using the Mo-DC Generation Toolbox

(Miltenyi, Cat#130-093-568). The cells were cultured with Mo-DC Differentiation Medium for 7 days, and were then induced to be mature DC with Mo-Dc Maturation medium for 2 days. To set up the MLR, for each reaction, 10^5 purified T-cells and 10^4 allogeneic mature DC cells were added in a total volume of 200 µl. The testing antibody was assayed at different concentrations as shown in **Figures 13a, 13b, 14a, 14b, 15a**, and **15b** (i.e., 20µg/mL, 2µg/mL, 0.2 µg/mL, 0.02 µg/mL, and 0.002 µg/mL). Either no antibody or an isotype control antibody was used as a negative control. The cells were cultured for 5 days at 37 ^{0}C. On day 6th, the levels of IFN-γ and IL-2 in the culture medium were measured using the IL-2 ELISA kit (eBioscience) and hIFN-γ ELISA kit (R&D, cat#DY285). The results are shown in **Figures 13a, 14a,** and **15a** for IL-2 secretion, and **Figures 13b, 14b,** and **15b** for IFN-γ secretion. The results of the study showed that hybridoma, chimeric, and humanized anti-PD-L1 antibodies promoted T-cell IFN-γ and IL-2 secretion in a concentration dependent manner. In contrast, cultures containing the isotype control antibody did not show increase in IFN-γ and IL-2 secretion.

Example 6: Effect of anti-PD-L1 antibody on the function of T regulatory cells

[00119] T regulatory cells (CD4+, CD25+) are lymphocytes that suppress the immune response. The effect of T regulatory cells on cytokine secretion of T effector cells in MLR was tested in the presence or absence of chimeric or humanized anti-PD-L1 antibodies. T regulatory cells (CD4+ CD25+) were purified from PBMC using a regulatory T cell isolation kit (Miltenyi Biotec, cat#130-091-301). Immature dendritic cells (DC) were derived from monocytes isolated from human PBMC using the Mo-DC Generation Toolbox (Miltenyi, cat#130-093-568). The cells were cultured with Mo-DC Differentiation Medium for 7 days, and were then induced to be mature DC with Mo-Dc Maturation medium for 2 days. T regulatory cells were added into a mixed lymphocyte reaction containing purified $CD4^+$ $CD25^-$ T cells and allogeneic dendritic cells in a 4:1 ratio of $CD4^+$ $CD25^-$ to T regulatory cells. For example: the reaction was added with 1x10^5cells/well of $CD4^+CD25^-$ cells, 1x10^4cells/well of mDC, and 0.25x10^5cells/well of $CD4^+CD25^+$ cells. Antibody was added to each reaction at a concentration of 10 µg/ml. Either no antibody or an isotype control antibody was used as a negative control. The cells were cultured for 5 days at 37° C. On the 5th day, 50 µl medium was taken to detect IL-2 and IFN-gamma concentration. After supplementing each well with 50µl culture medium, the cells were cultured for another 2 days before analyzed for cell proliferation by CTG (Promega, G7573). The

levels of IFN-γ and IL-2 in the culture medium were measured using a hIFN-γ ELISA kit (R&D, cat#DY285) and IL-2 ELISA kit (eBioscience). As shown in **Figure 16**, chimeric and humanized anti-PD-L1 antibodies, ch-13C5-hIgG1, ch-13C5-hIgG4, hu-13C5-IgG1, hu-13C5-IgG4, ch-5G11-IgG1, ch-5G11-IgG4, hu-5G11-IgG1, and hu-5G11-IgG4, can reduce the inhibitory effect of Treg cells on the secretion of IFN-γ by $CD4^+CD25^-$ T effector cells, suggesting that anti-PD-L1 antibodies can modulate the immune suppression function of T regulatory cells.

Example 7: Effect of humanized anti-PD-L1 antibody on autologous T cell activation

[00120] In this example, the effect of blocking PD-1/PD-L1 pathway by anti-PD-L1 antibody on T cell activation was examined. Purified human CD4+ T cells (Mitenyi Biotech, cat# 130-091-155) were activated with 1μg/ml soluble anti-CD3 antibody (R&D, cat#MAB100) in the presence of autologous monocyte-derived dendritic cells (DCs). After three days of activation in the presence or absence of titrated anti-PD-L1 antibody, culture medium was harvested and the concentration of IFNγ was measured with ELISA. The results are shown in **Figure 17** and suggest that PD-L1 blockage by humanized anti-PD-L1 antibodies enhanced IFN-γ secretion by T cells.

Example 8: Human recall T cell response to tetanus toxoid challenge is enhanced by humanized anti-PD-L1 antibody

[00121] To investigate whether the antigen-specific T cell receptor triggering was modulated by blocking PD-1/PD-L1 pathway with anti-PD-L1 antibodies, the human T-cell recall assay was employed using tetanus toxoid (TT) antigen to stimulate pre-existing memory T cells in the blood of healthy TT immunized donors. To this end, fresh PBMC from recently [<1 year] TT immunized donors were plated into 96-well round bottom plates (costar, cat#3799) at 4 x 10^5 cells/well using RPMI1640 (Invitrogen, cat# A10491-01) supplemented with 80 U/ml penicillin, 80 g/ml streptomycin and 30% autologous serum, added with humanized 5G11 or 13C5 at various concentrations, and stimulated with 0.1ug/ml SEB and 1μg/ml TT (Astarte Biologies). After co-culture for 7 days at 37°C, 5% CO_2, the supernatant was harvested and the concentration of IFN-γ was measured. **Figures 18a** and **18b** provide the results of the assay using PBMC from two separate donors. The results of the study demonstrate that, compared to TT antigen alone, PD-L1 blockage with anti-PD-L1 antibody resulted in enhanced IFN-γ secretion by memory T cells.

[00122] In summary, the humanized 5G11 and 13C5 antibody retained the functional activity of their parental antibodies during the humanization process.

CLAIMS

1. An isolated antibody or fragment thereof that binds to PD-L1, wherein the antibody or fragment thereof comprises

(i) a heavy chain CDR1, CDR2, and CDR3 sequence comprising SEQ ID NO: 81, 82, and 83, respectively, and a light chain CDR1, CDR2, and CDR3 sequence comprising SEQ ID NO: 84, 85, and 86, respectively;

(ii) a heavy chain CDR1, CDR2, and CDR3 sequence comprising SEQ ID NO: 93, 94, and 95, respectively, and a light chain CDR1, CDR2, and CDR3 sequence comprising SEQ ID NO: 96, 97, and 98, respectively;

(iii) a heavy chain CDR1, CDR2, and CDR3 sequence comprising SEQ ID NO: 99, 100, and 101, respectively, and a light chain CDR1, CDR2, and CDR3 sequence comprising SEQ ID NO: 102, 103, and 104, respectively;

(iv) a heavy chain CDR1, CDR2, and CDR3 sequence comprising SEQ ID NO: 111, 112, and 113, respectively, and a light chain CDR1, CDR2, and CDR3 sequence comprising SEQ ID NO: 114, 115, and 116, respectively; or

(v) a heavy chain CDR1, CDR2, and CDR3 sequence comprising SEQ ID NO: 123, 124, and 125, respectively, and a light chain CDR1, CDR2, and CDR3 sequence comprising SEQ ID NO: 126, 127, and 128, respectively.

2. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence according to SEQ ID NOs: 81, 82, and 83, respectively; and a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence according to SEQ ID NOs: 84, 85, and 86, respectively.

3. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence according to SEQ ID NOs: 93, 94, and 95, respectively; and a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence according to SEQ ID NOs: 96, 97, and 98, respectively.

4. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence according to SEQ ID NOs: 99, 100, and 101, respectively; and a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence according to SEQ ID NOs: 102, 103, and 104, respectively.

5. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence according to SEQ ID NOs: 111, 112, and 113, respectively; and a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence according to SEQ ID NOs: 114, 115, and 116, respectively.

6. The antibody or fragment of claim 1, wherein the antibody or fragment thereof comprises a heavy chain CDR1, CDR2, and CDR3 comprising an amino acid sequence according to SEQ ID NOs: 123, 124, and 125, respectively; and a light chain CDR1, CDR2, and CDR3 comprising an amino acid sequence according to SEQ ID NOs: 126, 127, and 128, respectively.

7. The isolated antibody or fragment thereof of any one of claims 1-6, wherein the antibody or fragment thereof is chimeric or humanized.

8. The isolated antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof comprises:

(i) a heavy chain variable region having at least 80% homology to SEQ ID NO: 2 and a light chain variable region having at least 80% homology to SEQ ID NO: 4;

(ii) a heavy chain variable region having at least 80% homology to SEQ ID NO: 10 and a light chain variable region having at least 80% homology to SEQ ID NO: 12;

(iii) a heavy chain variable region having at least 80% homology to SEQ ID NO: 14 and a light chain variable region having at least 80% homology to SEQ ID NO: 16;

(iv) a heavy chain variable region having at least 80% homology to SEQ ID NO: 22 and a light chain variable region having at least 80% homology to SEQ ID NO: 24;

(v) a heavy chain variable region having at least 80% homology to SEQ ID NO: 30 and a light chain variable region having at least 80% homology to SEQ ID NO: 32;

(vi) a heavy chain variable region having at least 80% homology to SEQ ID NO: 42 and a light chain variable region having at least 80% homology to SEQ ID NO: 44; or

(vii) a heavy chain variable region having at least 80% homology to SEQ ID NO: 46 and a light chain variable region having at least 80% homology to SEQ ID NO: 48.

9. The isolated antibody or fragment thereof of claim 8, wherein the antibody or fragment thereof comprises

(i) a heavy chain variable region comprising SEQ ID NO: 2 and a light chain variable region comprising SEQ ID NO: 4;

(ii) a heavy chain variable region comprising SEQ ID NO: 10 and a light chain variable region comprising SEQ ID NO: 12;

(iii) a heavy chain variable region comprising SEQ ID NO: 14 and a light chain variable region comprising SEQ ID NO: 16;

(iv) a heavy chain variable region comprising SEQ ID NO: 22 and a light chain variable region comprising SEQ ID NO: 24;

(v) a heavy chain variable region comprising SEQ ID NO: 30 and a light chain variable region comprising SEQ ID NO: 32;

(xi) a heavy chain variable region comprising SEQ ID NO: 42 and a light chain variable region comprising SEQ ID NO: 44; or

(xii) a heavy chain variable region comprising SEQ ID NO: 46 and a light chain variable region comprising SEQ ID NO: 48.

10. The isolated antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof comprises a heavy chain variable region according to SEQ ID NO: 42 and a light chain variable region according to SEQ ID NO: 44.

11. The isolated antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof comprises a heavy chain variable region according to SEQ ID NO: 46 and a light chain variable region according to SEQ ID NO: 48.

12. The isolated antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof comprises a heavy chain having at least 80% homology to SEQ ID NO: 70 and a light chain having at least 80% homology to SEQ ID NO: 74.

13. The isolated antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof comprises a heavy chain having at least 80% homology to SEQ ID NO: 72 and a light chain having at least 80% homology to SEQ ID NO: 74.

14. The isolated antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof comprises a heavy chain having at least 80% homology to SEQ ID NO: 76 and a light chain having at least 80% homology to SEQ ID NO: 80.

15. The isolated antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof comprises a heavy chain having at least 80% homology to SEQ ID NO: 78 and a light chain having at least 80% homology to SEQ ID NO: 80.

16. The isolated antibody or fragment thereof of claim 1, wherein the antibody comprises
(i) a heavy chain according to SEQ ID NO: 70 and a light chain according to SEQ ID NO: 74;
(ii) a heavy chain according to SEQ ID NO: 72 and a light chain according to SEQ ID NO: 74;
(iii) a heavy chain according to SEQ ID NO: 76 and a light chain according to SEQ ID NO: 80; or
(iv) a heavy chain according to SEQ ID NO: 78 and a light chain according to SEQ ID NO: 80.

17. The isolated antibody or fragment thereof of any one of claims 1-16, wherein the antibody or fragment thereof is selected from the group consisting of a monoclonal antibody, an scFv, a Fab fragment, an Fab' fragment, and an F(ab)' fragment.

18. An antibody or fragment thereof according to any one of claims 1-16, wherein the antibody or fragment thereof is linked or conjugated to a therapeutic agent.

19. The antibody or fragment thereof according to claim 18, wherein the therapeutic agent is a cytotoxic drug, a radioactive isotope, an immunomodulator, or an antibody.

20. The isolated antibody or fragment thereof according to any one of claims 1-16, wherein the antibody or fragment thereof has an affinity for PD-L1 of about 10 nM to about 0.01 nM.

21. The isolated antibody or fragment thereof according to claim 20, wherein the antibody or fragment thereof has an affinity for PD-L1 of about 10 nM or less.

22. The isolated antibody or fragment thereof according to claim 20, wherein the antibody or fragment thereof has an affinity for PD-L1 of about 1.0 nM or less.

23. The isolated antibody or fragment thereof according to any one of claims 1-16, wherein the antibody has a binding EC50 of about 5 ng/mL to about 1000 ng/mL.

24. The isolated antibody or fragment thereof according to any one of claims 1-16, wherein the antibody blocks binding of PD-L1 to PD-1.

25. The isolated antibody or fragment thereof of claim 24, wherein the antibody or fragment thereof blocks the binding of PD-L1 to PD-1 at an IC50 of about 5 ng/mL to about 1000 ng/mL.

26. The isolated antibody or fragment thereof according to any one of claims 1-16, wherein the antibody or fragment increases T cell activation as measured by inflammatory cytokine production.

27. The isolated antibody or fragment thereof according to claim 26, wherein the antibody or fragment thereof increases T cell production of IL-2 and IFNγ.

28. An isolated antibody or fragment thereof that binds to PD-L1, wherein the antibody or fragment thereof is produced by a hybridoma selected from the group consisting of 13C5, 5G11, 8C6, 4D1, or 8H4.

29. A composition comprising the antibody or fragment thereof according to any one of claims 1-28 and a pharmaceutically acceptable carrier.

30. An isolated polynucleotide encoding the antibody or fragment thereof according to any one of claims 1-28.

31. An expression vector comprising the isolated polynucleotide according to claim 30.

32. A host cell comprising the expression vector according to claim 31.

33. An isolated hybridoma cell line selected from the group consisting of 13C5, 5G11, 8C6, 4D1, or 8H4.

34. A method for increasing T cell activation, the method comprising contacting T cells with an antibody or fragment thereof according to any one of claims 1-28.

35. A method for reducing tumors or inhibiting the growth of tumor cells in a subject, the method comprising administering to the subject a therapeutically effective amount of the isolated antibody or fragment thereof according to any one of claims 1-28.

36. A method for treating a cancer in a subject in need thereof, the method comprising administering a therapeutically effective amount of the isolated antibody or fragment thereof according to any one of claims 1-28 to the subject.

37. The method according to claim 36, wherein the cancer is selected from the group consisting of lymphoma, leukemia, melanoma, glioma, breast cancer, lung cancer, colon cancer, bone cancer, ovarian cancer, bladder cancer, kidney cancer, liver cancer, stomach cancer, rectal cancer, testicular cancer, salivary cancer, thyroid cancer, thymic cancer, epithelial cancer, head or neck cancer, gastric cancer, pancreatic cancer, or a combination thereof.

38. A method for treating an infectious disease in a subject in need thereof, the method comprising administering a therapeutically effective amount of the isolated antibody or fragment thereof according to any one of claims 1-28 to the subject.

39. The method according to claim 38, wherein the infectious disease is selected from the group consisting of candidiasis, candidemia, aspergillosis, streptococcal pneumonia, streptococcal skin and oropharyngeal conditions, gram negative sepsis, tuberculosis, mononucleosis, influenza, respiratory illness caused by Respiratory Syncytial Virus, malaria, schistosomiasis, and trypanosomiasis.

Figure 1

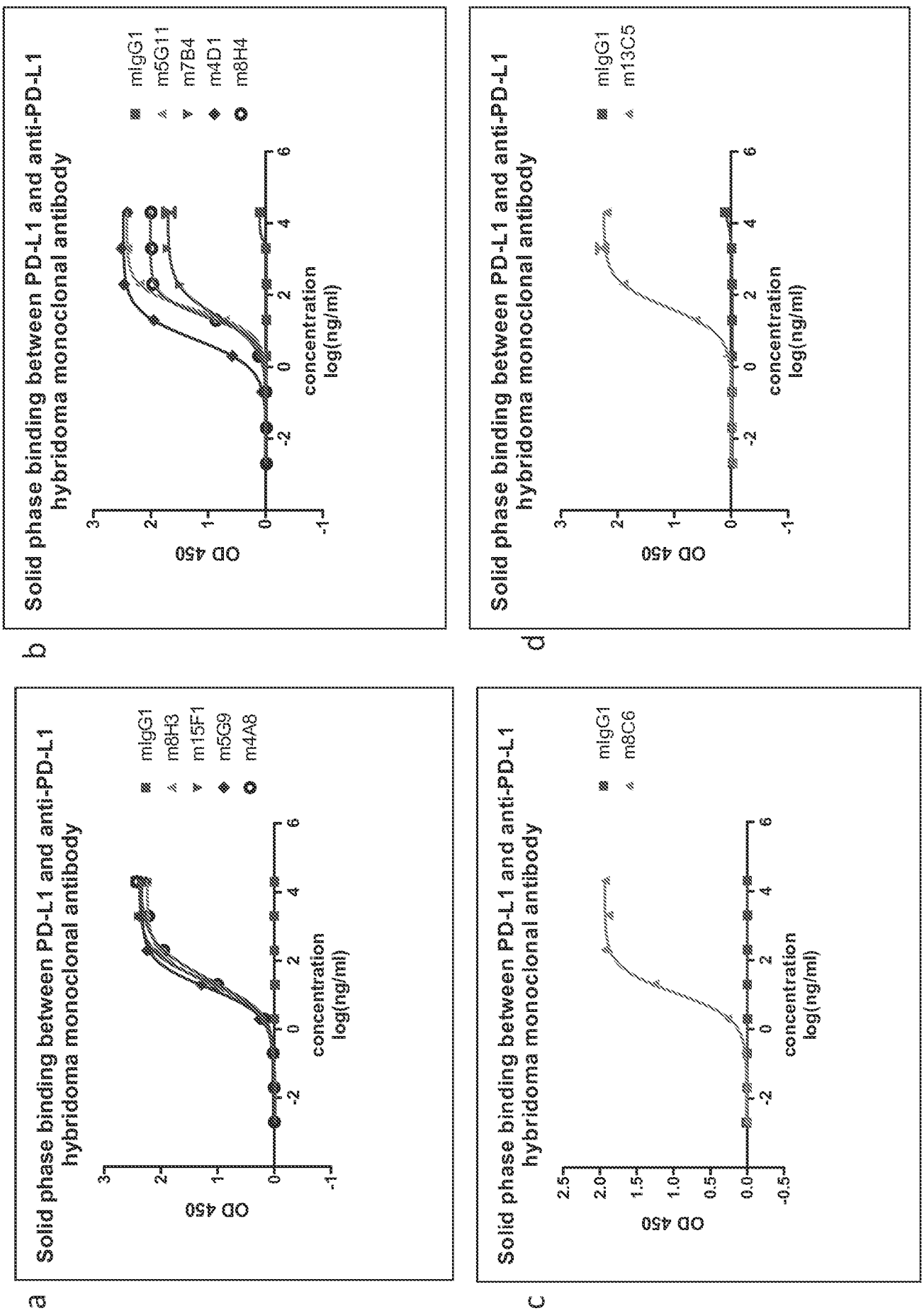

a
Solid phase binding between PD-L1 and anti-PD-L1
hybridoma monocional antibody
mIgG1
m8H3
m15F1
m5G9
m4A8
OD 450
concentration
log(ng/ml)
b
Solid phase binding between PD-L1 and anti-PD-L1
hybridoma monocional antibody
mIgG1
m5G11
m7B4
m4D1
m8H4
OD 450
concentration
log(ng/ml)
c
Solid phase binding between PD-L1 and anti-PD-L1
hybridoma monocional antibody
mIgG1
m8C6
OD 450
concentration
log(ng/ml)
d
Solid phase binding between PD-L1 and anti-PD-L1
hybridoma monocional antibody
mIgG1
m13C5
OD 450
concentration
log(ng/ml)

Figure 2

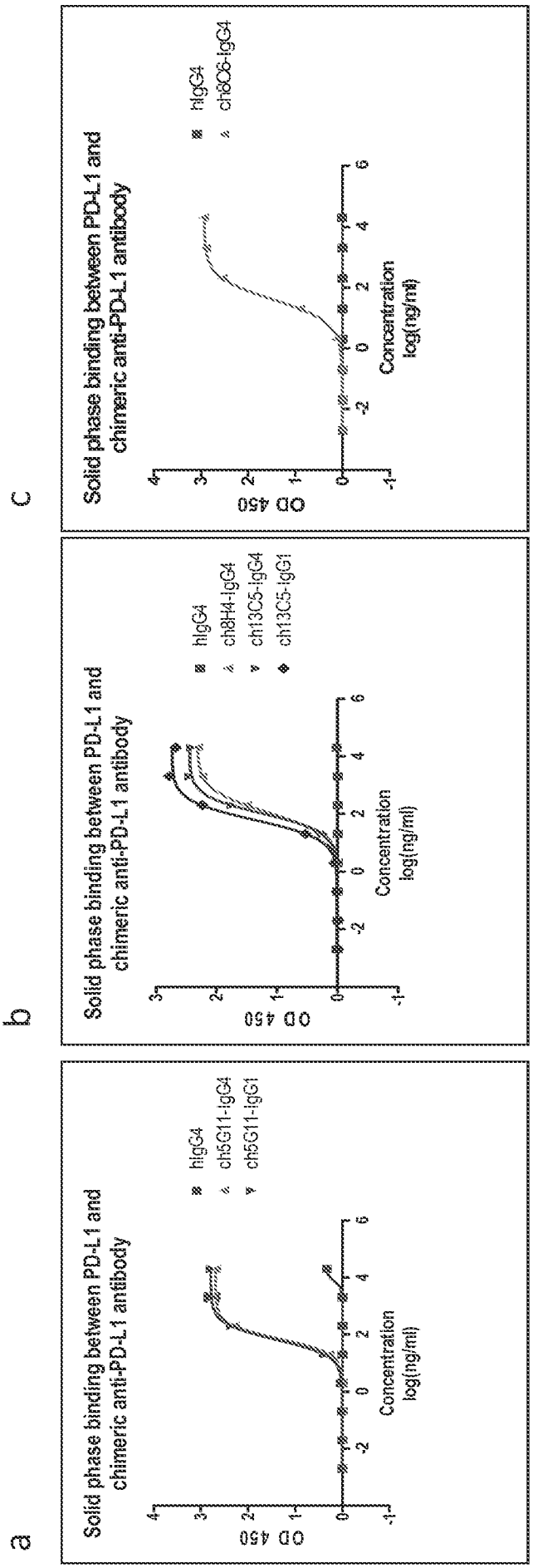

a
Solid phase binding between PD-L1 and chimeric anti-PD-L1 antibody
OD 450
Concentration log(ng/ml)
hIgG4
ch5G11-IgG4
ch5G11-IgG1
b
Solid phase binding between PD-L1 and chimeric anti-PD-L1 antibody
OD 450
Concentration log(ng/ml)
hIgG4
ch8H4-IgG4
ch13C5-IgG4
ch13C5-IgG1
c
Solid phase binding between PD-L1 and chimeric anti-PD-L1 antibody
OD 450
Concentration log(ng/ml)
hIgG4
ch6C6-IgG4

Figure 3

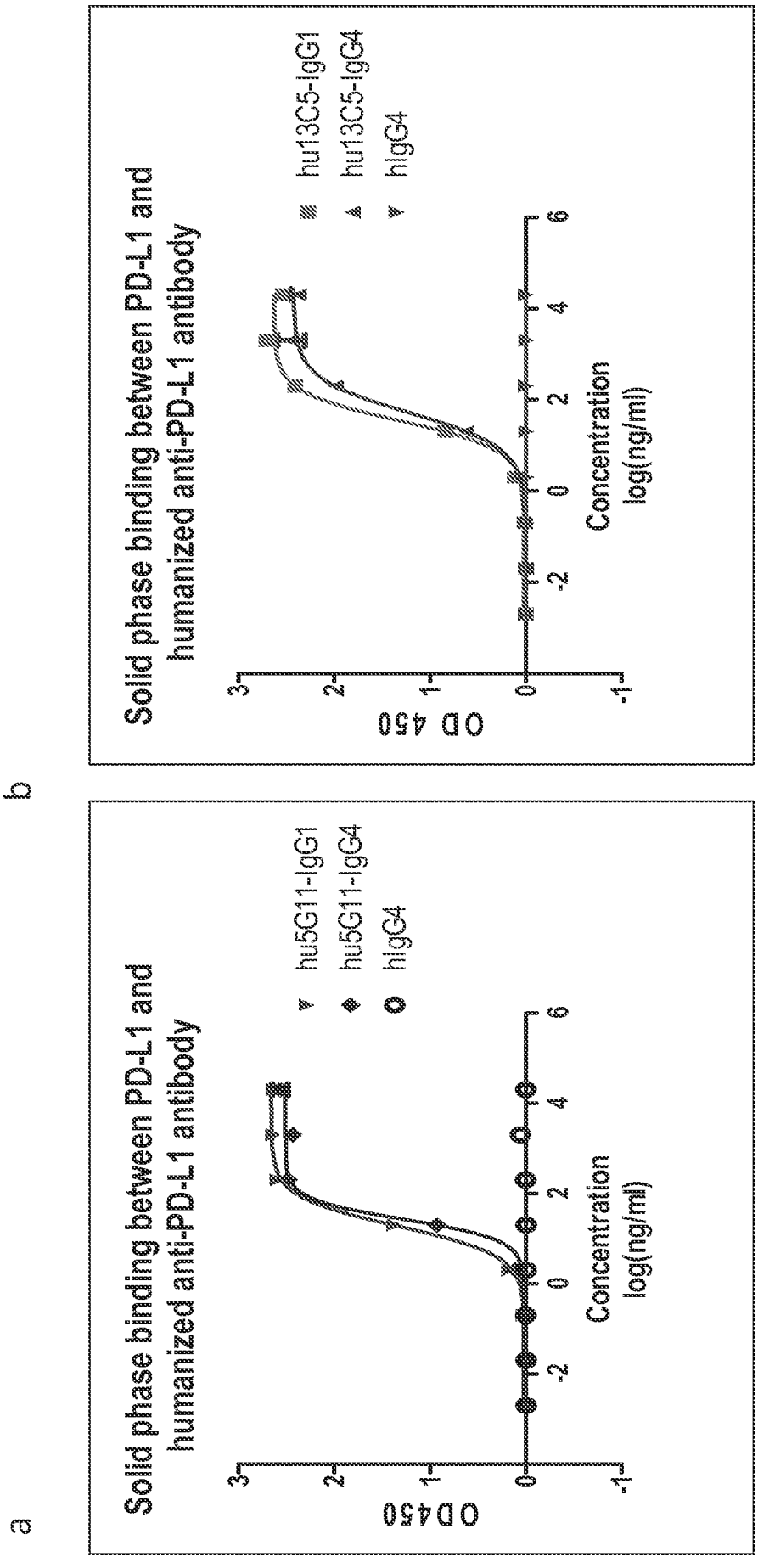

a
Solid phase binding between PD-L1 and humanized anti-PD-L1 antibody
hu5G11-IgG1
hu5G11-IgG4
hIgG4
OD450
Concentration log(ng/ml)
b
Solid phase binding between PD-L1 and humanized anti-PD-L1 antibody
hu13C5-IgG1
hu13C5-IgG4
hIgG4
OD 450
Concentration log(ng/ml)

Figure 4

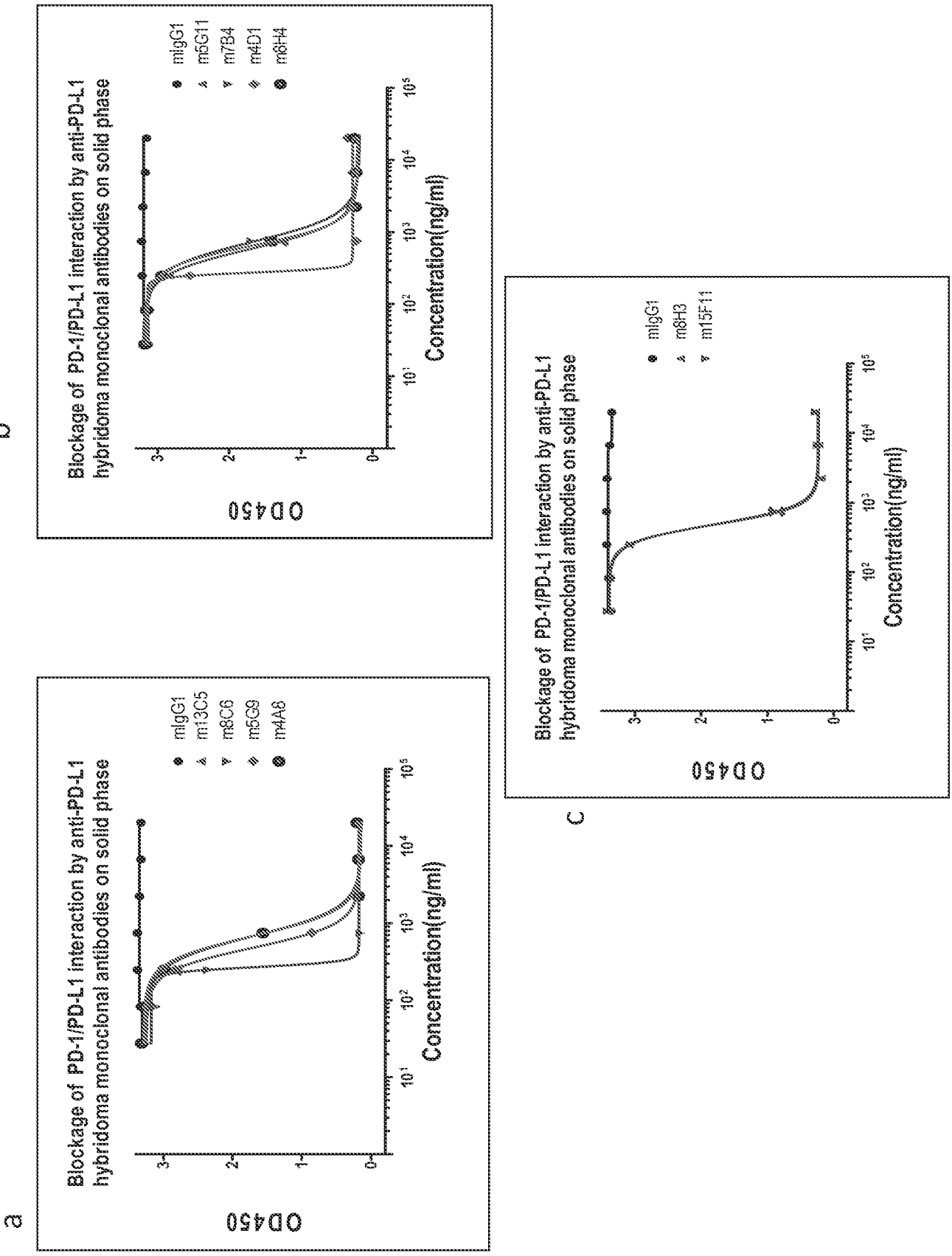

a
Blockage of PD-1/PD-L1 interaction by anti-PD-L1 hybridoma monocional antibodies on solid phase
OD450
Concentration(ng/ml)
mIgG1
m13C5
m8C6
m5G9
m4A8
b
Blockage of PD-1/PD-L1 interaction by anti-PD-L1 hybridoma monocional antibodies on solid phase
OD450
Concentration(ng/ml)
mIgG1
m5G11
m7B4
m4D1
m6H4
c
Blockage of PD-1/PD-L1 interaction by anti-PD-L1 hybridoma monocional antibodies on solid phase
OD450
Concentration(ng/ml)
mIgG1
m8H3
m15F11

Figure 5

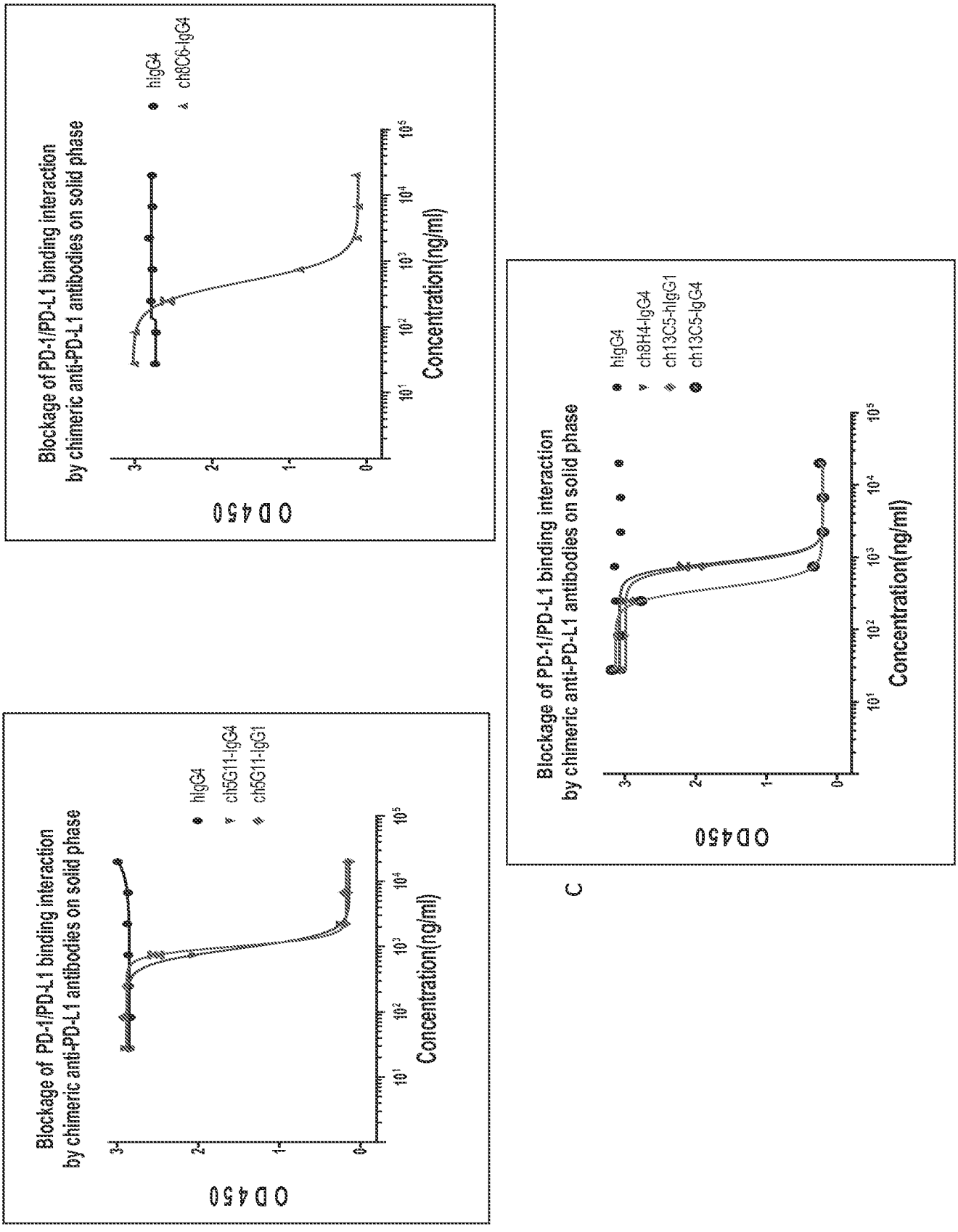
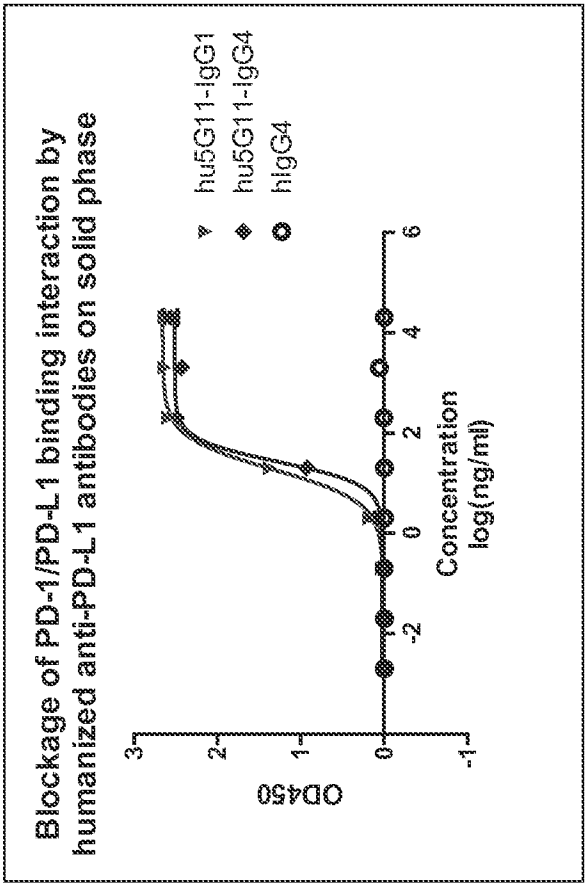

a
Blockage of PD-1/PD-L1 binding interaction
by chimeric anti-PD-L1 antibodies on solid phase
hIgG4
ch5G11-IgG4
ch5G11-IgG1
OD450
Concentration(ng/ml)
b
Blockage of PD-1/PD-L1 binding interaction
by chimeric anti-PD-L1 antibodies on solid phase
hIgG4
ch8C6-IgG4
OD450
Concentration(ng/ml)
C
Blockage of PD-1/PD-L1 binding interaction
by chimeric anti-PD-L1 antibodies on solid phase
hIgG4
ch8H4-IgG4
ch13C5-hIgG1
ch13C5-IgG4
OD450
Concentration(ng/ml)

Figure 6

a


Blockage of PD-1/PD-L1 binding interaction by humanized anti-PD-L1 antibodies on solid phase
hu5G11-IgG1
hu5G11-IgG4
hIgG4
OD450
Concentration log(ng/ml)

b

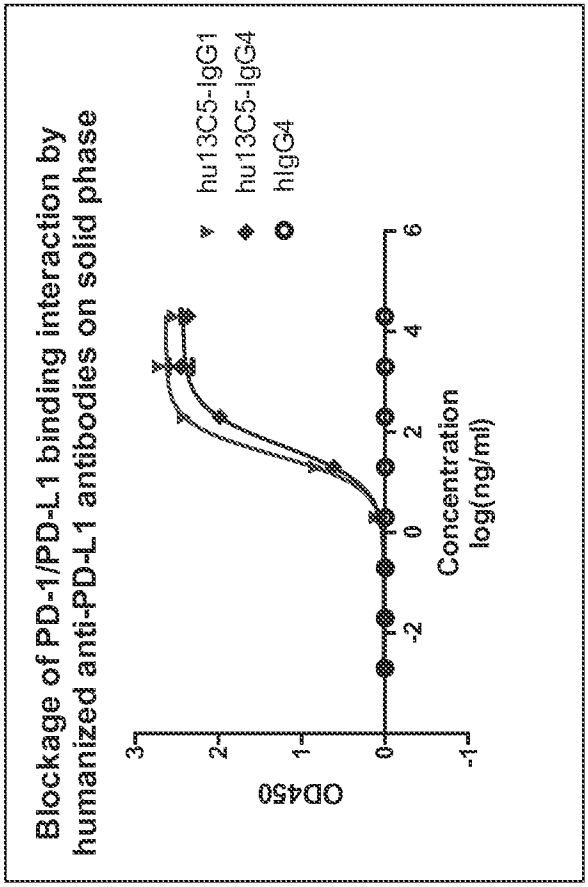
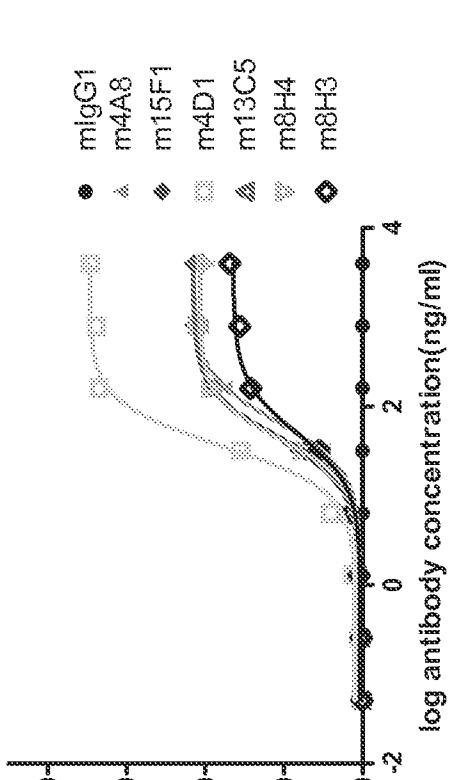

Blockage of PD-1/PD-L1 binding interaction by humanized anti-PD-L1 antibodies on solid phase
hu13C5-IgG1
hu13C5-IgG4
hIgG4
OD450
Concentration log(ng/ml)

Figure 7

a


Binding interaction between anti-PD-L1 hybridoma monoclonal antibody and the PD-L1 on cell surface
MFI
4000
3000
2000
1000
0
-2
0
2
4
log antibody concentration(ng/ml)
mIgG1
m4A8
m15F1
m4D1
m13C5
m8H4
m8H3

b

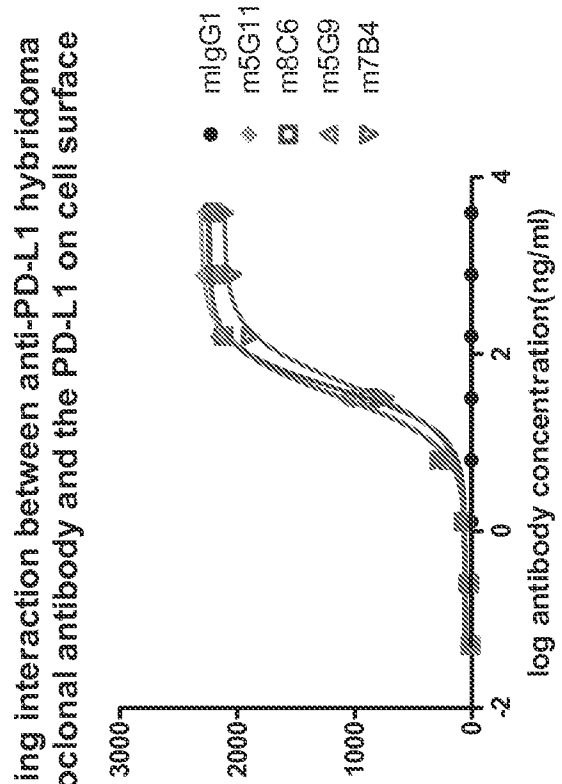

Binding interaction between anti-PD-L1 hybridoma monoclonal antibody and the PD-L1 on cell surface
MFI
3000
2000
1000
0
-2
0
2
4
log antibody concentration(ng/ml)
mIgG1
m5G11
m8C6
m5G9
m7B4

Figure 8

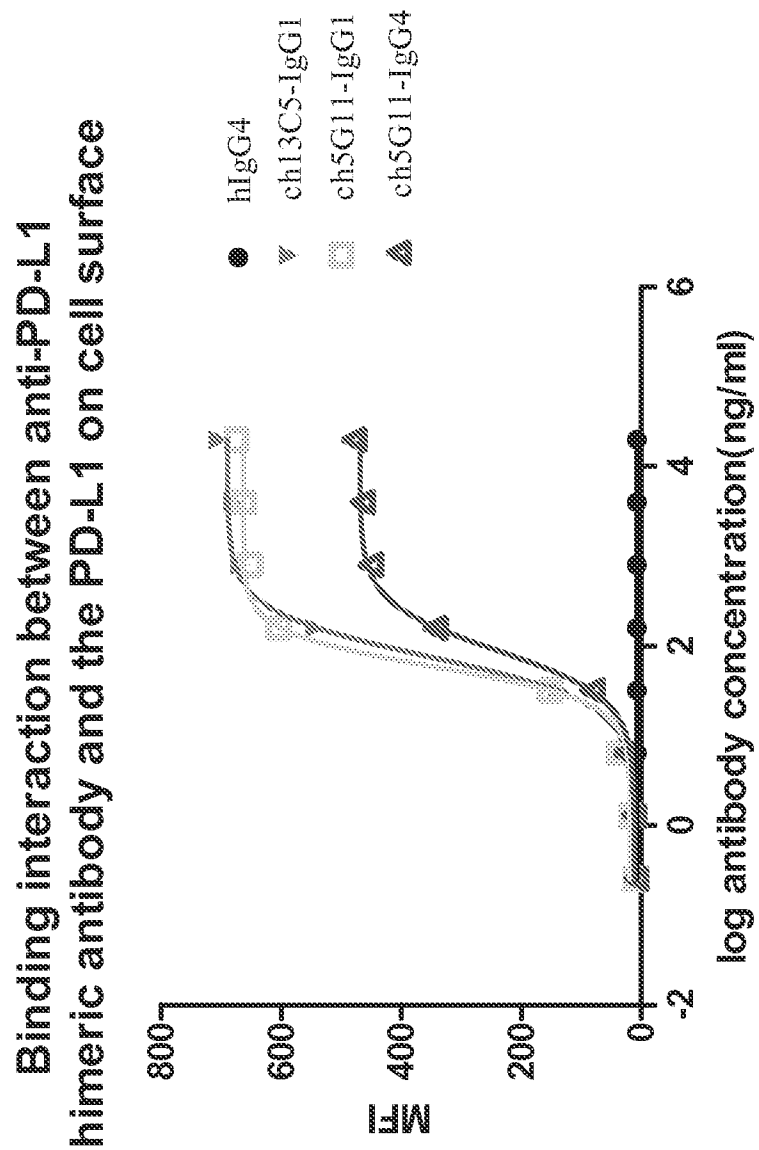

Binding interaction between anti-PD-L1
chimeric antibody and the PD-L1 on cell surface
hIgG4
ch13C5-IgG1
ch5G11-IgG1
ch5G11-IgG4
MFI
800
600
400
200
0
-2
0
2
4
6
log antibody concentration(ng/ml)

Figure 9

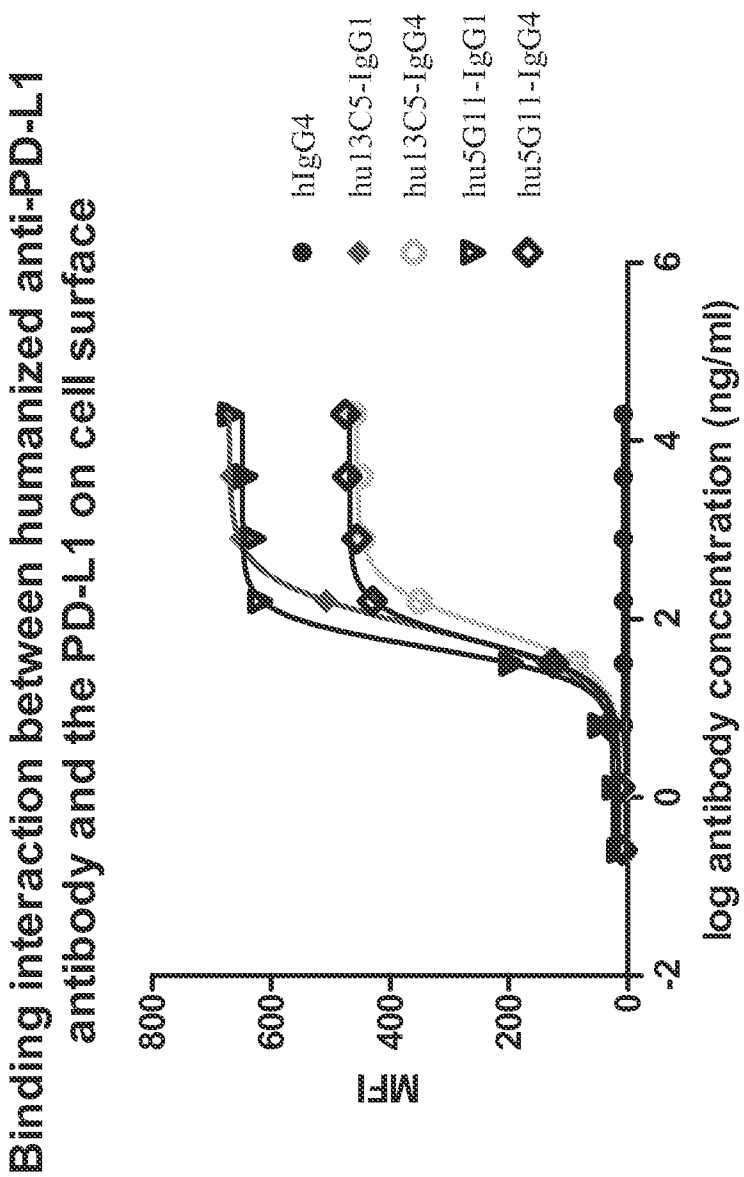

Binding interaction between humanized anti-PD-L1 antibody and the PD-L1 on cell surface
MFI
800
600
400
200
0
-2
0
2
4
6
log antibody concentration (ng/ml)
hIgG4
hu13C5-IgG1
hu13C5-IgG4
hu5G11-IgG1
hu5G11-IgG4

Figure 10

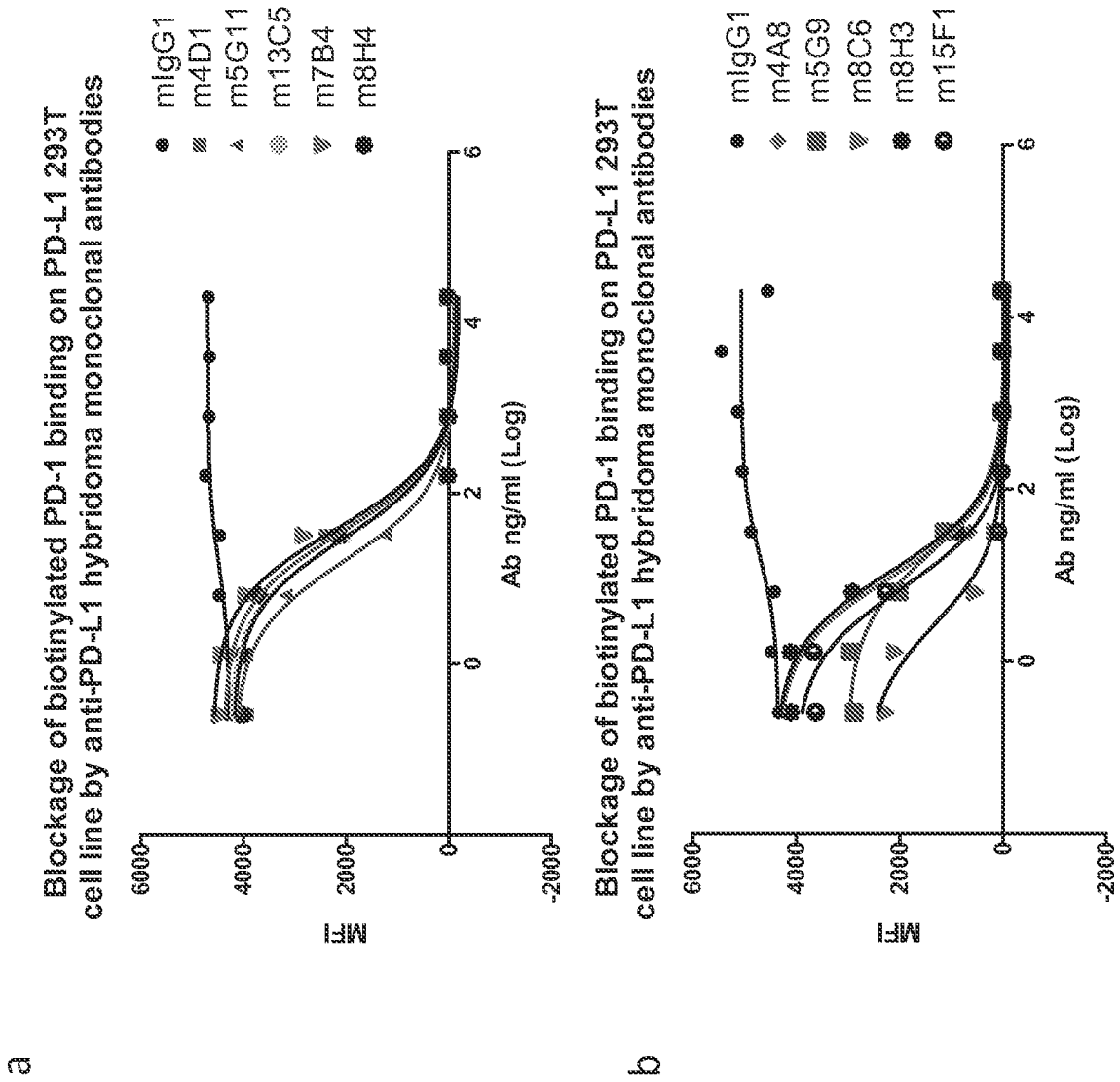

a
Blockage of biotinylated PD-1 binding on PD-L1 293T cell line by anti-PD-L1 hybridoma monoclonal antibodies
mIgG1
m4D1
m5G11
m13C5
m7B4
m8H4
MFI
6000
4000
2000
0
-2000
0
2
4
6
Ab ng/ml (Log)
b
Blockage of biotinylated PD-1 binding on PD-L1 293T cell line by anti-PD-L1 hybridoma monoclonal antibodies
mIgG1
m4A8
m5G9
m8C6
m8H3
m15F1
MFI
6000
4000
2000
0
-2000
0
2
4
6
Ab ng/ml (Log)

Figure 11

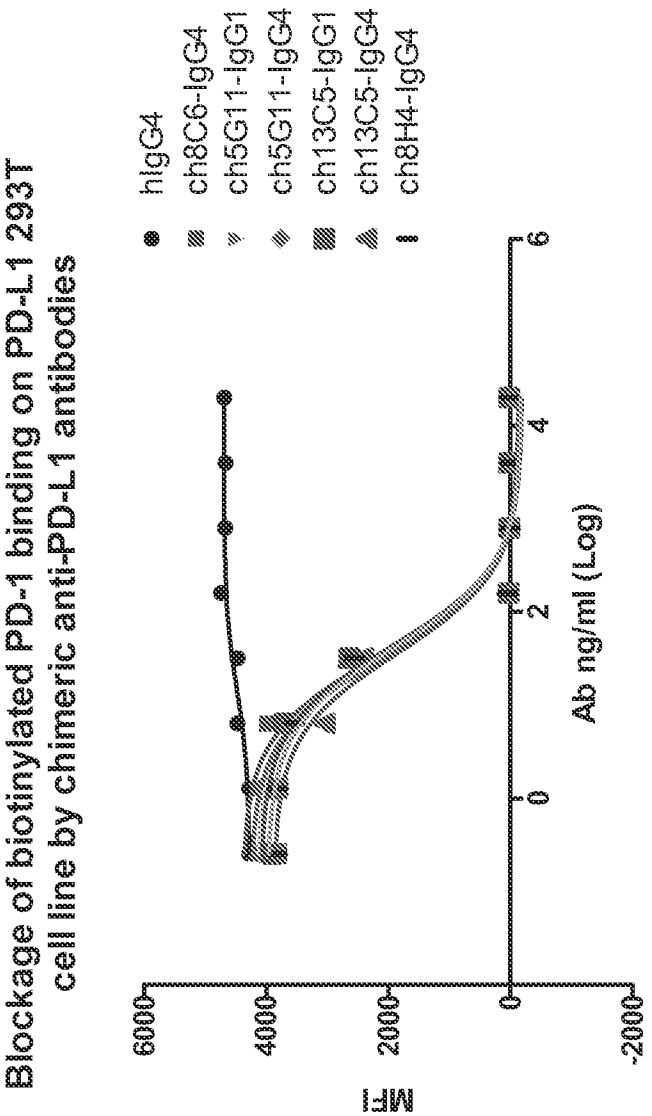

Blockage of biotinylated PD-1 binding on PD-L1 293T cell line by chimeric anti-PD-L1 antibodies
hIgG4
ch8C6-IgG4
ch5G11-IgG1
ch5G11-IgG4
ch13C5-IgG1
ch13C5-IgG4
ch8H4-IgG4
MFI
6000
4000
2000
0
-2000
0
2
4
6
Ab ng/ml (Log)

Figure 12

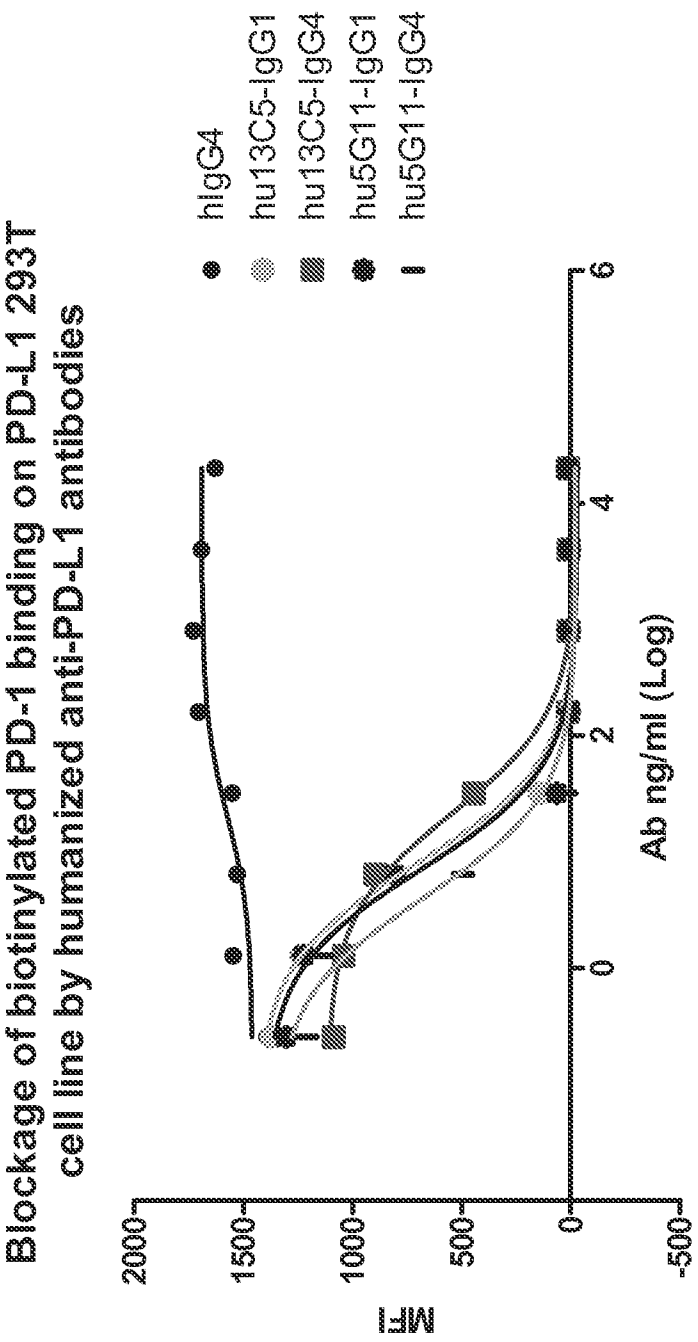

Blockage of biotinylated PD-1 binding on PD-L1 293T cell line by humanized anti-PD-L1 antibodies
hIgG4
hu13C5-IgG1
hu13C5-IgG4
hu5G11-IgG1
hu5G11-IgG4
MFI
2000
1500
1000
500
0
-500
0
2
4
6
Ab ng/ml (Log)

Figure 13a

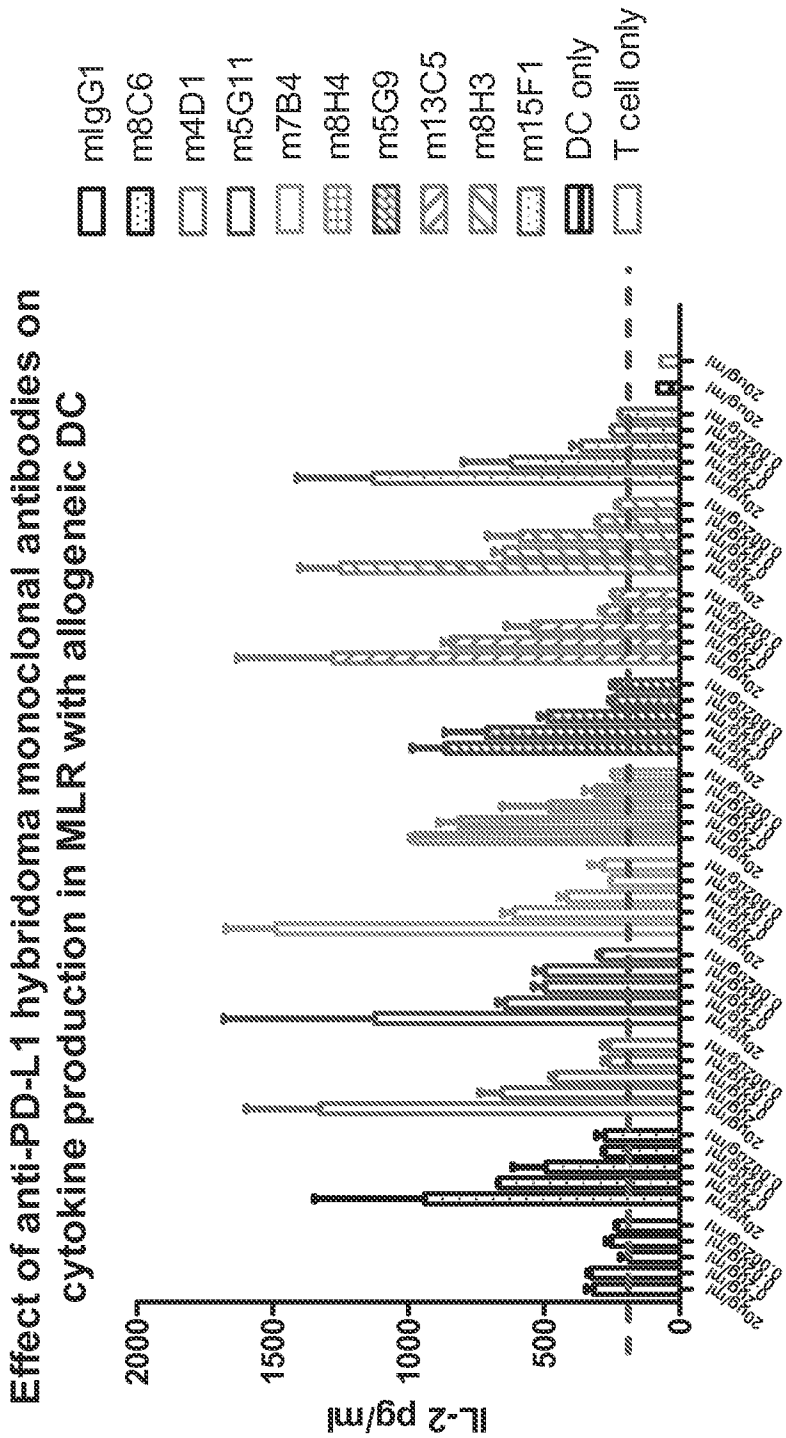

Effect of anti-PD-L1 hybridoma monoclonal antibodies on cytokine production in MLR with allogeneic DC
IL-2 pg/ml
2000
1500
1000
500
0
mIgG1
m8C6
m4D1
m5G11
m7B4
m8H4
m5G9
m13C5
m8H3
m15F1
DC only
T cell only

Figure 13b

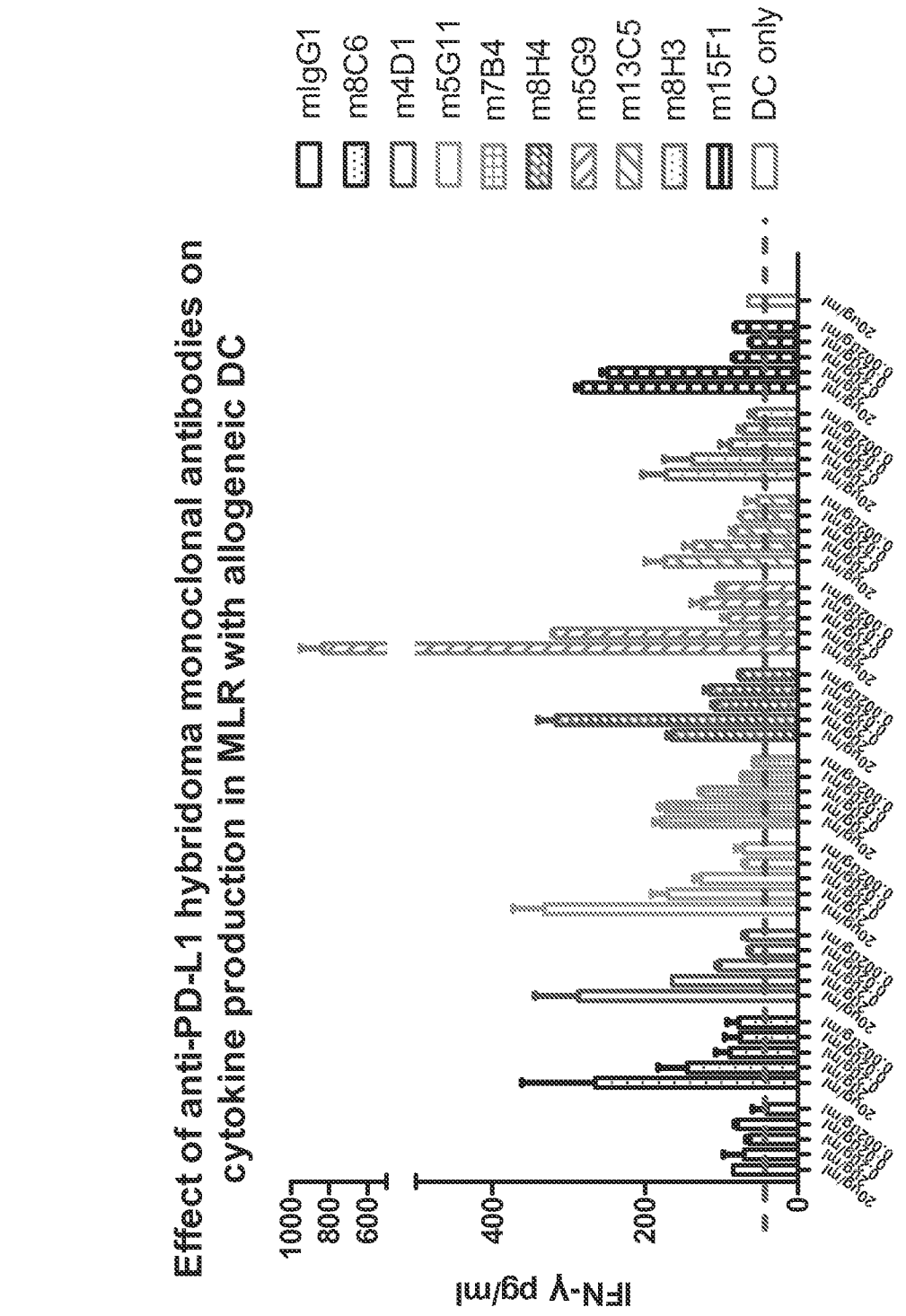

Effect of anti-PD-L1 hybridoma monoclonal antibodies on cytokine production in MLR with allogeneic DC
IFN-γ pg/ml
1000
800
600
400
200
0
mIgG1
m8C6
m4D1
m5G11
m7B4
m8H4
m5G9
m13C5
m8H3
m15F1
DC only

Figure 14

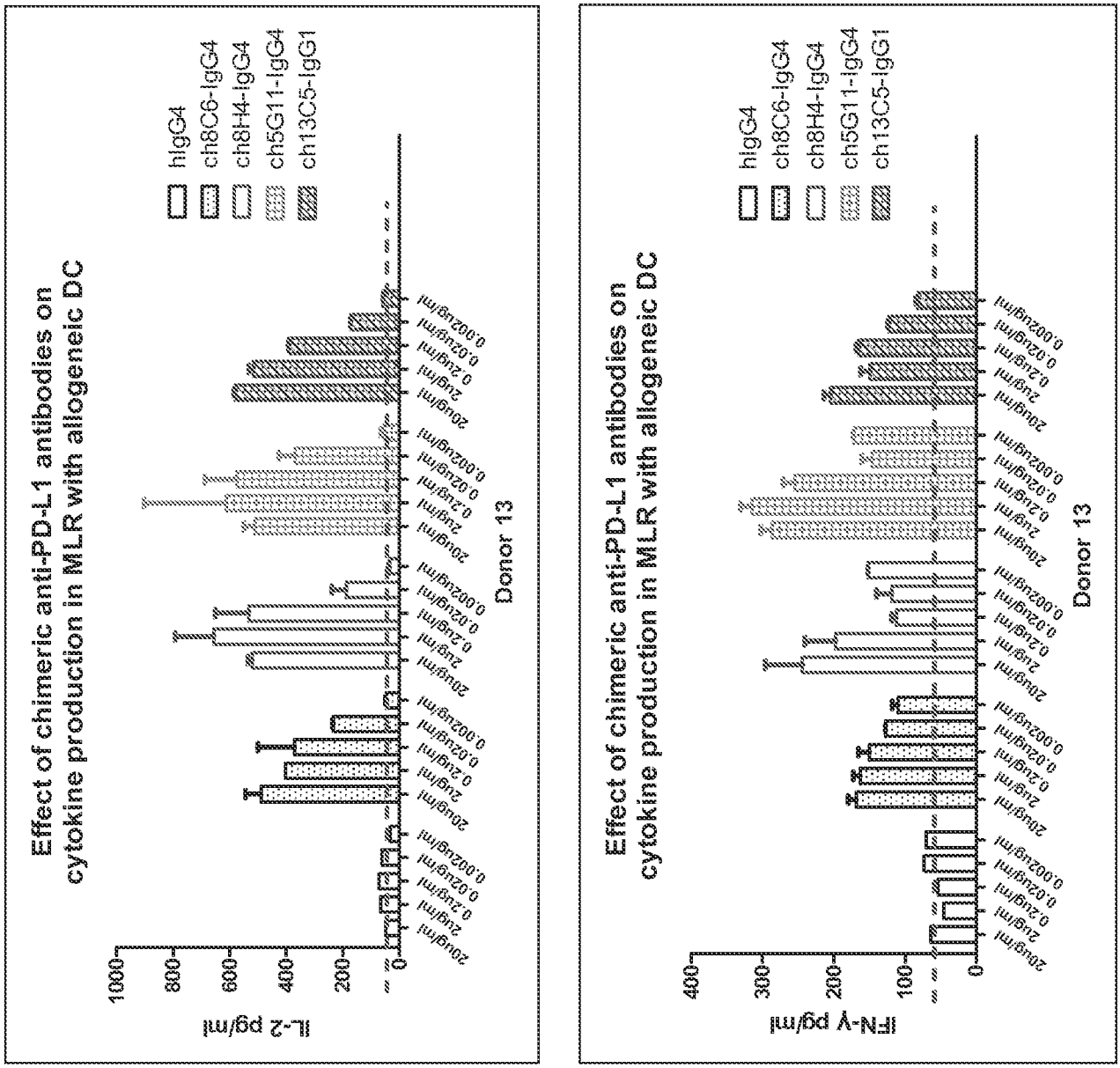

a
Effect of chimeric anti-PD-L1 antibodies on cytokine production in MLR with allogeneic DC
hIgG4
ch8C6-IgG4
ch8H4-IgG4
ch5G11-IgG4
ch13C5-IgG1
IL-2 pg/ml
1000
800
600
400
200
0
Donor 13
20ug/ml
2ug/ml
0.2ug/ml
0.02ug/ml
0.002ug/ml
b
Effect of chimeric anti-PD-L1 antibodies on cytokine production in MLR with allogeneic DC
hIgG4
ch8C6-IgG4
ch8H4-IgG4
ch5G11-IgG4
ch13C5-IgG1
IFN-γ pg/ml
400
300
200
100
0
Donor 13
20ug/ml
2ug/ml
0.2ug/ml
0.02ug/ml
0.002ug/ml

Figure 15

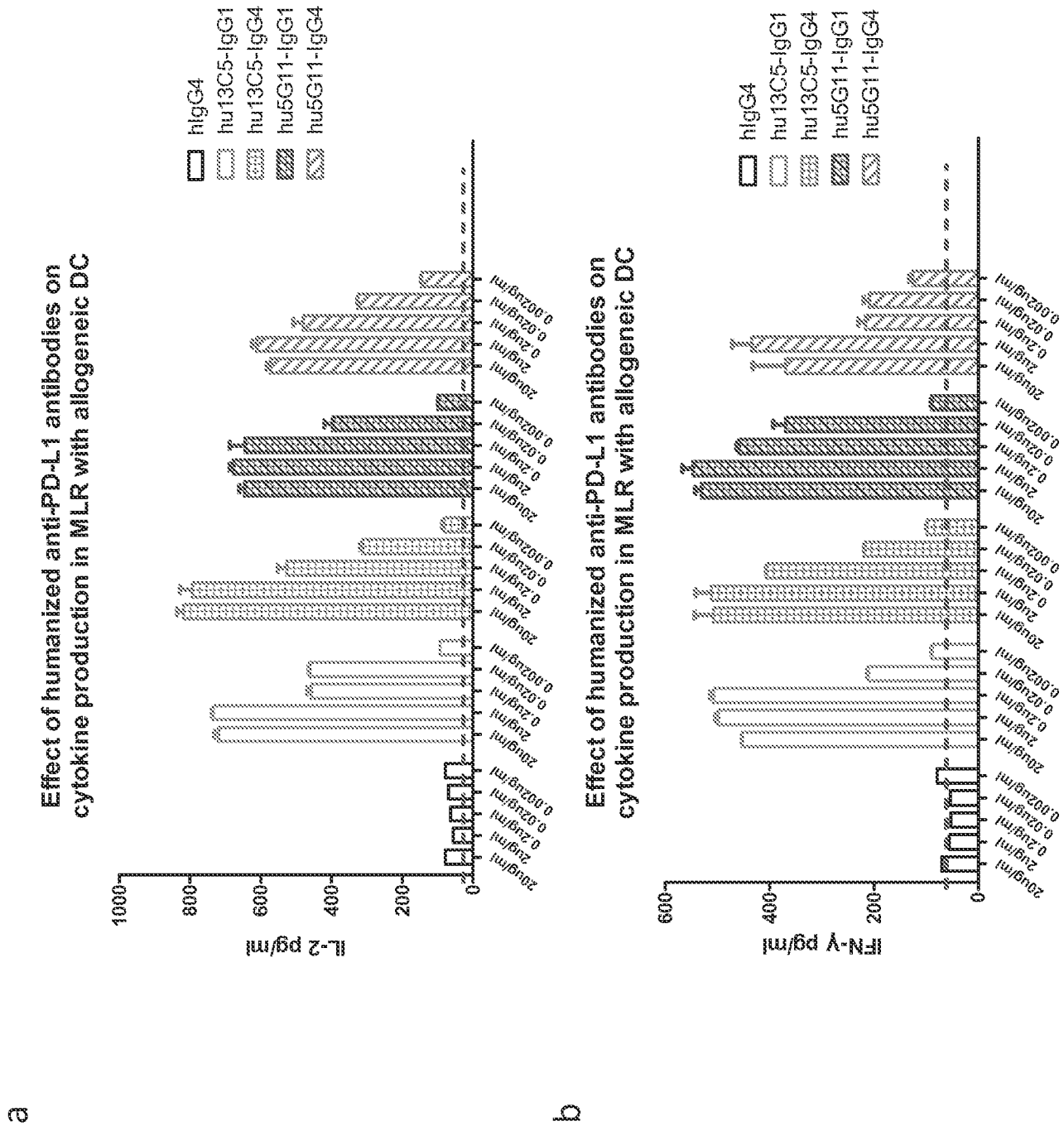

a
Effect of humanized anti-PD-L1 antibodies on cytokine production in MLR with allogeneic DC
hIgG4
hu13C5-IgG1
hu13C5-IgG4
hu5G11-IgG1
hu5G11-IgG4
IL-2 pg/ml
1000
800
600
400
200
0
20ug/ml
2ug/ml
0.2ug/ml
0.02ug/ml
0.002ug/ml
b
Effect of humanized anti-PD-L1 antibodies on cytokine production in MLR with allogeneic DC
hIgG4
hu13C5-IgG1
hu13C5-IgG4
hu5G11-IgG1
hu5G11-IgG4
IFN-γ pg/ml
600
400
200
0
20ug/ml
2ug/ml
0.2ug/ml
0.02ug/ml
0.002ug/ml

Figure 16

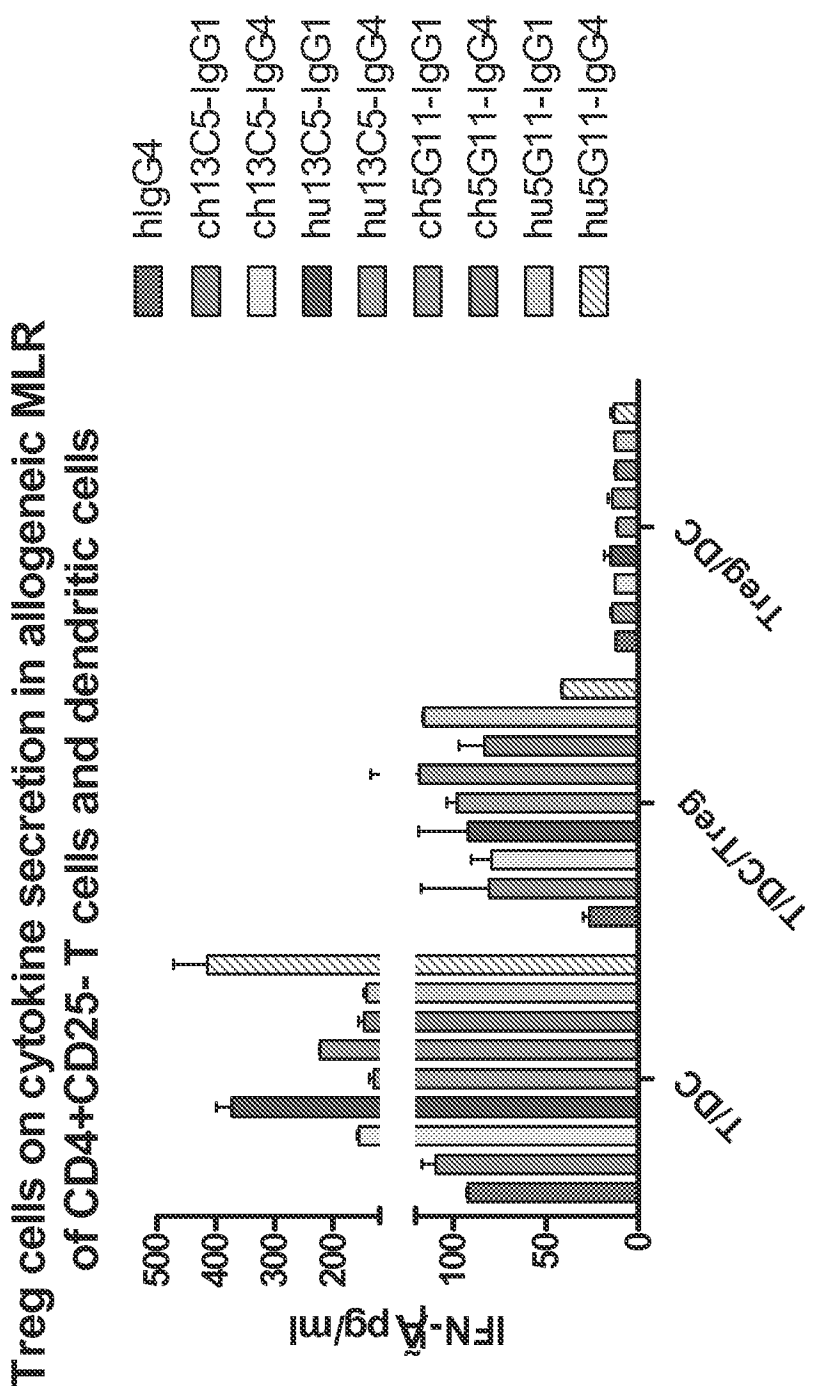

Treg cells on cytokine secretion in allogeneic MLR
of CD4+CD25- T cells and dendritic cells
hIgG4
ch13C5-IgG1
ch13C5-IgG4
hu13C5-IgG1
hu13C5-IgG4
ch5G11-IgG1
ch5G11-IgG4
hu5G11-IgG1
hu5G11-IgG4
IFN-γ pg/ml
500
400
300
200
100
50
0
T/DC
T/DC/Treg
Treg/DC

Figure 17

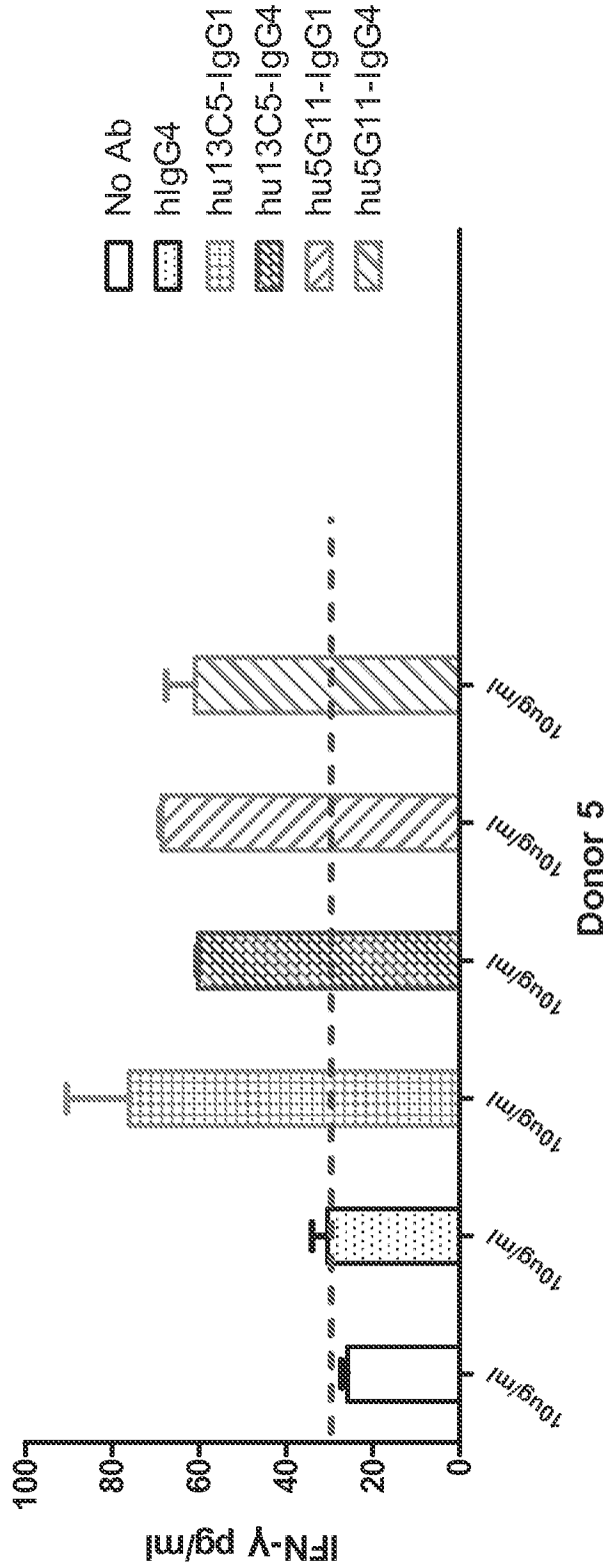
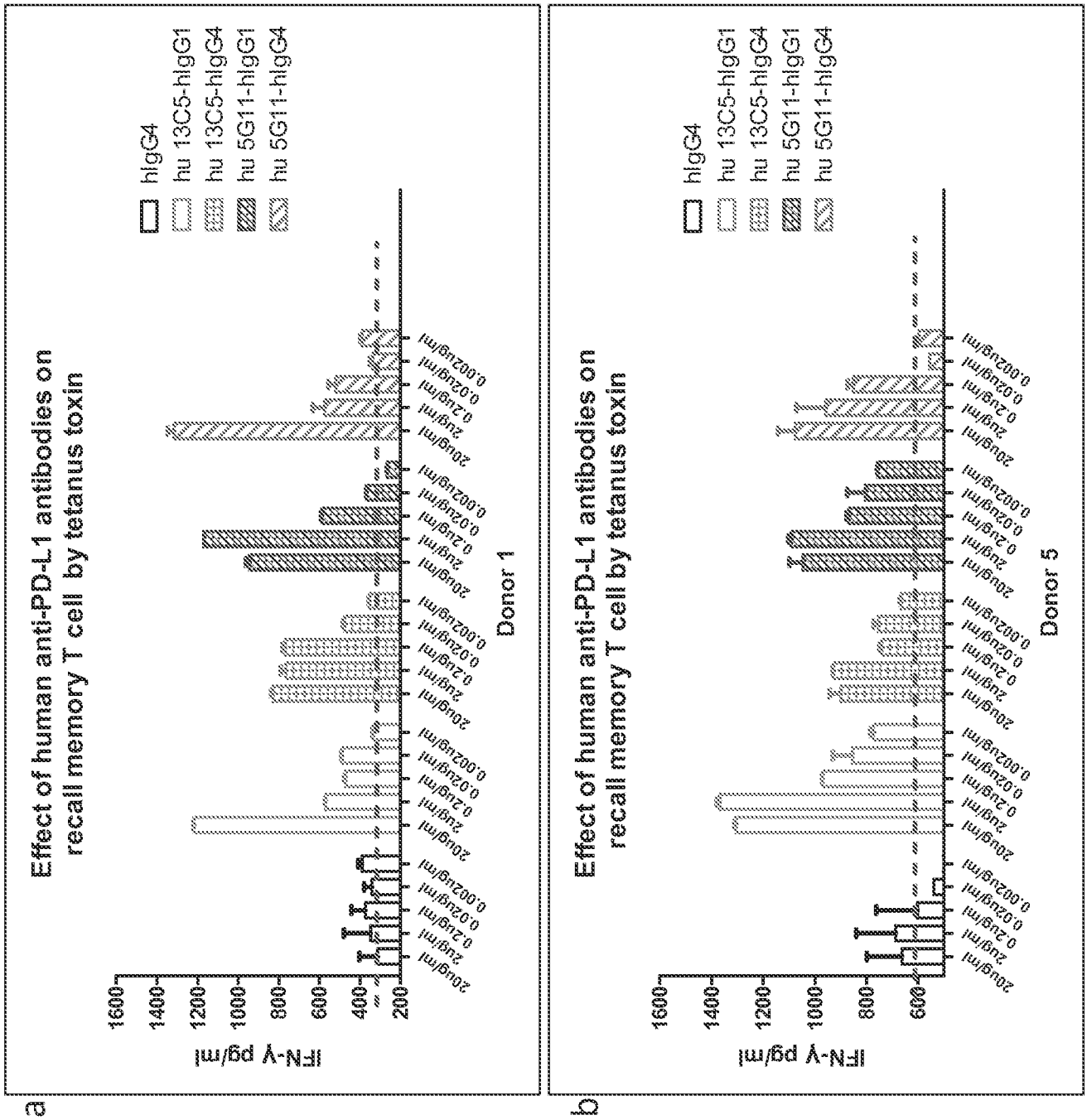

Effect of humanized anti-PD-L1 antibodies on cytokine production co-stimulated with autologous DCs and anti-CD3 antibody
No Ab
hIgG4
hu13C5-IgG1
hu13C5-IgG4
hu5G11-IgG1
hu5G11-IgG4
IFN-γ pg/ml
100
80
60
40
20
0
10ug/ml
10ug/ml
10ug/ml
10ug/ml
10ug/ml
10ug/ml
Donor 5

Figure 18


a
Effect of human anti-PD-L1 antibodies on recall memory T cell by tetanus toxin
hIgG4
hu 13C5-hIgG1
hu 13C5-hIgG4
hu 5G11-hIgG1
hu 5G11-hIgG4
IFN-γ pg/ml
1600
1400
1200
1000
800
600
400
200
20ug/ml
2ug/ml
0.2ug/ml
0.02ug/ml
0.002ug/ml
Donor 1
b
Effect of human anti-PD-L1 antibodies on recall memory T cell by tetanus toxin
hIgG4
hu 13C5-hIgG1
hu 13C5-hIgG4
hu 5G11-hIgG1
hu 5G11-hIgG4
IFN-γ pg/ml
1600
1400
1200
1000
800
600
20ug/ml
2ug/ml
0.2ug/ml
0.02ug/ml
0.002ug/ml
Donor 5

SEQUENCE LISTING

<110> Crown Bioscience, Inc.
Zha, Jiping
Sun, Ziyong
Qiu, Junzhuan

<120> ANTI PD-L1 ANTIBODIES

<130> CRBI-007/01WO

<150> PCT/CN2014/083715
<151> 2014-08-05

<160> 156

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ccagagaaga ggctggagtg ggtcgcatcc attagtagtg gtggtagcac ctactatcca 180

gacagtgtga agggccgatt caccatctcc agagataatg ccaggaacat cttgtacctg 240

caaatgagca gtctgaggtc tgaggacacg gccatgtatg actgtgcaag aggctatgat 300

tcggggtttg cttattgggg ccaagggact ctggtcactg tctctgaa 348

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<213> Mus sp.

<400> 2

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Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Arg Ser Tyr
20 25 30

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

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

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

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

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

Thr Val Ser Glu
115

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<211> 336
<212> DNA
<213> Mus sp.

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caacagaaac caggacagcc acccaaactc ctcatcaagt atgcatccaa cctagaatct 180

ggggtccctg ccaggttcag tggcagtggg tctgggacag acttcaccct caacatccat 240

cctgtggagg aggaggatac tgcaacatat tactgtcagc acagttggga gattccgtac 300

acgttcggag gggggaccaa gctggaaata aaacgg 336

<210> 4
<211> 112
<212> PRT
<213> Mus sp.

<400> 4

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1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Gln Ser Val Ser Thr Ser
20 25 30

Ser Ser Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Lys Tyr Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

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

Glu Ile Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100 105 110

<210> 5
<211> 348
<212> DNA
<213> Mus sp.

<400> 5

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tcctgtgcag cctctggatt cactttcaga agctatggca tgtcttgggt tcgccagact 120

ccagagaaga ggctggagtg ggtcgcatcc attagtagtg gtggtaccac ctactatcca 180

gacagtgtga agggccgatt catcatctcc agagataatg ccaggaacat cctgtacctg 240

caaatgagca gtctgaggtc tgaggacacg gccatgtatt attgtgcaaa aggctatgat 300

tcggggtttg cttactgggg ccaagggact ctggtcattg tctctgca 348

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<211> 116
<212> PRT
<213> Mus sp.

<400> 6

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1 5 10 15

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

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

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

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

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

Lys Gly Tyr Asp Ser Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Ile Val Ser Ala
115

<210> 7
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<212> DNA
<213> Mus sp.

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atctcatgca gggccagcca aagtgtcagt acatctagtt ctagttatat gcactggtac 120

caacagaaac caggacagcc tcccaaactc ctcatcaagt atgcatccaa cctagaatct 180

ggggtccctg ccaggttcag tggcagtggg tctgggacag acttcaccct caacatccat 240

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acgttcggag gggggaccaa gctggaaata aaa 333

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<211> 111
<212> PRT
<213> Mus sp.

<400> 8

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1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Gln Ser Val Ser Thr Ser
20 25 30

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

Lys Leu Leu Ile Lys Tyr Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

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

Glu Ile Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 9
<211> 348
<212> DNA
<213> Mus sp.

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acctgcacag tctctggttt ctcattaact acctatggtg tacactgggt tcgccagtct 120

ccaggaaagg gtctggaatg gctgggagtg atatggcgtg gtgtaaccac agactataat 180

gcagctttca tgtccagact gaccatcacc aaggacaatt ccaagagcca agttttcttt 240

aaaatgaaca gtctgcaagc taatgacaca gccatatatt actgtgccag actgggtttc 300

tatgctatgg actactgggg tcaaggaacc tcagtcaccg tctcctca 348

<210> 10
<211> 116
<212> PRT
<213> Mus sp.

<400> 10

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1 5 10 15

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Thr Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Arg Gly Val Thr Thr Asp Tyr Asn Ala Ala Phe Met
50 55 60

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

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

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

Thr Val Ser Ser
115

<210> 11
<211> 324
<212> DNA
<213> Mus sp.

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ataacctgca aggccagtca gagtgtgagt aatgatgtag cttggtacca gcagaagcca 120

gggcagtctc ctaaactgct gatatattat gcagccaatc gctacactgg agtccctgat 180

cgcttcactg gcagtggata tgggacggat ttcactttca ccatcagcat tgtgcaggct 240

gaagacctgg cagtttattt ctgtcagcag gattatacct ctccgtacac gttcggaggg 300

gggaccaagc tggaaataaa acgg 324

<210> 12
<211> 107
<212> PRT
<213> Mus sp.

<400> 12

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

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

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

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

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

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

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

<210> 13
<211> 348
<212> DNA
<213> Mus sp.

<400> 13
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acctgcacag tctctggttt ctcattaact agctatggtg tacactgggt tcgccagtct 120

ccaggaaagg gtctggagtg gctgggagtg atatggagtg gtggagtcac agactataat 180

gcagctttca tatccagact gagcatcagc aaggacaatt ccaagagcca agttttcttt 240

aaaatgaaca gtctgcaagc taatgacaca gccatatatt actgtgccag actcggtttc 300

tatgctatgg actactgggg tcaaggaacc tcagtcaccg tctcctca 348

<210> 14
<211> 116
<212> PRT
<213> Mus sp.

<400> 14

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1 5 10 15

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

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

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65 70 75 80

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

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

Thr Val Ser Ser
        115

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<212> DNA
<213> Mus sp.

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ataacctgca aggccagtca gagtgtgagt aatgatgtag gttggtacca acagaagcca 120

gggcagtctc ctaaactact gatatactat gcatccaatc gctactctgg agtccctgat 180

cgcttcactg gcagtggata tgggacggat ttcactttca ccatcagcac tgtgcaggct 240

gaagacctgg cagtttattt ctgtcaacaa gattatacct ctccgtacac gttcggaggg 300

gggaccaagc tggaaataaa a 321

<210> 16
<211> 107
<212> PRT
<213> Mus sp.

<400> 16

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1   5   10   15

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

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

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

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

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

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

<210> 17
<211> 357
<212> DNA
<213> Mus sp.

<400> 17
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tcctgtgtag cctcaggatt cgattttagt acatactgga tgcattgggt ccggcaggct 120

ccagggcaag ggctagaatg gattggacaa attaatccag atagcactac gataaactat 180

gcgccatctc taaaggatag attcatcatc tccagagaca acgccaaaaa tacgctgttc 240

ctgcaaatga gcaaagtgag atctgaggac actgcccttt attactgtgc aaaacccggg 300

gactatggtt acgactttga ctgctggggc caaggcacca ctctcacagt ctcctca 357

<210> 18
<211> 119
<212> PRT
<213> Mus sp.

<400> 18

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

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

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

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

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

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

Ala Lys Pro Gly Asp Tyr Gly Tyr Asp Phe Asp Cys Trp Gly Gln Gly
100 105 110

Thr Thr Leu Thr Val Ser Ser
115

<210> 19
<211> 339
<212> DNA
<213> Mus sp.

<400> 19
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atctcttgca gatctagtca gatcattgta catagtaatg caaacaccta tttagaatgg 120

ttcctgcaga aaccaggcca gtctccaaag ctcctgatct acaaagtttc caaccgattt 180

tctggggtcc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc 240

agcagagtgg aggctgagga tctgggagtt tattactgct ttcaaggttc acatgttccg 300

tacacgttcg gaggggggac caagctggaa ataaaacgg 339

<210> 20

<211> 112
<212> PRT
<213> Mus sp.

<400> 20

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

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

Asn Ala Asn Thr Tyr Leu Glu Trp Phe Leu Gln Lys Pro Gly Gln Ser
35 40 45

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

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

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

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 21
<211> 357
<212> DNA
<213> Mus sp.

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acctgttctg tcactggcga ctccatcacc agtggttact ggaactggat ccggaaattc 120

ccagggaata aacttgagta catggggtac ataagctaca gtggtagcac ttactacaat 180

ccatctctca aaagtcgaat ctccatcact cgagacacat ccaagaacca gtactacctg 240

cagttgaatt ctgtgactac tgaggacaca gccacatatt actgtgcaag aagtctacta 300

tggttctcta cggggtttgc ttactggggc caagggactc tggtcactgt ctctgca 357

<210> 22
<211> 119
<212> PRT
<213> Mus sp.

<400> 22

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1 5 10 15

Thr Leu Ser Leu Thr Cys Ser Val Thr Gly Asp Ser Ile Thr Ser Gly
20 25 30

Tyr Trp Asn Trp Ile Arg Lys Phe Pro Gly Asn Lys Leu Glu Tyr Met
35 40 45

Gly Tyr Ile Ser Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Ile Ser Ile Thr Arg Asp Thr Ser Lys Asn Gln Tyr Tyr Leu
65 70 75 80

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

Arg Ser Leu Leu Trp Phe Ser Thr Gly Phe Ala Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ala
115

<210> 23
<211> 324
<212> DNA
<213> Mus sp.

<400> 23
caaattgttc tcacccagtc tccagcaatc atgtctgcat ctcctgggga gaaggtcacc 60

ttgacctgca gtgccagctc aagtgtaagt tccagctact tgtactggaa ccagcagaag 120

ccaggatcct cccccaaagt ctggatttat aacacatcca acctggcttc tggagtccct 180

gctcgcttca gtggcagtgg gtctgggacc tcttactctc tcacaatcag cagcatggag 240

gctgaagatg ctgcctctta tttctgccat cagtggagaa gttacccacc cacgctcggt 300

gctgggacca agctggagct gaaa 324

<210> 24
<211> 108
<212> PRT
<213> Mus sp.

<400> 24

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

Glu Lys Val Thr Leu Thr Cys Ser Ala Ser Ser Ser Val Ser Ser Ser
20 25 30

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

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

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

Ala Glu Asp Ala Ala Ser Tyr Phe Cys His Gln Trp Arg Ser Tyr Pro
85 90 95

Pro Thr Leu Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105

<210> 25
<211> 348
<212> DNA
<213> Mus sp.

<400> 25
caggtgcagc tgaagcagtc aggacctggc ctagtgcagc cctcacagag cctgtccatc 60

acctgcacag tctctggttt ctcattaact agctatggtg tacactgggt tcgccagtct 120

ccaggaaagg gtctggagtg gctgggagtg atatggagtg gtggaatcac agactataat 180

gcagctttca aatccagact gagcatcagc aaggacaatt ccaagagcca agttttcttt 240

aagatgaaca gtctgcaagc taatgacaca gccatatatt tctgtgccag actgggtttt 300

tatgctatgg actactgggg tcaaggaacc tcagtcaccg tctcctca 348

<210> 26
<211> 116
<212> PRT
<213> Mus sp.

<400> 26

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

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Ser Gly Gly Ile Thr Asp Tyr Asn Ala Ala Phe Lys
50 55 60

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65 70 75 80

Lys Met Asn Ser Leu Gln Ala Asn Asp Thr Ala Ile Tyr Phe Cys Ala
85 90 95

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

Thr Val Ser Ser
115

<210> 27
<211> 318
<212> DNA
<213> Mus sp.

<400> 27
caaattgttc tcacccagtc tccagcaatc atgtctgcat ctccagggga gaaggtcacc 60

atgacctgca gtgccaactc aagtgtaagt tacatgcact ggtaccagca gaagtcaggc 120

acttccccca aaagatggat ttatgacaca tccaaactgg cttctggagt ccctgctcgc 180

ttcagtggca gtgggtctgg gacctcttac tctctcacaa tcagcagcat gggggctgaa 240

gatgctgcca cttattactg ccagcagtgg agtagtaacc catggacgtt cggtggaggc 300

accaagctgg aaatcaaa 318

<210> 28
<211> 106
<212> PRT
<213> Mus sp.

<400> 28

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

Glu Lys Val Thr Met Thr Cys Ser Ala Asn Ser Ser Val Ser Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
35 40 45

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

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

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Trp Thr
85 90 95

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

<210> 29
<211> 348
<212> DNA
<213> Mus sp.

<400> 29
gaagtgaaac tggtggagtc tgggggaggc ttagtgaagc ctggagggtc cctgaaactc 60

tcctgtgcag cctctggatt cactttcagg agctatggca tgtcttgggc tcgccagatt 120

ccagagaaga ggctggagtg ggtcgcatcc attagtagtg gtggaaccac ctactatcta 180

gggagtgtgc agggccgatt cacaatctcc agagataatg ccaggaacat cctgtacctg 240

caaatgagca gtctgaggtc tgaggacacg gccatgtatt attgtgcaag aggctatgat 300

gcgggatttg cttactgggg ccaagggact ctggtcagtg tctctgaa 348

<210> 30
<211> 116
<212> PRT
<213> Mus sp.

<400> 30

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

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

Gly Met Ser Trp Ala Arg Gln Ile Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Ser Ile Ser Ser Gly Gly Thr Thr Tyr Tyr Leu Gly Ser Val Gln
50 55 60

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

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

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

Ser Val Ser Glu
115

<210> 31
<211> 333
<212> DNA
<213> Mus sp.

<400> 31
gacattgtgc tgacacagtc tcctgcttcc ttagctgtat ctctggggca gagggccacc 60

atctcatgca gggccagcca aagtgtcagt acatctagct atagttatat gcactggtac 120

caacagaaac caggacagcc tcccaaactc ctcatcaagt atgcatccaa cctagaatct 180

ggggtccctg ccaggttcag tggcagtggg tctgggacag acttcaccct caacatccat 240

cctgtggagg aggaggatac tgcaacatat tactgtcaga acagttggga gattccgtac 300

acgttcggag gggggaccaa gctggaaata aaa 333

<210> 32
<211> 111
<212> PRT
<213> Mus sp.

<400> 32

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

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Gln Ser Val Ser Thr Ser
20 25 30

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

Lys Leu Leu Ile Lys Tyr Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

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

Glu Ile Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 33
<211> 351
<212> DNA
<213> Mus sp.

<400> 33
gaggtgcagc ttcaggagtc aggacctagc ctcgtcaaac cttctcagac tctgtccctc 60

acctgttctg tcactggcga ctccatcacc agtggttact ggacctggat ccggaaattc 120

ccagggaata aacttgaata catgggatac ataagctaca ctggtagcac ttactacaat 180

ccatctctca aaagtcgaat ctccatctct cgagacacat ccaagagcca gtactacctg 240

cagttgaatt ctgtgactac tgaggacaca gccacatatt actgtgcaag acagagggat 300

tggttagggt ttgcttactg gggccaaggg actctggtca ctgtctctgc a 351

<210> 34
<211> 117
<212> PRT
<213> Mus sp.

<400> 34

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

Thr Leu Ser Leu Thr Cys Ser Val Thr Gly Asp Ser Ile Thr Ser Gly
20 25 30

Tyr Trp Thr Trp Ile Arg Lys Phe Pro Gly Asn Lys Leu Glu Tyr Met
35 40 45

Gly Tyr Ile Ser Tyr Thr Gly Ser Thr Tyr Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Ile Ser Ile Ser Arg Asp Thr Ser Lys Ser Gln Tyr Tyr Leu
65 70 75 80

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

Arg Gln Arg Asp Trp Leu Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ala
115

<210> 35
<211> 339
<212> DNA
<213> Mus sp.

<400> 35
gatattgtga tgacacagac tccatcctcc ctagctgtgt cacttggaga gaaggttact 60

atgagctgca agtccagtca gagcctttta tatagtagca atcaaaagaa ctccttggcc 120

tggtaccagc agaaaccagg acagtctcct aaactgctga tttactgggc atccaatagg 180

gaatctgggg tccctgatcg cttcacaggc agtagctctg ggacagattt cactctcacc 240

atcagcagtg tgaaggctga agacctggca gtttattact gtcagcaata ttatagctat 300

ccgctcacgt tcggtgctgg gaccaagctg gagctgaaa 339

<210> 36
<211> 113
<212> PRT
<213> Mus sp.

<400> 36

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

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

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

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Asn Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Ser Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

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

Tyr Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
100 105 110

Lys

<210> 37
<211> 348
<212> DNA
<213> Mus sp.

<400> 37
gaagagaagc tggtggagtc tgggggaggc ttagtgaagc ctggagggtc cctgaaactc 60

tcctgtgcag cctctggatt cagtttcagt agttatggca tgtcttgggt tcgtcagact 120

ccagagaaga ggctggagtg ggtcgcatcc atcagtagtg gtggtagtat ctactatcca 180

gacagtgtga agggccgatt caccatctcc agagataatg ccaggaacat cctgtacctg 240

caaatgagca gtctgaggtc tgaggacacg gccatgtatt attgtgcaag aggctatgat 300

gcggggtttg ctttctgggg ccaagggaca ctggtcactg cctctgca 348

<210> 38
<211> 116
<212> PRT
<213> Mus sp.

<400> 38

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

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

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

Ala Ser Ile Ser Ser Gly Gly Ser Ile Tyr Tyr Pro Asp Ser Val Lys
50 55 60

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

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

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

Thr Ala Ser Ala
115

<210> 39
<211> 333
<212> DNA
<213> Mus sp.

<400> 39
gacattgtgc tgacacagtc tcctgcttcc ttagctgtat ctctggggca gagggccacc 60

atctcatgca gggccagcca aagtgtcagt acatctagtt atagttatgt gcactggtac 120

caacagaaac caggacagcc acccaaactc ctcatcaagt atgcatccaa cctagaatct 180

ggggtccctg ccaggttcag tggcagtggg tctgggacag acttcaccct caacatccat 240

cctgtggagg aggaggatac tgcaacatat tactgtcagc acagttggga gattccgtac 300

acgttcggag gggggaccaa gctggaaata aaa 333

<210> 40
<211> 111
<212> PRT
<213> Mus sp.

<400> 40

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

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Gln Ser Val Ser Thr Ser
20 25 30

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

Lys Leu Leu Ile Lys Tyr Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

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

Glu Ile Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 41
<211> 348
<212> DNA
<213> Artificial Sequence

<220>
<223> Humanized 5G11 antibody heavy chain variable region sequence

<400> 41
cagatcacac tgaaagaaag cggccctacc ctggtcaagc caactcagac cctgacactg 60

acttgcaccg tgtctgggtt ctctctgagt acatacggag tccactggat caggcagccc 120

cctggcaaag ctctggagtg gctgggagtg atttggcggg gcgtcaccac agactataac 180

gccgctttta tgtcaagact gacaatcact aaggataaca gcaaaaatca ggtggtcctg 240

accatgaaca atatggaccc cgtggatacc gcaacatact attgtgcccg gctggggttc 300

tacgccatgg actattgggg ccaggggact ctggtgaccg tctcgagc 348

<210> 42
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Humanized 5G11 antibody heavy chain variable region sequence

<400> 42

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

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

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

Gly Val Ile Trp Arg Gly Val Thr Thr Asp Tyr Asn Ala Ala Phe Met
50 55 60

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

Thr Met Asn Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Arg Leu Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 43
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> Humanized 5G11 antibody light chain variable region sequence

<400> 43
gatatccaga tgactcagtc tccaagcagc ctgtctgcat ctgtgggga cagggtcacc 60

atcacatgca aagcatctca gagtgtgtca aacgatgtcg cctggtacca gcagaagccc 120

ggaaaagctc ctaagctgct gatttactat gccgctaatc ggtacactgg cgtgccagac 180

agattcagcg gatccggata tggaaccgat ttcactttta ccatcagctc cctgcagcca 240

gaggacattg ccacatattt ctgtcagcag gattacacaa gcccctatac ttttggccag 300

gggaccaaac tggaaatcaa g 321

<210> 44
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Humanized 5G11 antibody light chain variable region sequence

<400> 44

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

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

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

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

Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

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

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

<210> 45
<211> 348
<212> DNA
<213> Artificial Sequence

<220>
<223> Humanized 13C5 antibody heavy chain variable region sequence

<400> 45
gaggtgcagc tggtcgagtc aggaggggg ctggtcaagc caggagggtc actgcgactg 60

agctgcgcag cttccgggtt catctttagg tcttatggca tgagttgggt gcgccaggca 120

ccagggaaag gactggagtg ggtcgcttca atcagctccg gaggcagcac ttactatcct 180

gactccgtga agggccggtt caccatttct agagataacg ccaaaaatag tctgtacctg 240

cagatgaact ctctgcgagc agaagacaca gccgtctacg attgtgctag aggatatgac 300

agcggctttg catactgggg ccaggggacc ctggtgacag tctcgagc 348

<210> 46
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Humanized 13C5 antibody heavy chain variable region sequence

<400> 46

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Arg Ser Tyr
20 25 30

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

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

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

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

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

Thr Val Ser Ser
115

<210> 47
<211> 333
<212> DNA
<213> Artificial Sequence

<220>
<223> Humanized 13C5 antibody light chain variable region sequence

<400> 47
gacattgtgc tgactcagag ccccgcttca ctggcagtgt ctccagggca gcgggcaacc 60

atcacatgca gagcctcaca gagcgtctcc accagctcct ctagtttcat gcactggtac 120

cagcagaagc ccggacagcc ccctaagctg ctgatcaaat atgctagcaa cctggagtcc 180

ggcgtgccag ccaggttctc tggcagtggg tcaggaaccg actttactct gaccattaat 240

cccgtcgaag ccaacgatac agctaattac tattgtcagc attcctggga gatcccttac 300

acatttggcc aggggactaa gctggagatc aag 333

<210> 48
<211> 111
<212> PRT
<213> Artificial Sequence

<220>
<223> Humanized 13C5 antibody light chain variable region sequence

<400> 48

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

Gln Arg Ala Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Ser Thr Ser
20 25 30

Ser Ser Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Lys Tyr Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
50 55 60

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

Pro Val Glu Ala Asn Asp Thr Ala Asn Tyr Tyr Cys Gln His Ser Trp
85 90 95

Glu Ile Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 49
<211> 1329
<212> DNA
<213> Artificial Sequence

<220>
<223> 8C6-IgG4 (F234A/L235A) chimeric antibody heavy chain full length sequence

<400> 49
caggtgcagc tgaagcagtc aggacctggc ctagtgcagc cctcacagag cctgtccatc 60

acctgcacag tctctggttt ctcattaact agctatggtg tacactgggt tcgccagtct 120

ccaggaaagg gtctggagtg gctgggagtg atatggagtg gtggagtcac agactataat 180

gcagctttca tatccagact gagcatcagc aaggacaatt ccaagagcca agttttcttt 240

aaaatgaaca gtctgcaagc taatgacaca gccatatatt actgtgccag actcggtttc 300

tatgctatgg actactgggg tcaaggaacc tcagtcaccg tctcgagcgc ctccaccaag 360

ggacccagcg tgtttccccc ggccccctgt tccagatcca cctccgaaag cacagccgct 420

ctcggctgcc tggtcaagga ttacttccct gagcccgtga cagtctcctg gaatagcggc 480

gctctgacct ccggcgtgca taccttccct gctgtgctgc aatcctccgg actgtacagc 540

ctgagcagcg tggtcaccgt gccttcctcc agcctgggaa ccaaaaccta cacatgcaac 600

gtggaccaca agcccagcaa caccaaagtg gacaagaggg tggagtccaa gtacggaccc 660

ccttgtcctc cctgccctgc tcctgaagcc gctggaggac ctagcgtgtt cctgtttccc 720

cccaagccca aggacaccct catgatctcc aggacccccg aggtgacctg tgtcgtggtg 780

gacgtgagcc aagaggaccc cgaggtgcag ttcaactggt acgtggatgg cgtcgaggtc 840

cataacgcca agaccaagcc tagggaggag cagttcaaca gcacctacag agtggtgagc 900

gtcctgaccg tgctccacca agactggctg aacggcaagg aatacaagtg caaggtctcc 960

aacaagggac tcccttcctc catcgagaag accatcagca aggccaaggg ccagcccaga 1020

gaaccccaag tctacacact gccccccagc caagaggaaa tgaccaagaa ccaggtgagc 1080

ctgacctgcc tggtgaaagg cttctacccc agcgacattg ctgtcgaatg ggagagcaac 1140

ggccaacccg agaacaacta caagaccacc ccccctgtgc tcgacagcga cggctccttc 1200

ttcctctaca gcaggctgac agtggacaag tccaggtggc aagagggcaa tgtcttcagc 1260

tgtagcgtca tgcacgaggc cctccacaac cactacaccc agaagagcct gtccctctcc 1320

ctgggctga 1329

<210> 50
<211> 442
<212> PRT
<213> Artificial Sequence

<220>
<223> 8C6-IgG4 (F234A/L235A) chimeric antibody heavy chain full length sequence

<400> 50

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

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

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

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65 70 75 80

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

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

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115 120 125

Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu
130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145 150 155 160

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Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
                165                 170                 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
            180                 185                 190

Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr
        195                 200                 205

Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro
    210                 215                 220

Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro
225                 230                 235                 240

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                245                 250                 255

Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
            260                 265                 270

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        275                 280                 285

Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    290                 295                 300

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
305                 310                 315                 320

Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
                325                 330                 335

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
            340                 345                 350

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        355                 360                 365

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    370                 375                 380

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
385                 390                 395                 400

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
                405                 410                 415

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
            420                 425                 430
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Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly
435 440

<210> 51
<211> 645
<212> DNA
<213> Artificial Sequence

<220>
<223> 8C6 chimeric antibody light chain full length sequence

<400> 51
agtattgtga tgacccagac tcccaaattc ctacttgtat cagcaggaga cagggttacc 60

ataacctgca aggccagtca gagtgtgagt aatgatgtag gttggtacca acagaagcca 120

gggcagtctc ctaaactact gatatactat gcatccaatc gctactctgg agtccctgat 180

cgcttcactg gcagtggata tgggacggat ttcactttca ccatcagcac tgtgcaggct 240

gaagacctgg cagtttattt ctgtcaacaa gattatacct ctccgtacac gttcggaggg 300

gggaccaagc tggaaataaa acgtacggtg gccgcaccaa gcgtcttcat cttcccgcca 360

tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat 420

cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag 480

gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 540

ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 600

ctgagctcgc ccgtcacaaa gagctttaac agaggcgagt gctga 645

<210> 52
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> 8C6 chimeric antibody light chain full length sequence

<400> 52

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

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

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

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

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

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

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

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

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 53
<211> 1329
<212> DNA
<213> Artificial Sequence

<220>
<223> 8H4-IgG4 (F234A/L235A) chimeric antibody heavy chain full length sequence

<400> 53
gaagtgaaac tggtggagtc tgggggaggc ttagtgaagc ctggagggtc cctgaaactc 60

tcctgtgcag cctctggatt cactttcagg agctatggca tgtcttgggc tcgccagatt 120

ccagagaaga ggctggagtg ggtcgcatcc attagtagtg gtggaaccac ctactatcta 180

gggagtgtgc agggccgatt cacaatctcc agagataatg ccaggaacat cctgtacctg 240

caaatgagca gtctgaggtc tgaggacacg gccatgtatt attgtgcaag aggctatgat 300

gcgggatttg cttactgggg ccaagggact ctggtcagtg tctcgagcgc ctccaccaag 360

ggacccagcg tgtttccccc ggcccccctgt tccagatcca cctccgaaag cacagccgct 420

ctcggctgcc tggtcaagga ttacttccct gagcccgtga cagtctcctg gaatagcggc 480

gctctgacct ccggcgtgca taccttccct gctgtgctgc aatcctccgg actgtacagc 540

ctgagcagcg tggtcaccgt gccttcctcc agcctgggaa ccaaaaccta cacatgcaac 600

gtggaccaca agcccagcaa caccaaagtg gacaagaggg tggagtccaa gtacggaccc 660

ccttgtcctc cctgccctgc tcctgaagcc gctggaggac ctagcgtgtt cctgtttccc 720

cccaagccca aggacaccct catgatctcc aggacccccg aggtgacctg tgtcgtggtg 780

gacgtgagcc aagaggaccc cgaggtgcag ttcaactggt acgtggatgg cgtcgaggtc 840

cataacgcca agaccaagcc tagggaggag cagttcaaca gcacctacag agtggtgagc 900

gtcctgaccg tgctccacca agactggctg aacggcaagg aatacaagtg caaggtctcc 960

aacaagggac tcccttcctc catcgagaag accatcagca aggccaaggg ccagcccaga 1020

gaaccccaag tctacacact gccccccagc caagaggaaa tgaccaagaa ccaggtgagc 1080

ctgacctgcc tggtgaaagg cttctacccc agcgacattg ctgtcgaatg ggagagcaac 1140

ggccaacccg agaacaacta caagaccacc ccccctgtgc tcgacagcga cggctccttc 1200

ttcctctaca gcaggctgac agtggacaag tccaggtggc aagagggcaa tgtcttcagc 1260

tgtagcgtca tgcacgaggc cctccacaac cactacaccc agaagagcct gtccctctcc 1320

ctgggctga 1329

<210> 54
<211> 442
<212> PRT
<213> Artificial Sequence

<220>
<223> 8H4-IgG4 (F234A/L235A) chimeric antibody heavy chain full length sequence

<400> 54

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

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

Gly Met Ser Trp Ala Arg Gln Ile Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Ser Ile Ser Ser Gly Gly Thr Thr Tyr Tyr Leu Gly Ser Val Gln
50 55 60

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

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

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

Ser Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115 120 125

Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu
130 135 140

```
Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145                 150                 155                 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
                165                 170                 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
            180                 185                 190

Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr
        195                 200                 205

Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro
    210                 215                 220

Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro
225                 230                 235                 240

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                245                 250                 255

Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
            260                 265                 270

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        275                 280                 285

Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    290                 295                 300

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
305                 310                 315                 320

Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
                325                 330                 335

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
            340                 345                 350

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        355                 360                 365

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    370                 375                 380

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
385                 390                 395                 400

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
                405                 410                 415
```

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
420 425 430

Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly
435 440

<210> 55
<211> 657
<212> DNA
<213> Artificial Sequence

<220>
<223> 8H4 chimeric antibody light chain full length sequence

<400> 55
gacattgtgc tgacacagtc tcctgcttcc ttagctgtat ctctggggca gagggccacc 60

atctcatgca gggccagcca aagtgtcagt acatctagct atagttatat gcactggtac 120

caacagaaac caggacagcc tcccaaactc ctcatcaagt atgcatccaa cctagaatct 180

ggggtccctg ccaggttcag tggcagtggg tctgggacag acttcaccct caacatccat 240

cctgtggagg aggaggatac tgcaacatat tactgtcaga acagttggga gattccgtac 300

acgttcggag gggggaccaa gctggaaata aaacgtacgg tggccgcacc aagcgtcttc 360

atcttcccgc catctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg 420

aataacttct atcccagaga ggccaaagta cagtggaagg tggataacgc cctccaatcg 480

ggtaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc 540

agcaccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc 600

acccatcagg gcctgagctc gcccgtcaca aagagcttta acagaggcga gtgctga 657

<210> 56
<211> 218
<212> PRT
<213> Artificial Sequence

<220>
<223> 8H4 chimeric antibody light chain full length sequence

<400> 56

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

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Gln Ser Val Ser Thr Ser
20 25 30

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

Lys Leu Leu Ile Lys Tyr Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

```
Pro Val Glu Glu Glu Asp Thr Ala Thr Tyr Tyr Cys Gln Asn Ser Trp
                85                  90                  95

Glu Ile Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
            100                 105                 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
        115                 120                 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
    130                 135                 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145                 150                 155                 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
                165                 170                 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
            180                 185                 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
        195                 200                 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> 57
<211> 1341
<212> DNA
<213> Artificial Sequence

<220>
<223> 5G11-IgG1 (D265A) chimeric antibody heavy chain full length sequence

<400> 57

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caggtgcagc tgaagcagtc aggacctggc ctagtgcagc cctcacagag cctgtccatc       60

acctgcacag tctctggttt ctcattaact acctatggtg tacactgggt tcgccagtct      120

ccaggaaagg gtctggaatg gctgggagtg atatggcgtg gtgtaaccac agactataat      180

gcagctttca tgtccagact gaccatcacc aaggacaatt ccaagagcca agttttcttt      240

aaaatgaaca gtctgcaagc taatgacaca gccatatatt actgtgccag actgggtttc      300

tatgctatgg actactgggg tcaaggaacc tcagtcaccg tctcgagcgc ctccactaag      360

ggcccatccg tgttccctct ggcaccctcc agcaagagca caagcggagg caccgccgca      420

ctgggctgcc tcgtgaagga ctacttccca gaacccgtga ccgtcagctg gaatagcggc      480

gctctgacca gcggagtcca cactttcccc gcagtgctgc agtccagcgg cctgtacagc      540

ctgagcagcg tggtcactgt gccaagcagc agcctgggca ctcagaccta catctgcaac      600

gtcaaccaca agcccagcaa cacaaaggtg gacaagaagg tcgagcccaa gtcctgcgat      660
```

aagacccaca cctgccctcc atgtcccgcc cccgagctgc tgggaggacc cagcgtcttc 720

ctgtttcccc ccaagccaaa ggacaccctg atgatcagca ggacccccga agtgacctgc 780

gtcgtggtgg ccgtgagcca cgaagatccc gaggtgaagt tcaactggta cgtggacggc 840

gtggaagtgc acaacgccaa gacaaaaccc agggaggagc agtatgccag cacctacagg 900

gtcgtgagcg tcctgaccgt gctgcaccaa gactggctga acggcaagga gtataagtgc 960

aaggtgagca acaaggcact gcccgccccc atcgagaaga ccatttccaa ggccaagggg 1020

caacctaggg agccacaggt ctacactctg ccccctagca gggacgagct gaccaagaac 1080

caggtctccc tgacttgcct ggtgaagggg ttttatccca gcgacatcgc cgtcgagtgg 1140

gagagcaatg gccagcccga aaacaactac aagaccacac cccctgtgct ggacagcgac 1200

ggcagcttct ttctgtatag caaactgaca gtggataaga gcagatggca gcagggcaac 1260

gtgttctcct gctccgtgat gcacgaggcc ctgcacaatc actacaccca gaagtccctg 1320

agcctgtccc ccggaaaatg a 1341

<210> 58
<211> 446
<212> PRT
<213> Artificial Sequence

<220>
<223> 5G11-IgG1 (D265A) chimeric antibody heavy chain full length sequence

<400> 58

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

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Thr Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Arg Gly Val Thr Thr Asp Tyr Asn Ala Ala Phe Met
50 55 60

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

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

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

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115 120 125

```
Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
    130                 135                 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145                 150                 155                 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
                165                 170                 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
            180                 185                 190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
        195                 200                 205

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
    210                 215                 220

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
225                 230                 235                 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
                245                 250                 255

Glu Val Thr Cys Val Val Val Ala Val Ser His Glu Asp Pro Glu Val
            260                 265                 270

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
        275                 280                 285

Lys Pro Arg Glu Glu Gln Tyr Ala Ser Thr Tyr Arg Val Val Ser Val
    290                 295                 300

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305                 310                 315                 320

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
                325                 330                 335

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
            340                 345                 350

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
        355                 360                 365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
    370                 375                 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
385                 390                 395                 400
```

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
405 410 415

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
420 425 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 59
<211> 1329
<212> DNA
<213> Artificial Sequence

<220>
<223> 5G11-IgG4 (F234A/L235A) chimeric antibody heavy chain full length sequence

<400> 59
caggtgcagc tgaagcagtc aggacctggc ctagtgcagc cctcacagag cctgtccatc 60

acctgcacag tctctggttt ctcattaact acctatggtg tacactgggt tcgccagtct 120

ccaggaaagg gtctggaatg gctgggagtg atatggcgtg gtgtaaccac agactataat 180

gcagctttca tgtccagact gaccatcacc aaggacaatt ccaagagcca agttttcttt 240

aaaatgaaca gtctgcaagc taatgacaca gccatatatt actgtgccag actgggtttc 300

tatgctatgg actactgggg tcaaggaacc tcagtcaccg tctcgagcgc ctccaccaag 360

ggacccagcg tgtttccccc ggccccctgt tccagatcca cctccgaaag cacagccgct 420

ctcggctgcc tggtcaagga ttacttccct gagcccgtga cagtctcctg gaatagcggc 480

gctctgacct ccggcgtgca taccttccct gctgtgctgc aatcctccgg actgtacagc 540

ctgagcagcg tggtcaccgt gccttcctcc agcctgggaa ccaaaaccta cacatgcaac 600

gtggaccaca agcccagcaa caccaaagtg gacaagaggg tggagtccaa gtacggaccc 660

ccttgtcctc cctgccctgc tcctgaagcc gctggaggac ctagcgtgtt cctgtttccc 720

cccaagccca aggacaccct catgatctcc aggacccccg aggtgacctg tgtcgtggtg 780

gacgtgagcc aagaggaccc cgaggtgcag ttcaactggt acgtggatgg cgtcgaggtc 840

cataacgcca agaccaagcc tagggaggag cagttcaaca gcacctacag agtggtgagc 900

gtcctgaccg tgctccacca agactggctg aacggcaagg aatacaagtg caaggtctcc 960

aacaagggac tcccttcctc catcgagaag accatcagca aggccaaggg ccagcccaga 1020

gaaccccaag tctacacact gccccccagc caagaggaaa tgaccaagaa ccaggtgagc 1080

ctgacctgcc tggtgaaagg cttctacccc agcgacattg ctgtcgaatg ggagagcaac 1140

ggccaacccg agaacaacta caagaccacc ccccctgtgc tcgacagcga cggctccttc 1200

ttcctctaca gcaggctgac agtggacaag tccaggtggc aagagggcaa tgtcttcagc 1260

tgtagcgtca tgcacgaggc cctccacaac cactacaccc agaagagcct gtccctctcc 1320

ctgggctga 1329

<210> 60
<211> 442
<212> PRT
<213> Artificial Sequence

<220>
<223> 5G11-IgG4 (F234A/L235A) chimeric antibody heavy chain full length sequence

<400> 60

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

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Thr Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Arg Gly Val Thr Thr Asp Tyr Asn Ala Ala Phe Met
50 55 60

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

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

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

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115 120 125

Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu
130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
180 185 190

Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr
195 200 205

Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro
210 215 220

```
Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro
225                 230                 235                 240

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                245                 250                 255

Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
            260                 265                 270

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        275                 280                 285

Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    290                 295                 300

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
305                 310                 315                 320

Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
                325                 330                 335

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
            340                 345                 350

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        355                 360                 365

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    370                 375                 380

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
385                 390                 395                 400

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
                405                 410                 415

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
            420                 425                 430

Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly
        435                 440


<210>  61
<211>  645
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  5G11 chimeric antibody light chain full length sequence

<400>  61
agtattgtga tgacccagac tcccaaattc ctgcttgtat cagcaggaga cagggttacc       60

ataacctgca aggccagtca gagtgtgagt aatgatgtag cttggtacca gcagaagcca      120
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gggcagtctc ctaaactgct gatatattat gcagccaatc gctacactgg agtccctgat 180

cgcttcactg gcagtggata tgggacggat ttcactttca ccatcagcat tgtgcaggct 240

gaagacctgg cagtttattt ctgtcagcag gattatacct ctccgtacac gttcggaggg 300

gggaccaagc tggaaataaa acgtacggtg gccgcaccaa gcgtcttcat cttcccgcca 360

tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat 420

cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag 480

gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 540

ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 600

ctgagctcgc ccgtcacaaa gagctttaac agaggcgagt gctga 645

<210> 62
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> 5G11 chimeric antibody light chain full length sequence

<400> 62

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

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

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

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

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

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

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

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

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 63
<211> 1341
<212> DNA
<213> Artificial Sequence

<220>
<223> 13C5-IgG1 (D265A) chimeric antibody heavy chain full length sequence

<400> 63
gaagtgaagc tggtggagtc tgggggaggc ttagtgaagc ctggagggtc cctgaaactc 60

tcctgtgcag cctctggatt cattttcaga agctatggca tgtcttgggt tcgccagact 120

ccagagaaga ggctggagtg ggtcgcatcc attagtagtg gtggtagcac ctactatcca 180

gacagtgtga agggccgatt caccatctcc agagataatg ccaggaacat cttgtacctg 240

caaatgagca gtctgaggtc tgaggacacg gccatgtatg actgtgcaag aggctatgat 300

tcggggtttg cttattgggg ccaagggact ctggtcactg tctcgagcgc ctccactaag 360

ggcccatccg tgttccctct ggcaccctcc agcaagagca caagcggagg caccgccgca 420

ctgggctgcc tcgtgaagga ctacttccca gaacccgtga ccgtcagctg gaatagcggc 480

gctctgacca gcggagtcca cactttcccc gcagtgctgc agtccagcgg cctgtacagc 540

ctgagcagcg tggtcactgt gccaagcagc agcctgggca ctcagaccta catctgcaac 600

gtcaaccaca agcccagcaa cacaaaggtg gacaagaagg tcgagcccaa gtcctgcgat 660

aagacccaca cctgccctcc atgtcccgcc cccgagctgc tgggaggacc cagcgtcttc 720

ctgtttcccc ccaagccaaa ggacaccctg atgatcagca ggacccccga agtgacctgc 780

gtcgtggtgg ccgtgagcca cgaagatccc gaggtgaagt tcaactggta cgtggacggc 840

gtggaagtgc acaacgccaa gacaaaaccc agggaggagc agtatgccag cacctacagg 900

gtcgtgagcg tcctgaccgt gctgcaccaa gactggctga acggcaagga gtataagtgc 960

aaggtgagca acaaggcact gcccgccccc atcgagaaga ccatttccaa ggccaagggg 1020

caacctaggg agccacaggt ctacactctg ccccctagca gggacgagct gaccaagaac 1080

caggtctccc tgacttgcct ggtgaagggg ttttatccca gcgacatcgc cgtcgagtgg 1140

gagagcaatg gccagcccga aaacaactac aagaccacac cccctgtgct ggacagcgac 1200

ggcagcttct ttctgtatag caaactgaca gtggataaga gcagatggca gcagggcaac 1260

gtgttctcct gctccgtgat gcacgaggcc ctgcacaatc actacaccca gaagtccctg 1320

agcctgtccc ccggaaaatg a 1341

<210> 64
<211> 446
<212> PRT
<213> Artificial Sequence

<220>
<223> 13C5-IgG1 (D265A) chimeric antibody heavy chain full length sequence

<400> 64

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

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Arg Ser Tyr
20 25 30

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

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

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

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

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

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115 120 125

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

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
180 185 190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
195 200 205

```
Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
    210                 215                 220

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
225                 230                 235                 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
                245                 250                 255

Glu Val Thr Cys Val Val Val Ala Val Ser His Glu Asp Pro Glu Val
            260                 265                 270

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
        275                 280                 285

Lys Pro Arg Glu Glu Gln Tyr Ala Ser Thr Tyr Arg Val Val Ser Val
    290                 295                 300

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305                 310                 315                 320

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
                325                 330                 335

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
            340                 345                 350

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
        355                 360                 365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
    370                 375                 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
385                 390                 395                 400

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
                405                 410                 415

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
            420                 425                 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 65
<211> 1329
<212> DNA
<213> Artificial Sequence

<220>
<223> 13C5-IgG4 (F234A/L235A) chimeric antibody heavy chain full length sequence

<400> 65
gaagtgaagc tggtggagtc tgggggaggc ttagtgaagc ctggagggtc cctgaaactc 60

tcctgtgcag cctctggatt cattttcaga agctatggca tgtcttgggt tcgccagact 120

ccagagaaga ggctggagtg ggtcgcatcc attagtagtg gtggtagcac ctactatcca 180

gacagtgtga agggccgatt caccatctcc agagataatg ccaggaacat cttgtacctg 240

caaatgagca gtctgaggtc tgaggacacg gccatgtatg actgtgcaag aggctatgat 300

tcggggtttg cttattgggg ccaagggact ctggtcactg tctcgagcgc ctccaccaag 360

ggacccagcg tgtttcccct ggcccctgt tccagatcca cctccgaaag cacagccgct 420

ctcggctgcc tggtcaagga ttacttccct gagcccgtga cagtctcctg gaatagcggc 480

gctctgacct ccggcgtgca taccttccct gctgtgctgc aatcctccgg actgtacagc 540

ctgagcagcg tggtcaccgt gccttcctcc agcctgggaa ccaaaaccta cacatgcaac 600

gtggaccaca agcccagcaa caccaaagtg gacaagaggg tggagtccaa gtacggaccc 660

ccttgtcctc cctgccctgc tcctgaagcc gctggaggac ctagcgtgtt cctgtttccc 720

cccaagccca aggacaccct catgatctcc aggacccccg aggtgacctg tgtcgtggtg 780

gacgtgagcc aagaggaccc cgaggtgcag ttcaactggt acgtggatgg cgtcgaggtc 840

cataacgcca agaccaagcc tagggaggag cagttcaaca gcacctacag agtggtgagc 900

gtcctgaccg tgctccacca agactggctg aacggcaagg aatacaagtg caaggtctcc 960

aacaagggac tcccttcctc catcgagaag accatcagca aggccaaggg ccagcccaga 1020

gaaccccaag tctacacact gccccccagc caagaggaaa tgaccaagaa ccaggtgagc 1080

ctgacctgcc tggtgaaagg cttctacccc agcgacattg ctgtcgaatg ggagagcaac 1140

ggccaacccg agaacaacta caagaccacc ccccctgtgc tcgacagcga cggctccttc 1200

ttcctctaca gcaggctgac agtggacaag tccaggtggc aagagggcaa tgtcttcagc 1260

tgtagcgtca tgcacgaggc cctccacaac cactacaccc agaagagcct gtccctctcc 1320

ctgggctga 1329

<210> 66
<211> 442
<212> PRT
<213> Artificial Sequence

<220>
<223> 13C5-IgG4 (F234A/L235A) chimeric antibody heavy chain full length sequence

<400> 66

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

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Arg Ser Tyr
20 25 30

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

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

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

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

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

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
        115                 120                 125

Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu
    130                 135                 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145                 150                 155                 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
                165                 170                 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
            180                 185                 190

Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr
        195                 200                 205

Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro
    210                 215                 220

Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro
225                 230                 235                 240

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                245                 250                 255

Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
            260                 265                 270

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        275                 280                 285

Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    290                 295                 300
```

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
305 310 315 320

Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
325 330 335

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
340 345 350

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
355 360 365

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
370 375 380

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
385 390 395 400

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
405 410 415

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
420 425 430

Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly
435 440

<210> 67
<211> 663
<212> DNA
<213> Artificial Sequence

<220>
<223> 13C5 chimeric antibody light chain full length sequence

<400> 67
gacattgtgc tgacacagtc tcctgcttcc ttagctgttt ctctggggca gagggccacc 60

atctcatgca gggccagcca aagtgtcagt acttctagct ctagttttat gcactggtac 120

caacagaaac caggacagcc acccaaactc ctcatcaagt atgcatccaa cctagaatct 180

ggggtccctg ccaggttcag tggcagtggg tctgggacag acttcaccct caacatccat 240

cctgtggagg aggaggatac tgcaacatat tactgtcagc acagttggga gattccgtac 300

acgttcggag gggggaccaa gctggaaata aaacgtacgc gtacggtggc cgcaccaagc 360

gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420

ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgccctc 480

caatcgggta actcccagga gagtgtcaca gagcaggaca gcaaggacag cacctacagc 540

ctcagcagca ccctgacgct gagcaaagca gactacgaga aacacaaagt ctacgcctgc 600

gaagtcaccc atcagggcct gagctcgccc gtcacaaaga gctttaacag aggcgagtgc 660

tga 663

<210> 68
<211> 220
<212> PRT
<213> Artificial Sequence

<220>
<223> 13C5 chimeric antibody light chain full length sequence

<400> 68

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

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Gln Ser Val Ser Thr Ser
            20                  25                  30

Ser Ser Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45

Lys Leu Leu Ile Lys Tyr Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
    50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65                  70                  75                  80

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

Glu Ile Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
            100                 105                 110

Thr Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
        115                 120                 125

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
    130                 135                 140

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
145                 150                 155                 160

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
                165                 170                 175

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
            180                 185                 190

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
        195                 200                 205

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215                 220
```

<210> 69
<211> 1341
<212> DNA
<213> Artificial Sequence

<220>
<223> Humanized 5G11-IgG1 (D265A) antibody heavy chain full length sequence

<400> 69
cagatcacac tgaaagaaag cggccctacc ctggtcaagc caactcagac cctgacactg 60

acttgcaccg tgtctgggtt ctctctgagt acatacggag tccactggat caggcagccc 120

cctggcaaag ctctggagtg gctgggagtg atttggcggg gcgtcaccac agactataac 180

gccgctttta tgtcaagact gacaatcact aaggataaca gcaaaaatca ggtggtcctg 240

accatgaaca atatggaccc cgtggatacc gcaacatact attgtgcccg gctggggttc 300

tacgccatgg actattgggg ccaggggact ctggtgaccg tctcgagcgc ctccactaag 360

ggcccatccg tgttccctct ggcaccctcc agcaagagca caagcggagg caccgccgca 420

ctgggctgcc tcgtgaagga ctacttccca gaacccgtga ccgtcagctg gaatagcggc 480

gctctgacca gcggagtcca cactttcccc gcagtgctgc agtccagcgg cctgtacagc 540

ctgagcagcg tggtcactgt gccaagcagc agcctgggca ctcagaccta catctgcaac 600

gtcaaccaca agcccagcaa cacaaaggtg gacaagaagg tcgagcccaa gtcctgcgat 660

aagacccaca cctgccctcc atgtcccgcc cccgagctgc tgggaggacc cagcgtcttc 720

ctgtttcccc ccaagccaaa ggacaccctg atgatcagca ggacccccga agtgacctgc 780

gtcgtggtgg ccgtgagcca cgaagatccc gaggtgaagt tcaactggta cgtggacggc 840

gtggaagtgc acaacgccaa gacaaaaccc agggaggagc agtataacag cacctacagg 900

gtcgtgagcg tcctgaccgt gctgcaccaa gactggctga acggcaagga gtataagtgc 960

aaggtgagca acaaggcact gcccgccccc atcgagaaga ccatttccaa ggccaagggg 1020

caacctaggg agccacaggt ctacactctg ccccctagca gggacgagct gaccaagaac 1080

caggtctccc tgacttgcct ggtgaagggg ttttatccca gcgacatcgc cgtcgagtgg 1140

gagagcaatg gccagcccga aaacaactac aagaccacac cccctgtgct ggacagcgac 1200

ggcagcttct ttctgtatag caaactgaca gtggataaga gcagatggca gcagggcaac 1260

gtgttctcct gctccgtgat gcacgaggcc ctgcacaatc actacaccca gaagtccctg 1320

agcctgtccc ccggaaaatg a 1341

<210> 70
<211> 446
<212> PRT
<213> Artificial Sequence

<220>
<223> Humanized 5G11-IgG1 (D265A) antibody heavy chain full length sequence

<400> 70

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

```
Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Thr Tyr
            20                  25                  30

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

Gly Val Ile Trp Arg Gly Val Thr Thr Asp Tyr Asn Ala Ala Phe Met
    50                  55                  60

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

Thr Met Asn Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
                85                  90                  95

Arg Leu Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
            100                 105                 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
        115                 120                 125

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

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145                 150                 155                 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
                165                 170                 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
            180                 185                 190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
        195                 200                 205

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
    210                 215                 220

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
225                 230                 235                 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
                245                 250                 255

Glu Val Thr Cys Val Val Val Ala Val Ser His Glu Asp Pro Glu Val
            260                 265                 270

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
        275                 280                 285
```

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
290 295 300

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305 310 315 320

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
325 330 335

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
340 345 350

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
355 360 365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
370 375 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
385 390 395 400

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
405 410 415

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
420 425 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 71
<211> 1329
<212> DNA
<213> Artificial Sequence

<220>
<223> Humanized 5G11-IgG4 (F234A/L235A) antibody heavy chain full length sequence

<400> 71
cagatcacac tgaaagaaag cggccctacc ctggtcaagc caactcagac cctgacactg 60

acttgcaccg tgtctgggtt ctctctgagt acatacggag tccactggat caggcagccc 120

cctggcaaag ctctggagtg gctgggagtg atttggcggg gcgtcaccac agactataac 180

gccgctttta tgtcaagact gacaatcact aaggataaca gcaaaaatca ggtggtcctg 240

accatgaaca atatggaccc cgtggatacc gcaacatact attgtgcccg gctggggttc 300

tacgccatgg actattgggg ccaggggact ctggtgaccg tctcgagcgc ctccaccaag 360

ggacccagcg tgtttccccc ggcccccctgt tccagatcca cctccgaaag cacagccgct 420

ctcggctgcc tggtcaagga ttacttccct gagcccgtga cagtctcctg gaatagcggc 480

gctctgacct ccggcgtgca taccttccct gctgtgctgc aatcctccgg actgtacagc 540

ctgagcagcg tggtcaccgt gccttcctcc agcctgggaa ccaaaaccta cacatgcaac 600

gtggaccaca agcccagcaa caccaaagtg gacaagaggg tggagtccaa gtacggaccc 660

ccttgtcctc cctgccctgc tcctgaagcc gctggaggac ctagcgtgtt cctgtttccc 720

cccaagccca aggacaccct catgatctcc aggacccccg aggtgacctg tgtcgtggtg 780

gacgtgagcc aagaggaccc cgaggtgcag ttcaactggt acgtggatgg cgtcgaggtc 840

cataacgcca agaccaagcc tagggaggag cagttcaaca gcacctacag agtggtgagc 900

gtcctgaccg tgctccacca agactggctg aacggcaagg aatacaagtg caaggtctcc 960

aacaagggac tcccttcctc catcgagaag accatcagca aggccaaggg ccagcccaga 1020

gaaccccaag tctacacact gccccccagc caagaggaaa tgaccaagaa ccaggtgagc 1080

ctgacctgcc tggtgaaagg cttctacccc agcgacattg ctgtcgaatg ggagagcaac 1140

ggccaacccg agaacaacta caagaccacc ccccctgtgc tcgacagcga cggctccttc 1200

ttcctctaca gcaggctgac agtggacaag tccaggtggc aagagggcaa tgtcttcagc 1260

tgtagcgtca tgcacgaggc cctccacaac cactacaccc agaagagcct gtccctctcc 1320

ctgggctga 1329

<210> 72
<211> 442
<212> PRT
<213> Artificial Sequence

<220>
<223> Humanized 5G11-IgG4 (F234A/L235A) antibody heavy chain full length sequence

<400> 72

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

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

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

Gly Val Ile Trp Arg Gly Val Thr Thr Asp Tyr Asn Ala Ala Phe Met
50 55 60

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

Thr Met Asn Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala
85 90 95

Arg Leu Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

```
Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
        115                 120                 125

Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu
    130                 135                 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145                 150                 155                 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
                165                 170                 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
            180                 185                 190

Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr
        195                 200                 205

Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro
    210                 215                 220

Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro
225                 230                 235                 240

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                245                 250                 255

Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
            260                 265                 270

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        275                 280                 285

Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    290                 295                 300

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
305                 310                 315                 320

Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
                325                 330                 335

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
            340                 345                 350

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        355                 360                 365

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    370                 375                 380
```

```
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
385                 390                 395                 400


Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
                405                 410                 415


Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
            420                 425                 430


Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly
        435                 440


<210>  73
<211>  645
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Humanized 5G11 antibody light chain full length sequence

<400>  73
gatatccaga tgactcagtc tccaagcagc ctgtctgcat ctgtggggga cagggtcacc       60

atcacatgca aagcatctca gagtgtgtca aacgatgtcg cctggtacca gcagaagccc      120

ggaaaagctc ctaagctgct gatttactat gccgctaatc ggtacactgg cgtgccagac      180

agattcagcg gatccggata tggaaccgat ttcactttta ccatcagctc cctgcagcca      240

gaggacattg ccacatattt ctgtcagcag gattacacaa gcccctatac ttttggccag      300

gggaccaaac tggaaatcaa gcgtacggtg gccgcaccaa gcgtcttcat cttcccgcca      360

tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat      420

cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag      480

gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg      540

ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc      600

ctgagctcgc ccgtcacaaa gagctttaac agaggcgagt gctga                      645


<210>  74
<211>  214
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Humanized 5G11 antibody light chain full length sequence

<400>  74

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


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

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

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

Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

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

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

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

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

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 75
<211> 1341
<212> DNA
<213> Artificial Sequence

<220>
<223> Humanized 13C5-IgG1 (D265A) antibody heavy chain full length sequence

<400> 75
gaggtgcagc tggtcgagtc aggaggggg ctggtcaagc caggagggtc actgcgactg 60

agctgcgcag cttccgggtt catctttagg tcttatggca tgagttgggt gcgccaggca 120

ccagggaaag gactggagtg ggtcgcttca atcagctccg gaggcagcac ttactatcct 180

gactccgtga agggccggtt caccatttct agagataacg ccaaaaatag tctgtacctg 240

cagatgaact ctctgcgagc agaagacaca gccgtctacg attgtgctag aggatatgac 300

agcggctttg catactgggg ccaggggacc ctggtgacag tctcgagcgc ctccactaag 360

ggcccatccg tgttccctct ggcaccctcc agcaagagca caagcggagg caccgccgca 420

ctgggctgcc tcgtgaagga ctacttccca gaacccgtga ccgtcagctg gaatagcggc 480

gctctgacca gcggagtcca cactttcccc gcagtgctgc agtccagcgg cctgtacagc 540

ctgagcagcg tggtcactgt gccaagcagc agcctgggca ctcagaccta catctgcaac 600

gtcaaccaca agcccagcaa cacaaaggtg gacaagaagg tcgagcccaa gtcctgcgat 660

aagacccaca cctgccctcc atgtcccgcc cccgagctgc tgggaggacc cagcgtcttc 720

ctgtttcccc ccaagccaaa ggacaccctg atgatcagca ggacccccga agtgacctgc 780

gtcgtggtgg ccgtgagcca cgaagatccc gaggtgaagt tcaactggta cgtggacggc 840

gtggaagtgc acaacgccaa gacaaaaccc agggaggagc agtataacag cacctacagg 900

gtcgtgagcg tcctgaccgt gctgcaccaa gactggctga acggcaagga gtataagtgc 960

aaggtgagca acaaggcact gcccgccccc atcgagaaga ccatttccaa ggccaagggg 1020

caacctaggg agccacaggt ctacactctg ccccctagca gggacgagct gaccaagaac 1080

caggtctccc tgacttgcct ggtgaagggg ttttatccca gcgacatcgc cgtcgagtgg 1140

gagagcaatg gccagcccga aaacaactac aagaccacac cccctgtgct ggacagcgac 1200

ggcagcttct ttctgtatag caaactgaca gtggataaga gcagatggca gcagggcaac 1260

gtgttctcct gctccgtgat gcacgaggcc ctgcacaatc actacaccca gaagtccctg 1320

agcctgtccc ccggaaaatg a 1341

<210> 76
<211> 446
<212> PRT
<213> Artificial Sequence

<220>
<223> Humanized 13C5-IgG1 (D265A) antibody heavy chain full length sequence

<400> 76

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Arg Ser Tyr
20 25 30

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

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

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

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

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

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
        115                 120                 125

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

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145                 150                 155                 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
                165                 170                 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
            180                 185                 190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
        195                 200                 205

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
    210                 215                 220

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
225                 230                 235                 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
                245                 250                 255

Glu Val Thr Cys Val Val Val Ala Val Ser His Glu Asp Pro Glu Val
            260                 265                 270

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
        275                 280                 285

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
    290                 295                 300

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305                 310                 315                 320

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
                325                 330                 335

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
            340                 345                 350

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
        355                 360                 365
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Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
370 375 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
385 390 395 400

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
405 410 415

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
420 425 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 77
<211> 1329
<212> DNA
<213> Artificial Sequence

<220>
<223> Humanized 13C5-IgG4 (F234A/L235A) antibody heavy chain full length sequence

<400> 77
gaggtgcagc tggtcgagtc aggaggggg ctggtcaagc caggagggtc actgcgactg 60

agctgcgcag cttccgggtt catctttagg tcttatggca tgagttgggt gcgccaggca 120

ccagggaaag gactggagtg ggtcgcttca atcagctccg gaggcagcac ttactatcct 180

gactccgtga agggccggtt caccatttct agagataacg ccaaaaatag tctgtacctg 240

cagatgaact ctctgcgagc agaagacaca gccgtctacg attgtgctag aggatatgac 300

agcggctttg catactgggg ccaggggacc ctggtgacag tctcgagcgc ctccaccaag 360

ggacccagcg tgtttcccct ggcccctgt tccagatcca cctccgaaag cacagccgct 420

ctcggctgcc tggtcaagga ttacttccct gagcccgtga cagtctcctg gaatagcggc 480

gctctgacct ccggcgtgca taccttccct gctgtgctgc aatcctccgg actgtacagc 540

ctgagcagcg tggtcaccgt gccttcctcc agcctgggaa ccaaaaccta cacatgcaac 600

gtggaccaca agcccagcaa caccaaagtg gacaagaggg tggagtccaa gtacggaccc 660

ccttgtcctc cctgccctgc tcctgaagcc gctggaggac ctagcgtgtt cctgtttccc 720

cccaagccca aggacaccct catgatctcc aggacccccg aggtgacctg tgtcgtggtg 780

gacgtgagcc aagaggaccc cgaggtgcag ttcaactggt acgtggatgg cgtcgaggtc 840

cataacgcca agaccaagcc tagggaggag cagttcaaca gcacctacag agtggtgagc 900

gtcctgaccg tgctccacca agactggctg aacggcaagg aatacaagtg caaggtctcc 960

aacaagggac tcccttcctc catcgagaag accatcagca aggccaaggg ccagcccaga 1020

gaaccccaag tctacacact gccccccagc caagaggaaa tgaccaagaa ccaggtgagc 1080

ctgacctgcc tggtgaaagg cttctacccc agcgacattg ctgtcgaatg ggagagcaac 1140

ggccaacccg agaacaacta caagaccacc ccccctgtgc tcgacagcga cggctccttc 1200

ttcctctaca gcaggctgac agtggacaag tccaggtggc aagagggcaa tgtcttcagc 1260

tgtagcgtca tgcacgaggc cctccacaac cactacaccc agaagagcct gtccctctcc 1320

ctgggctga 1329

<210> 78
<211> 442
<212> PRT
<213> Artificial Sequence

<220>
<223> Humanized 13C5-IgG4 (F234A/L235A) antibody heavy chain full length sequence

<400> 78

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Arg Ser Tyr
20 25 30

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

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

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

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

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

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115 120 125

Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu
130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
180 185 190

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Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr
        195                 200                 205

Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro
    210                 215                 220

Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro
225                 230                 235                 240

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                245                 250                 255

Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn
            260                 265                 270

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        275                 280                 285

Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    290                 295                 300

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
305                 310                 315                 320

Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
                325                 330                 335

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
            340                 345                 350

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        355                 360                 365

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    370                 375                 380

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
385                 390                 395                 400

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
                405                 410                 415

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
            420                 425                 430

Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly
        435                 440
```

<210> 79
<211> 657

<212> DNA
<213> Artificial Sequence

<220>
<223> Humanized 13C5 antibody light chain full length sequence

<400> 79
gacattgtgc tgactcagag ccccgcttca ctggcagtgt ctccagggca gcgggcaacc 60

atcacatgca gagcctcaca gagcgtctcc accagctcct ctagtttcat gcactggtac 120

cagcagaagc ccggacagcc ccctaagctg ctgatcaaat atgctagcaa cctggagtcc 180

ggcgtgccag ccaggttctc tggcagtggg tcaggaaccg actttactct gaccattaat 240

cccgtcgaag ccaacgatac agctaattac tattgtcagc attcctggga gatcccttac 300

acatttggcc aggggactaa gctggagatc aagcgtacgg tggccgcacc aagcgtcttc 360

atcttcccgc catctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg 420

aataacttct atcccagaga ggccaaagta cagtggaagg tggataacgc cctccaatcg 480

ggtaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc 540

agcaccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc 600

acccatcagg gcctgagctc gcccgtcaca aagagcttta acagaggcga gtgctga 657

<210> 80
<211> 218
<212> PRT
<213> Artificial Sequence

<220>
<223> Humanized 13C5 antibody light chain full length sequence

<400> 80

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

Gln Arg Ala Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Ser Thr Ser
20 25 30

Ser Ser Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Lys Tyr Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
50 55 60

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

Pro Val Glu Ala Asn Asp Thr Ala Asn Tyr Tyr Cys Gln His Ser Trp
85 90 95

Glu Ile Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 81
<211> 5
<212> PRT
<213> Mus sp.

<400> 81

Ser Tyr Gly Met Ser
1 5

<210> 82
<211> 16
<212> PRT
<213> Mus sp.

<400> 82

Ser Ile Ser Ser Gly Gly Ser Thr Tyr Tyr Pro Asp Ser Val Lys Gly
1 5 10 15

<210> 83
<211> 8
<212> PRT
<213> Mus sp.

<400> 83

Gly Tyr Asp Ser Gly Phe Ala Tyr
1 5

<210> 84
<211> 15
<212> PRT
<213> Mus sp.

<400> 84

Arg Ala Ser Gln Ser Val Ser Thr Ser Ser Ser Ser Phe Met His
1 5 10 15

<210> 85
<211> 7
<212> PRT
<213> Mus sp.

<400> 85

Tyr Ala Ser Asn Leu Glu Ser
1 5

<210> 86
<211> 9
<212> PRT
<213> Mus sp.

<400> 86

Gln His Ser Trp Glu Ile Pro Tyr Thr
1 5

<210> 87
<211> 5
<212> PRT
<213> Mus sp.

<400> 87

Ser Tyr Gly Met Ser
1 5

<210> 88
<211> 16
<212> PRT
<213> Mus sp.

<400> 88

Ser Ile Ser Ser Gly Gly Thr Thr Tyr Tyr Pro Asp Ser Val Lys Gly
1 5 10 15

<210> 89
<211> 8
<212> PRT
<213> Mus sp.

<400> 89

Gly Tyr Asp Ser Gly Phe Ala Tyr
1 5

<210> 90
<211> 15
<212> PRT
<213> Mus sp.

<400> 90

Arg Ala Ser Gln Ser Val Ser Thr Ser Ser Ser Ser Tyr Met His
1 5 10 15

<210> 91
<211> 7
<212> PRT
<213> Mus sp.

<400> 91

Tyr Ala Ser Asn Leu Glu Ser
1 5

<210> 92
<211> 9
<212> PRT
<213> Mus sp.

<400> 92

Gln His Ser Trp Glu Ile Pro Tyr Thr
1 5

<210> 93
<211> 5
<212> PRT
<213> Mus sp.

<400> 93

Thr Tyr Gly Val His
1 5

<210> 94
<211> 16
<212> PRT
<213> Mus sp.

<400> 94

Val Ile Trp Arg Gly Val Thr Thr Asp Tyr Asn Ala Ala Phe Met Ser
1 5 10 15

<210> 95
<211> 8
<212> PRT
<213> Mus sp.

<400> 95

Leu Gly Phe Tyr Ala Met Asp Tyr
1 5

<210> 96
<211> 11
<212> PRT
<213> Mus sp.

<400> 96

Lys Ala Ser Gln Ser Val Ser Asn Asp Val Ala
1 5 10

<210> 97
<211> 7
<212> PRT
<213> Mus sp.

<400> 97

Tyr Ala Ala Asn Arg Tyr Thr
1 5

<210> 98
<211> 9
<212> PRT
<213> Mus sp.

<400> 98

Gln Gln Asp Tyr Thr Ser Pro Tyr Thr
1 5

<210> 99
<211> 5
<212> PRT
<213> Mus sp.

<400> 99

Ser Tyr Gly Val His
1 5

<210> 100
<211> 16
<212> PRT
<213> Mus sp.

<400> 100

Val Ile Trp Ser Gly Gly Val Thr Asp Tyr Asn Ala Ala Phe Ile Ser
1 5 10 15

<210> 101
<211> 8
<212> PRT
<213> Mus sp.

<400> 101

Leu Gly Phe Tyr Ala Met Asp Tyr
1 5

<210> 102
<211> 11
<212> PRT
<213> Mus sp.

<400> 102

Lys Ala Ser Gln Ser Val Ser Asn Asp Val Gly
1 5 10

<210> 103
<211> 7

<212> PRT
<213> Mus sp.

<400> 103

Tyr Ala Ser Asn Arg Tyr Ser
1 5

<210> 104
<211> 9
<212> PRT
<213> Mus sp.

<400> 104

Gln Gln Asp Tyr Thr Ser Pro Tyr Thr
1 5

<210> 105
<211> 5
<212> PRT
<213> Mus sp.

<400> 105

Thr Tyr Trp Met His
1 5

<210> 106
<211> 17
<212> PRT
<213> Mus sp.

<400> 106

Gln Ile Asn Pro Asp Ser Thr Thr Ile Asn Tyr Ala Pro Ser Leu Lys
1 5 10 15

Asp

<210> 107
<211> 10
<212> PRT
<213> Mus sp.

<400> 107

Pro Gly Asp Tyr Gly Tyr Asp Phe Asp Cys
1 5 10

<210> 108
<211> 16
<212> PRT
<213> Mus sp.

<400> 108

Arg Ser Ser Gln Ile Ile Val His Ser Asn Ala Asn Thr Tyr Leu Glu
1 5 10 15

<210> 109
<211> 7
<212> PRT
<213> Mus sp.

<400> 109

Lys Val Ser Asn Arg Phe Ser
1 5

<210> 110
<211> 9
<212> PRT
<213> Mus sp.

<400> 110

Phe Gln Gly Ser His Val Pro Tyr Thr
1 5

<210> 111
<211> 5
<212> PRT
<213> Mus sp.

<400> 111

Ser Gly Tyr Trp Asn
1 5

<210> 112
<211> 16
<212> PRT
<213> Mus sp.

<400> 112

Tyr Ile Ser Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> 113
<211> 11
<212> PRT
<213> Mus sp.

<400> 113

Ser Leu Leu Trp Phe Ser Thr Gly Phe Ala Tyr
1 5 10

<210> 114
<211> 12
<212> PRT
<213> Mus sp.

<400> 114

Ser Ala Ser Ser Ser Val Ser Ser Ser Tyr Leu Tyr
1 5 10

<210> 115
<211> 7

<212> PRT
<213> Mus sp.

<400> 115

Asn Thr Ser Asn Leu Ala Ser
1 5

<210> 116
<211> 9
<212> PRT
<213> Mus sp.

<400> 116

His Gln Trp Arg Ser Tyr Pro Pro Thr
1 5

<210> 117
<211> 5
<212> PRT
<213> Mus sp.

<400> 117

Ser Tyr Gly Val His
1 5

<210> 118
<211> 16
<212> PRT
<213> Mus sp.

<400> 118

Val Ile Trp Ser Gly Gly Ile Thr Asp Tyr Asn Ala Ala Phe Lys Ser
1 5 10 15

<210> 119
<211> 8
<212> PRT
<213> Mus sp.

<400> 119

Leu Gly Phe Tyr Ala Met Asp Tyr
1 5

<210> 120
<211> 10
<212> PRT
<213> Mus sp.

<400> 120

Ser Ala Asn Ser Ser Val Ser Tyr Met His
1 5 10

<210> 121
<211> 7
<212> PRT
<213> Mus sp.

<400> 121

Asp Thr Ser Lys Leu Ala Ser
1 5

<210> 122
<211> 9
<212> PRT
<213> Mus sp.

<400> 122

Gln Gln Trp Ser Ser Asn Pro Trp Thr
1 5

<210> 123
<211> 5
<212> PRT
<213> Mus sp.

<400> 123

Ser Tyr Gly Met Ser
1 5

<210> 124
<211> 16
<212> PRT
<213> Mus sp.

<400> 124

Ser Ile Ser Ser Gly Gly Thr Thr Tyr Tyr Leu Gly Ser Val Gln Gly
1 5 10 15

<210> 125
<211> 8
<212> PRT
<213> Mus sp.

<400> 125

Gly Tyr Asp Ala Gly Phe Ala Tyr
1 5

<210> 126
<211> 15
<212> PRT
<213> Mus sp.

<400> 126

Arg Ala Ser Gln Ser Val Ser Thr Ser Ser Tyr Ser Tyr Met His
1 5 10 15

<210> 127
<211> 7
<212> PRT
<213> Mus sp.

<400> 127

Tyr Ala Ser Asn Leu Glu Ser
1 5

<210> 128
<211> 9
<212> PRT
<213> Mus sp.

<400> 128

Gln Asn Ser Trp Glu Ile Pro Tyr Thr
1 5

<210> 129
<211> 5
<212> PRT
<213> Mus sp.

<400> 129

Ser Gly Tyr Trp Thr
1 5

<210> 130
<211> 16
<212> PRT
<213> Mus sp.

<400> 130

Tyr Ile Ser Tyr Thr Gly Ser Thr Tyr Tyr Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> 131
<211> 9
<212> PRT
<213> Mus sp.

<400> 131

Gln Arg Asp Trp Leu Gly Phe Ala Tyr
1 5

<210> 132
<211> 17
<212> PRT
<213> Mus sp.

<400> 132

Lys Ser Ser Gln Ser Leu Leu Tyr Ser Ser Asn Gln Lys Asn Ser Leu
1 5 10 15

Ala

<210> 133
<211> 7
<212> PRT
<213> Mus sp.

<400> 133

Trp Ala Ser Asn Arg Glu Ser
1 5

<210> 134
<211> 9
<212> PRT
<213> Mus sp.

<400> 134

Gln Gln Tyr Tyr Ser Tyr Pro Leu Thr
1 5

<210> 135
<211> 5
<212> PRT
<213> Mus sp.

<400> 135

Ser Tyr Gly Met Ser
1 5

<210> 136
<211> 16
<212> PRT
<213> Mus sp.

<400> 136

Ser Ile Ser Ser Gly Gly Ser Ile Tyr Tyr Pro Asp Ser Val Lys Gly
1 5 10 15

<210> 137
<211> 8
<212> PRT
<213> Mus sp.

<400> 137

Gly Tyr Asp Ala Gly Phe Ala Phe
1 5

<210> 138
<211> 15
<212> PRT
<213> Mus sp.

<400> 138

Arg Ala Ser Gln Ser Val Ser Thr Ser Ser Tyr Ser Tyr Val His
1 5 10 15

<210> 139
<211> 7
<212> PRT
<213> Mus sp.

<400> 139

Tyr Ala Ser Asn Leu Glu Ser
1 5

<210> 140
<211> 9
<212> PRT
<213> Mus sp.

<400> 140

Gln His Ser Trp Glu Ile Pro Tyr Thr
1 5

<210> 141
<211> 660
<212> DNA
<213> Homo sapiens

<400> 141
tttactgtca cggttcccaa ggacctatat gtggtagagt atggtagcaa tatgacaatt 60

gaatgcaaat tcccagtaga aaaacaatta gacctggctg cactaattgt ctattgggaa 120

atggaggata agaacattat tcaatttgtg catggagagg aagacctgaa ggttcagcat 180

agtagctaca gacagagggc ccggctgttg aaggaccagc tctccctggg aaatgctgca 240

cttcagatca cagatgtgaa attgcaggat gcaggggtgt accgctgcat gatcagctat 300

ggtggtgccg actacaagcg aattactgtg aaagtcaatg ccccatacaa caaaatcaac 360

caaagaattt tggttgtgga tccagtcacc tctgaacatg aactgacatg tcaggctgag 420

ggctacccca aggccgaagt catctggaca agcagtgacc atcaagtcct gagtggtaag 480

accaccacca ccaattccaa gagagaggag aagcttttca atgtgaccag cacactgaga 540

atcaacacaa caactaatga gattttctac tgcacttta ggagattaga tcctgaggaa 600

aaccatacag ctgaattggt catcccagaa ctacctctgg cacatcctcc aaatgaaagg 660

<210> 142
<211> 220
<212> PRT
<213> Homo sapiens

<400> 142

Phe Thr Val Thr Val Pro Lys Asp Leu Tyr Val Val Glu Tyr Gly Ser
1 5 10 15

Asn Met Thr Ile Glu Cys Lys Phe Pro Val Glu Lys Gln Leu Asp Leu
20 25 30

Ala Ala Leu Ile Val Tyr Trp Glu Met Glu Asp Lys Asn Ile Ile Gln
35 40 45

Phe Val His Gly Glu Glu Asp Leu Lys Val Gln His Ser Ser Tyr Arg
50 55 60

```
Gln Arg Ala Arg Leu Leu Lys Asp Gln Leu Ser Leu Gly Asn Ala Ala
65                  70                  75                  80

Leu Gln Ile Thr Asp Val Lys Leu Gln Asp Ala Gly Val Tyr Arg Cys
                85                  90                  95

Met Ile Ser Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Val Lys Val
            100                 105                 110

Asn Ala Pro Tyr Asn Lys Ile Asn Gln Arg Ile Leu Val Val Asp Pro
        115                 120                 125

Val Thr Ser Glu His Glu Leu Thr Cys Gln Ala Glu Gly Tyr Pro Lys
    130                 135                 140

Ala Glu Val Ile Trp Thr Ser Ser Asp His Gln Val Leu Ser Gly Lys
145                 150                 155                 160

Thr Thr Thr Thr Asn Ser Lys Arg Glu Glu Lys Leu Phe Asn Val Thr
                165                 170                 175

Ser Thr Leu Arg Ile Asn Thr Thr Thr Asn Glu Ile Phe Tyr Cys Thr
            180                 185                 190

Phe Arg Arg Leu Asp Pro Glu Glu Asn His Thr Ala Glu Leu Val Ile
        195                 200                 205

Pro Glu Leu Pro Leu Ala His Pro Pro Asn Glu Arg
    210                 215                 220
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<210> 143
<211> 681
<212> DNA
<213> Homo sapiens

<400> 143

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tttactgtca cggttcccaa ggacctatat gtggtagagt atggtagcaa tatgacaatt       60

gaatgcaaat tcccagtaga aaaacaatta gacctggctg cactaattgt ctattgggaa      120

atggaggata agaacattat tcaatttgtg catggagagg aagacctgaa ggttcagcat      180

agtagctaca gacagagggc ccggctgttg aaggaccagc tctccctggg aaatgctgca      240

cttcagatca cagatgtgaa attgcaggat gcaggggtgt accgctgcat gatcagctat      300

ggtggtgccg actacaagcg aattactgtg aaagtcaatg ccccatacaa caaaatcaac      360

caaagaattt tggttgtgga tccagtcacc tctgaacatg aactgacatg tcaggctgag      420

ggctacccca aggccgaagt catctggaca agcagtgacc atcaagtcct gagtggtaag      480

accaccacca ccaattccaa gagagaggag aagcttttca atgtgaccag cacactgaga      540

atcaacacaa caactaatga gattttctac tgcacttta ggagattaga tcctgaggaa      600

aaccatacag ctgaattggt catcccagaa ctacctctgg cacatcctcc aaatgaaagg      660
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catcatcacc accatcacta a 681

<210> 144
<211> 226
<212> PRT
<213> Homo sapiens

<400> 144

Phe Thr Val Thr Val Pro Lys Asp Leu Tyr Val Val Glu Tyr Gly Ser
1 5 10 15

Asn Met Thr Ile Glu Cys Lys Phe Pro Val Glu Lys Gln Leu Asp Leu
20 25 30

Ala Ala Leu Ile Val Tyr Trp Glu Met Glu Asp Lys Asn Ile Ile Gln
35 40 45

Phe Val His Gly Glu Glu Asp Leu Lys Val Gln His Ser Ser Tyr Arg
50 55 60

Gln Arg Ala Arg Leu Leu Lys Asp Gln Leu Ser Leu Gly Asn Ala Ala
65 70 75 80

Leu Gln Ile Thr Asp Val Lys Leu Gln Asp Ala Gly Val Tyr Arg Cys
85 90 95

Met Ile Ser Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Val Lys Val
100 105 110

Asn Ala Pro Tyr Asn Lys Ile Asn Gln Arg Ile Leu Val Val Asp Pro
115 120 125

Val Thr Ser Glu His Glu Leu Thr Cys Gln Ala Glu Gly Tyr Pro Lys
130 135 140

Ala Glu Val Ile Trp Thr Ser Ser Asp His Gln Val Leu Ser Gly Lys
145 150 155 160

Thr Thr Thr Thr Asn Ser Lys Arg Glu Glu Lys Leu Phe Asn Val Thr
165 170 175

Ser Thr Leu Arg Ile Asn Thr Thr Thr Asn Glu Ile Phe Tyr Cys Thr
180 185 190

Phe Arg Arg Leu Asp Pro Glu Glu Asn His Thr Ala Glu Leu Val Ile
195 200 205

Pro Glu Leu Pro Leu Ala His Pro Pro Asn Glu Arg His His His His
210 215 220

His His
225

<210> 145
<211> 1344
<212> DNA
<213> Artificial Sequence

<220>
<223> Human PD-L1-mFc sequence

<400> 145
tttactgtca cggttcccaa ggacctatat gtggtagagt atggtagcaa tatgacaatt 60

gaatgcaaat tcccagtaga aaaacaatta gacctggctg cactaattgt ctattgggaa 120

atggaggata agaacattat tcaatttgtg catggagagg aagacctgaa ggttcagcat 180

agtagctaca gacagagggc ccggctgttg aaggaccagc tctccctggg aaatgctgca 240

cttcagatca cagatgtgaa attgcaggat gcaggggtgt accgctgcat gatcagctat 300

ggtggtgccg actacaagcg aattactgtg aaagtcaatg ccccatacaa caaaatcaac 360

caaagaattt tggttgtgga tccagtcacc tctgaacatg aactgacatg tcaggctgag 420

ggctacccca aggccgaagt catctggaca agcagtgacc atcaagtcct gagtggtaag 480

accaccacca ccaattccaa gagagaggag aagctttca atgtgaccag cacactgaga 540

atcaacacaa caactaatga gattttctac tgcactttta ggagattaga tcctgaggaa 600

aaccatacag ctgaattggt catcccagaa ctacctctgg cacatcctcc aaatgaaagg 660

ggtaccagat ctagaggctg caaaccctgt atctgcacag tgcccgaggt gagctccgtg 720

ttcatctttc cccccaagcc caaggacgtg ctgaccatca cactcacacc caaggtcacc 780

tgcgtggtcg tggacatctc caaggacgac cccgaagtcc agttcagctg gttcgtggac 840

gacgtggagg tgcacaccgc tcagacccaa cccagagagg agcagtttaa ctccaccttc 900

aggtccgtgt ccgagctccc catcatgcac caggactggc tgaatggcaa ggagttcaag 960

tgcagggtga actccgctgc tttccccgcc cccattgaga agaccatctc caagaccaag 1020

ggaaggccca aggcccccca ggtgtacacc attcccccctc ccaaggagca gatggccaag 1080

gacaaggtgt ccctgacctg tatgatcacc gacttctttc ccgaggacat caccgtcgaa 1140

tggcagtgga acggccagcc cgccgagaac tataagaaca cccaacccat catggacacc 1200

gacggcagct acttcgtgta tagcaagctc aacgtgcaga agagcaactg ggaagccgga 1260

aataccttca cctgctccgt cctgcacgag ggcctgcaca accaccatac cgaaaagagc 1320

ctgagccaca gccccggaaa gtaa 1344

<210> 146
<211> 447
<212> PRT
<213> Artificial Sequence

<220>
<223> Human PD-L1-mFc sequence

<400> 146

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Phe Thr Val Thr Val Pro Lys Asp Leu Tyr Val Val Glu Tyr Gly Ser
1               5                  10                  15

Asn Met Thr Ile Glu Cys Lys Phe Pro Val Glu Lys Gln Leu Asp Leu
            20                  25                  30

Ala Ala Leu Ile Val Tyr Trp Glu Met Glu Asp Lys Asn Ile Ile Gln
        35                  40                  45

Phe Val His Gly Glu Glu Asp Leu Lys Val Gln His Ser Ser Tyr Arg
    50                  55                  60

Gln Arg Ala Arg Leu Leu Lys Asp Gln Leu Ser Leu Gly Asn Ala Ala
65                  70                  75                  80

Leu Gln Ile Thr Asp Val Lys Leu Gln Asp Ala Gly Val Tyr Arg Cys
                85                  90                  95

Met Ile Ser Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Val Lys Val
            100                 105                 110

Asn Ala Pro Tyr Asn Lys Ile Asn Gln Arg Ile Leu Val Val Asp Pro
        115                 120                 125

Val Thr Ser Glu His Glu Leu Thr Cys Gln Ala Glu Gly Tyr Pro Lys
    130                 135                 140

Ala Glu Val Ile Trp Thr Ser Ser Asp His Gln Val Leu Ser Gly Lys
145                 150                 155                 160

Thr Thr Thr Thr Asn Ser Lys Arg Glu Glu Lys Leu Phe Asn Val Thr
                165                 170                 175

Ser Thr Leu Arg Ile Asn Thr Thr Thr Asn Glu Ile Phe Tyr Cys Thr
            180                 185                 190

Phe Arg Arg Leu Asp Pro Glu Glu Asn His Thr Ala Glu Leu Val Ile
        195                 200                 205

Pro Glu Leu Pro Leu Ala His Pro Pro Asn Glu Arg Gly Thr Arg Ser
    210                 215                 220

Arg Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val
225                 230                 235                 240

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

Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu
            260                 265                 270
```

Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln
275 280 285

Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser
290 295 300

Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys
305 310 315 320

Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile
325 330 335

Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro
340 345 350

Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met
355 360 365

Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn
370 375 380

Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr
385 390 395 400

Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn
405 410 415

Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu
420 425 430

His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys
435 440 445

<210> 147
<211> 1374
<212> DNA
<213> Artificial Sequence

<220>
<223> Human PD-L1-hFc sequence

<400> 147
tttactgtca cggttcccaa ggacctatat gtggtagagt atggtagcaa tatgacaatt 60

gaatgcaaat tcccagtaga aaaacaatta gacctggctg cactaattgt ctattgggaa 120

atggaggata agaacattat tcaatttgtg catggagagg aagacctgaa ggttcagcat 180

agtagctaca gacagagggc ccggctgttg aaggaccagc tctccctggg aaatgctgca 240

cttcagatca cagatgtgaa attgcaggat gcaggggtgt accgctgcat gatcagctat 300

ggtggtgccg actacaagcg aattactgtg aaagtcaatg ccccatacaa caaaatcaac 360

caaagaattt tggttgtgga tccagtcacc tctgaacatg aactgacatg tcaggctgag 420

ggctacccca aggccgaagt catctggaca agcagtgacc atcaagtcct gagtggtaag 480

accaccacca ccaattccaa gagagaggag aagcttttca atgtgaccag cacactgaga 540

atcaacacaa caactaatga gattttctac tgcactttta ggagattaga tcctgaggaa 600

aaccatacag ctgaattggt catcccagaa ctacctctgg cacatcctcc aaatgaaagg 660

ggtaccagat ctagagagcc caaatcttct gacaaaactc acacatgccc accgtgccca 720

gcacctgaat tcgagggtgc accgtcagtc ttcctcttcc ccccaaaacc caaggacacc 780

ctcatgatct cccggactcc tgaggtcaca tgcgtggtgg tggacgtaag ccacgaagac 840

cctgaggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag 900

ccgcgggagg agcagtacaa cagcacgtac cgtgtggtca gcgtcctcac cgtcctgcac 960

caggactggc tgaatggcaa ggagtacaag tgcaaggtct ccaacaaagc cctcccaacc 1020

cccatcgaga aaaccatctc caaagccaaa gggcagcccc gagaaccaca ggtgtacacc 1080

ctgcccccat cccgggatga gctgaccaag aaccaggtca gcctgacctg cctggtcaaa 1140

ggcttctatc caagcgacat cgccgtggag tgggagagca atgggcagcc ggagaacaac 1200

tacaagacca cgcctcccgt gctggactcc gacggctcct tcttcctcta cagcaagctc 1260

accgtggaca agagcaggtg gcagcagggg aacgtcttct catgctccgt gatgcatgag 1320

gctctgcaca accactacac gcagaagagc ctctccctgt ctccgggtaa atga 1374

<210> 148
<211> 457
<212> PRT
<213> Artificial Sequence

<220>
<223> Human PD-L1-hFc sequence

<400> 148

Phe Thr Val Thr Val Pro Lys Asp Leu Tyr Val Val Glu Tyr Gly Ser
1 5 10 15

Asn Met Thr Ile Glu Cys Lys Phe Pro Val Glu Lys Gln Leu Asp Leu
20 25 30

Ala Ala Leu Ile Val Tyr Trp Glu Met Glu Asp Lys Asn Ile Ile Gln
35 40 45

Phe Val His Gly Glu Glu Asp Leu Lys Val Gln His Ser Ser Tyr Arg
50 55 60

Gln Arg Ala Arg Leu Leu Lys Asp Gln Leu Ser Leu Gly Asn Ala Ala
65 70 75 80

Leu Gln Ile Thr Asp Val Lys Leu Gln Asp Ala Gly Val Tyr Arg Cys
85 90 95

Met Ile Ser Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Val Lys Val
100 105 110

```
Asn Ala Pro Tyr Asn Lys Ile Asn Gln Arg Ile Leu Val Val Asp Pro
        115                 120                 125

Val Thr Ser Glu His Glu Leu Thr Cys Gln Ala Glu Gly Tyr Pro Lys
    130                 135                 140

Ala Glu Val Ile Trp Thr Ser Ser Asp His Gln Val Leu Ser Gly Lys
145                 150                 155                 160

Thr Thr Thr Thr Asn Ser Lys Arg Glu Glu Lys Leu Phe Asn Val Thr
                165                 170                 175

Ser Thr Leu Arg Ile Asn Thr Thr Thr Asn Glu Ile Phe Tyr Cys Thr
            180                 185                 190

Phe Arg Arg Leu Asp Pro Glu Glu Asn His Thr Ala Glu Leu Val Ile
        195                 200                 205

Pro Glu Leu Pro Leu Ala His Pro Pro Asn Glu Arg Gly Thr Arg Ser
    210                 215                 220

Arg Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro
225                 230                 235                 240

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

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

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
        275                 280                 285

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    290                 295                 300

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
305                 310                 315                 320

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                325                 330                 335

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

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
        355                 360                 365

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
    370                 375                 380
```

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

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

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

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

Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455

<210> 149
<211> 681
<212> DNA
<213> Artificial Sequence

<220>
<223> Cyno-PD-L1-HisTag

<400> 149
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gaatgcaaat tcccagtaga aaaacaatta gacctgactt cactaattgt ctattgggaa 120

atggaggata agaacattat tcaatttgtg catggagagg aagacctgaa ggttcagcat 180

agtaactaca gacagagggc ccagctgttg aaggaccagc tctccctggg aaatgctgca 240

cttcggatca cagatgtgaa attgcaggat gcaggggttt accgctgcat gatcagctat 300

ggtggtgccg actacaagcg gattaccgtg aaagtcaatg ctccatacaa caaaatcaac 360

caaagaattt tggttgtcga tccagtcacc tctgaacatg aactaacatg tcaggctgag 420

ggctacccca aggccgaagt catttggaca agcagtgacc atcaagtcct gagtggtaag 480

accaccacca ccaattccaa gagagaggag aagcttttaa atgtgaccag cacactgaga 540

atcaacacaa cagctaatga gattttctac tgcatttta ggagattaga tcctgaggaa 600

aaccatacag ctgaattggt catcccagaa ctacctctgg cgcttcctcc aaatgaaagg 660

catcatcacc accatcacta a 681

<210> 150
<211> 226
<212> PRT
<213> Artificial Sequence

<220>
<223> Cyno-PD-L1-HisTag

<400> 150

Phe Thr Val Thr Val Pro Lys Asp Leu Tyr Val Val Glu Tyr Gly Ser
1 5 10 15

```
Asn Met Thr Ile Glu Cys Lys Phe Pro Val Glu Lys Gln Leu Asp Leu
            20                  25                  30

Thr Ser Leu Ile Val Tyr Trp Glu Met Glu Asp Lys Asn Ile Ile Gln
        35                  40                  45

Phe Val His Gly Glu Glu Asp Leu Lys Val Gln His Ser Asn Tyr Arg
    50                  55                  60

Gln Arg Ala Gln Leu Leu Lys Asp Gln Leu Ser Leu Gly Asn Ala Ala
65                  70                  75                  80

Leu Arg Ile Thr Asp Val Lys Leu Gln Asp Ala Gly Val Tyr Arg Cys
                85                  90                  95

Met Ile Ser Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Val Lys Val
            100                 105                 110

Asn Ala Pro Tyr Asn Lys Ile Asn Gln Arg Ile Leu Val Val Asp Pro
        115                 120                 125

Val Thr Ser Glu His Glu Leu Thr Cys Gln Ala Glu Gly Tyr Pro Lys
    130                 135                 140

Ala Glu Val Ile Trp Thr Ser Ser Asp His Gln Val Leu Ser Gly Lys
145                 150                 155                 160

Thr Thr Thr Thr Asn Ser Lys Arg Glu Glu Lys Leu Leu Asn Val Thr
                165                 170                 175

Ser Thr Leu Arg Ile Asn Thr Thr Ala Asn Glu Ile Phe Tyr Cys Ile
            180                 185                 190

Phe Arg Arg Leu Asp Pro Glu Glu Asn His Thr Ala Glu Leu Val Ile
        195                 200                 205

Pro Glu Leu Pro Leu Ala Leu Pro Pro Asn Glu Arg His His His His
    210                 215                 220

His His
225
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<210> 151
<211> 450
<212> DNA
<213> Homo sapiens

<400> 151
ccaggatggt tcttagactc cccagacagg ccctggaacc cccccacctt ctccccagcc 60

ctgctcgtgg tgaccgaagg ggacaacgcc accttcacct gcagcttctc caacacatcg 120

gagagcttcg tgctaaactg gtaccgcatg agccccagca accagacgga caagctggcc 180

gccttccccg aggaccgcag ccagcccggc caggactgcc gcttccgtgt cacacaactg 240

cccaacgggc gtgacttcca catgagcgtg gtcagggccc ggcgcaatga cagcggcacc 300

tacctctgtg gggccatctc cctggccccc aaggcgcaga tcaaagagag cctgcgggca 360

gagctcaggg tgacagagag aagggcagaa gtgcccacag cccaccccag cccctcaccc 420

aggccagccg gccagttcca aaccctggtg 450

<210> 152
<211> 150
<212> PRT
<213> Homo sapiens

<400> 152

Pro Gly Trp Phe Leu Asp Ser Pro Asp Arg Pro Trp Asn Pro Pro Thr
1 5 10 15

Phe Ser Pro Ala Leu Leu Val Val Thr Glu Gly Asp Asn Ala Thr Phe
20 25 30

Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val Leu Asn Trp Tyr
35 40 45

Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala Ala Phe Pro Glu
50 55 60

Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg Val Thr Gln Leu
65 70 75 80

Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg Ala Arg Arg Asn
85 90 95

Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu Ala Pro Lys Ala
100 105 110

Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val Thr Glu Arg Arg
115 120 125

Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro Arg Pro Ala Gly
130 135 140

Gln Phe Gln Thr Leu Val
145 150

<210> 153
<211> 1134
<212> DNA
<213> Artificial Sequence

<220>
<223> Human PD-1-mFc sequence

<400> 153
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ctgctcgtgg tgaccgaagg ggacaacgcc accttcacct gcagcttctc caacacatcg 120

gagagcttcg tgctaaactg gtaccgcatg agccccagca accagacgga caagctggcc 180

gccttccccg aggaccgcag ccagcccggc caggactgcc gcttccgtgt cacacaactg 240

cccaacgggc gtgacttcca catgagcgtg gtcagggccc ggcgcaatga cagcggcacc 300

tacctctgtg gggccatctc cctggccccc aaggcgcaga tcaaagagag cctgcgggca 360

gagctcaggg tgacagagag aagggcagaa gtgcccacag cccaccccag cccctcaccc 420

aggccagccg gccagttcca aaccctggtg ggtaccagat ctagaggctg caaaccctgt 480

atctgcacag tgcccgaggt gagctccgtg ttcatctttc cccccaagcc caaggacgtg 540

ctgaccatca cactcacacc caaggtcacc tgcgtggtcg tggacatctc caaggacgac 600

cccgaagtcc agttcagctg gttcgtggac gacgtggagg tgcacaccgc tcagacccaa 660

cccagagagg agcagtttaa ctccaccttc aggtccgtgt ccgagctccc catcatgcac 720

caggactggc tgaatggcaa ggagttcaag tgcagggtga actccgctgc tttccccgcc 780

cccattgaga agaccatctc caagaccaag ggaaggccca aggcccccca ggtgtacacc 840

attccccctc ccaaggagca gatggccaag gacaaggtgt ccctgacctg tatgatcacc 900

gacttctttc ccgaggacat caccgtcgaa tggcagtgga acggccagcc cgccgagaac 960

tataagaaca cccaacccat catggacacc gacggcagct acttcgtgta tagcaagctc 1020

aacgtgcaga agagcaactg ggaagccgga aataccttca cctgctccgt cctgcacgag 1080

ggcctgcaca accaccatac cgaaaagagc ctgagccaca gccccggaaa gtaa 1134

<210> 154
<211> 377
<212> PRT
<213> Artificial Sequence

<220>
<223> Human PD-1-mFc sequence

<400> 154

Pro Gly Trp Phe Leu Asp Ser Pro Asp Arg Pro Trp Asn Pro Pro Thr
1 5 10 15

Phe Ser Pro Ala Leu Leu Val Val Thr Glu Gly Asp Asn Ala Thr Phe
20 25 30

Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val Leu Asn Trp Tyr
35 40 45

Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala Ala Phe Pro Glu
50 55 60

Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg Val Thr Gln Leu
65 70 75 80

```
Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg Ala Arg Arg Asn
                85                  90                  95

Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu Ala Pro Lys Ala
            100                 105                 110

Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val Thr Glu Arg Arg
        115                 120                 125

Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro Arg Pro Ala Gly
    130                 135                 140

Gln Phe Gln Thr Leu Val Gly Thr Arg Ser Arg Gly Cys Lys Pro Cys
145                 150                 155                 160

Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys
                165                 170                 175

Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val
            180                 185                 190

Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe
        195                 200                 205

Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu
    210                 215                 220

Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His
225                 230                 235                 240

Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala
                245                 250                 255

Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg
            260                 265                 270

Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met
        275                 280                 285

Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro
    290                 295                 300

Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn
305                 310                 315                 320

Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val
                325                 330                 335

Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr
            340                 345                 350
```

Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His His Thr Glu
355 360 365

Lys Ser Leu Ser His Ser Pro Gly Lys
370 375

<210> 155
<211> 1164
<212> DNA
<213> Artificial Sequence

<220>
<223> Human PD-1-hFc sequence

<400> 155
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ctgctcgtgg tgaccgaagg ggacaacgcc accttcacct gcagcttctc caacacatcg 120

gagagcttcg tgctaaactg gtaccgcatg agccccagca accagacgga caagctggcc 180

gccttccccg aggaccgcag ccagcccggc caggactgcc gcttccgtgt cacacaactg 240

cccaacgggc gtgacttcca catgagcgtg gtcagggccc ggcgcaatga cagcggcacc 300

tacctctgtg gggccatctc cctggccccc aaggcgcaga tcaaagagag cctgcgggca 360

gagctcaggg tgacagagag aagggcagaa gtgcccacag cccaccccag cccctcaccc 420

aggccagccg gccagttcca aaccctggtg ggtaccagat ctagagagcc caaatcttct 480

gacaaaactc acacatgccc accgtgccca gcacctgaat tcgagggtgc accgtcagtc 540

ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggactcc tgaggtcaca 600

tgcgtggtgg tggacgtaag ccacgaagac cctgaggtca agttcaactg gtacgtggac 660

ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 720

cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 780

tgcaaggtct ccaacaaagc cctcccaacc cccatcgaga aaaccatctc caaagccaaa 840

gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggatga gctgaccaag 900

aaccaggtca gcctgacctg cctggtcaaa ggcttctatc caagcgacat cgccgtggag 960

tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc 1020

gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg 1080

aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc 1140

ctctccctgt ctccgggtaa atga 1164

<210> 156
<211> 222
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Pro Gly Trp Phe Leu Asp Ser Pro Asp Arg Pro Trp Asn Pro Pro Thr
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Phe Ser Pro Ala Leu Leu Val Val Thr Glu Gly Asp Asn Ala Thr Phe
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Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val Leu Asn Trp Tyr
        35                  40                  45

Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala Ala Phe Pro Glu
    50                  55                  60

Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg Val Thr Gln Leu
65                  70                  75                  80

Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg Ala Arg Arg Asn
                85                  90                  95

Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu Ala Pro Lys Ala
            100                 105                 110

Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val Thr Glu Arg Arg
        115                 120                 125

Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro Arg Pro Ala Gly
    130                 135                 140

Gln Phe Gln Thr Leu Val Gly Thr Arg Ser Arg Glu Pro Lys Ser Ser
145                 150                 155                 160

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Phe Glu Gly
                165                 170                 175

Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            180                 185                 190

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        195                 200                 205

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
    210                 215                 220
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