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(54) Title: ANTIBODIES THAT SPECIFICALLY BIND PD-1 AND METHODS OF USE

(57) Abstract: Antibodies that specifically bind PD-1 or antigen binding fragments thereof, polynucleotides encoding the antibodies or fragments, and methods of making and using the foregoing are useful in the treatment of an inflammatory or immune disorders.

Figure 1A.

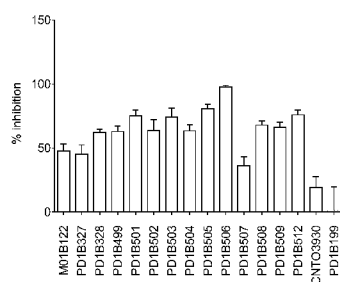
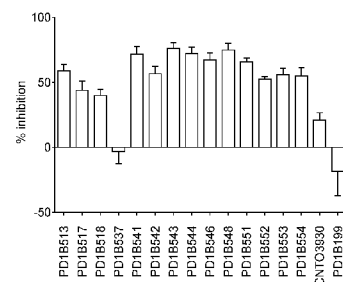


Figure 1B.



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Antibodies That Specifically Bind PD-1 and Methods of Use

Sequence Listing

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 30, 2018, is named JBI5131WOPCT_ST25.txt and is 220 kilobytes in size.

Field of the Invention

The present invention relates to antibodies that specifically bind PD-1, polynucleotides encoding the antibodies or antigen binding fragments, and methods of making and using the foregoing.

Background of the Invention

PD-1 (Programmed Death-1; *PDCD1*) is an inhibitory receptor that belongs to the CD28/CTLA-4 family. PD-1 is a type I transmembrane glycoprotein that contains a single extracellular domain, and a cytoplasmic domain containing both an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). PD-1 is expressed on activated T cells, B cells, NK cells, and thymocytes, and on resting memory T cells including follicular helper T cells (T_{FH}) and peripheral helper T cells (T_{PH}). PD-1, upon engagement by its ligands PD-L1 or PD-L2, suppresses T cell functions through multiple mechanisms (Pauken & Wherry (2015) *Trends in Immunology* 36(4): 265–276). PD-1 engagement directly inhibits T cell receptor (TCR) signaling through co-localization with the TCR and subsequent induction of dephosphorylation of TCR proximal signaling molecules, inhibition of Ras/MEK/ERK pathway leading to inhibition of the cell cycle progression and T cell proliferation, inhibition of cell growth and survival and reprogramming of T cell metabolism through suppression of PI3K/AKT pathway, leading to the upregulation of the BATF transcription factor, and modulation of development, maintenance and function of regulatory T cells. PD-1 has also been proposed to increase T cell motility and to limit duration of interaction between T cells and target cells, thereby reducing the extent of T cell activation (Honda *et al.*, (2014) *Immunity* 40(2):235-47).

Studies in PD-1-deficient mice have indicated that this pathway is important for both central and peripheral tolerance. PD-1 deficient mice on a C57Bl/6 background can develop spontaneous autoimmune disease symptoms including autoantibody production, glomerulonephritis and arthritis (Nishimura et al., Immunity 1999). These data indicate that PD-1 is negatively regulating immune responses.

Monoclonal antibodies against PD-1 and PD-L1 are approved therapies for the treatment of cancers such as advanced melanoma, advanced non-small cell lung cancer, and classical Hodgkin lymphoma. PD-L1 has been found to be upregulated on many different tumor types, and is able to inhibit the tumor-infiltrating PD-1⁺ T cells. Antagonist PD-1 or PD-L1 monoclonal antibodies reverse this suppression, allowing the T cells to become activated and attack the tumor. Thus, immune checkpoint blockade provides a way to enhance anti-tumor immune responses.

Although biologic anti-inflammatory therapeutics are available, there remains a need for improved anti-inflammatory drugs that can effectively suppress inflammation for the treatment of various immune disorders, for example rheumatoid arthritis, in which a significant portion of patients still do not respond adequately to therapy.

Summary of the invention

The invention provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, a HCDR3, a light chain complementarity determining region 1 (LCDR1), a LCDR2 and a LCDR3 of SEQ ID NOs: 2, 165, 4, 166, 6 and 7, respectively.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 3, 4, 5, 6 and 7, respectively; the VH of SEQ ID NOs: 8, 9 or 10 and the VL of SEQ ID NOs: 14, 15 or 16; and/or a heavy chain (HC) of SEQ ID NO: 20, 21 or 22 and a light chain (LC) of SEQ ID NO: 26, 27 or 28.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 145, 4, 5, 6, and 7, respectively;

the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 16; and/or the HC of SEQ ID NO: 150 and the LC of SEQ ID NO: 28.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 146, 4, 5, 6, and 7, respectively; the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 16; and/or the HC of SEQ ID NO: 151 and the LC of SEQ ID NO: 28.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 147, 4, 5, 6, and 7, respectively; the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 16; and/or the HC of SEQ ID NO: 152 and the LC of SEQ ID NO: 28.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 3, 4, 148, 6 and 7, respectively; the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 143; and/or the HC of SEQ ID NO: 22 and the LC of SEQ ID NO: 153.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 3, 4, 149, 6 and 7, respectively; the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 144; and/or the HC of SEQ ID NO: 22 and the LC of SEQ ID NO: 154.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 145, 4, 148, 6 and 7, respectively; the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 143; and/or the HC of SEQ ID NO: 150 and the LC of SEQ ID NO: 153.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 146, 4, 148, 6 and 7, respectively; the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 143; and/or the HC of SEQ ID NO: 151 and the LC of SEQ ID NO: 153.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 147, 4, 148, 6 and 7, respectively; the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 143; and/or the HC of SEQ ID NO: 152 and the LC of SEQ ID NO: 153.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 145, 4, 149, 6 and 7, respectively; the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 144; and/or the HC of SEQ ID NO: 150 and the LC of SEQ ID NO: 154.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 146, 4, 149, 6 and 7, respectively; the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 144; and/or the HC of SEQ ID NO: 151 and the LC of SEQ ID NO: 154.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 147, 4, 149, 6 and 7, respectively. the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 144; and/or the HC of SEQ ID NO: 152 and the LC of SEQ ID NO: 154.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, a HCDR3, a light chain complementarity determining region 1 (LCDR1), a LCDR2 and a LCDR3 of SEQ ID NOs: 32, 124, 40, 41, 42 and 43, respectively.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: SEQ ID NOs: 32, 33, 40, 41, 42 and 43, respectively; the VH of SEQ ID NOs: 44 or 45 and the VL of SEQ ID NOs: 60, 61 or 62; and/or the HC of SEQ ID NOs: 66 or 67 and the LC of SEQ ID NOs: 82, 83 or 84.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the

LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 34, 40, 41, 42 and 43, respectively; the VH of SEQ ID NO: 46 and the VL of SEQ ID NO: 61; and/or the HC of SEQ ID NO: 68 and the LC of SEQ ID NO: 83.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 35, 40, 41, 42 and 43, respectively; the VH of SEQ ID NO: 47 and the VL of SEQ ID NO: 61; and/or the HC of SEQ ID NO: 69 and the LC of SEQ ID NO: 83.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 36, 40, 41, 42 and 43, respectively; the VH of SEQ ID NO: 48 and the VL of SEQ ID NO: 61; and/or the HC of SEQ ID NO: 70 and the LC of SEQ ID NO: 83.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 37, 40, 41, 42 and 43, respectively; the VH of SEQ ID NO: 49 and the VL of SEQ ID NO: 61; and/or the HC of SEQ ID NO: 71 and the LC of SEQ ID NO: 83.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 38, 40, 41, 42 and 43, respectively; the VH of SEQ ID NO: 50 and the VL of SEQ ID NO: 61; and/or the HC of SEQ ID NO: 72 and the LC of SEQ ID NO: 83.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 39, 40, 41, 42 and 43, respectively; the VH of SEQ ID NO: 51 and the VL of SEQ ID NO: 61; and/or the HC of SEQ ID NO: 73 and the LC of SEQ ID NO: 83.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, a HCDR3, a light chain complementarity determining

region 1 (LCDR1), a LCDR2 and a LCDR3 of SEQ ID NOs: 88, 89, 90, 91, 92 and 93, respectively.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, wherein the antibody or the antigen binding fragment thereof competes for binding to PD-1 with the antibody of the invention.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, wherein the antibody or the antigen binding fragment thereof binds to the same PD-1 epitope as the antibody of the invention.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, wherein the antibody VH and the antibody VL or the antibody HC and the antibody LC are encoded by certain polynucleotides recited herein.

The invention also provides a polynucleotide encoding the antibody of the invention.

The invention also provides a vector comprising at least one polynucleotide of the invention

The invention also provides a host cell comprising the vector of the invention.

The invention also provides a method of making the antibody or the antigen binding fragment thereof of the invention, comprising culturing the host cell of the invention in conditions that the antibody or the antigen binding fragment thereof is expressed, and isolating the antibody or the antigen binding fragment thereof.

The invention also provides a pharmaceutical composition comprising the antibody or the antigen binding fragment thereof of the invention.

The invention also provides a kit comprising the antibody or the antigen binding fragment thereof of the invention.

The invention also provides a method of suppressing activation of a PD-1 expressing T cell in a subject, comprising administering to a subject the isolated antibody or the antigen binding fragment thereof of the invention for a time sufficient to suppress activation of the PD-1 expressing T cell.

The invention also provides a method of downmodulating an immune response comprising administering to a subject in need thereof a therapeutically effective amount of the isolated antibody or the antigen binding fragment thereof of the invention for a time sufficient to downmodulate the immune response.

The invention also provides a method of treating an immune disorder comprising administering to a subject in need thereof a therapeutically effective amount of the isolated antibody or the antigen binding fragment thereof of the invention for a time sufficient to treat the immune disorder.

The invention also provides an anti-idiotypic antibody that specifically binds the antibody or the antigen-binding fragment of the invention.

The invention also provides an immunoconjugate comprising the antibody or the antigen binding fragment of the invention conjugated to a heterologous molecule.

Brief Description of the Figures

Figure 1A shows that select generated antibodies inhibited T cell activation in CMV recall assay at a level of over 50% or more. CNTO3930: isotype control. PD1B199: an antagonistic PD-1 mAb.

Figure 1B shows that select generated antibodies inhibited T cell activation in CMV recall assay at a level of over 50% or more. CNTO3930: isotype control. PD1B199: an antagonistic PD-1 mAb.

Figure 2A shows the alignment of PD1B505 mAb lineage VH regions PD1H93 (SEQ ID NO: 8) PD1H384 (SEQ ID NO: 9), PD1H405 (SEQ ID NO: 10), PD1H585 (SEQ ID NO: 140), PD1H586 (SEQ ID NO: 141) and PD1H587 (SEQ ID NO: 142). CDR regions are underlined. The SEQ ID NOs: of the VH chains are shown after the chain name in the Figure (e.g. PD1H93_8; _8 indicates the SEQ ID NO: 8)

Figure 2B shows the alignment of PD1B505 mAb lineage VL regions PD1L30 (SEQ ID NO: 14) PD1L468 (SEQ ID NO: 15), PD1L469 (SEQ ID NO: 16), PD1L651 (SEQ ID NO: 143) and PD1L652 (SEQ ID NO: 144). CDR regions are underlined. The SEQ ID NOs: of the VL chains are shown after the chain name in the Figure (e.g. PD1L30_14; _14 indicates the SEQ ID NO: 14)

Figure 3A shows the alignment of PD1B506 mAb lineage VH regions PD1H90 (SEQ ID NO: 44) PD1H388 (SEQ ID NO: 45), PD1H399 (SEQ ID NO: 46), PD1H400 (SEQ ID NO: 47), PD1H401 (SEQ ID NO: 48), PD1H402 (SEQ ID NO: 49), PD1H403 (SEQ ID NO: 50) and PD1H404 (SEQ ID NO: 51). CDR regions are underlined.

Figure 3B shows the alignment of PD1B506 mAb lineage VL regions PD1L28 (SEQ ID NO: 60) PD1L470 (SEQ ID NO: 61) and PD1L471 (SEQ ID NO: 62). CDR regions are underlined.

Figure 4A shows the alignment of PD1B512 mAb lineage VH regions PD1H81 (SEQ ID NO: 94) and PD1H389 (SEQ ID NO: 95). CDR regions are underlined.

Figure 4B shows the alignment of PD1B512 mAb lineage VL regions PD1L43 (SEQ ID NO: 98) PD1L472 (SEQ ID NO: 99) and PD1L473 (SEQ ID NO: 100). CDR regions are underlined.

Figure 5A shows that PD1B505 and PD1B506 inhibited activation of antigen specific T cells. The Figure shows the mean % inhibition and STDEV of T cell proliferation in CMV recall assay. IgG1: isotype control.

Figure 5B shows that PD1B743, PD1B750 and PD1B756 inhibited activation of antigen specific T cells. The Figure shows the mean % inhibition and STDEV of T cell proliferation in CMV recall assay. IgG1: isotype control.

Figure 5C shows that PD1B878 and PD1B849 inhibited activation of antigen specific T cells in a CMV-specific recall assay. The Figure shows the mean % inhibition and STDEV of T cell proliferation in CMV recall assay. IgG1: isotype control.

Figure 6A shows that PD1B743 and PD1B756 did not block PD-L1 binding to PD-1 whereas PD1B750 blocked the interaction in an assay evaluating degree of clustering of PD-1 and PD-L1 expressing cells in the presence or absence of indicated antibodies using percent (%) double positive events as readout for clustering. Positive control mAb blocked PD-L1/PD-1 interaction.

Figure 6B shows that PD1B743 and PD1B756 did not block PD-L2 binding to PD-1 whereas PD1B750 blocked the interaction in an assay evaluating degree of clustering of PD-1 and PD-L2 expressing cells in the presence or absence of indicated antibodies using percent (%) double positive events as readout for clustering. Positive control mAb blocked PD-L2/PD-1 interaction.

Figure 7 shows a schematic of five distinct epitope bins of the generated PD-1 antibodies. Bin5 mAbs blocked PD-L1/PD-1 interaction whereas mAbs within bins 1-4 did not.

Figure 8A shows that PD-1 expression was higher on the CD4⁺ CD45RO⁺ or CD8⁺ CD45RO⁺ memory T cells stimulated with CMV as compared to the T cells stimulated with

TT (inset, geometric mean fluorescent intensity). PD-1 antibody: solid lines; isotype control: dashed lines.

Figure 8B shows that PD1B878 and PD1B849 inhibited activation of CMV-specific T cells in a CMV-specific recall assay. The Figure shows the mean percent (%) inhibition and STDEV of T cell proliferation in the assay.

Figure 9A shows that PD1B849 and PD1B878 elicited ADCC of activated memory T cells in the presence of NK cells as effector cells. Low fucose (LF) versions of the antibodies (PD1B849-LF and PD1B878-LF) demonstrated enhanced ADCC activity.

Figure 9B shows that PD1B849 and PD1B878 elicited ADCC of activated memory T cells in the presence of PBMCs as effector cells. Low fucose (LF) versions of the antibodies (PD1B849-LF and PD1B878-LF) demonstrated enhanced ADCC activity.

Figure 10A shows lack of PD1B849 and PD1B878 mediated ADCC in resting memory T cells which express low levels of PD1 in the presence of NK cells as effector cells. Low fucose (LF) versions of the antibodies (PD1B849-LF and PD1B878-LF) mediated some ADCC.

Figure 10B shows lack of PD1B849 and PD1B878 mediated ADCC in resting memory T cells which express low levels of PD1 in the presence of PBMCs as effector cells. Low fucose (LF) versions of the antibodies (PD1B849-LF and PD1B878-LF) mediated some ADCC.

Figure 11 shows that PD1B849 and PD1B878 did not mediate measurable CDC of activated T cells using rabbit complement. OKT3: a mouse anti-human CD3 antibody (positive control); huIgG1: isotype control, muIgG2a: isotype control.

Figure 12 shows that PD1B878, PD1B1090 and PD1B1094 did not block PD-L1 binding to PD1 on cells.

Figure 13A shows that PD1B505-mIgG2a and PD1B506-mIgG2a prevented disease development in the mouse model of graft vs host disease (GvHD). Antibodies were dosed at 10 mg/kg i.p. on Days 0, 4, 7, 11, 14 & 18 and clinical score was recorded over time.

Figure 13B shows that PD1B505-mIgG2a and PD1B506-mIgG2a mAbs (mIgG2a) prevented weight loss in the mouse model of GvHD. Antibodies were dosed at 10 mg/kg i.p. on Days 0, 4, 7, 11, 14 & 18.

Figure 14A shows that PD1B849-mIgG2a and PD1B878-mIgG2a prevented disease development in the mouse model of graft vs host disease (GvHD). Antibodies were dosed at 10 mg/kg i.p. on Days 0, 4, 7, 11, 14 & 18 and clinical score was recorded over time.

Figure 14B shows that PD1B849-mIgG2a and PD1B878-mIgG2a prevented weight loss in the mouse model of GvHD. Antibodies were dosed at 10 mg/kg i.p. on Days 0, 4, 7, 11, 14 & 18.

Figure 15 shows that PD1B849-mIgG2a and PD1B878-mIgG2a increased the frequency of regulatory T cells (Tregs) in spleens in the mouse model of GvHD.

Figure 16 shows that select anti-PD1 antibodies deplete T_{FH}/T_{PH} populations. LF: low fucose.

Detailed Description of the Invention

All publications, including but not limited to patents and patent applications cited in this specification are herein incorporated by reference as though fully set forth.

It is to be understood that the terminology used herein is for the purpose of describing embodiments only and is not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

Although any methods and materials similar or equivalent to those described herein may be used in the practice for testing of the present invention, exemplary materials and methods are described herein. In describing and claiming the present invention, the following terminology will be used.

As used herein and in the claims, the singular forms “a,” “and,” and “the” include plural reference unless the context clearly dictates otherwise.

Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise,” “comprising,” and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”.

“Specifically binds,” “specific binding” or “binds” refers to antibody binding to an antigen or an epitope within the antigen with greater affinity than for other antigens or epitopes. Typically, the antibody binds to the antigen or the epitope within the antigen with an equilibrium dissociation constant (K_D) of about 1×10^{-7} M or less, for example about

1×10^{-8} M or less, about 1×10^{-9} M or less, about 1×10^{-10} M or less, about 1×10^{-11} M or less, or about 1×10^{-12} M or less, typically with a K_D that is at least one hundred-fold less than its K_D for binding to a non-specific antigen (e.g., BSA, casein). The K_D may be measured using standard procedures. Antibodies that specifically bind to the antigen or the epitope within the antigen may, however, have cross-reactivity to other related antigens, for example to the same antigen from other species (homologs), such as human or monkey, for example *Macaca fascicularis* (cynomolgus, cyno), *Pan troglodytes* (chimpanzee, chimp) or *Callithrix jacchus* (common marmoset, marmoset). While a monospecific antibody specifically binds one antigen or one epitope, a bispecific antibody specifically binds two distinct antigens or two distinct epitopes.

"Agonist" or "agonistic" refers to an antibody which upon binding to PD-1 induces at least one biological activity that is induced by PD-1 ligand PD-L1. The antibody is an agonist when the at least one biological activity is induced by at least about 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% greater than in the absence of the agonist (e.g., negative control), or when the induction is statistically significant when compared to the induction in the absence of the agonist. A typical biological activity that is induced by PD-L1 binding to PD-1 is inhibition of antigen-specific $CD4^+$ and/or $CD8^+$ T cells, resulting in suppression of immune responses.

PD-1 refers to human programmed cell death protein 1, PD-1. PD-1 is also known as CD279 or PDCD1. The amino acid sequence of the mature human PD-1 (without signal sequence) is shown in **SEQ ID NO: 131**. The extracellular domain spans residues 1-150, the transmembrane domain spans residues 151-171 and the cytoplasmic domain spans residues 172-268 of **SEQ ID NO: 1**. Throughout the specification "the extracellular domain of human PD-1" or "huPD1-ECD" refers to protein having amino acid sequence of residues 1-150 of **SEQ ID NO: 1**.

"Antibodies" is meant in a broad sense and includes immunoglobulin molecules belonging to any class, IgA, IgD, IgE, IgG and IgM, or sub-class IgA₁, IgA₂, IgG₁, IgG₂, IgG₃ and IgG₄ and including either kappa (κ) and lambda (λ) light chain. Antibodies include monoclonal antibodies, full length antibodies, antigen binding fragments, bispecific or multispecific antibodies, dimeric, tetrameric or multimeric antibodies, single chain antibodies, domain antibodies and any other modified configuration of the immunoglobulin molecule that comprises an antigen binding fragment of the required specificity. "Full

length antibodies” are comprised of two heavy chains (HC) and two light chains (LC) interconnected by disulfide bonds as well as multimers thereof (e.g. IgM). Each heavy chain is comprised of a heavy chain variable region (VH) and a heavy chain constant region (comprised of domains CH1, hinge, CH2 and CH3). Each light chain is comprised of a light chain variable region (VL) and a light chain constant region (CL). The VH and the VL may be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with framework regions (FR). Each VH and VL is composed of three CDRs and four FR segments, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.

“Complementarity determining regions (CDR)” are antibody regions that bind an antigen. There are three CDRs in the VH (HCDR1, HCDR2, HCDR3) and three CDRs in the VL (LCDR1, LCDR2, LCDR3). CDRs may be defined using various delineations such as Kabat (Wu et al. (1970) *J Exp Med* 132: 211-50) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991), Chothia (Chothia et al. (1987) *J Mol Biol* 196: 901-17), IMGT (Lefranc et al. (2003) *Dev Comp Immunol* 27: 55-77) and AbM (Martin and Thornton *J Biol Biol* 263: 800-15, 1996). The correspondence between the various delineations and variable region numbering are described (see e.g. Lefranc et al. (2003) *Dev Comp Immunol* 27: 55-77; Honegger and Pluckthun, *J Mol Biol* (2001) 309:657-70; International ImMunoGeneTics (IMGT) database; Web resources, <http://www.imgt.org>). Available programs such as abYsis by UCL Business PLC may be used to delineate CDRs. The term “CDR”, “HCDR1”, “HCDR2”, “HCDR3”, “LCDR1”, “LCDR2” and “LCDR3” as used herein includes CDRs defined by any of the methods described supra, Kabat, Chothia, IMGT or AbM, unless otherwise explicitly stated in the specification.

“Antigen binding fragment” refers to a portion of an immunoglobulin molecule that binds an antigen. Antigen binding fragments may be synthetic, enzymatically obtainable or genetically engineered polypeptides and include the VH, the VL, the VH and the VL, Fab, F(ab')₂, Fd and Fv fragments, domain antibodies (dAb) consisting of one VH domain or one VL domain, shark variable IgNAR domains, camelized VH domains, minimal recognition units consisting of the amino acid residues that mimic the CDRs of an antibody, such as FR3-CDR3-FR4 portions, the HCDR1, the HCDR2 and/or the HCDR3 and the LCDR1, the LCDR2 and/or the LCDR3. VH and VL domains may be linked

together via a synthetic linker to form various types of single chain antibody designs where the VH/VL domains may pair intramolecularly, or intermolecularly in those cases when the VH and VL domains are expressed by separate single chain antibody constructs, to form a monovalent antigen binding site, such as single chain Fv (scFv) or diabody; described for example in Int. Patent Publ. Nos. WO1998/44001, WO1988/01649, WO1994/13804 and WO1992/01047.

“Monoclonal antibody” refers to an antibody obtained from a substantially homogenous population of antibody molecules, i.e., the individual antibodies comprising the population are identical except for possible well-known alterations such as removal of C-terminal lysine from the antibody heavy chain or post-translational modifications such as amino acid isomerization or deamidation, methionine oxidation or asparagine or glutamine deamidation. Monoclonal antibodies typically bind one antigenic epitope. A bispecific monoclonal antibody binds two distinct antigenic epitopes. Monoclonal antibodies may have heterogeneous glycosylation within the antibody population. Monoclonal antibody may be monospecific or multispecific such as bispecific, monovalent, bivalent or multivalent.

“Isolated” refers to a homogenous population of molecules (such as synthetic polynucleotides or a protein such as an antibody) which have been substantially separated and/or purified away from other components of the system the molecules are produced in, such as a recombinant cell, as well as a protein that has been subjected to at least one purification or isolation step. “Isolated antibody” refers to an antibody that is substantially free of other cellular material and/or chemicals and encompasses antibodies that are isolated to a higher purity, such as to 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% purity.

“Antibodies” include antibodies generated using various technologies, including antibodies generated from immunized animals such as mice, rats, rabbits or chickens, or identified from phage or mammalian display libraries as described herein.

“Humanized antibody” refers to an antibody in which at least one CDR is derived from non-human species and at least one framework is derived from human immunoglobulin sequences. Humanized antibody may include substitutions in the frameworks so that the frameworks may not be exact copies of expressed human immunoglobulin or human immunoglobulin germline gene sequences.

“Human antibody” refers to an antibody that is optimized to have minimal immune response when administered to a human subject. Variable regions of human antibody are derived from human germline immunoglobulin sequences. If the antibody contains a constant region or a portion of the constant region, the constant region is also derived from human germline immunoglobulin sequences.

Human antibody comprises heavy or light chain variable regions that are “derived from” human germline immunoglobulin sequences if the variable regions of the antibody are obtained from a system that uses human germline immunoglobulin genes. Such exemplary systems are human immunoglobulin gene libraries displayed on phage or mammalian cells, and transgenic non-human animals such as mice, rats or chickens carrying human immunoglobulin loci. “Human antibody” typically contains amino acid differences when compared to the immunoglobulins expressed in humans due to differences between the systems used to obtain the antibody and human immunoglobulin loci, introduction of naturally occurring somatic mutations, intentional introduction of substitutions into the framework or CDRs. “Human antibody” is typically about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical in amino acid sequence to an amino acid sequence encoded by human germline immunoglobulin sequences. In some cases, “human antibody” may contain consensus framework sequences derived from human framework sequence analyses, for example as described in (Knappik et al. (2000) J Mol Biol 296: 57-86), or synthetic HCDR3 incorporated into human immunoglobulin gene libraries displayed on phage, for example as described in (Shi et al. (2010) J Mol Biol 397: 385-96), and in Int. Patent Publ. No. WO2009/085462. Antibodies in which CDRs are derived from a non-human species are not included in the definition of “human antibody”.

“Recombinant” refers to antibodies and other proteins that are prepared, expressed, created or isolated by recombinant means.

"Epitope" refers to a portion of an antigen to which an antibody specifically binds. Epitopes usually consist of chemically active (such as polar, non-polar or hydrophobic) surface groupings of moieties such as amino acids or polysaccharide side chains and may have specific three-dimensional structural characteristics, as well as specific charge characteristics. An epitope may be composed of contiguous and/or discontinuous amino acids that form a conformational spatial unit. For a discontinuous epitope, amino acids

from differing portions of the linear sequence of the antigen come in close proximity in 3-dimensional space through the folding of the protein molecule.

“Multispecific” refers to a protein, such as an antibody, that specifically binds two or more distinct antigens or two or more distinct epitopes within the same antigen. The multispecific protein may have cross-reactivity to other related antigens, for example to the same antigen from other species (homologs), such as human or monkey, for example *Macaca fascicularis* (cynomolgus, cyno), *Pan troglodytes* (chimpanzee, chimp) or *Callithrix jacchus* (common marmoset, marmoset), or may bind an epitope that is shared between two or more distinct antigens.

“Bispecific” refers to a protein, such as an antibody, that specifically binds two distinct antigens or two distinct epitopes within the same antigen. The bispecific protein may have cross-reactivity to other related antigens, for example to the same antigen from other species (homologs), such as human or monkey, for example *Macaca fascicularis* (cynomolgus, cyno), *Pan troglodytes* (chimpanzee, chimp) or *Callithrix jacchus* (common marmoset, marmoset), or may bind an epitope that is shared between two or more distinct antigens.

“In combination with” means that the drugs or therapeutics are administered to a subject such as human together in a mixture, concurrently as single agents or sequentially as single agents in any order.

“Treat” or “treatment” refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder. Beneficial or desired clinical results include alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable.

“Treatment” can also mean prolonging survival as compared to expected survival if a subject was not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

“Therapeutically effective amount” refers to an amount effective, at doses and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount may vary depending on factors such as the disease state, age, sex, and

weight of the individual, and the ability of a therapeutic or a combination of therapeutics to elicit a desired response in the individual. Exemplary indicators of an effective therapeutic or combination of therapeutics that include, for example, improved well-being of the patient.

“Immune response” includes T cell mediated and/or B cell mediated immune responses. Exemplary immune responses include T cell responses, e.g., cytokine production and cellular cytotoxicity. In addition, the term immune response includes immune responses that are indirectly affected by T cell activation, e.g., antibody production (humoral responses) and activation of cytokine responsive cells, e.g., macrophages.

“Downmodulate” or “downmodulating” refers to a detectable decrease in the level of an immune response in a subject compared with the level of a response in the subject in the absence of a treatment or compound, and/or compared with the level of a response in an otherwise identical but untreated subject.

“Immune disorder” refers to any disease, disorder or disease symptom caused by an activity of the immune system, including autoimmune diseases, inflammatory diseases and allergies.

“Subject” includes any human or nonhuman animal. “Nonhuman animal” includes all vertebrates, e.g., mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc. The terms “subject” and “patient” can be used interchangeably herein.

“Vector” means a polynucleotide capable of being duplicated within a biological system or that can be moved between such systems. Vector polynucleotides typically contain elements, such as origins of replication, polyadenylation signal or selection markers, that function to facilitate the duplication or maintenance of these polynucleotides in a biological system. Examples of such biological systems may include a cell, virus, animal, plant, and reconstituted biological systems utilizing biological components capable of duplicating a vector. The polynucleotide comprising a vector may be DNA or RNA molecules or a hybrid of these.

“Expression vector” means a vector that can be utilized in a biological system or in a reconstituted biological system to direct the translation of a polypeptide encoded by a polynucleotide sequence present in the expression vector.

“Polynucleotide” refers to a synthetic molecule comprising a chain of nucleotides covalently linked by a sugar-phosphate backbone or other equivalent covalent chemistry. cDNA is an exemplary synthetic polynucleotide.

“Polypeptide” or “protein” means a molecule that comprises at least two amino acid residues linked by a peptide bond to form a polypeptide. Small polypeptides of less than 50 amino acids may be referred to as “peptides”.

“About” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. Unless explicitly stated otherwise within the Examples or elsewhere in the Specification in the context of a particular assay, result or embodiment, “about” means within one standard deviation per the practice in the art, or a range of up to 5%, whichever is larger.

“Sample” refers to a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Exemplary samples are biological fluids such as blood, serum and serosal fluids, plasma, lymph, urine, saliva, cystic fluid, tear drops, feces, sputum, mucosal secretions of the secretory tissues and organs, vaginal secretions, ascites fluids, fluids of the pleural, pericardial, peritoneal, abdominal and other body cavities, fluids collected by bronchial lavage, synovial fluid, liquid solutions contacted with a subject or biological source, for example, cell and organ culture medium including cell or organ conditioned medium, lavage fluids and the like, tissue biopsies, fine needle aspirations, surgically resected tissue, organ cultures or cell cultures.

Conventional one and three-letter amino acid codes are used herein as shown in

Table 1.

Table 1.

Amino acid	Three-letter code	One-letter code		Amino acid	Three-letter code	One-letter code
Alanine	Ala	A		Leucine	Leu	L
Arginine	Arg	R		Lysine	Lys	K
Asparagine	Asn	N		Methionine	Met	M
Aspartate	Asp	D		Phenylalanine	Phe	F
Cysteine	Cys	C		Proline	Pro	P
Glutamate	Gln	E		Serine	Ser	S
Glutamine	Glu	Q		Threonine	Thr	T

Glycine	Gly	G		Tryptophan	Trp	W
Histidine	His	H		Tyrosine	Tyr	Y
Isoleucine	Ile	I		Valine	Val	V

Compositions of matter

Provided herein are antibodies that specifically bind PD-1 or antigen binding fragments thereof, polynucleotides encoding the antibodies provided herein, vectors, host cells and methods of making and using the antibodies. The antibodies or antigen binding fragments thereof may be agonistic antibodies.

Some cancer patients treated with immune checkpoint inhibitors including PD-1 antagonists, develop autoimmune-related adverse events, such as symptoms of arthritis, colitis, or psoriasis. One hypothesis to explain this observation is that self-reactive T cells in these patients were being actively suppressed through PD-1, and were “unleashed” in the presence of a PD-1 antagonist. It is then reasonable to reverse this hypothesis and believe that it is likely that “already-unleashed” T cells in patients who have autoimmune disease could be suppressed through PD-1 ligation/agonism.

SNPs in the PD-1 gene PDCD1 have been found to be associated with a variety of autoimmune diseases including rheumatoid arthritis, lupus, and ankylosing spondylitis (summarized by Zamani et al., Cell Immunol 2016 310:27-41). Though functions have not yet been elucidated for the PD-1 SNPs, these associations may indicate that a reduction in PD-1 activity may lead to a reduction in T cell suppression, which could increase susceptibility to autoimmune disease.

There is a need for therapeutics to suppress autoreactive T cells in autoimmune diseases. PD-1⁺ T cells have been found in tissues from patients with autoimmune diseases, including rheumatoid arthritis and Sjogren’s Syndrome (Wan et al., J Immunol 2006 177(12):8844-50.; Kobayashi et al., J Rheumatol 2005 32(11):2156-63). An antibody capable of agonizing PD-1 could be used to suppress T cell proliferation and cytokine release, to limit damage within tissues and restore immune homeostasis. A PD-1 agonist mAb would target activated instead of resting naïve and memory T cells and B cells. In this way the therapeutic would suppress immune responses towards self-antigens in patients with autoimmune diseases without compromising immune memory responses to pathogens. Two T cell types that express high levels of PD-1, T_{FH} and T_{PH}, promote B cell responses and antibody production (Rao et al., Nature 2017; 542: 110-114). The frequency of these cells is increased in

autoimmune diseases driven by autoantibody production, including rheumatoid arthritis, systemic lupus erythematosus, and Sjogren's Syndrome (Rao *et al.*, *Nature* 2017; 542: 110-114; He *et al.*, *Immunity* 2013; 39: 770-781; Verstappen *et al.*, *Arthr & Rheum* 2017; 69(9): 1850-1861). The antibodies of the invention, in addition to providing suppression of activated T cells may selectively deplete cells exhibiting high PD-1 expression, such as T_{FH} and T_{PH} cells.

The invention provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, and HCDR3, a light chain complementarity determining region 1 (LCDR1), a LCDR2 and/or a LCDR3 of any one of antibodies PD1B505, PD1B742, PD1B743, PD1B878, PD1B506, PD1B750, PD1B751, PD1B845, PD1B846, PD1B847, PD1B848, PD1B849, PD1B850, PD1B512, PD1B756, PD1B757, PD1B1085, PD1B1086, PD1B1087, PD1B1088, PD1B1089, PD1B1090, PD1B1091, PD1B1092, PD1B1093, PD1B1094 or PD1B1095.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising a heavy chain variable region (VH) framework and/or a light chain variable region (VL) framework of any one of antibodies PD1B505, PD1B742, PD1B743, PD1B878, PD1B506, PD1B750, PD1B751, PD1B845, PD1B846, PD1B847, PD1B848, PD1B849, PD1B850, PD1B512, PD1B756, PD1B757, PD1B1085, PD1B1086, PD1B1087, PD1B1088, PD1B1089, PD1B1090, PD1B1091, PD1B1092, PD1B1093, PD1B1094 or PD1B1095.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the VH and/or the VL of any one of antibodies PD1B505, PD1B742, PD1B743, PD1B878, PD1B506, PD1B750, PD1B751, PD1B845, PD1B846, PD1B847, PD1B848, PD1B849, PD1B850, PD1B512, PD1B756, PD1B757, PD1B1085, PD1B1086, PD1B1087, PD1B1088, PD1B1089, PD1B1090, PD1B1091, PD1B1092, PD1B1093, PD1B1094 or PD1B1095.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof is an agonistic antibody.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof mediates ADCC of PD-1 expressing cells.

In some embodiments, PD-1 expressing cells are activated memory T cells, T follicular helper cells (T_{FH}) or T peripheral helper cells (T_{PH}), or any combination thereof.

T_{FH} cells may be identified as: live, CD19-CD56-
/CD4+CD45RO+/HLADR+/CXCR5+/ICOS+PD1+;

T_{PH} cells may be identified as: live, CD19-CD56-/CD4+CD45RO+/HLADR+/CXCR5-
/ICOS+PD1+; combination of T_{FH}/T_{PH} cells may be identified as: live, CD19-CD56-

/CD4+CD45RO+/HLADR+/ICOS+PD1+. Memory T cells may be identified as CD4⁺ CD45RO⁺ or CD8⁺ CD45RO⁺.

PD1B505 lineage antibodies

mAbs PD1B505, PD1B742, PD1B743, PD1B878, PD1B1085, PD1B1086, PD1B1087, PD1B1088, PD1B1089, PD1B1090, PD1B1091, PD1B1092, PD1B1093, PD1B1094 or PD1B1095 are exemplary antibodies of PD1B505 mAb lineage. These mAbs have identical CDR regions except that some antibodies have one amino acid difference in the HCDR2 and one or two amino acid differences in the LCDR1. The VH region identity is between 82-100% and the VL region identity between 78-100%. PD1B505 lineage mAbs are ligand non-blocking.

The lineage is characterized by the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 165, 4, 166, 6 and 7, respectively, and by the VH genus sequence of SEQ ID NO: 118 and the VL genus sequence of SEQ ID NO: 119.

SEQ ID NO: 165 (HCDR2 genus)

WINIETGXPT;

wherein X is E, Y, H or W.

SEQ ID NO: 166 (LCDR1 genus)

TASSSX₁X₂SSYLH;

wherein

X₁ is V or F; and

X₂ is S or P.

The invention also provides and isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 165, 4, 166, 6 and 7, respectively.

In some embodiments, the isolated antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the HCDR2 of SEQ ID NOs: 3, 145, 146 or 147 and/or the LCDR1 of SEQ ID NOs: 5, 148 or 149.

In some embodiments, the antibody or the antigen binding fragment thereof has one, two, three, four or five of the following properties:
does not block PD-L1 binding to PD-1, wherein lack of blocking is measured by inability of the antibody to inhibit clustering of PD-L1 expressing and PD-1 expressing cells as described in Example 1;

binds PD-1 with an equilibrium dissociation constant (K_D) of about 5×10^{-8} M or less,

wherein the K_D is measured using a ProteOn XPR36 system at +25°C;

binds PD-1 with an association constant (k_a) of about 3×10^4 1/Ms or more, wherein the k_a is measured using a ProteOn XPR36 system at +25°C;

binds PD-1 with a dissociation constant (k_d) of about 3×10^{-3} 1/s or less, wherein the k_d is measured using a ProteOn XPR36 system at +25°C; or

inhibits proliferation of antigen specific T cells; wherein proliferation is assessed in a CMV-PBMC assay.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the VH framework derived from IGHV7-4-1*1 (SEQ ID NO: 125).

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the VL framework derived from IGKV3D-20*1 (SEQ ID NO: 126).

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the VH framework derived from IGHV7-4-1*1 (SEQ ID NO: 125) and the VL framework derived from IGKV3D-20*1 (SEQ ID NO: 126).

SEQ ID NO: 125 IGHV7-4-1*1

QVQLVQSGSELKKPGASVKVSCASGYTFTSYAMNWVRQAPGQGLEWMGWINT
NTGNPTYAQGFTGRFVFSLDTSVSTAYLQICSLKAEDTAVYYCAR

SEQ ID NO: 126 IGKV3D-20*1

EIVLTQSPATLSLSPGERATLSCGASQSVSSSYLAWYQQKPG LAPRLLIYDASSRAT
GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSP

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising a heavy chain variable region (VH) of SEQ ID NO: 118. SEQ ID NO: 118 is a VH genus sequence of PD1B505 lineage mAbs.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising a light chain variable region (VL) of SEQ ID NO: 119. SEQ ID NO: 118 is a VL genus sequence of PD1B505 lineage mAbs.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the VH of SEQ ID NO: 118 and the VL of SEQ ID NO: 119. The CDRs are shown in bold in SEQ ID NO: 118 and SEQ ID NO: 119.

SEQ ID NO: 118 (PD1B505 lineage VH genus)

X₁VQLX₂X₃SGX₄ELKKPGX₅X₆VKX₇SCKASGYTFTDYSMHVWX₈QAPGX₉GLX₁₀W
MGWINIETGX₁₁**PTYAX**₁₂X₁₃FX₁₄GRFX₁₅FSLX₁₆TSX₁₇STAYLQIX₁₈X₁₉LKX₂₀EDTA
X₂₁YFCARDYYGTYFYAMDYWGQGTXX₂₂X₂₃TVSS; wherein

X₁ is D or Q;

X₂ is Q or V;

X₃ is E or Q;

X₄ is P or S;

X₅ is E or A;

X₆ is T or S;

X₇ is I or V;

X₈ is K or R;

X₉ is K or Q;

X₁₀ is K or E;

X₁₁ is E, Y, H or W;

X₁₂ is D or Q;

X₁₃ is D or G;

X₁₄ is K or T;

X₁₅ is A or V;

X₁₆ is E or D;

X₁₇ is A or V;

X₁₈ is N, C or S;

X₁₉ is N or S;

X₂₀ is N or A;

X₂₁ is T or V;

X₂₂ is T or L; or

X₂₃ is L or V.

SEQ ID NO: 119 (PD1B505 lineage VL genus)

X₁IVLTQSPAX₂X₃SX₄SX₅GERX₆TX₇X₈CTASSSX₉X₁₀SSYLHWYQQKPGX₁₁X₁₂PX₁₃L
X₁₄IYSTSNLASGX₁₅PX₁₆RFSGSGSGTX₁₇X₁₈X₁₉LTISX₂₀X₂₁EX₂₂EDX₂₃AX₂₄YYCH
QYHRSPLTFGX₂₅GTKLEX₂₆K; wherein

X₁ is Q or E;

X₂ is I or T;

X₃ is M or L;

X₄ is A or L;

X₅ is L or P;

X₆ is V or A;

X₇ is M or L;

X₈ is T or S;

X₉ is V or F;

X₁₀ is S or P;

X₁₁ is S or L;

X₁₂ is S or A;

X₁₃ is K or R;

X₁₄ is W or L;

X₁₅ is V or I;
X₁₆ is A or D;
X₁₇ is S or D;
X₁₈ is Y or F;
X₁₉ is S or T;
X₂₀ is S or R;
X₂₁ is M or L;
X₂₂ is A or P;
X₂₃ is A or F;
X₂₄ is T or V;
X₂₅ is A or Q; and
X₂₆ is L or I.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the VH of SEQ ID NOs: 8, 9, 10, 140, 141 or 142.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the VL of SEQ ID NOs: 14, 15, 16, 143 or 144.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the VH of SEQ ID NOs: 8, 9, 10, 140, 141 or 142 and the VL of SEQ ID NOs: 14, 15, 16, 143 or 144.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 3, 4, 5, 6 and 7, respectively.

In some embodiments, the antibody comprises the VH of SEQ ID NOs: 8, 9 or 10 and the VL of SEQ ID NOs: 14, 15 or 16.

In some embodiments, the antibody comprises a heavy chain (HC) of SEQ ID NO: 20, 21 or 22 and a light chain (LC) of SEQ ID NO: 26, 27 or 28.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 145, 4, 5, 6, and 7, respectively.

In some embodiments, the antibody comprises the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 16. In some embodiments, the antibody comprises the HC of SEQ ID NO:

150 and the LC of SEQ ID NO: 28. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 146, 4, 5, 6, and 7, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 16. In some embodiments, the antibody comprises the HC of SEQ ID NO: 151 and the LC of SEQ ID NO: 28. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 147, 4, 5, 6, and 7, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 16. In some embodiments, the antibody comprises the HC of SEQ ID NO: 152 and the LC of SEQ ID NO: 28. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 3, 4, 148, 6 and 7, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 143. In some embodiments, the antibody comprises the HC of SEQ ID NO: 22 and the LC of SEQ ID NO: 153. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 3, 4, 149, 6 and 7, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 144. In some embodiments, the antibody comprises the HC of SEQ ID NO:

22 and the LC of SEQ ID NO: 154. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 145, 4, 148, 6 and 7, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 143. In some embodiments, the antibody comprises the HC of SEQ ID NO: 150 and the LC of SEQ ID NO: 153. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 146, 4, 148, 6 and 7, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 143. In some embodiments, the antibody comprises the HC of SEQ ID NO: 151 and the LC of SEQ ID NO: 153. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 147, 4, 148, 6 and 7, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 143. In some embodiments, the antibody comprises the HC of SEQ ID NO: 152 and the LC of SEQ ID NO: 153. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 145, 4, 149, 6 and 7, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 144. In some embodiments, the antibody comprises the HC of SEQ ID NO:

150 and the LC of SEQ ID NO: 154. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 146, 4, 149, 6 and 7, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 144. In some embodiments, the antibody comprises the HC of SEQ ID NO: 151 and the LC of SEQ ID NO: 154. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 147, 4, 149, 6 and 7, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 144. In some embodiments, the antibody comprises the HC of SEQ ID NO: 152 and the LC of SEQ ID NO: 154. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof is an agonistic antibody.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, wherein the antibody competes for binding to PD-1 with the antibody or the antigen binding fragment comprising

- the VH of SEQ ID NO: 8 and the VL of SEQ ID NO: 14;
- the VH of SEQ ID NO: 9 and the VL of SEQ ID NO: 15;
- the VH of SEQ ID NO: 9 and the VL of SEQ ID NO: 16;
- the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 16;
- the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 16;
- the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 16;
- the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 16;
- the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 143;

the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 144;
the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 144;
the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 144; or
the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 144.

The invention also provides herein an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, wherein the antibody binds the same epitope that is bound by the antibody comprising

the VH of SEQ ID NO: 8 and the VL of SEQ ID NO: 14;
the VH of SEQ ID NO: 9 and the VL of SEQ ID NO: 15;
the VH of SEQ ID NO: 9 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 144;
the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 144;
the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 144; or
the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 144.

In some embodiments, the VH and the VL or the HC and the LC of the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein are encoded by polynucleotide comprising the polynucleotide sequence of

SEQ ID NOs: 11 and 17, respectively;
SEQ ID NOs: 12 and 18, respectively;
SEQ ID NOs: 12 and 19, respectively;
SEQ ID NOs: 13 and 19, respectively;

SEQ ID NOs: 23 and 29, respectively;
SEQ ID NOs: 24 and 30, respectively;
SEQ ID NOs: 24 and 31, respectively;
SEQ ID NOs: 25 and 31, respectively;
SEQ ID NOs: 132 and 133, respectively; or
SEQ ID NOs: 134 and 135, respectively;
SEQ ID NOs: 155 and 19, respectively;
SEQ ID NOs: 156 and 19, respectively;
SEQ ID NOs: 157 and 19, respectively;
SEQ ID NOs: 13 and 158, respectively;
SEQ ID NOs: 13 and 159, respectively;
SEQ ID NOs: 155 and 158, respectively;
SEQ ID NOs: 156 and 158, respectively;
SEQ ID NOs: 157 and 158, respectively;
SEQ ID NOs: 155 and 159, respectively;
SEQ ID NOs: 156 and 159, respectively;
SEQ ID NOs: 157 and 159, respectively;
SEQ ID NOs: 160 and 31, respectively;
SEQ ID NOs: 161 and 31, respectively;
SEQ ID NOs: 162 and 31, respectively;
SEQ ID NOs: 25 and 163, respectively;
SEQ ID NOs: 25 and 164, respectively;
SEQ ID NOs: 160 and 163, respectively;
SEQ ID NOs: 161 and 163, respectively;
SEQ ID NOs: 162 and 163, respectively;
SEQ ID NOs: 160 and 164, respectively;
SEQ ID NOs: 161 and 164, respectively; or
SEQ ID NOs: 162 and 164, respectively.

PD1B506 lineage antibodies

mAbs PD1B506, PD1B750, PD1B751, PD1B845, PD1B846, PD1B847, PD1B848, PD1B849 and PD1B850 are exemplary antibodies of the PD1B506 mAb lineage. These

mAbs have identical HCDR1, HCDR3, LCDR1, LCDR2 and LCDR3, and a variant HCDR2. The VH region identity is between 80-100% and the VL region identity about 98%. PD1B506 lineage mAbs are ligand blocking. The lineage is characterized by the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 124, 40, 41, 42 and 43, respectively, and by the VH genus sequence of SEQ ID NO: 120 and the VL genus sequence of SEQ ID NO: 121.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2 and the HCDR3 of SEQ ID NOs: 32, 124, 40, 41, 42 and 43, respectively.

SEQ ID NO: 124 (PD1B506 lineage HCDR2 genus)

EINPNX₁X₂GIN; wherein

X₁ is N, D, Q, K or E; and

X₂ is G, A or I.

In some embodiments, the isolated antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the HCDR2 of SEQ ID NOs: 33, 34, 35, 36, 37, 38 or 39.

In some embodiments, the antibody or the antigen binding fragment thereof provided herein has one, two, three, four or five of the following properties:

- blocks PD-L1 binding to PD-1, wherein blocking is measured by ability of the antibody to inhibit clustering of PD-L1 expressing and PD-1 expressing cells as described in Example 1;
- binds PD-1 with an equilibrium dissociation constant (K_D) of about 5×10^{-8} M or less, wherein the K_D is measured using a ProteOn XPR36 system at +25°C;
- binds PD-1 with an association constant (k_a) of about 4×10^5 1/Ms or more, wherein the k_a is measured using a ProteOn XPR36 system at +25°C;
- binds PD-1 with a dissociation constant (k_d) of about 1×10^{-2} 1/s or less, wherein the k_d is measured using a ProteOn XPR36 system at +25°C; or
- inhibit proliferation of antigen specific T cells; wherein proliferation is assessed in a CMV-PBMC assay as described in Example 1.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the VH framework derived from IGHV1-2*02 (SEQ ID NO: 127).

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the VL framework derived from IGKV1D-16*1 (SEQ ID NO: 128).

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the VH framework derived from IGHV1-2*02 (SEQ ID NO: 127) and the VL framework derived from IG IGKV1D-16*1 (SEQ ID NO: 128).

SEQ ID NO: 127 IGHV1-2*02

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINP
NSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAR

SEQ ID NO: 128 IGKV1D-16*1

DIQMTQSPSSLSASVGDRVTITCRASQGSSWLAWYQQKPEKAPKSLIYAASSLQSG
VPSRFGSGSGTDFTLTISSLQPEDFATYYCQQYNSTP

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising a heavy chain variable region (VH) of SEQ ID NO: 120. SEQ ID NO: 120 is a VH genus sequence of PD1B506 lineage mAbs.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising a light chain variable region (VL) of SEQ ID NO: 121. SEQ ID NO: 121 is a VL genus sequence of PD1B506 lineage mAbs.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the VH of SEQ ID NO: 120 and the VL of SEQ ID NO: 121. The CDRs are bolded in SEQ ID NO: 120 and SEQ ID NO: 121.

SEQ ID NO: 120 (PD1B506 lineage VH genus)

QVQLX₁QX₂GAEX₃X₄KPGASVKX₅SCKASGYTFTTYWMHWVX₆QX₇PGQGLEWX₈
GEINPNX₉X₁₀**GINY** X₁₁X₁₂KFX₁₃X₁₄X₁₅X₁₆TLTVDKSX₁₇STAYMX₁₈LSX₁₉LX₂₀SX₂₁D
X₂₂AVYYCTIDYYDYGGYWGQGTX₂₃X₂₄TVSS; wherein

X₁ is Q or V;
 X₂ is S or P;
 X₃ is L or V;
 X₄ is V or K;
 X₅ is L or V;
 X₆ is K or R;
 X₇ is R or A;
 X₈ is I or M;
 X₉ is N, D, Q, K or E;
 X₁₀ is G, A or I;
 X₁₁ is N or A;
 X₁₂ is E or Q;
 X₁₃ is K or Q;
 X₁₄ is K or G;
 X₁₅ is K or R;
 X₁₆ is A or V;
 X₁₇ is S or I;
 X₁₈ is Q or E;
 X₁₉ is S or R;
 X₂₀ is T or R;
 X₂₁ is E or D;
 X₂₂ is S or T;
 X₂₃ is T or L; and
 X₂₄ is L or V.

SEQ ID NO: 121 (PD1B506 lineage VL genus)

DIX₁MTQ₂SX₃X₄X₅SX₆SVX₇DRVX₈X₉TCKASQNVGTNVAWYQQKPX₁₀X₁₁X₁₂PKX₁₃LIYSASYRYS₁₄GV₁₅VPX₁₆RFX₁₇GSGSGTDFTLTIX₁₈X₁₉QX₂₀EDX₂₁AX₂₂YX₂₃CQQYN
 IYPYTFGX₂₄GTKLEX₂₅K; wherein

X₁ is V or Q;
 X₂ is Q or P;
 X₃ is K or S;

X₄ is F or S;
X₅ is M or L;
X₆ is T or A;
X₇ is R or G;
X₈ is S or T;
X₉ is V or I;
X₁₀ is G or E;
X₁₁ is Q or K;
X₁₂ is S or A;
X₁₃ is A or S;
X₁₄ is D or S;
X₁₅ is T or S;
X₁₆ is T or S;
X₁₇ is N or S;
X₁₈ is V or L;
X₁₉ is S or P;
X₂₀ is L or F;
X₂₁ is E or T;
X₂₂ is F or Y;
X₂₃ is S or Q; and
X₂₄ is M or I.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the VH of SEQ ID NOs: 44, 45, 46, 47, 48, 49, 50 or 51.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the VL of SEQ ID NOs: 60, 61 or 62.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the VH of SEQ ID NOs: 44, 45, 46, 47, 48, 49, 50 or 51 and the VL of SEQ ID NOs: 60, 61 or 62.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 33, 40, 41, 42 and 43,

respectively. In some embodiments, the antibody comprises the VH of SEQ ID NOs: 44 or 45 and the VL of SEQ ID NOs: 60, 61 or 62. In some embodiments, the antibody comprises the HC of SEQ ID NOs: 66 or 67 and the LC of SEQ ID NOs: 82, 83 or 84. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 34, 40, 41, 42 and 43, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 46 and the VL of SEQ ID NO: 61. In some embodiments, the antibody comprises the HC of SEQ ID NO: 68 and the LC of SEQ ID NO: 83. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 35, 40, 41, 42 and 43, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 47 and the VL of SEQ ID NO: 61. In some embodiments, the antibody comprises the HC of SEQ ID NO: 69 and the LC of SEQ ID NO: 83. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 36, 40, 41, 42 and 43, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 48 and the VL of SEQ ID NO: 61. In some embodiments, the antibody comprises the HC of SEQ ID NO: 70 and the LC of SEQ ID NO: 83. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 37, 40, 41, 42 and 43,

respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 49 and the VL of SEQ ID NO: 61. In some embodiments, the antibody comprises the HC of SEQ ID NO: 71 and the LC of SEQ ID NO: 83. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 38, 40, 41, 42 and 43, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 50 and the VL of SEQ ID NO: 61. In some embodiments, the antibody comprises the HC of SEQ ID NO: 72 and the LC of SEQ ID NO: 83. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 39, 40, 41, 42 and 43, respectively. In some embodiments, the antibody comprises the VH of SEQ ID NO: 51 and the VL of SEQ ID NO: 61. In some embodiments, the antibody comprises the HC of SEQ ID NO: 73 and the LC of SEQ ID NO: 83. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

In some embodiments, the antibody is an agonistic antibody.

The invention also provides herein an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, wherein the antibody competes for binding to PD-1 with the antibody or the antigen binding fragment comprising

- the VH of SEQ ID NO: 44 and the VL of SEQ ID NO: 60;
- the VH of SEQ ID NO: 45 and the VL of SEQ ID NO: 61;
- the VH of SEQ ID NO: 45 and the VL of SEQ ID NO: 62;
- the VH of SEQ ID NO: 46 and the VL of SEQ ID NO: 61;
- the VH of SEQ ID NO: 47 and the VL of SEQ ID NO: 61;
- the VH of SEQ ID NO: 48 and the VL of SEQ ID NO: 61;
- the VH of SEQ ID NO: 49 and the VL of SEQ ID NO: 61;

the VH of SEQ ID NO: 50 and the VL of SEQ ID NO: 61; or
the VH of SEQ ID NO: 51 and the VL of SEQ ID NO: 61.

The invention also provides herein an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, wherein the antibody binds the same epitope that is bound by the antibody comprising

the VH of SEQ ID NO: 44 and the VL of SEQ ID NO: 60;
the VH of SEQ ID NO: 45 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 45 and the VL of SEQ ID NO: 62;
the VH of SEQ ID NO: 46 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 47 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 48 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 49 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 50 and the VL of SEQ ID NO: 61; or
the VH of SEQ ID NO: 51 and the VL of SEQ ID NO: 61.

In some embodiments, the VH and the VL or the HC and the LC of the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein are encoded by polynucleotide comprising the polynucleotide sequence of

SEQ ID NOs: 52 and 63, respectively;
SEQ ID NOs: 53 and 64, respectively;
SEQ ID NOs: 53 and 65, respectively;
SEQ ID NOs: 54 and 64, respectively;
SEQ ID NOs: 55 and 64, respectively;
SEQ ID NOs: 56 and 64, respectively;
SEQ ID NOs: 57 and 64, respectively;
SEQ ID NOs: 58 and 64, respectively;
SEQ ID NOs: 59 and 64, respectively;
SEQ ID NOs: 74 and 85, respectively;
SEQ ID NOs: 75 and 86, respectively;
SEQ ID NOs: 75 and 87, respectively;
SEQ ID NOs: 76 and 86, respectively;
SEQ ID NOs: 77 and 86, respectively;
SEQ ID NOs: 78 and 86, respectively;

SEQ ID NOs: 79 and 86, respectively;
SEQ ID NOs: 80 and 86, respectively;
SEQ ID NOs: 81 and 86, respectively;
SEQ ID NOs: 136 and 137, respectively; or
SEQ ID NOs: 138 and 139, respectively.

PD1B512 lineage antibodies

mAbs PD1B505, PD1B756 and PD1B757 are exemplary antibodies of PD1B512 mAb lineage. These mAbs have identical CDR regions with the VH region identity of about 84% and the VL region identity between 90-99%. PD1B512 lineage mAbs are ligand non-blocking. The lineage is characterized by the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 88, 89, 90, 91, 92 and 93, respectively, and by the VH genus sequence of SEQ ID NO: 122 and the VL genus sequence of SEQ ID NO: 123.

The invention also provides and isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 88, 89, 90, 91, 92 and 93, respectively.

In some embodiments, the antibody or the antigen binding fragment thereof does not block PD-L1 binding to PD-1, wherein lack of blocking is measured by inability of the antibody to inhibit clustering of PD-L1 expressing and PD-1 expressing cells as described in Example 1.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein comprises the VH framework derived from IGHV2-5*04 (SEQ ID NO: 129).

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein comprises the VL framework derived from IGKV2-28*01 (SEQ ID NO: 130).

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein comprises the VH framework derived from IGHV2-5*04 (SEQ ID NO: 129) and the VL framework derived from IGKV2-28*01 (SEQ ID NO: 130).

SEQ ID NO: 129 IGHV2-5*04

QITLKESGPTLVKPTQTLTLCTFSGFSLSTSGVGVGWIRQPPGKALEWLALIYWND
DKRYSPSLKSRLTITKDTSKNQVVLMTNMDPVDGTYYCV

SEQ ID NO: 130 IGKV2-28*01

DIVMTQSPLSLPVTGPGEPAISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLG
NRASGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTP

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising a heavy chain variable region (VH) of SEQ ID NO: 122. SEQ ID NO: 122 is a VH genus sequence of PD1B512 lineage mAbs.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising a light chain variable region (VL) of SEQ ID NO: 123. SEQ ID NO: 123 is a VL genus sequence of PD1B512 lineage mAbs.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the VH of SEQ ID NO: 122 and the VL of SEQ ID NO: 123. The CDRs are bolded in SEQ ID NO: 122 and SEQ ID NO: 123.

SEQ ID NO: 122 (PD1B112 lineage VH genus)

QX₁TLKESGPX₂LX₃X₄PX₅QTLX₆LTCX₇FSGFSLSTSGMGVSWIRQPX₈GKX₉LEWL
AHYWDDDKRYX₁₀PSLKSRLTIX₁₁KDTSX₁₂NQVX₁₃LX₁₄X₁₅TX₁₆X₁₇DX₁₈X₁₉DTGT
YYCVRKGGYYDYGYYVMDYWGQGTGX₂₀VTVSS, wherein

X₁ is V or I;

X₂ is G or T;

X₃ is L or V;

X₄ is Q or K;

X₅ is S or T;

X₆ is S or T;

X₇ is S or T;

X₈ is S or P;

X₉ is G or A;
X₁₀ is N or S;
X₁₁ is S or T;
X₁₂ is S or K;
X₁₃ is F or V;
X₁₄ is K or T;
X₁₅ is I or M;
X₁₆ is S or N;
X₁₇ is V or M;
X₁₈ is T or P;
X₁₉ is A or V; and
X₂₀ is T or L.

SEQ ID NO: 123 (PD1B112 lineage VL genus)

DIVMTQX₁X₂LSX₃PVTX₄GX₅X₆ASISCRSSKSLHNSNGITYLWNWYLQKPGQSPQLLI
YQMSNLASGVDPDRFSX₇SGSGTDFTLX₈ISRVEAEDVGVYYCAQNLELPLTFGX₉G
TKX₁₀EX₁₁K, wherein

X₁ is A or S;
X₂ is A or P;
X₃ is N or L;
X₄ is L or P;
X₅ is T or E;
X₆ is S or P;
X₇ is S or G;
X₈ is R or K;
X₉ is S or G;
X₁₀ is L or V; and
X₁₁ is M or I.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the VH of SEQ ID NOs: 94 or 95.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises the VL of SEQ ID NOs: 98, 99 or 100.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the VH of SEQ ID NOs: 94 or 95 and the VL of SEQ ID NOs: 98, 99 or 100.

The invention also provides an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof comprising the VH of SEQ ID NO: 94 and the VL of SEQ ID NO: 98. In some embodiments, the antibody comprises the HC of SEQ ID NO: 104 and the LC of SEQ ID NO: 108. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein comprises the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 99. In some embodiments, the antibody comprises the HC of SEQ ID NO: 105 and the LC of SEQ ID NO: 109. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein comprises the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 100. In some embodiments, the antibody comprises the HC of SEQ ID NO: 105 and the LC of SEQ ID NO: 110. The antibody is also provided for use in therapy, such as in treatment of an immune disorder, rheumatoid arthritis, lupus, systemic lupus erythematosus or graft versus host disease.

In some embodiments, the antibody is an agonistic antibody.

The invention also provides herein an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, wherein the antibody competes for binding to PD-1 with the antibody or the antigen binding fragment comprising

the VH of SEQ ID NO: 94 and the VL of SEQ ID NO: 98;

the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 99; or

the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 100.

The invention also provides herein an isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, wherein the antibody binds the same epitope that is bound by the antibody comprising

the VH of SEQ ID NO: 94 and the VL of SEQ ID NO: 98;
the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 99; or
the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 100.

In some embodiments, the VH and the VL or the HC and the LC of the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein are encoded by polynucleotide comprising the polynucleotide sequence of

SEQ ID NOs: 96 and 101, respectively;
SEQ ID NOs: 97 and 102, respectively;
SEQ ID NOs: 97 and 103, respectively;
SEQ ID NOs: 106 and 111, respectively;
SEQ ID NOs: 107 and 112, respectively; or
SEQ ID NOs: 107 and 113, respectively.

Homologous antibodies and antibodies with conservative mutations

Variants of the antibodies that specifically bind PD-1 or the antigen binding fragments thereof provided herein are within the scope of the invention. For example, variants may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29 amino acid substitutions in the VH and/or the VL as long as the variant antibodies retain or have improved functional properties when compared to the parental antibodies. In some embodiments, the sequence identity may be about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% to the VH and/or the VL amino acid sequence of the invention. In some embodiments, the variation is in the framework regions. In some embodiments, variants are generated by conservative substitutions.

For example, PD1B505 lineage antibodies may comprise substitutions at VH residue positions 1, 5, 6, 9, 16, 17, 20, 38, 43, 46, 57, 62, 63, 65, 69, 73, 76, 84, 85, 88, 93, 116 and/or 117 (residue numbering according to SEQ ID NO: 8) and at VL residue positions 1, 10, 11, 13, 15, 19, 21, 22, 29, 30, 43, 44, 46, 48, 59, 61, 71, 72, 73, 78, 79, 81, 84, 86, 101 and/or 107 (residue numbering according to SEQ ID NO: 14). PD1B506

lineage antibodies may comprises substitutions at VH residue positions 5, 7, 11, 12, 20, 38, 40, 48, 55, 56, 61, 62, 65, 66, 67, 68, 76, 82, 85, 87, 89, 91, 112 and/or 113 (residue numbering according to SEQ ID NO: 44) and at VL residue positions 3, 8, 9, 10, 11, 13, 16, 20, 21, 41, 42, 43, 46, 60, 63, 76, 77, 78, 80, 83, 85, 87, 100 and/or 106 (residue numbering according to SEQ ID NO: 60). PD1B512 lineage antibodies may comprise substitutions at VH residue positions 2, 10, 12, 13, 15, 19, 23, 43, 46, 62, 72, 77, 81, 83, 84, 86, 87, 89, 90 and/or 117 and at VL residue positions 7, 8, 11, 15, 17, 18, 69, 79, 105, 109 and/or 111. Conservative substitutions may be made at any indicated positions and the resulting variant antibodies tested for their desired characteristics in the assays described herein. Alternatively, substitutions in one lineage mAb may be made at indicated positions by substitution with corresponding amino acid at the particular position present in the other antibodies within the lineage.

Also provided are antibodies that specifically bind PD-1 or antigen binding fragments thereof comprising the VH and the VL which are at least 80% identical to

- the VH of SEQ ID NO: 8 and the VL of SEQ ID NO: 14;
- the VH of SEQ ID NO: 9 and the VL of SEQ ID NO: 15;
- the VH of SEQ ID NO: 9 and the VL of SEQ ID NO: 16;
- the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 16;
- the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 16;
- the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 16;
- the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 16;
- the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 143;
- the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 144;
- the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 143;
- the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 143;
- the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 143;
- the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 144;
- the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 144;
- the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 144;
- the VH of SEQ ID NO: 44 and the VL of SEQ ID NO: 60;
- the VH of SEQ ID NO: 45 and the VL of SEQ ID NO: 61;
- the VH of SEQ ID NO: 45 and the VL of SEQ ID NO: 62;

the VH of SEQ ID NO: 46 and the VL of SEQ ID NO: 61;
 the VH of SEQ ID NO: 47 and the VL of SEQ ID NO: 61;
 the VH of SEQ ID NO: 48 and the VL of SEQ ID NO: 61;
 the VH of SEQ ID NO: 49 and the VL of SEQ ID NO: 61;
 the VH of SEQ ID NO: 50 and the VL of SEQ ID NO: 61;
 the VH of SEQ ID NO: 51 and the VL of SEQ ID NO: 61;
 the VH of SEQ ID NO: 94 and the VL of SEQ ID NO: 98;
 the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 99; or
 the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 100.

In some embodiments, the identity is 85%. In some embodiments, the identity is 90%. In some embodiments, the identity is 91%. In some embodiments, the identity is 91%. In some embodiments, the identity is 92%. In some embodiments, the identity is 93%. In some embodiments, the identity is 94%. In some embodiments, the identity is 94%. In some embodiments, the identity is 96%. In some embodiments, the identity is 97%. In some embodiments, the identity is 98%. In some embodiments, the identity is 99%.

The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = number of identical positions/total number of positions × 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The percent identity between two amino acid sequences may be determined using the algorithm of E. Meyers and W. Miller (*Comput Appl Biosci* 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences may be determined using the Needleman and Wunsch (*J Mol Biol* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

In some embodiments, variant antibodies comprise one or two conservative substitutions in any of the CDR regions, wherein the antibodies retain the desired functional properties of the parental antibodies.

“Conservative modifications” refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid modifications. Conservative modifications include amino acid substitutions, additions and deletions. Conservative amino acid substitutions are those in which the amino acid is replaced with an amino acid residue having a similar side chain. The families of amino acid residues having similar side chains are well defined and include amino acids with acidic side chains (e.g., aspartic acid, glutamic acid), basic side chains (e.g., lysine, arginine, histidine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), uncharged polar side chains (e.g., glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine, tryptophan), aromatic side chains (e.g., phenylalanine, tryptophan, histidine, tyrosine), aliphatic side chains (e.g., glycine, alanine, valine, leucine, isoleucine, serine, threonine), amide (e.g., asparagine, glutamine), beta-branched side chains (e.g., threonine, valine, isoleucine) and sulfur-containing side chains (cysteine, methionine). Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for alanine scanning mutagenesis (MacLennan *et al.*, (1988) *Acta Physiol Scand Suppl* 643:55-67; Sasaki *et al.*, (1988) *Adv Biophys* 35:1-24). Amino acid substitutions to the antibodies of the invention may be made by known methods for example by PCR mutagenesis (US Pat. No. 4,683,195). Alternatively, libraries of variants may be generated for example using random (NNK) or non-random codons, for example DVK codons, which encode 11 amino acids (Ala, Cys, Asp, Glu, Gly, Lys, Asn, Arg, Ser, Tyr, Trp). The resulting antibody variants may be tested for their characteristics using assays described herein.

Engineered and modified antibodies

The antibodies that specifically bind PD-1 or the antigen binding fragments thereof provided herein may be further engineered to generate modified antibodies with similar or altered properties when compared to the parental antibodies. The VH, the VL, the VH and the VL, the constant regions, the heavy chain framework, the light chain framework, or any or all the six CDRs may be engineered in the antibodies of the invention.

The antibodies that specifically bind PD-1 or the antigen binding fragments thereof may be engineered by CDR grafting. One or more CDR sequences of the antibodies of the invention may be grafted to a different framework sequence. CDR grafting may be done using known methods and methods described herein.

The framework sequences that may be used may be obtained from public DNA databases or published references that include germline antibody gene sequences. For example, germline DNA and the encoded protein sequences for human heavy and light chain variable domain genes may be found at IMGT®, the international ImMunoGeneTics information system® <http://www-imgt.org>. Framework sequences that may be used to replace the existing framework sequences of the antibodies of the invention may be those that show the highest percent (%) identity to the parental variable domains over the entire length of the VH or the VL, or over the length of FR1, FR2, FR3 and FR4. In addition, suitable frameworks may further be selected based on the VH and the VL CDR1 and CDR2 lengths or identical LCDR1, LCDR2, LCDR3, HCDR1 and HCDR2 canonical structure. Suitable frameworks may be selected using known methods, such as human framework adaptation described in U.S. Patent No. 8,748,356 or superhumanization described in U.S. Patent No. 7,709, 226.

The framework sequences of the parental and engineered antibodies may further be modified, for example by backmutations to restore and/or improve binding of the generated antibodies to the antigen as described for example in U.S. Patent No. 6,180,370. The framework sequences of the parental or engineered antibodies may further be modified by mutating one or more residues within the framework region (or alternatively within one or more CDR regions) to remove T-cell epitopes to thereby reduce the potential immunogenicity of the antibody. This approach is also referred to as “deimmunization” and described in further detail in U.S. Patent Publ. No. US20070014796.

The CDR residues of the antibodies or the antigen-binding fragments thereof provided herein may be mutated to improve affinity of the antibodies to PD-1.

The CDR residues of the antibodies or the antigen-binding fragments thereof provided herein may be mutated to minimize risk of post-translational modifications. Amino acid residues of putative motifs for deamination (NS), acid-catalyzed hydrolysis (DP), isomerization (DS), or oxidation (W) may be substituted with any of the naturally

occurring amino acids to mutagenize the motifs, and the resulting antibodies may be tested for their functionality and stability using methods described herein.

The antibodies that specifically bind PD-1 or the antigen binding fragments thereof provided herein which are modified to improve stability, selectivity, cross-reactivity, affinity, immunogenicity or other desirable biological or biophysical property are within the scope of the invention. Stability of an antibody is influenced by a number of factors, including (1) core packing of individual domains that affects their intrinsic stability, (2) protein/protein interface interactions that have impact upon the HC and LC pairing, (3) burial of polar and charged residues, (4) H-bonding network for polar and charged residues; and (5) surface charge and polar residue distribution among other intra- and inter-molecular forces (Worn *et al.*, (2001) *J Mol Biol* 305:989-1010). Potential structure destabilizing residues may be identified based upon the crystal structure of the antibody or by molecular modeling in certain cases, and the effect of the residues on antibody stability may be tested by generating and evaluating variants harboring mutations in the identified residues. One of the ways to increase antibody stability is to raise the thermal transition midpoint (T_m) as measured by differential scanning calorimetry (DSC). In general, the protein T_m is correlated with its stability and inversely correlated with its susceptibility to unfolding and denaturation in solution and the degradation processes that depend on the tendency of the protein to unfold (Remmele *et al.*, (2000) *Biopharm* 13:36-46). A number of studies have found correlation between the ranking of the physical stability of formulations measured as thermal stability by DSC and physical stability measured by other methods (Gupta *et al.*, (2003) *AAPS PharmSci* 5E8; Zhang *et al.*, (2004) *J Pharm Sci* 93:3076-89; Maa *et al.*, (1996) *Int J Pharm* 140:155-68; Bedu-Addo *et al.*, (2004) *Pharm Res* 21:1353-61; Remmele *et al.*, (1997) *Pharm Res* 15:200-8). Formulation studies suggest that a Fab T_m has implication for long-term physical stability of a corresponding mAb.

Antibody isotypes, allotypes and Fc engineered antibodies

The antibodies that specifically bind PD-1 or the antigen binding fragments thereof provided herein may be of any known isotype or allotype with wild-type or engineered Fc.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein is an IgG1 isotype.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein is an IgG2 isotype.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein is an IgG3 isotype.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein is an IgG4 isotype.

C-terminal lysine (CTL) may be removed from injected antibodies by endogenous circulating carboxypeptidases in the blood stream (Cai *et al.*, (2011) *Biotechnol Bioeng* 108:404-412). During manufacturing, CTL removal may be controlled to less than the maximum level by control of concentration of extracellular Zn^{2+} , EDTA or EDTA – Fe^{3+} as described in U.S. Patent Publ. No. US20140273092. CTL content in antibodies may be measured using known methods.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein has a C-terminal lysine content from about 10% to about 90%. In some embodiments, the C-terminal lysine content is from about 20% to about 80%. In some embodiments, the C-terminal lysine content is from about 40% to about 70%. In some embodiments, the C-terminal lysine content is from about 55% to about 70%. In some embodiments, the C-terminal lysine content is about 60%.

Immunogenicity of therapeutic antibodies is associated with increased risk of infusion reactions and decreased duration of therapeutic response (Baert *et al.*, (2003) *N Engl J Med* 348:602-08). The extent to which therapeutic antibodies induce an immune response in the host may be determined in part by the allotype of the antibody (Stickler *et al.*, (2011) *Genes and Immunity* 12:213-21). Antibody allotype is related to amino acid sequence variations at specific locations in the constant region sequences of the antibody. **Table 2** shows select IgG1, IgG2 and IgG4 allotypes.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein is an G2m(n) allotype.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein is an G2m(n-) allotype.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein is an G2m(n)/(n-) allotype.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein is an nG4m(a) allotype.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof provided herein is an G1m(17,1) allotype.

Table 2.

Allotype	Amino acid residue at position of diversity (residue numbering: EU Index)							
	IgG2		IgG4		IgG1			
	189	282	309	422	214	356	358	431
G2m(n)	T	M						
G2m(n-)	P	V						
G2m(n)/(n-)	T	V						
nG4m(a)			L	R				
G1m(17)					K	E	M	A
G1m(17,1)					K	D	L	A

Fc mutations may be made to the antibodies that specifically bind PD-1 or the antigen binding fragments thereof provided herein to modulate antibody effector functions such as ADCC, ADCP and/or ADCP and/or pharmacokinetic properties. This may be achieved by introducing mutation(s) into the Fc that modulate binding of the mutated Fc to activating FcγRs (FcγRI, FcγRIIa, FcγRIII), inhibitory FcγRIIb and/or to FcRn.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprise at least one mutation in the antibody Fc.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprise one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen mutations in the Fc.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprise at least one mutation in the Fc that modulates binding of the antibody to FcRn.

Fc positions that may be mutated to modulate antibody half-life (e.g. binding to FcRn) include positions 250, 252, 253, 254, 256, 257, 307, 376, 380, 428, 434 and 435. Exemplary mutations that may be made singularly or in combination are mutations T250Q, M252Y, I253A, S254T, T256E, P257I, T307A, D376V, E380A, M428L, H433K, N434S,

N434A, N434H, N434F, H435A and H435R. Exemplary singular or combination mutations that may be made to increase the half-life of the antibodies provided herein are mutations M428L/N434S, M252Y/S254T/T256E, T250Q/M428L, N434A and T307A/E380A/N434A. Exemplary singular or combination mutations that may be made to reduce the half-life of the antibodies provided herein are mutations H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises a mutation M252Y/S254T/T256E.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises at least one mutation in the antibody Fc that reduces binding of the antibody to an activating Fc γ receptor (Fc γ R) and/or reduces Fc effector functions such as C1q binding, complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) or phagocytosis (ADCP).

Fc positions that may be mutated to reduce binding of the antibody to the activating Fc γ R and subsequently to reduce effector function include positions 214, 233, 234, 235, 236, 237, 238, 265, 267, 268, 270, 295, 297, 309, 327, 328, 329, 330, 331 and 365. Exemplary mutations that may be made singularly or in combination are mutations K214T, E233P, L234V, L234A, deletion of G236, V234A, F234A, L235A, G237A, P238A, P238S, D265A, S267E, H268A, H268Q, Q268A, N297A, A327Q, P329A, D270A, Q295A, V309L, A327S, L328F, A330S and P331S in IgG1, IgG2, IgG3 or IgG4. Exemplary combination mutations that result in antibodies with reduced ADCC are mutations L234A/L235A on IgG1, V234A/G237A/ P238S/H268A/V309L/A330S/P331S on IgG2, F234A/L235A on IgG4, S228P/F234A/ L235A on IgG4, N297A on all Ig isotypes, V234A/G237A on IgG2, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M on IgG1, H268Q/V309L/A330S/P331S on IgG2, S267E/L328F on IgG1, L234F/L235E/D265A on IgG1, L234A/L235A/G237A/P238S/H268A/A330S/P331S on IgG1, S228P/F234A/L235A/G237A/P238S on IgG4, and S228P/F234A/L235A/G236-deleted/G237A/P238S on IgG4. Hybrid IgG2/4 Fc domains may also be used, such as Fc with residues 117-260 from IgG2 and residues 261-447 from IgG4.

Exemplary mutation that result in antibodies with reduced CDC is a K322A mutation.

Well-known S228P mutation may be made in IgG4 antibodies to enhance IgG4 stability.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises a mutation in at least one residue position 214, 233, 234, 235, 236, 237, 238, 265, 267, 268, 270, 295, 297, 309, 322, 327, 328, 329, 330, 331 or 365.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises at least one mutation selected from the group consisting of K214T, E233P, L234V, L234A, deletion of G236, V234A, F234A, L235A, G237A, P238A, P238S, D265A, S267E, H268A, H268Q, Q268A, N297A, A327Q, P329A, D270A, Q295A, V309L, A327S, L328F, K322, A330S and P331S.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises a mutation in at least one residue position 228, 234, 235, 237, 238, 268, 322, 330 or 331.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises a K322A mutation.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises a S228P mutation.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises at least one mutation in an antibody Fc that enhances binding of the antibody to an Fc γ receptor (Fc γ R) and/or enhances Fc effector functions such as C1q binding, complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and/or phagocytosis (ADCP).

Fc positions that may be mutated to increase binding of the antibody to the activating Fc γ R and/or enhance antibody effector functions include positions 236, 239, 243, 256, 290, 292, 298, 300, 305, 312, 326, 330, 332, 333, 334, 345, 360, 339, 378, 396 or 430 (residue numbering according to the EU index). Exemplary mutations that may be made singularly or in combination are a G236A mutation, a S239D mutation, a F243L mutation, a T256A mutation, a K290A mutation, a R292P mutation, a S298A mutation, an Y300L mutation, a V305L mutation, a K326A mutation, an A330K mutation, an I332E mutation, an E333A mutation, a K334A mutation, an A339T mutation and a P396L mutation. Exemplary combination mutations that result in antibodies with increased ADCC or ADCP

are a S239D/I332E mutation, a S298A/E333A/K334A mutation, a F243L/R292P/Y300L mutation, a F243L/R292P/Y300L/P396L mutation, a F243L/R292P/Y300L/V305I/P396L mutation and a G236A/S239D/I332E mutation on IgG1.

Fc positions that may be mutated to enhance CDC of the antibody include positions 267, 268, 324, 326, 333, 345 and 430. Exemplary mutations that may be made singularly or in combination are a S267E mutation, a F1268F mutation, a S324T mutation, a K326A mutation, a K326W mutation, an E333A mutation, an E345K mutation, an E345Q mutation, an E345R mutation, an E345Y mutation, an E430S mutation, an E430F mutation and an E430T mutation. Exemplary combination mutations that result in antibodies with increased CDC are a K326A/E333A mutation, a K326W/E333A mutation, a H268F/S324T mutation, a S267E/H268F mutation, a S267E/S324T mutation and a S267E/H268F/S324T mutation on IgG1.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises at least one mutation in the Fc region that enhances binding of the antibody to FcγRIIb. Enhanced binding to FcγRIIb may result in attenuation of FcγRIIb+ B cells, and/or result in clustering of the antibody and subsequent activation of PD-1 downstream signaling pathways.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises a S267E mutation.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises a S267D mutation.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises a S267E/I332E mutation.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises a S267E/L328F mutation.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises a G236D/S267E mutation.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises a P238D/E233D/G237D/H268D/P271G/A330R mutation.

In some embodiments, the antibody that specifically binds PD-1 or the antigen binding fragment thereof comprises a P238D mutation.

"Antibody-dependent cellular cytotoxicity", "antibody-dependent cell-mediated cytotoxicity" or "ADCC" is a mechanism for inducing cell death that depends upon the interaction of antibody-coated target cells with effector cells possessing lytic activity, such as natural killer cells (NK), monocytes, macrophages and neutrophils via Fc gamma receptors (FcγR) expressed on effector cells. For example, NK cells express FcγRIIIa, whereas monocytes express FcγRI, FcγRII and FcγRIIIa. ADCC activity of the antibodies provided herein may be assessed using an *in vitro* assay using PD-1 expressing cells as target cells and NK cells as effector cells. Cytolysis may be detected by the release of label (e.g. radioactive substrates, fluorescent dyes or natural intracellular proteins) from the lysed cells. In an exemplary assay, target cells are used with a ratio of 1 target cell to 4 effector cells. Target cells are pre-labeled with BATDA and combined with effector cells and the test antibody. The samples are incubated for 2 hours and cell lysis measured by measuring released BATDA into the supernatant. Data is normalized to maximal cytotoxicity with 0.67% Triton X-100 (Sigma Aldrich) and minimal control determined by spontaneous release of BATDA from target cells in the absence of any antibody.

"Antibody-dependent cellular phagocytosis" ("ADCP") refers to a mechanism of elimination of antibody-coated target cells by internalization by phagocytic cells, such as macrophages or dendritic cells. ADCP may be evaluated by using monocyte-derived macrophages as effector cells and Daudi cells (ATCC® CCL-213™) or any other PD-1 expressing cells as target cells engineered to express GFP or other labeled molecule. In an exemplary assay, effector:target cell ratio may be for example 4:1. Effector cells may be incubated with target cells for 4 hours with or without the antibody of the invention. After incubation, cells may be detached using accutase. Macrophages may be identified with anti-CD11b and anti-CD14 antibodies coupled to a fluorescent label, and percent phagocytosis may be determined based on % GFP fluorescence in the CD11⁺CD14⁺ macrophages using standard methods.

"Complement-dependent cytotoxicity", or "CDC", refers to a mechanism for inducing cell death in which the Fc effector domain of a target-bound antibody binds and activates complement component C1q which in turn activates the complement cascade leading to target cell death. Activation of complement may also result in deposition of complement components on the target cell surface that facilitate CDC by binding complement receptors (e.g., CR3) on leukocytes. CDC of cells may be measured for

example by plating Daudi cells at 1×10^5 cells/well (50 μ L/well) in RPMI-B (RPMI supplemented with 1% BSA), adding 50 μ L of test antibodies to the wells at final concentration between 0-100 μ g/mL, incubating the reaction for 15 min at room temperature, adding 11 μ L of pooled human serum to the wells, and incubation the reaction for 45 min at 37° C. Percentage (%) lysed cells may be detected as % propidium iodide stained cells in FACS assay using standard methods.

Binding of the antibody to Fc γ R or FcRn may be assessed on cells engineered to express each receptor using flow cytometry. In an exemplary binding assay, 2×10^5 cells per well are seeded in 96-well plate and blocked in BSA Stain Buffer (BD Biosciences, San Jose, USA) for 30 min at 4°C. Cells are incubated with a test antibody on ice for 1.5 hour at 4°C. After being washed twice with BSA stain buffer, the cells are incubated with R-PE labeled anti-human IgG secondary antibody (Jackson ImmunoResearch Laboratories) for 45 min at 4°C. The cells are washed twice in stain buffer and then resuspended in 150 μ L of Stain Buffer containing 1:200 diluted DRAQ7 live/dead stain (Cell Signaling Technology, Danvers, USA). PE and DRAQ7 signals of the stained cells are detected by Miltenyi MACSQuant flow cytometer (Miltenyi Biotec, Auburn, USA) using B2 and B4 channel respectively. Live cells are gated on DRAQ7 exclusion and the geometric mean fluorescence signals are determined for at least 10,000 live events collected. FlowJo software (Tree Star) is used for analysis. Data is plotted as the logarithm of antibody concentration versus mean fluorescence signals. Nonlinear regression analysis is performed.

“Enhance” or “enhanced” refers to enhanced effector function (e.g. ADCC, CDC and/or ADCP) or enhanced binding to an Fc γ receptor (Fc γ R) or FcRn of the antibody of the invention having at least one mutation in the Fc region when compared to the parental antibody without the mutation. “Enhanced” may be an enhancement of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or more, or a statistically significant enhancement.

“Reduce” or “reduced” refers to reduced effector function (e.g. ADCC, CDC and/or ADCP) or reduced binding to an Fc γ receptor (Fc γ R) or FcRn of the antibody of the invention having at least one mutation in the Fc region when compared to the parental antibody without the mutation. “Reduced” may be a reduction of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or more, or a statistically significant reduction.

“Modulate” refers to either enhanced or reduced effector function (e.g. ADCC, CDC and/or ADCCP) or enhanced or reduced binding to an Fc γ receptor (Fc γ R) or FcRn of the antibody of the invention having at least one mutation in the Fc region when compared to the parental antibody without the mutation.

Glycoengineered antibodies

The ability of monoclonal antibodies to induce ADCC can be enhanced by engineering their oligosaccharide component. Human IgG1 or IgG3 are N-glycosylated at Asn297 with the majority of the glycans in the well-known biantennary G0, G0F, G1, G1F, G2 or G2F forms. Antibodies produced by non-engineered CHO cells typically have a glycan fucose content of about at least 85%. The removal of the core fucose from the biantennary complex-type oligosaccharides attached to the Fc regions enhances the ADCC of antibodies via improved Fc γ RIIIa binding without altering antigen binding or CDC activity. Such mAbs can be achieved using different methods reported to lead to the successful expression of relatively high defucosylated antibodies bearing the biantennary complex-type of Fc oligosaccharides such as control of culture osmolality (Konno *et al.*, *Cytotechnology* 64:249-65, 2012), application of a variant CHO line Lec13 as the host cell line (Shields *et al.*, *J Biol Chem* 277:26733-26740, 2002), application of a variant CHO line EB66 as the host cell line (Olivier *et al.*, *MAbs*;2(4): 405-415, 2010; PMID:20562582), application of a rat hybridoma cell line YB2/0 as the host cell line (Shinkawa *et al.*, *J Biol Chem* 278:3466-3473, 2003), introduction of small interfering RNA specifically against the α 1,6-fucosyltransferase (*FUT8*) gene (Mori *et al.*, *Biotechnol Bioeng* 88:901-908, 2004), or coexpression of β -1,4-*N*-acetylglucosaminyltransferase III and Golgi α -mannosidase II or a potent α -mannosidase I inhibitor, kifunensine (Ferrara *et al.*, *J Biol Chem* 281:5032-5036, 2006, Ferrara *et al.*, *Biotechnol Bioeng* 93:851-861, 2006; Xhou *et al.*, *Biotechnol Bioeng* 99:652-65, 2008).

In some embodiments described herein, the antibodies of the invention have a biantennary glycan structure with fucose content of about between 1% to about 15%, for example about 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1%. In some embodiments, the antibodies of the invention have a glycan structure with fucose content of about 50%, 40%, 45%, 40%, 35%, 30%, 25%, or 20%.

“Fucose content” means the amount of the fucose monosaccharide within the sugar chain at Asn297. The relative amount of fucose is the percentage of fucose-containing structures related to all glycostructures. These may be characterized and quantified by multiple methods, for example: 1) using MALDI-TOF of N-glycosidase F treated sample (e.g. complex, hybrid and oligo- and high-mannose structures) as described in Int Pat. Publ. No. WO2008/077546 2); 2) by enzymatic release of the Asn297 glycans with subsequent derivatization and detection/ quantitation by HPLC (UPLC) with fluorescence detection and/or HPLC-MS (UPLC-MS); 3) intact protein analysis of the native or reduced mAb, with or without treatment of the Asn297 glycans with Endo S or other enzyme that cleaves between the first and the second GlcNAc monosaccharides, leaving the fucose attached to the first GlcNAc; 4) digestion of the mAb to constituent peptides by enzymatic digestion (e.g., trypsin or endopeptidase Lys-C), and subsequent separation, detection and quantitation by HPLC-MS (UPLC-MS); 5) Separation of the mAb oligosaccharides from the mAb protein by specific enzymatic deglycosylation with PNGase F at Asn 297. The oligosaccharides thus released can be labeled with a fluorophore, separated and identified by various complementary techniques which allow: fine characterization of the glycan structures by matrix-assisted laser desorption ionization (MALDI) mass spectrometry by comparison of the experimental masses with the theoretical masses, determination of the degree of sialylation by ion exchange HPLC (GlycoSep C), separation and quantification of the oligosaccharide forms according to hydrophilicity criteria by normal-phase HPLC (GlycoSep N), and separation and quantification of the oligosaccharides by high performance capillary electrophoresis-laser induced fluorescence (HPCE-LIF).

“Low fucose” or “low fucose content” as used herein refers to antibodies with fucose content of about between 1%-15%.

“Normal fucose” or ‘normal fucose content’ as used herein refers to antibodies with fucose content of about over 50%, typically about over 80% or over 85%.

Anti-idiotypic antibodies

Anti-idiotypic antibodies are antibodies that specifically bind to the antibodies that specifically bind PD-1 disclosed herein.

The invention also provides an anti-idiotypic antibody that specifically binds to the antibodies that specifically bind PD-1 provided herein.

The invention also provides an anti-idiotypic antibody that specifically binds to the antibody comprising

the VH of SEQ ID NO: 8 and the VL of SEQ ID NO: 14;
the VH of SEQ ID NO: 9 and the VL of SEQ ID NO: 15;
the VH of SEQ ID NO: 9 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 144;
the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 144;
the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 144;
the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 144;
the VH of SEQ ID NO: 44 and the VL of SEQ ID NO: 60;
the VH of SEQ ID NO: 45 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 45 and the VL of SEQ ID NO: 62;
the VH of SEQ ID NO: 46 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 47 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 48 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 49 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 50 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 51 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 94 and the VL of SEQ ID NO: 98;
the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 99; or
the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 100.

An anti-idiotypic (Id) antibody is an antibody which recognizes the antigenic determinants (*e.g.* the paratope or CDRs) of the antibody. The Id antibody may be antigen-blocking or non-blocking. The antigen-blocking Id may be used to detect the free antibody

in a sample (e.g. anti-PD-1 antibody of the invention described herein). The non-blocking Id may be used to detect the total antibody (free, partially bound to antigen, or fully bound to antigen) in a sample. An Id antibody may be prepared by immunizing an animal with the antibody to which an anti-Id is being prepared.

An anti-Id antibody may also be used as an immunogen to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. An anti-anti-Id may be epitopically identical to the original mAb, which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity. Anti-Id antibodies may be varied (thereby producing anti-Id antibody variants) and/or derivatized by any suitable technique, such as those described elsewhere herein with respect to the antibodies that specifically bind PD-1.

Conjugates of the antibodies that specifically bind PD-1 provided herein

The invention also provides an immunoconjugate comprising an isolated antibody or an antigen-binding fragment thereof that specifically binds PD-1 conjugated to a heterologous molecule.

In some embodiments, the heterologous molecule is a detectable label or a cytotoxic agent.

The invention also provides an antibody or an antigen-binding fragment thereof that specifically binds PD-1 conjugated to a detectable label.

The invention also provides an antibody or an antigen-binding fragment thereof that specifically binds PD-1 conjugated to a cytotoxic agent.

Antibodies or antigen-binding fragments thereof that bind PD-1 may be used to direct therapeutics to PD-1 expressing cells. Cells such as activated T cells that overexpress PD-1 may be targeted with an antibody that specifically binds PD-1 conjugated to a cytotoxic agent that kills the cell upon internalization of the PD-1 antibody. Alternatively, PD-1 expressing cells could be targeted with a PD-1 antibody coupled to a therapeutic intended to modify cell function once internalized.

In some embodiments, the detectable label is also a cytotoxic agent.

The isolated antibody that specifically binds PD-1 or the antigen-binding fragment thereof provided herein conjugated to a detectable label may be used to evaluate expression of PD-1 on a variety of samples.

Detectable label includes compositions that when conjugated to the isolated antibody that specifically binds PD-1 or the antigen-binding fragment thereof provided herein renders the latter detectable, via spectroscopic, photochemical, biochemical, immunochemical, or chemical means.

Exemplary detectable labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes (for example, as commonly used in an ELISA), biotin, digoxigenin, haptens, luminescent molecules, chemiluminescent molecules, fluorochromes, fluorophores, fluorescent quenching agents, colored molecules, radioactive isotopes, scintillates, avidin, streptavidin, protein A, protein G, antibodies or fragments thereof, polyhistidine, Ni²⁺, Flag tags, myc tags, heavy metals, enzymes, alkaline phosphatase, peroxidase, luciferase, electron donors/acceptors, acridinium esters, and colorimetric substrates.

A detectable label may emit a signal spontaneously, such as when the detectable label is a radioactive isotope. In other cases, the detectable label emits a signal as a result of being stimulated by an external field.

Exemplary radioactive isotopes may be γ -emitting, Auger-emitting, β -emitting, an alpha-emitting or positron-emitting radioactive isotope. Exemplary radioactive isotopes include ³H, ¹¹C, ¹³C, ¹⁵N, ¹⁸F, ¹⁹F, ⁵⁵Co, ⁵⁷Co, ⁶⁰Co, ⁶¹Cu, ⁶²Cu, ⁶⁴Cu, ⁶⁷Cu, ⁶⁸Ga, ⁷²As, ⁷⁵Br, ⁸⁶Y, ⁸⁹Zr, ⁹⁰Sr, ^{94m}Tc, ^{99m}Tc, ¹¹⁵In, ¹²³I, ¹²⁴I, ¹²⁵I, ¹³¹I, ²¹¹At, ²¹²Bi, ²¹³Bi, ²²³Ra, ²²⁶Ra, ²²⁵Ac and ²²⁷Ac.

Exemplary metal atoms are metals with an atomic number greater than 20, such as calcium atoms, scandium atoms, titanium atoms, vanadium atoms, chromium atoms, manganese atoms, iron atoms, cobalt atoms, nickel atoms, copper atoms, zinc atoms, gallium atoms, germanium atoms, arsenic atoms, selenium atoms, bromine atoms, krypton atoms, rubidium atoms, strontium atoms, yttrium atoms, zirconium atoms, niobium atoms, molybdenum atoms, technetium atoms, ruthenium atoms, rhodium atoms, palladium atoms, silver atoms, cadmium atoms, indium atoms, tin atoms, antimony atoms, tellurium atoms, iodine atoms, xenon atoms, cesium atoms, barium atoms, lanthanum atoms, hafnium atoms, tantalum atoms, tungsten atoms, rhenium atoms, osmium atoms, iridium atoms, platinum

atoms, gold atoms, mercury atoms, thallium atoms, lead atoms, bismuth atoms, francium atoms, radium atoms, actinium atoms, cerium atoms, praseodymium atoms, neodymium atoms, promethium atoms, samarium atoms, europium atoms, gadolinium atoms, terbium atoms, dysprosium atoms, holmium atoms, erbium atoms, thulium atoms, ytterbium atoms, lutetium atoms, thorium atoms, protactinium atoms, uranium atoms, neptunium atoms, plutonium atoms, americium atoms, curium atoms, berkelium atoms, californium atoms, einsteinium atoms, fermium atoms, mendelevium atoms, nobelium atoms, or lawrencium atoms.

In some embodiments, the metal atoms may be alkaline earth metals with an atomic number greater than twenty.

In some embodiments, the metal atoms may be lanthanides.

In some embodiments, the metal atoms may be actinides.

In some embodiments, the metal atoms may be transition metals.

In some embodiments, the metal atoms may be poor metals.

In some embodiments, the metal atoms may be gold atoms, bismuth atoms, tantalum atoms, and gadolinium atoms.

In some embodiments, the metal atoms may be metals with an atomic number of 53 (i.e. iodine) to 83 (i.e. bismuth).

In some embodiments, the metal atoms may be atoms suitable for magnetic resonance imaging.

The metal atoms may be metal ions in the form of +1, +2, or +3 oxidation states, such as Ba^{2+} , Bi^{3+} , Cs^+ , Ca^{2+} , Cr^{2+} , Cr^{3+} , Cr^{6+} , Co^{2+} , Co^{3+} , Cu^+ , Cu^{2+} , Cu^{3+} , Ga^{3+} , Gd^{3+} , Au^+ , Au^{3+} , Fe^{2+} , Fe^{3+} , F^{3+} , Pb^{2+} , Mn^{2+} , Mn^{3+} , Mn^{4+} , Mn^{7+} , Hg^{2+} , Ni^{2+} , Ni^{3+} , Ag^+ , Sr^{2+} , Sn^{2+} , Sn^{4+} , and Zn^{2+} . The metal atoms may comprise a metal oxide, such as iron oxide, manganese oxide, or gadolinium oxide.

Suitable dyes include any commercially available dyes such as, for example, 5(6)-carboxyfluorescein, IRDye 680RD maleimide or IRDye 800CW, ruthenium polypyridyl dyes, and the like.

Suitable fluorophores are fluorescein isothiocyanate (FITC), fluorescein thiosemicarbazide, rhodamine, Texas Red, CyDyes (e.g., Cy3, Cy5, Cy5.5), Alexa Fluors (e.g., Alexa488, Alexa555, Alexa594; Alexa647), near infrared (NIR) (700-900 nm) fluorescent dyes, and carbocyanine and aminostyryl dyes.

The isolated antibody that specifically binds PD-1 or the antigen-binding fragment thereof provided herein conjugated to a detectable label may be used as an imaging agent.

In some embodiments, the isolated antibody that specifically binds PD-1 or the antigen-binding fragment thereof provided herein is conjugated to a cytotoxic agent.

In some embodiments, the cytotoxic agent is a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

In some embodiments, the cytotoxic agent is daunomycin, doxorubicin, methotrexate, vindesine, bacterial toxins such as diphtheria toxin, ricin, geldanamycin, maytansinoids or calicheamicin. The cytotoxic agent may elicit their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition.

In some embodiments, the cytotoxic agent is an enzymatically active toxin such as diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcin, croton, *sapaonaria officinalis* inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In some embodiments, the cytotoxic agent is a radionuclide, such as ^{212}Bi , ^{131}I , ^{131}In , ^{90}Y , and ^{186}Re .

In some embodiments, the cytotoxic agent is dolastatins or dolostatin peptidic analogs and derivatives, auristatin or monomethyl auristatin phenylalanine. Exemplary molecules are disclosed in U.S. Pat No. 5,635,483 and 5,780,588. Dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001) Antimicrob Agents and Chemother. 45(12):3580-3584) and have anticancer and antifungal activity. The dolastatin or auristatin drug moiety may be attached to the antibody of the invention through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO02/088172), or via any cysteine engineered into the antibody.

The isolated antibody that specifically binds PD-1 or the antigen-binding fragment thereof provided herein may be conjugated to a detectable label using known methods.

In some embodiments, the detectable label is complexed with a chelating agent.

In some embodiments, the detectable label is conjugated to the antibody that specifically binds PD-1 or the antigen-binding fragment thereof provided herein via a linker.

The detectable label or the cytotoxic moiety may be linked directly, or indirectly, to the antibody that specifically binds PD-1 or the antigen-binding fragment thereof provided herein using known methods. Suitable linkers are known in the art and include, for example, prosthetic groups, non-phenolic linkers (derivatives of N-succinimidyl-benzoates; dodecaborate), chelating moieties of both macrocyclics and acyclic chelators, such as derivatives of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), derivatives of diethylenetriaminepentaacetic acid (DTPA), derivatives of S-2-(4-Isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) and derivatives of 1,4,8,11-tetraazacyclododecane-1,4,8,11-tetraacetic acid (TETA), N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl)hexanediamine), bis-diazonium derivatives (such as bis(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene) and other chelating moieties. Suitable peptide linkers are well known.

In some embodiments, the antibody that specifically binds PD-1 or the antigen-binding fragment thereof that provided herein are removed from the blood via renal clearance.

Kits

The invention also provides a kit comprising the antibody that specifically binds PD-1 or the antigen-binding fragment thereof disclosed herein.

The kit may be used for therapeutic uses and as diagnostic kits.

The kit may be used to detect the presence of PD-1 in a sample.

In some embodiments, the kit comprises the antibody that specifically binds PD-1 or the antigen-binding fragment thereof provided herein and reagents for detecting the antibody. The kit can include one or more other elements including: instructions for use; other reagents, e.g., a label, a therapeutic agent, or an agent useful for chelating, or

otherwise coupling, an antibody to a label or therapeutic agent, or a radioprotective composition; devices or other materials for preparing the antibody for administration; pharmaceutically acceptable carriers; and devices or other materials for administration to a subject.

In some embodiments, the kit comprises the antibody that specifically binds PD-1 or the antigen-binding fragment thereof provided herein in a container and instructions for use of the kit.

In some embodiments, the antibody in the kit is labeled.

In some embodiments, the kit comprises the antibody that specifically binds PD-1 or the antigen-binding fragment thereof comprising

the VH of SEQ ID NO: 8 and the VL of SEQ ID NO: 14;
the VH of SEQ ID NO: 9 and the VL of SEQ ID NO: 15;
the VH of SEQ ID NO: 9 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 16;
the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 144;
the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 143;
the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 144;
the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 144;
the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 144;
the VH of SEQ ID NO: 44 and the VL of SEQ ID NO: 60;
the VH of SEQ ID NO: 45 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 45 and the VL of SEQ ID NO: 62;
the VH of SEQ ID NO: 46 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 47 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 48 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 49 and the VL of SEQ ID NO: 61;

the VH of SEQ ID NO: 50 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 51 and the VL of SEQ ID NO: 61;
the VH of SEQ ID NO: 94 and the VL of SEQ ID NO: 98;
the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 99; or
the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 100.

Methods of detecting PD-1

The invention also provides a method of detecting PD-1 in a sample, comprising obtaining the sample, contacting the sample with the antibody that specifically binds PD-1 or the antigen-binding fragment thereof provided herein, and detecting the antibody bound to PD-1 in the sample.

In some embodiments, the sample may be derived from urine, blood, serum, plasma, saliva, ascites, circulating cells, synovial fluid, circulating cells, cells that are not tissue associated (*i.e.*, free cells), tissues (*e.g.*, surgically resected tissue, biopsies, including fine needle aspiration), histological preparations, and the like.

The antibodies or the antigen-binding fragments thereof of the invention bound to PD-1 may be detected using known methods. Exemplary methods include direct labeling of the antibodies using fluorescent or chemiluminescent labels, or radiolabels, or attaching to the antibodies of the invention a moiety which is readily detectable, such as biotin, enzymes or epitope tags. Exemplary labels and moieties are ruthenium, ¹¹¹In-DOTA, ¹¹¹In-diethylenetriaminepentaacetic acid (DTPA), horseradish peroxidase, alkaline phosphatase and beta-galactosidase, poly-histidine (HIS tag), acridine dyes, cyanine dyes, fluorone dyes, oxazin dyes, phenanthridine dyes, rhodamine dyes and Alexafluor® dyes.

The antibodies of the invention may be used in a variety of assays to detect PD-1 in the sample. Exemplary assays are western blot analysis, radioimmunoassay, surface plasmon resonance, immunoprecipitation, equilibrium dialysis, immunodiffusion, electrochemiluminescence (ECL) immunoassay, immunohistochemistry, fluorescence-activated cell sorting (FACS) or ELISA assay.

Methods generating antibodies

Antibodies that specifically bind PD-1 or antigen-binding fragments thereof provided herein may be generated using various technologies. For example, the hybridoma

method of Kohler and Milstein may be used to generate monoclonal antibodies. In the hybridoma method, a mouse or other host animal, such as a hamster, rat or chicken is immunized with human and/or cyno PD-1 antigens, such as the extracellular domain of PD-1, followed by fusion of spleen cells from immunized animals with myeloma cells using standard methods to form hybridoma cells. Colonies arising from single immortalized hybridoma cells may be screened for production of antibodies with desired properties, such as specificity of binding, cross-reactivity or lack thereof, affinity for the antigen, and functionality such as agonistic activity.

Exemplary humanization techniques including selection of human acceptor frameworks include CDR grafting (U.S. Patent No. 5,225,539), SDR grafting (U.S. Patent No. 6,818,749), Resurfacing (Padlan, (1991) *Mol Immunol* 28:489-499), Specificity Determining Residues Resurfacing (U.S. Patent Publ. No. 2010/0261620), human framework adaptation (U.S. Patent No. 8,748,356) or superhumanization (U.S. Patent No. 7,709, 226). In these methods, CDRs or a subset of CDR residues of parental antibodies are transferred onto human frameworks that may be selected based on their overall homology to the parental frameworks, based on similarity in CDR length, or canonical structure identity, or a combination thereof.

Humanized antibodies may be further optimized to improve their selectivity or affinity to a desired antigen by incorporating altered framework support residues to preserve binding affinity (backmutations) by techniques such as those described in Int. Patent Publ. Nos. WO1090/007861 and WO1992/22653, or by introducing variation at any of the CDRs for example to improve affinity of the antibody.

Transgenic animals, such as mice, rat or chicken carrying human immunoglobulin (Ig) loci in their genome may be used to generate antibodies against PD-1, and are described in for example U.S. Patent No. 6,150,584, Int. Patent Publ. No. WO1999/45962, Int. Patent Publ. Nos. WO2002/066630, WO2002/43478, WO2002/043478 and WO1990/04036. The endogenous immunoglobulin loci in such animal may be disrupted or deleted, and at least one complete or partial human immunoglobulin locus may be inserted into the genome of the animal using homologous or non-homologous recombination, using transchromosomes, or using minigenes. Companies such as Regeneron (http://_www_regeneron_com), Harbour Antibodies (http://_www_harbourantibodies_com), Open Monoclonal Technology, Inc. (OMT)

(http://_www_omtinc_net), KyMab (http://_www_kymab_com), Trianni (http://_www.trianni_com) and Ablexis (http://_www_ablexis_com) may be engaged to provide human antibodies directed against a selected antigen using technologies as described above.

Antibodies may be selected from a phage display library, where the phage is engineered to express human immunoglobulins or portions thereof such as Fabs, single chain antibodies (scFv), or unpaired or paired antibody variable regions. The antibodies of the invention may be isolated for example from phage display library expressing antibody heavy and light chain variable regions as fusion proteins with bacteriophage pIX coat protein as described in Shi *et al.*, (2010) *J Mol Biol* 397:385-96, and Int. Patent Publ. No. WO09/085462). The libraries may be screened for phage binding to human and/or cyno PD-1 and the obtained positive clones may be further characterized, the Fabs isolated from the clone lysates, and expressed as full length IgGs.

Preparation of immunogenic antigens and monoclonal antibody production may be performed using any suitable technique, such as recombinant protein production. The immunogenic antigens may be administered to an animal in the form of purified protein, or protein mixtures including whole cells or cell or tissue extracts, or the antigen may be formed *de novo* in the animal's body from nucleic acids encoding said antigen or a portion thereof.

In some embodiments, the antibody that specifically binds PD-1 or the antigen-binding fragment thereof of the invention is a bispecific antibody.

In some embodiments, the antibody or the antigen-binding fragment thereof of the invention is a multispecific antibody.

The monospecific antibodies that specifically bind PD-1 provided herein may be engineered into bispecific antibodies which are also encompassed within the scope of the invention.

Full length bispecific antibodies may be generated for example using Fab arm exchange (e.g., half-molecule exchange, exchanging one heavy chain-light chain pair) between two monospecific bivalent antibodies by introducing substitutions at the heavy chain CH3 interface in each half molecule to favor heterodimer formation of two antibody half molecules having distinct specificity either *in vitro* in cell-free environment or using co-expression. The Fab arm exchange reaction is the result of a disulfide-bond

isomerization reaction and dissociation-association of CH3 domains. The heavy chain disulfide bonds in the hinge regions of the parental monospecific antibodies are reduced. The resulting free cysteines of one of the parental monospecific antibodies form an inter heavy-chain disulfide bond with cysteine residues of a second parental monospecific antibody molecule and simultaneously CH3 domains of the parental antibodies release and reform by dissociation-association. The CH3 domains of the Fab arms may be engineered to favor heterodimerization over homodimerization. The resulting product is a bispecific antibody having two Fab arms or half molecules which each bind a distinct epitope.

Bispecific antibodies may also be generated using designs such as the Triomab/Quadroma (Trion Pharma/Fresenius Biotech), Knob-in-Hole (Genentech), CrossMAbs (Roche) and the electrostatically-induced CH3 interaction (Chugai, Amgen, NovoNordisk, Oncomed), the LUZ-Y (Genentech), the Strand Exchange Engineered Domain body (SEEDbody) (EMD Serono), the Biclonic (Merus) and as DuoBody® Products (Genmab A/S).

The Triomab quadroma technology may be used to generate full length bispecific antibodies. Triomab technology promotes Fab arm exchange between two parental chimeric antibodies, one parental mAb having IgG2a and the second parental mAb having rat IgG2b constant regions, yielding chimeric bispecific antibodies.

The “knob-in-hole” strategy may be used to generate full length bispecific antibodies. Briefly, selected amino acids forming the interface of the CH3 domains in human IgG can be mutated at positions affecting CH3 domain interactions to promote heterodimer formation. An amino acid with a small side chain (hole) is introduced into a heavy chain of an antibody specifically binding a first antigen and an amino acid with a large side chain (knob) is introduced into a heavy chain of an antibody specifically binding a second antigen. After co-expression of the two antibodies, a heterodimer is formed as a result of the preferential interaction of the heavy chain with a “hole” with the heavy chain with a “knob”. Exemplary CH3 substitution pairs forming a knob and a hole are (expressed as modified position in the first CH3 domain of the first heavy chain/ modified position in the second CH3 domain of the second heavy chain): T366Y/F405A, T366W/F405W, F405W/Y407A, T394W/Y407T, T394S/Y407A, T366W/T394S, F405W/T394S and T366W/T366S_L368A_Y407V.

The CrossMAb technology may be used to generate full length bispecific antibodies. CrossMAbs, in addition to utilizing the “knob-in-hole” strategy to promote Fab arm exchange, have in one of the half arms the CH1 and the CL domains exchanged to ensure correct light chain pairing of the resulting bispecific antibody (see e.g. U.S. Patent No. 8,242,247).

Other cross-over strategies may be used to generate full length bispecific antibodies by exchanging variable or constant, or both domains between the heavy chain and the light chain or within the heavy chain of the bispecific antibodies, either in one or both arms. These exchanges include for example VH-CH1 with VL-CL, VH with VL, CH3 with CL and CH3 with CH1 as described in Int. Patent Publ. Nos. WO2009/080254, WO2009/080251, WO2009/018386 and WO2009/080252.

Other strategies such as promoting heavy chain heterodimerization using electrostatic interactions by substituting positively charged residues at one CH3 surface and negatively charged residues at a second CH3 surface may be used, as described in US Patent Publ. No. US2010/0015133; US Patent Publ. No. US2009/0182127; US Patent Publ. No. US2010/028637 or US Patent Publ. No. US2011/0123532. In other strategies, heterodimerization may be promoted by following substitutions (expressed as modified position in the first CH3 domain of the first heavy chain/ modified position in the second CH3 domain of the second heavy chain): L351Y_F405A_Y407V/T394W, T366I_K392M_T394W/F405A_Y407V, T366L_K392M_T394W/F405A_Y407V, L351Y_Y407A/T366A_K409F, L351Y_Y407A/T366V_K409F, Y407A/T366A_K409F, or T350V_L351Y_F405A_Y407V/T350V_T366L_K392L_T394W as described in U.S. Patent Publ. No. US2012/0149876 or U.S. Patent Publ. No. US2013/0195849.

LUZ-Y technology may be utilized to generate bispecific antibodies. In this technology, a leucine zipper is added into the C terminus of the CH3 domains to drive the heterodimer assembly from parental mAbs that is removed post-purification.

SEEDbody technology may be utilized to generate bispecific antibodies. SEEDbodies have, in their constant domains, select IgG residues substituted with IgA residues to promote heterodimerization as described in U.S. Patent No. US20070287170.

Bispecific antibodies may be generated *in vitro* in a cell-free environment by introducing asymmetrical mutations in the CH3 regions of two monospecific homodimeric antibodies and forming the bispecific heterodimeric antibody from two parent monospecific

homodimeric antibodies in reducing conditions to allow disulfide bond isomerization according to methods described in Int. Patent Publ. No. WO2011/131746. In the methods, the first monospecific bivalent antibody and the second monospecific bivalent antibody are engineered to have certain substitutions at the CH3 domain that promote heterodimer stability; the antibodies are incubated together under reducing conditions sufficient to allow the cysteines in the hinge region to undergo disulfide bond isomerization; thereby generating the bispecific antibody by Fab arm exchange. Substitutions that may be used are F405L in one heavy chain and K409R in the other heavy chain in IgG1 antibodies. In IgG4 antibodies, one heavy chain may be a wild-type IgG4 having F at position 405 and R at position 409 and the other heavy chain may have F405L and R409K substitutions. The incubation conditions may optimally be restored to non-reducing. Exemplary reducing agents that may be used are 2-mercaptoethylamine (2-MEA), dithiothreitol (DTT), dithioerythritol (DTE), glutathione, tris(2-carboxyethyl)phosphine (TCEP), L-cysteine and beta-mercaptoethanol. For example, incubation for at least 90 min at a temperature of at least 20°C in the presence of at least 25 mM 2-MEA or in the presence of at least 0.5 mM dithiothreitol at a pH of from 5-8, for example at pH of 7.0 or at pH of 7.4 may be used.

In some embodiments, the bispecific antibodies include recombinant IgG-like dual targeting molecules, wherein the two sides of the molecule each contain the Fab fragment or part of the Fab fragment of at least two different antibodies; IgG fusion molecules, wherein full length IgG antibodies are fused to an extra Fab fragment or parts of Fab fragment; Fc fusion molecules, wherein single chain Fv molecules or stabilized diabodies are fused to heavy-chain constant-domains, Fc-regions or parts thereof; Fab fusion molecules, wherein different Fab-fragments are fused together; ScFv- and diabody-based and heavy chain antibodies (e.g., domain antibodies, nanobodies) wherein different single chain Fv molecules or different diabodies or different heavy-chain antibodies (e.g. domain antibodies, nanobodies) are fused to each other or to another protein or carrier molecule.

Substitutions are typically made at the DNA level to a molecule such as the constant domain of the antibody using standard methods.

The antibodies of the invention may be engineered into various well-known antibody forms.

For example, a bispecific PD-1/CD3 antibody can be generated using the VH/VL domains of the PD-1 antibodies described herein and any VH/VL regions of published anti-CD3 antibodies.

Another embodiment of the invention is a bispecific antibody comprising a first domain that binds PD-1 and a second domain that binds CD3.

Methods of characterizing antibodies

Agonistic antibodies

A typical biological activity induced by the agonistic antibodies provided herein is inhibition (e.g. suppression) of antigen-specific CD4⁺ or CD8⁺ T cells. Various readouts may be used to assess the agonism of the antibodies provided herein, such as reduced proliferation or reduced production of interferon- γ (IFN- γ), IL-17 IL-2, IL-6, IL-22, IL-23 or GM-CSF by antigen-specific CD4⁺ or CD8⁺ T cells. In an exemplary assay, the effect of antibodies on T cells from normal donor that are stimulated by allogeneic dendritic cells or specific antigens, such as Tetanus toxoid or CMV are used. In this setting, changes in T cell function with antibody treatment can be detected by measuring supernatant cytokine levels or markers of T cell activation. In an exemplary assay, PBMCs determined to be reactive to CMV antigens are used as source of antigen-specific CD4⁺ or CD8⁺ T cells. 1.5×10^6 cells/mL or 2×10^6 cells/mL of CMV-reactive PBMCs are plated onto culture plates and 0.1 -0.2 μ g/mL CMV peptides added to cultures. CMV peptides may be purchased for example from JPT Technologies. Test antibodies are added at single dose of 10 μ g/mL, plates incubated for 6 days, and cell proliferation assessed by addition of 1 μ Ci/well methyl-3H-thymidine (PerkinElmer) for 6 hours and radioactivity measured in each sample. Alternatively, cytokine production by cells is measured using ELISA or known multiplex assays.

Antibodies that block PD-L1 and/or PD-L2 binding to PD-1

The antibodies of the invention provided herein (such as agonistic antibodies that specifically bind PD-1) may be ligand blocking or non-blocking. Ability of the agonistic antibodies provided herein may be assessed for their ability to block ligand, e.g. PD-L1 or PD-L2 or both using competition assays such as cell clustering assay.

In an exemplary assay, differentially labeled HEK cells overexpressing either PD-1 or PD-L1 (or PD-L2) are mixed in a 1:1 ratio, and the ability of the antibodies to inhibit clustering of PD-1 and PD-L1 or PD-1 and PD-L2 expressing cells is quantified. Cells overexpressing human PD-1 may be labeled with Violet Cell Trace stain and cells overexpressing PD-L1 or PD-L2 may be labeled with Far Red Cell Trace Stain (Life Technologies). PD-1 expressing cells are mixed with the test antibody and after a brief incubation PD-L1 expressing cells are added into the mixture. The mixture is incubated for one hour, and the percentage of double positive events (e.g. cell clusters positive with Violet Cell Trace stain and Far Rd Cell Trace stain) is evaluated using flow cytometry. The level of clustering is measured by the percentage of double positive events, and the percentage of clustering in the presence of test antibodies is compared to positive, and negative isotype control antibodies. The antibody provided herein blocks PD-L1 (or PD-L2) binding to PD-1 when the antibody inhibits double positive events of PD-1 and PD-L1 (or PD-L2) expressing cells in a statistically significant manner when compared to the isotype control using a p value of ≤ 0.01 as a measure of significance. The antibody provided herein does not block PD-L1 (or PD-L2) binding to PD-1 when the antibody inhibits double positive events of PD-1 and PD-L1 (or PD-L2) in a statistically insignificant manner, e.g. $p > 0.01$.

Antibody affinity measurements

The affinity of an antibody to human or non-human such as cynomolgus PD-1 may be determined experimentally using any suitable method. Such methods may utilize ProteOn XPR36, Biacore 3000 or KinExA instrumentation, ELISA or competitive binding assays known to those skilled in the art. The measured affinity of a particular antibody/PD-1 interaction may vary if measured under different conditions (e.g., osmolarity, pH). Thus, measurements of affinity and other binding parameters (e.g., K_D , K_{on} , K_{off}) are typically made with standardized conditions and a standardized buffer, such as the buffer described herein in Example 1. Skilled in the art will appreciate that the internal error for affinity measurements for example using Biacore 3000 or ProteOn (measured as standard deviation, SD) may typically be within 5-33% for measurements within the typical limits of detection. Therefore, the term “about” in the context of K_D reflects the typical standard deviation in the assay. For example, the typical SD for a K_D of 1×10^{-9} M is up to

$\pm 0.33 \times 10^{-9}$ M.

Antibodies competing for binding to PD-1 with a reference antibody

Competition between binding to PD-1 with antibodies of the invention (e.g. reference antibodies) comprising certain VH and VL sequences may be assayed *in vitro* on an Octet Red384 platform (Forte Bio). Histidine-tagged PD1 antigen is loaded onto HIS sensors and the sensors are exposed to 20 $\mu\text{g/mL}$ reference anti-PD1 antibody, followed by exposure to an equal concentration of a test anti-PD1 antibody. Additional binding by the test antibody after saturation with the reference antibody indicates simultaneous binding of the two antibodies to PD-1, indicating the reference and the test antibody do not compete for binding to PD-1. Alternatively, no additional binding of the test antibody indicates that the two antibodies compete for binding to PD-1.

Antibodies that compete for binding to PD-1 with a reference antibody may be generated by isolating antibodies that specifically bind PD-1 using phage display libraries, and screening the generated antibodies for their ability to compete for binding to PD-1 with the aforementioned reference antibodies.

The test antibody competes with binding to PD-1 with the reference antibody when the test antibody binds PD-1 at saturating concentration of the reference antibody. Binding can be detected using bio-layer interferometry (such as Octet) by recording a wavelength shift due to the bound antibody increasing the optical density of the biosensor tip over time.

Antibody epitope

The PD-1 epitope the antibody of the invention binds to may be resolved for example using hydrogen/deuterium exchange (H/D exchange) or by analyzing a crystal structure of the antibody in complex with PD-1. Two PD-1 antibodies “bind the same epitope on PD-1” when about 70% or more PD-1 amino acid residues protected by the antibody by at least 5% difference in deuteration levels through H/D exchange are identical between the two antibodies, or when 70% or more PD-1 surface exposed amino acid residues determined to bind the antibody in a crystal structure of a complex of the antibody and PD-1 are identical between the two antibodies. In the crystal structure of a complex of the antibody and PD-1 the epitope residues are those PD-1 residues that reside within 4 Å distance or less from any of the antibody CDR residues.

In an H/D exchange assay, PD-1 protein is incubated in the presence or absence of the antibody in deuterated water for predetermined times resulting in deuterium incorporation at exchangeable hydrogen atoms which are unprotected by the antibody, followed by protease digestion of the protein and analyses of the peptide fragments using LC-MS. In an exemplary assay, 5 μ L of the test antibody (10 μ g) or 5 μ L of the complex of PD-1 and the test antibody (10 & 7.35 μ g, respectively) is incubated with 120 μ L deuterium oxide labeling buffer (50mM phosphate, 100mM sodium chloride at pH 7.4) for 0 sec, 60 sec, 300 sec, 1800 sec, 7200 sec, and 14400 sec. Deuterium exchange is quenched by adding 63 μ L of 5 M guanidine hydrochloride and final pH is 2.5. The quenched sample is subjected to on-column pepsin/protease type XIII digestion and LC-MS analysis. For pepsin/protease type XIII digestion, 5 μ g of the samples in 125 μ L control buffer (50mM phosphate, 100mM sodium chloride at pH 7.4) are denatured by adding 63 μ L of 5 M guanidine hydrochloride (final pH is 2.5) and incubating the mixture for 3 min. Then, the mixture is subjected to on-column pepsin/protease type XIII digestion and the resultant peptides analyzed using an UPLC-MS system comprised of a Waters Acquity UPLC coupled to a Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo). Raw MS data is processed using HDX WorkBench, software for the analysis of H/D exchange MS data. The deuterium levels are calculated using the average mass difference between the deuterated peptide and its native form (t_0). Peptide identification is done through searching MS/MS data against the PD-1 sequence with Mascot. The mass tolerance for the precursor and product ions is 20 ppm and 0.05 Da, respectively.

For X-ray crystallography, PD-1 and the test antibody are expressed and purified using standard protocols. The PD-1/test antibody complex is incubated overnight at 4°C, concentrated, and separated from the uncomplexed species using size-exclusion chromatography. The complex is crystallized by the vapor-diffusion method from various known test solutions for example solutions containing PEG3350, ammonium citrate and 2-(N-morpholino)ethanesulfonic acid (MES).

Antibodies binding the same epitope on PD-1 as a reference antibody may be generated by isolating antibodies binding PD-1 using phage display libraries, selecting those antibodies that compete with the reference antibody for binding to PD-1 by 100%, and identifying the antibody epitope by H/D exchange or by X-ray crystallography.

Alternatively, mice or rabbits may be immunized using peptides encompassing the epitope residues, and the generated antibodies may be evaluated for their binding within the recited region.

Polynucleotides, vectors, host cells

The invention also provides an isolated polynucleotide encoding any of the antibodies of the invention.

The invention also provides an isolated polynucleotide encoding any of the antibody heavy chain variable regions, any of the antibody light chain variable regions, or any of the antibody heavy chains and/or the antibody light chains of the invention.

The invention also provides for an isolated polynucleotide encoding the VH of SEQ ID NOs: 8, 9, 10, 140, 141 or 142.

The invention also provides for an isolated polynucleotide encoding the VL of SEQ ID NOs: 14, 15, 16, 143 or 144.

The invention also provides for an isolated polynucleotide encoding the VH of SEQ ID NOs: 8, 9, 10, 140, 141 or 142 and the VL of SEQ ID NOs: 14, 15, 16, 143 or 144.

The invention also provides for an isolated polynucleotide encoding the heavy chain (HC) of SEQ ID NOs: 20, 21, 22, 150, 151 or 152.

The invention also provides for an isolated polynucleotide encoding the light chain (LC) of SEQ ID NOs: 26, 27, 28, 153 or 154.

The invention also provides for an isolated polynucleotide encoding the HC of SEQ ID NOs: 20, 21, 22, 150, 151 or 152 and the LC of SEQ ID NOs: 26, 27, 28, 153 or 154.

The invention also provides for an isolated polynucleotide comprising the polynucleotide sequence of SEQ ID NOs: 11, 12, 13, 17, 18, 19, 23, 24, 25, 29, 30, 31, 132, 133, 134, 135, 155, 156, 157, 158, 159, 160, 161, 162, 163 or 164.

The invention also provides for an isolated polynucleotide encoding the VH of SEQ ID NOs: 44, 45, 46, 47, 48, 49, 50 or 51.

The invention also provides for an isolated polynucleotide encoding the VL of SEQ ID NOs: 60, 61 or 62.

The invention also provides for an isolated polynucleotide encoding the VH of SEQ ID NOs: 44, 45, 46, 47, 48, 49, 50 or 51 and the VL of SEQ ID NOs: 60, 61 or 62.

The invention also provides for an isolated polynucleotide encoding the heavy chain of SEQ ID NOs: 66, 67, 68, 69, 70, 71, 72 or 73.

The invention also provides for an isolated polynucleotide encoding the light chain of SEQ ID NOs: 82, 83 or 84.

The invention also provides for an isolated polynucleotide encoding the HC of SEQ ID NOs: 66, 67, 68, 69, 70, 71, 72 or 73 and the LC of SEQ ID NOs: 82, 83 or 84.

The invention also provides for an isolated polynucleotide comprising the polynucleotide sequence of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 63, 64, 65, 74, 75, 76, 77, 78, 79, 80, 81, 85, 86, 87, 136, 137, 138 or 139.

The invention also provides for an isolated polynucleotide encoding the VH of SEQ ID NOs: 94 or 95.

The invention also provides for an isolated polynucleotide encoding the VL of SEQ ID NOs: 98, 99 or 100.

The invention also provides for an isolated polynucleotide encoding the VH of SEQ ID NOs: 94 or 95 and the VL of SEQ ID NOs: 98, 99 or 100.

The invention also provides for an isolated polynucleotide encoding the HC of SEQ ID NOs: 104 or 105.

The invention also provides for an isolated polynucleotide encoding the LC of SEQ ID NOs: 108, 109 or 110.

The invention also provides for an isolated polynucleotide encoding the HC of SEQ ID NOs: 104 or 105 and the LC of SEQ ID NOs: 108, 109 or 110.

The invention also provides for an isolated polynucleotide comprising the polynucleotide sequence of SEQ ID NOs: 96, 97, 101, 102, 103, 106, 107, 111, 112 or 113.

The polynucleotide sequences encoding the VH and/or the VL or antigen binding fragments thereof of the antibodies of the invention or the heavy chain and/or the light chain of the antibodies of the invention may be operably linked to one or more regulatory elements, such as a promoter or enhancer, that allow expression of the nucleotide sequence in the intended host cell. The polynucleotide may be a cDNA.

The invention also provides for a vector comprising the polynucleotide of the invention. Such vectors may be plasmid vectors, viral vectors, vectors for baculovirus expression, transposon based vectors or any other vector suitable for introduction of the polynucleotide of the invention into a given organism or genetic background by any means.

For example, polynucleotides encoding light and/or heavy chain variable regions of the antibodies of the invention, optionally linked to constant regions, are inserted into expression vectors. The light and/or heavy chains may be cloned in the same or different expression vectors. The DNA segments encoding the VH, the VL, the HC and/or the LC or antigen binding fragments thereof may be operably linked to control sequences in the expression vector(s) that ensure the expression of the polypeptides. Such control sequences include signal sequences, promoters (e.g. naturally associated or heterologous promoters), enhancer elements, and transcription termination sequences, and are chosen to be compatible with the host cell chosen to express the antibody. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the proteins encoded by the incorporated polynucleotides.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 11 and/or the polynucleotide of SEQ ID NO: 17.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 12 and/or the polynucleotide of SEQ ID NO: 18.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 12 and/or the polynucleotide of SEQ ID NO: 19.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 13 and/or the polynucleotide of SEQ ID NO: 19.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 52 and/or the polynucleotide of SEQ ID NO: 63.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 53 and/or the polynucleotide of SEQ ID NO: 64.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 53 and/or the polynucleotide of SEQ ID NO: 65.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 54 and/or the polynucleotide of SEQ ID NO: 64.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 55 and/or the polynucleotide of SEQ ID NO: 64.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 56 and/or the polynucleotide of SEQ ID NO: 64.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 57 and/or the polynucleotide of SEQ ID NO: 64.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 58 and/or the polynucleotide of SEQ ID NO: 64.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 59 and/or the polynucleotide of SEQ ID NO: 64.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 96 and/or the polynucleotide of SEQ ID NO: 101.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 97 and/or the polynucleotide of SEQ ID NO: 102.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 97 and/or the polynucleotide of SEQ ID NO: 103.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 132 and/or the polynucleotide of SEQ ID NO: 133.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 134 and/or the polynucleotide of SEQ ID NO: 135.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 136 and/or the polynucleotide of SEQ ID NO: 137.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 138 and/or the polynucleotide of SEQ ID NO: 139.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 155 and/or the polynucleotide of SEQ ID NO: 19.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 156 and/or the polynucleotide of SEQ ID NO: 19.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 157 and/or the polynucleotide of SEQ ID NO: 19.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 13 and/or the polynucleotide of SEQ ID NO: 158.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 13 and/or the polynucleotide of SEQ ID NO: 159.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 155 and/or the polynucleotide of SEQ ID NO: 158.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 156 and/or the polynucleotide of SEQ ID NO: 158.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 157 and/or the polynucleotide of SEQ ID NO: 158.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 155 and/or the polynucleotide of SEQ ID NO: 159.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 156 and/or the polynucleotide of SEQ ID NO: 159.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 157 and/or the polynucleotide of SEQ ID NO: 159.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 160 and/or the polynucleotide of SEQ ID NO: 31.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 161 and/or the polynucleotide of SEQ ID NO: 31.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 162 and/or the polynucleotide of SEQ ID NO: 31.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 25 and/or the polynucleotide of SEQ ID NO: 163.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 25 and/or the polynucleotide of SEQ ID NO: 164.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 160 and/or the polynucleotide of SEQ ID NO: 163.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 161 and/or the polynucleotide of SEQ ID NO: 163.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 162 and/or the polynucleotide of SEQ ID NO: 163.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 160 and/or the polynucleotide of SEQ ID NO: 164.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 161 and/or the polynucleotide of SEQ ID NO: 164.

In some embodiments, the vector comprises the polynucleotide of SEQ ID NO: 162 and/or the polynucleotide of SEQ ID NO: 164.

Suitable expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors contain selection markers such as ampicillin-resistance, hygromycin-resistance, tetracycline resistance, kanamycin resistance or neomycin resistance to permit detection of those cells transformed with the desired DNA sequences. Glutamine synthetase system may be used to express recombinant proteins such as antibodies in cells.

Suitable promoter and enhancer elements are known in the art. For expression in a eukaryotic cell, exemplary promoters include light and/or heavy chain immunoglobulin gene promoter and enhancer elements; cytomegalovirus immediate early promoter; herpes simplex virus thymidine kinase promoter; early and late SV40 promoters; promoter present in long terminal repeats from a retrovirus; mouse metallothionein-I promoter; and various art-known tissue specific promoters. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

Large numbers of suitable vectors and promoters are known to those of skill in the art; many are commercially available for generating recombinant constructs. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene, La Jolla, Calif., USA); pTrc99A, pKK223-3, pKK233-3, pDR540, and pRIT5 (Pharmacia, Uppsala, Sweden). Eukaryotic: pWLneo, pSV2cat, pOG44, PXR1, pSG (Stratagene) pSVK3, pBPV, pMSG and pSVL (Pharmacia), and pEE6.1 and pEE14.1 (Lonza).

The invention also provides for a host cell comprising one or more vectors of the invention. "Host cell" refers to a cell into which a vector has been introduced. It is understood that the term host cell is intended to refer not only to the particular subject cell but to the progeny of such a cell, and also to a stable cell line generated from the particular subject cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. Such host cells may be eukaryotic cells, prokaryotic cells, plant cells or archeal cells.

Escherichia coli, bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species are examples of prokaryotic host cells. Other microbes, such as yeast, are also useful for expression. *Saccharomyces* (e.g., *S. cerevisiae*) and *Pichia* are examples of suitable yeast host cells.

Exemplary eukaryotic cells may be of mammalian, insect, avian or other animal origins. Mammalian eukaryotic cells include immortalized cell lines such as hybridomas or myeloma cell lines such as SP2/0 (American Type Culture Collection (ATCC), Manassas, VA, CRL-1581), NS0 (European Collection of Cell Cultures (ECACC), Salisbury, Wiltshire, UK, ECACC No. 85110503), FO (ATCC CRL-1646) and Ag653 (ATCC CRL-1580) murine cell lines. An exemplary human myeloma cell line is U266 (ATCC CRL-TIB-196). Other useful cell lines include those derived from Chinese Hamster Ovary (CHO) cells such as CHO-K1SV (Lonza Biologics, Walkersville, MD), CHO-K1 (ATCC CRL-61) or DG44.

The invention also provides for a method of producing the antibody of the invention comprising culturing the host cell of the invention in conditions that the antibody is expressed, and recovering the antibody produced by the host cell. Methods of making antibodies and purifying them are known. Once synthesized (either chemically or recombinantly), the whole antibodies, their dimers, individual light and/or heavy chains, or other antibody fragments such as VH and/or VL, may be purified according to standard procedures, including ammonium sulfate precipitation, affinity columns, column chromatography, high performance liquid chromatography (HPLC) purification, gel electrophoresis, and the like (see generally Scopes, Protein Purification (Springer-Verlag, N.Y., (1982)). A subject antibody may be substantially pure, e.g., at least about 80% to 85% pure, at least about 85% to 90% pure, at least about 90% to 95% pure, or at least about 98% to 99%, or more, pure, e.g., free from contaminants such as cell debris, macromolecules, etc. other than the subject antibody.

The invention also provides for a method for producing an antibody that specifically binds PD-1, comprising:

- incorporating the first polynucleotide encoding the VH of the antibody and the second polynucleotide encoding the VL of the antibody into an expression vector;
- transforming a host cell with the expression vector;
- culturing the host cell in culture medium under conditions wherein the VL and the VH are expressed and form the antibody; and
- recovering the antibody from the host cell or culture medium.

The polynucleotides encoding certain VH or VL sequences of the invention may be incorporated into vectors using standard molecular biology methods. Host cell

transformation, culture, antibody expression and purification are done using well known methods.

Pharmaceutical compositions/Administration

The invention also provides pharmaceutical compositions comprising the antibodies or the antigen binding fragments thereof of the invention and a pharmaceutically acceptable carrier. For therapeutic use, the antibodies of the invention may be prepared as pharmaceutical compositions containing an effective amount of the antibodies as an active ingredient in a pharmaceutically acceptable carrier. "Carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the antibody of the invention is administered. Such vehicles may be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. For example, 0.4% saline and 0.3% glycine may be used. These solutions are sterile and generally free of particulate matter. They may be sterilized by conventional, well-known sterilization techniques (*e.g.*, filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, stabilizing, thickening, lubricating and coloring agents, etc. The concentration of the antibodies or the antigen-binding fragments thereof of the invention in such pharmaceutical formulation may vary, from less than about 0.5%, usually to at least about 1% to as much as 15 or 20% by weight and may be selected primarily based on required dose, fluid volumes, viscosities, etc., according to the particular mode of administration selected. Suitable vehicles and formulations, inclusive of other human proteins, *e.g.*, human serum albumin, are described, for example, in *e.g.* Remington: The Science and Practice of Pharmacy, 21st Edition, Troy, D.B. ed., Lipincott Williams and Wilkins, Philadelphia, PA 2006, Part 5, Pharmaceutical Manufacturing pp 691-1092, See especially pp. 958-989.

The mode of administration for therapeutic use of the antibodies or the antigen-binding fragments thereof of the invention may be any suitable route that delivers the antibody to a subject, such as parenteral administration, *e.g.*, intradermal, intramuscular, intraperitoneal, intravenous or subcutaneous, pulmonary, transmucosal (oral, intranasal, intravaginal, rectal), using a formulation in a tablet, capsule, solution, powder, gel, particle; and contained in a syringe, an implanted device, osmotic pump, cartridge, micropump; or

other means appreciated by the skilled artisan, as well known in the art. Site specific administration may be achieved by for example intratumoral, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelical, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intracardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravascular, intravesical, intralesional, vaginal, rectal, buccal, sublingual, intranasal, or transdermal delivery.

The antibodies or the antigen binding fragments thereof of the invention may also be administered prophylactically in order to reduce the risk of developing an autoimmune disease and/or delay the onset of the symptoms.

The antibodies or the antigen binding fragments thereof of the invention may be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional protein preparations and well known lyophilization and reconstitution techniques can be employed.

Methods and Uses

The antibodies or the antigen binding fragments thereof of the invention have *in vitro* and *in vivo* diagnostic, as well as therapeutic and prophylactic utilities. For example, the antibodies or the antigen binding fragments thereof of the invention may be administered to cells in culture, *in vitro* or *ex vivo*, or to a subject to treat, prevent, and/or diagnose a variety of diseases, such as immune disorder or any conditions in which attenuation of PD-1 expressing T cell activity and/or downmodulation of immune response is desired.

The invention also provides a method of suppressing activation of a PD-1 expressing T cell in a subject, comprising administering to the subject the isolated antibody or the antigen binding fragment thereof of the invention for a time sufficient to suppress activation of the PD-1 expressing T cell.

In some embodiments, the PD-1 expressing T cell is an antigen-specific CD4⁺ T cell.

In some embodiments, the PD-1 expressing T cell is an antigen-specific CD8⁺ T cell.

“Suppress activation” refers to the ability of the antibodies provided herein to inhibit activation of PD-1 expressing T cells, for example inhibit proliferation or IFN- γ production by antigen specific CD4⁺ and/or CD8⁺ T cells. The antibody suppresses activation of PD-1 expressing T cells when the antibody inhibits proliferation or IFN- γ production by antigen specific CD4⁺ and/or CD8⁺ T cells by 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% greater than in the absence of the antibody (*e.g.*, negative control), or when the inhibition is statistically significant when compared to the inhibition in the absence of the antibody.

The PD-1 expressing T cell may be located at the vicinity of an inappropriate inflammatory response. The antibodies or the antigen binding fragments thereof of the invention may suppress activation of the PD-1 expressing T cell by augmenting PD-1 downstream signaling resulting in inhibition of TCR signaling and inhibition of T cell activation, proliferation and/or survival. The antibodies of the invention may alternatively mediate killing of the PD-1 positive T cells by antibody-mediated effector functions ADCC, ADCP and/or CDC.

Also provided is a method of downmodulating an immune response comprising administering to a subject in need thereof a therapeutically effective amount of the isolated antibody or the antigen binding fragment thereof of the invention to downmodulate the immune response.

Also provided is a method of treating an immune disorder comprising administering to a subject in need thereof a therapeutically effective amount of the isolated antibody or the antigen binding fragment thereof of the invention to treat the immune disorder.

The immune disorder may be chronic or acute, such as chronic inflammatory disease or acute inflammatory disease.

In some embodiments, the immune disorder is, arthritis, rheumatoid arthritis, asthma, COPD, pelvic inflammatory disease, Alzheimer's Disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, Peyronie's Disease, coeliac disease, gallbladder disease, Pilonidal disease, peritonitis, psoriasis, psoriatic arthritis, vasculitis, surgical adhesions, stroke, Type I Diabetes, Lyme disease, meningoencephalitis, autoimmune uveitis, multiple sclerosis, lupus (such as systemic lupus erythematosus), Guillain-Barr syndrome, Atopic dermatitis, autoimmune hepatitis, fibrosing alveolitis, Grave's disease,

IgA nephropathy, idiopathic thrombocytopenic purpura, Meniere's disease, pemphigus, primary biliary cirrhosis, sarcoidosis, scleroderma, Wegener's granulomatosis, other autoimmune disorders, pancreatitis, trauma (surgery), graft-versus-host disease, transplant rejection, heart disease including ischaemic diseases such as myocardial infarction as well as atherosclerosis, intravascular coagulation, bone resorption, osteoporosis, osteoarthritis, periodontitis and hypochlorhydria, infertility related to lack of fetal-maternal tolerance, Sjogren's Syndrome, vitiligo, myasthenia gravis or systemic sclerosis.

In some embodiments, the immune disorder is rheumatoid arthritis.

In some embodiments, the immune disorder is graft-versus-host disease.

In some embodiments, the immune disorder is arthritis.

In some embodiments, the immune disorder is asthma.

In some embodiments, the immune disorder is COPD.

In some embodiments, the immune disorder is pelvic inflammatory disease.

In some embodiments, the immune disorder is Alzheimer's Disease.

In some embodiments, the immune disorder is inflammatory bowel disease.

In some embodiments, the immune disorder is Crohn's disease.

In some embodiments, the immune disorder is ulcerative colitis.

In some embodiments, the immune disorder is Peyronie's Disease.

In some embodiments, the immune disorder is coeliac disease.

In some embodiments, the immune disorder is gallbladder disease.

In some embodiments, the immune disorder is Pilonidal disease.

In some embodiments, the immune disorder is peritonitis.

In some embodiments, the immune disorder is psoriasis.

In some embodiments, the immune disorder is psoriatic arthritis.

In some embodiments, the immune disorder is vasculitis.

In some embodiments, the immune disorder is surgical adhesion.

In some embodiments, the immune disorder is stroke.

In some embodiments, the immune disorder is type I diabetes.

In some embodiments, the immune disorder is lyme disease.

In some embodiments, the immune disorder is meningoencephalitis.

In some embodiments, the immune disorder is autoimmune uveitis.

In some embodiments, the immune disorder is multiple sclerosis.

In some embodiments, the immune disorder is lupus.

In some embodiments, the immune disorder is systemic lupus erythematosus.

In some embodiments, the immune disorder is Guillain-Barr syndrome.

In some embodiments, the immune disorder is Atopic dermatitis.

In some embodiments, the immune disorder is autoimmune hepatitis.

In some embodiments, the immune disorder is fibrosing alveolitis.

In some embodiments, the immune disorder is Grave's disease.

In some embodiments, the immune disorder is IgA nephropathy.

In some embodiments, the immune disorder is idiopathic thrombocytopenic purpura.

In some embodiments, the immune disorder is Meniere's disease.

In some embodiments, the immune disorder is pemphigus.

In some embodiments, the immune disorder is primary biliary cirrhosis.

In some embodiments, the immune disorder is sarcoidosis.

In some embodiments, the immune disorder is scleroderma.

In some embodiments, the immune disorder is Wegener's granulomatosis.

In some embodiments, the immune disorder is pancreatitis.

In some embodiments, the immune disorder is transplant rejection.

In some embodiments, the immune disorder is heart disease including ischaemic diseases such as myocardial infarction.

In some embodiments, the immune disorder is atherosclerosis.

In some embodiments, the immune disorder is intravascular coagulation.

In some embodiments, the immune disorder is bone resorption.

In some embodiments, the immune disorder is osteoporosis.

In some embodiments, the immune disorder is osteoarthritis.

In some embodiments, the immune disorder is periodontitis.

In some embodiments, the immune disorder is an hypochlorhydia.

In some embodiments, the immune disorder is Sjogren's Syndrome.

In some embodiments, the immune disorder is vitiligo.

In some embodiments, the immune disorder is myasthenia gravis.

In some embodiments, the immune disorder is systemic sclerosis.

Also provided is a method of treating pain associated with inflammation comprising administering to a subject in need thereof a therapeutically effective amount of the isolated antibody or the antigen binding fragment thereof of the invention to treat the pain associated with inflammation.

The invention also provides the antibody that specifically binds PD-1 of the invention for use in therapy.

The invention also provides the antibody that specifically binds PD-1 of the invention for use in treating an immune disorder.

The invention also provides the antibody that specifically binds PD-1 of the invention for use in treating rheumatoid arthritis.

The invention also provides the antibody that specifically binds PD-1 of the invention for use in treating lupus, such as systemic lupus erythematosus.

The invention also provides the antibody that specifically binds PD-1 of the invention for use in treating graft versus host disease.

The invention also provides the use of the antibody that specifically binds PD-1 of the invention in the manufacture of a medicament for the treatment or prophylaxis of an immune disorder.

Combination therapies

The antibodies provided herein may be administered in combination with a second therapeutic agent.

The second therapeutic agent may be any known therapy for an immune disorder such as autoimmune or inflammatory diseases, including any agent or combination of agents that are known to be useful, or which have been used or are currently in use, for treatment of these diseases. Such therapies and therapeutic agents include surgery or surgical procedures (e.g. splenectomy, lymphadenectomy, thyroidectomy, plasmapheresis, leukapheresis, cell, tissue, or organ transplantation, intestinal procedures, organ perfusion, and the like), radiation therapy, therapy such as steroid therapy and non-steroidal therapy, hormone therapy, cytokine therapy, therapy with dermatological agents (for example, topical agents used to treat skin conditions such as allergies, contact dermatitis, and psoriasis), immunosuppressive therapy, and other anti-inflammatory monoclonal antibody therapy.

The second therapeutic agent may be a corticosteroid, an antimalarial drug, an immunosuppressant, a cytotoxic drug, or a B-cell modulator.

In some embodiments, the second therapeutic agent is prednisone, prednisolone, methylprednisolone, deflazcort, hydroxychloroquine, azathioprine, methotrexate, cyclophosphamide, mycophenolate mofetil (MMF), mycophenolate sodium, cyclosporine, leflunomide, tacrolimus, RITUXAN[®] (rituximab), or BENLYSTA[®] (belimumab).

In some embodiments, the antibodies of the invention are administered in combination with a second therapeutic agent. Exemplary second therapeutic agents are corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), salicylates, hydroxychloroquine, sulfasalazine, cytotoxic drugs, immunosuppressive drugs, immunomodulatory antibodies, methotrexate, cyclophosphamide, mizoribine, chlorambucil, cyclosporine, tacrolimus (FK506; ProGrafrM), mycophenolate mofetil, and azathioprine (6-mercaptopurine), sirolimus (rapamycin), deoxyspergualin, leflunomide and its malononitriloamide analogs; anti-CTLA4 antibodies and Ig fusions, anti-B lymphocyte stimulator antibodies (e.g., LYMPHOSTAT-BTM) and CTLA4-Ig fusions (BLyS-Ig), anti-CD80 antibodies, anti-T cell antibodies such as anti-CD3 (OKT3), anti-CD4, corticosteroids such as, for example, clobetasol, halobetasol, hydrocortisone, triamcinolone, betamethasone, fluocinole, fluocinonide, prednisone, prednisolone, methylprednisolone; non-steroidal anti-inflammatory drugs (NSAIDs) such as, for example, sulfasalazine, medications containing mesalamine (known as 5-ASA agents), celecoxib, diclofenac, etodolac, fenprofen, flurbiprofen, ibuprofen, ketoprofen, meclofamate, meloxicam, nabumetone, naproxen, oxaprozin, piroxicam, rofecoxib, salicylates, sulindac, and tolmetin; phosphodiesterase-4 inhibitors, anti-TNF α antibodies REMICADE[®] (infliximab), SIMPONI[®] (golimumab) and HUMIRA[®] (adalimumab), thalidomide or its analogs such as lenalidomide.

The antibodies of the invention may be administered in combination with a second therapeutic agent simultaneously, sequentially or separately.

Treatment effectiveness of RA may be assessed using effectiveness as measured by clinical responses defined by the American College of Rheumatology criteria, the European League of Rheumatism criteria, or any other criteria. See for example, Felson et al. (1995) *Arthritis Rheum.* 38: 727-35 and van Gestel et al. (1996) *Arthritis Rheum.* 39: 34-40.

While having described the invention in general terms, the embodiments of the invention will be further disclosed in the following examples that should not be construed as limiting the scope of the claims.

Example 1. Methods

Affinity measurements using Surface Plasmon Resonance (SPR)

Affinity measurements were performed using a ProteOn XPR36 system (BioRad). A biosensor surface was prepared by coupling anti-Human IgG Fc (Jackson cat#109-005-098) to the modified alginate polymer layer surface of a GLC chip (BioRad, Cat#176-5011) using the manufacturer instructions for amine-coupling chemistry. Approximately 5000 RU (response units) of mAbs were immobilized. The kinetic experiments were performed at 25°C in running buffer (DPBS+0.01% P20+100 µg/mL BSA). To perform kinetic experiments, 200 RU of mAbs were captured followed by injections of analytes (human and cyno PD-1) at concentrations ranging from 1.563 nM to 400 nM (in a 4-fold serial dilution). The association phase was monitored for 3 minutes at 50 µL/min, then followed by 10 or 15 minutes of buffer flow (dissociation phase). The chip surface was regenerated with two 18 second pulses of 100 mM H₃PO₄ (Sigma, Cat#7961) at 100 µL/min.

The collected data were processed using ProteOn Manager software. First, the data was corrected for background using inter-spots. Then, double reference subtraction of the data was performed by using the buffer injection for analyte injections. The kinetic analysis of the data was performed using a Langmuir 1:1 binding model. The result for each mAb was reported in the format of k_a (On-rate), k_d (Off-rate) and K_D (equilibrium dissociation constant).

Inhibition of antigen specific T cells: Cytomegalovirus (CMV) induced peripheral blood mononuclear cell (PBMC) activation assay ("CMV-PBMC")

An assay utilizing CMV-specific recall response was used to assess the ability of the generated antibodies to inhibit T cell activation measured by inhibition of cell proliferation upon treatment of CMV-reactive donors' PBMC with CMV peptide mix (JPT Technologies).

PBMC (Astarte Biologics, Hemacare, Precision for Medicine) were determined to be reactive to CMV antigens by the respective vendors. Frozen vials of PBMC were

purchased from vendors and kept in liquid nitrogen. On the day of experiment an aliquot of frozen PBMC was thawed and the cells were resuspended in 10 ml of assay media (RPMI-1640/Glutamax/HEPES containing 1% Penicillin/Streptomycin, 1% sodium pyruvate, 1% non-essential amino acids (NEAA) solution, 10% heat-inactivated fetal bovine serum (all purchased from Thermo Fisher Scientific). The cells were centrifuged 200g for 15 minutes at room temperature. After centrifugation, the supernatant was discarded, cells were resuspended in 10 ml assay media, and spun down at 250g for 10 minutes. After centrifugation, cells were resuspended in 10 ml assay media, passed through 70 μ m strainer, and counted. Cell concentration was adjusted to 1.5×10^6 cells/ml or 2×10^6 cells/mL, and the cells were plated at 100 μ l/well using tissue culture treated round bottom 96-well plates (Corning). Plating cell density was donor specific and pre-determined in preliminary experiments to maximize assay window. The CMV peptide mix was prepared according to the manufacturer's instructions. Briefly, 40 μ L dimethyl sulfoxide (Sigma) was added to a vial containing 25 μ g CMV peptide mix lyophilized powder and pipetted gently to dissolve the reagent. DMSO stock of CMV peptide mix was diluted in PBS to prepare 50 μ g/mL solution and left at room temperature for 10 min. Further dilution was prepared an assay media at 4x final concentration, and 50 μ L of CMV peptide solution was added to each stimulated well; while unstimulated control wells received unsupplemented media. Final concentration was optimized for a specific donor PBMC in preliminary experiments (0.1-0.2 μ g/mL). The anti-PD-1 antibody was added at single dose of 10 μ g/mL of serially diluted in assay media at 4x final concentration, and 50 μ L of the antibody dilution was added to each well, while "no antibody" control wells receive 50 μ L media. The plates were incubated at 37°C/5% CO₂ for 6 days. After incubation, 100 μ L of supernatant was collected from each well, and 100 μ L of assay media containing 1 μ Ci/well methyl-3H-thymidine (PerkinElmer) was added to each well, and the plates were incubated for 6 hours at 37°C/5% CO₂. The cells were harvested onto Unifilter-96, GF/C plates (PerkinElmer), which were allowed to dry overnight at room temperature. Fifty μ L of Microscint-20 (PerkinElmer) was added to each well. The plates were sealed and counted using the TopCount NXT (PerkinElmer).

Inhibition of cell clustering to determine receptor-ligand blocking ability of antibodies

An assay utilizing a 1:1 mixture of human embryonic kidney (HEK) cells overexpressing either PD-1 (PD-1-HEK cells) or PD-1 ligand PD-L1 (PD-L1-HEK cells) or PD-L2 (PD-L2-HEK cells) was used to assess the ability of the generated antibodies to inhibit receptor:ligand interaction in a cell based context, measured by inhibition of cell clustering as quantified by flow cytometry.

HEK cells overexpressing human PD1 (Crown Bio) were labeled with Violet Cell Trace stain (Life Technologies). HEK cells overexpressing cynomolgus monkey PD-L1 or PD-L2 were labeled with Far Red Cell Trace Stain (Life technologies). Briefly, dyes were solubilized in 20 μ L DMSO to make a 5 mM stock then 5 μ L was diluted into 10 mL PBS to make a 2.5 μ M solution. Cells were counted and 50×10^6 cells were centrifuged at 1000 rpm for 5 minutes at room temperature. Cells were washed once in PBS, and 1×10^6 cells were set aside as unstained controls. After repeat centrifugation the supernatant was discarded, and cells were resuspended in the appropriate dye solutions described above and incubated for 15 minutes at room temperature. Next 4 mL ice cold fetal bovine serum (FBS) was added and incubated for another 5 minutes. Cells were then centrifuged as above, washed once in assay buffer (PBS, 10 % FBS, 1 mM EDTA), centrifuged again and supernatant aspirated, then each cell type was resuspended in assay buffer at 3×10^6 cells/mL. Test antibodies were diluted to three times the final desired concentration (60 μ g/mL) in assay buffer. To prepare the final cell/antibody samples (in triplicate), 100 μ L PD-1-HEK cells were mixed with 100 μ L antibody. After 10 minute incubation, 100 μ L PD-L1-HEK or PD-L2-HEK cells were added and the final mixed samples were incubated on ice for one hour minimum. Finally, 5 μ L propidium iodide was added and samples were mixed gently, transferred to polystyrene round bottom tubes and analyzed on an LSRII flow cytometer (BD Biosciences). After gating events on live cells, the percentage of double positive events, for Pacific Blue and APC channels, were determined using Flowjo software and graphed in GraphPad Prism 6. The level of clustering as measured by the percentage of double positive events, for anti-PD-1 mAbs was compared to positive, and negative isotype control antibodies.

Octet Epitope Binning

Epitope binning of the antibodies with a competition binding assay was performed on an Octet Red384 platform (Forte Bio), which is based on bio-layer interferometry. This technique measures binding of an initial antibody to the PD-1-coated biosensor as a wavelength shift due to the bound antibody increasing the optical density of the biosensor tip over time. Briefly, histidine-tagged PD-1 antigen was loaded onto HIS sensors. The sensors were then exposed to 20 µg/mL primary anti-PD-1 antibody, followed by exposure to an equal concentration of a second anti-PD-1 antibody. Data was processed using ForteBio Data Analysis Software. Additional binding by the second antibody after saturation with the first antibody indicates simultaneous binding of the two antibodies which necessarily have unique non-overlapping epitopes. Alternatively, no additional binding indicates that the two antibodies compete for binding to the PD-1 antigen.

Antibody-Dependent Cellular Cytotoxicity (ADCC)

Activation of human Memory T Cells (target cells):

Frozen aliquots of human Memory T Cells (AllCells LLC) were thawed in 37°C water bath and re-suspended in 40ml culture medium (RPMI + 10% FBS or 5%HuSAB + 50µM βME (1:1000). + 1% GlutaMax + 10mM Hepes. The cells were centrifuged 250g for 15 minutes at room temperature. After centrifugation, the supernatant was discarded, cells were resuspended in 10 ml culture media and spun down at 250g for 10 minutes. After centrifugation, cells were resuspended in 10 ml assay media, and counted. Cell concentration was adjusted to 1.0×10^6 cells/ml and 10mls of the cell suspension was transferred to tissue culture plates pre-coated with anti CD3 antibody (eBioscience, 5ug/ml in PBS, 1 hr at 37°C). Culture media was supplemented with 2 µg/mL anti-CD28 Ab (eBioscience) and a cocktail of IL-2, IL-15 and IL-7 cytokines (R&D Systems and Peprotech) at 100ng/ml conc. Preliminary experiments demonstrated that PD-1 expression peaked on day 5 post-activation and therefore this time point was chosen for the assay. Freshly isolated resting memory T Cells (not activated by CD3/CD28 stimulation) were also included in the analysis as a control, because they express low levels of PD-1.

Preparation of Effector Cells (NK92.CD16 and PBMCs):

The NK-92 cells were grown in tissue culture flasks stored upright and kept at a density of $0.5\text{--}1.0 \times 10^6$ cells/mL in 40 mL media (Myelocult H5100 (StemCell Technologies), 1X Sodium Pyruvate/ Non-Essential Amino Acids/Pen Strep (Invitrogen), $4\mu\text{M}$ Hydrocortisone (StemCell Technologies), 100ng/ml rhIL-2 (R&D Systems)). Frozen PBMC cells (Hemacare) were thawed one day before the experiment and resuspended in XVIVO-10 Media (Lonza), 10% HI FBS (Invitrogen) and 100ng/ml IL2 (R&D Systems). The cells were centrifuged 250g for 15 minutes at room temperature. After centrifugation, the supernatant was discarded, cells were resuspended in 10 mL culture media, and spun down at 250g for 10 minutes. After centrifugation, cells were resuspended in 10 mL culture media and counted. Cell concentration was adjusted to 1.0×10^6 cells/mL and the required number of cells were plated in TC dish.

On the day of the assay, PBMCs and NK cells were harvested and resuspended at 1×10^7 cells/mL and 4×10^6 cells/mL respectively in assay media (RPMI, 10% FBS, 1 mM Sodium pyruvate, 0.1 mM NEAA). Memory T Cells were washed once, resuspended at 1×10^6 cells/mL and labeled with 6 μL of BATDA (Perkin Elmer) per mL of cells at 37°C for 30min. BATDA-labeled cells were washed three times with excess cold assay media and their density adjusted to 0.2×10^6 cells/mL. Serial dilutions of the test Abs starting at 20 $\mu\text{g/mL}$ were prepared in assay media were delivered (100 μL , 2x final concentration) to U-Bottom 96-Well assay plates. BATDA-labeled target cells (50 μL) were added at 0.2×10^6 cells/mL and incubated with test mAbs. PBMC (50 μL) were added at 1×10^7 cells/mL for a 50:1 Effector:Target cell ratio whereas NK cells (50 μL) were added at 4×10^6 cells/mL for a 20:1 Effector:Target cell ratio. Maximum lysis control wells containing labeled target cells and 20 μL 2% Triton X-100 were set up in triplicate. Minimum (background BATDA release) control wells containing labeled target cells in assay media were also set up in triplicate. The final volume in all assay plate wells was 200 μL . Well contents were mixed gently by pipette. Plates were spun down briefly and incubated at 37°C in a 5% CO_2 incubator for 2.5 hours. Following incubation, cells were spun at 1200 rpm for 5 minutes. Supernatants (30 μL) were transferred to 96-well white opaque Nunc plates (ThermoFisher, 136101) containing 200 μL Europium solution (PerkinElmer, C135-100). Plates were covered to protect from light and mixed for 15 min on a shaker. Samples were read on an Envision MultiLabel Reader (PerkinElmer). Percent lysis was calculated as follows:

$100 \times (\text{Experimental release} - \text{Background release}) / (\text{Maximum release} - \text{Background release})$.

Complement-Dependent Cytotoxicity (CDC)

Frozen aliquots of human Pan T Cells (Biological Specialty, LSII 49301C) were thawed in 37°C water bath and re-suspended in 40 ml prewarmed RPMI 1640 + Glutamax + 25 mM HEPES (Life Technologies, Cat# 72400-047) + 10% FBS (Gibco, 160000-36). Cells were centrifuged at 250g for 5 minutes at RT. After centrifugation, the supernatant was discarded, cells were resuspended in 10 mL-20 mL media and counted. The human Pan T Cells (Biological Specialty, LSII 49301C) were activated for 5-6 days prior to CDC assay using Human T-Activator CD3/CD28 Dynabeads™ (Life Technologies, Cat# 11132D). Briefly, 75 µL prewashed Dynabeads were mixed with 6.0×10^6 cells/flask of T cells, added to T175 in 10-20 mL of media and incubated for 6 days in a 37°C/5% CO₂ incubator. After 6 days, beads were removed from the mixture using EasySep™ Magnets (STEMCELL Technologies, 18000).

In preparation for CDC assays, cells were centrifuged at 250g for 5 minutes at room temperature. After centrifugation, the supernatant was discarded, cells were resuspended in 10 ml serum-free culture media, and counted. Cell concentration was adjusted to 1.6×10^6 cells/mL in serum-free media and 50 µL/well were plated in 96-well U-Bottom plates (Falcon, 353077). Test antibodies were serially diluted 1:3 through eleven points in serum-free media, starting at 25 µg/mL (3x). 50 µL/well of test antibodies were added to the appropriate target-cell wells. 50 µL/well of serum-free media were added to background and lysis-control wells. Plates were covered and incubated for 1 hour at room temperature (RT). 50 µL/well of 10% (3x) Rabbit Complement (Invitrogen, 31038-100) diluted in serum-free media was added to test wells. 50 µL/well of serum-free media were added to background control wells. 50 µL/well of 2% Triton-X in serum-free media was added to the lysis control wells. Plates were incubated at 37°C/5% CO₂ for 60 minutes. Cells were spun down at 250g for 5 minutes. 50 µL/well of supernatant was removed and transferred to a 96-well Flat-Bottom UV-Vis plates (Corning, 3635). 50 µL/well of LDH detection reagent (Roche, 11-644-793-001) was added to each sample; plates were covered and incubated for 15 minutes at RT. Absorbance at 490 and 650 nm was recorded on a SpectraMax Plus M5 (Molecular Devices). Statistical analyses were performed using

Microsoft Excel and GraphPad Prism 6. The absorbance at 650 nm was subtracted from that at 490 nm to normalize for turbidity. Percent (%) Cytotoxicity was determined for each sample using the following formula:

$$(\text{Experimental Value} - \text{Low Control}) / (\text{High Control} - \text{Low Control}) \times 100$$

Where the high control is the average of the Triton-X lysis control wells, and the low control is the average of the 'media only' background control wells. A four-parameter logistic curve fitting model, [log(agonist) vs. response – Variable slope (four parameters)], was applied in GraphPad Prism to the log₁₀ of the Ab concentration versus the calculated % Cytotoxicity. Samples were run in duplicate, and the assay was performed twice.

To confirm target expression, PD-1 levels on activated Human Pan T cells were measured by flow cytometry on Day 5 or Day 6 post-activation. Briefly, the T cells were centrifuged at 250g for 5 minutes, and resuspended at 1×10^6 cells/mL in BSA Stain Buffer (BD Biosciences, 554657). 100-200K cells were incubated in 100 μ L total volume with saturating concentrations of PE-PD-1 Ab (Biolegend, 329906). Cells were washed 2x w/ BSA Stain Buffer, and resuspended in an equal volume of buffer containing DRAQ7 live/dead stain (Cell Signaling Technology, 7406S). The Median fluorescent intensity was recorded for 5K live cell events on a MACSQuant Analyzer 10 flow cytometer. Receptor levels were determined using a standard curve generated using Quantibrite™ PE Beads (BD, 340495) and expressed as antibodies bound per cell.

Example 2. Generation of agonistic antibodies that specifically bind PD-1 and their structural characterization

Three Balb/c and three C3H mice were immunized with extracellular domain (ECD) of human PD-1 (SEQ ID NO: 1) conjugated to Fc (huPD-1-Fc) and the hybridomas generated using standard protocols. The hybridomas were screened by ELISA for binding to recombinant PD-1 (ECD). Hits were defined as samples giving an ELISA signal greater than five times the negative control average. Positive clones were cross-screened against an irrelevant Fc fusion protein and for binding to mouse PD-1. Supernatants from single cell cloned hybridomas were tested for binding to human and cyno PD-1 protein. Hits were defined as signal greater than the average + 3 S.D. of the negative controls.

Select mouse antibodies were cloned as chimeric mAbs into human IgG1 and tested for their ability to inhibit antigen specific T cell activation in the CMV recall assay according to protocol described in Example 1 (CMV-PBMC assay). **Figure 1A** and **Figure 1B** shows that the majority of tested antibodies inhibited T cell proliferation at a level of over 50% or more. PD1B199 is an antagonistic anti-PD1 mAb. CNT03930: isotype control.

SEQ ID NO: 1 PD-1 ECD

PGWFLDSPDRPWNPPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQT
DKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPK
AQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV

SEQ ID NO: 131 FL mature PD-1

PGWFLDSPDRPWNPPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQT
DKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPK
AQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLVVGVVGGLLGSLVLLVWV
LAVICSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPPVPCVP
EQTEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL

Example 3. Humanization of anti-PD-1 antibodies

Several parental antibodies including PD1B505, PD1B506 and PD1B512 were humanized. To find the best combination of humanized VH and VL, one or more human germline heavy and light V-region sequences were selected for each of the antibodies. Human J segments for VL and VH of each parental antibody were chosen by comparing the parental J segment sequence to human J segment sequences to maximize sequence identity. All VH/VL humanized pairs of each antibody were made and tested in matrix as crude supernatants for antigen binding and protein expression. Based on these data, antibodies demonstrating comparable or improved PD-1 binding than the parental mouse antibody were purified and tested for their efficacy in the CMV-PBMC assay.

The generated antibodies were analyzed for possible unwanted post-translational modification risks. High risk deamidation motifs located in the CDRs and free cysteines

anywhere in the antibody were removed by mutagenesis and the resulting antibodies tested for their binding to PD-1 and efficacy in the CMV-PBMC assay.

The humanized antibodies and their variants were cloned as IgG1.

Table 3 shows the generated antibodies. **Table 4** shows the SEQ ID NOs: of the VH, the VL, the HC and the LC amino acid sequences of the antibodies. **Table 5** shows the SEQ ID NOs: of the polynucleotide sequences encoding the VH, the VL, the HC and the LC of the antibodies. **Table 6** shows the SEQ ID NOs: of the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 amino acid sequences of the antibodies. **Table 7** shows the HCDR1, the HCDR2 and the HCDR3 amino acid sequences of the antibodies. **Table 8** shows the LCDR1, the LCDR2 and the LCDR3 amino acid sequences of the antibodies. **Table 9** shows the VH and the VL amino acid sequences of the antibodies. **Table 10** shows the polynucleotide sequences encoding the VH of the antibodies. **Table 11** shows the polynucleotide sequences encoding the VL of the antibodies. **Table 12** shows the HC amino acid sequences. **Table 13** shows the LC amino acid sequences. **Table 14** shows the polynucleotide sequences encoding the HC of the antibodies. **Table 15** shows the polynucleotide sequences encoding the LC of the antibodies.

Table 3.

mAb	Antibody origin	VH name	VL name
PD1B505	Parental	PD1H93	PD1L30
PD1B742	Humanized PD1B505	PD1H384	PD1L468
PD1B743	Humanized PD1B505	PD1H384	PD1L469
PD1B878	C84S variant of PD1B743	PD1H405	PD1L469
PD1B506	Parental	PD1H90	PD1L28
PD1B750	Humanized PD1B506	PD1H388	PD1L470
PD1B751	Humanized PD1B506	PD1H388	PD1L471
PD1B845	G56A variant of PD1B750	PD1H399	PD1L470
PD1B846	N55D, G56A variant of PD1B750	PD1H400	PD1L470
PD1B847	N55Q variant of PD1B750	PD1H401	PD1L470
PD1B848	N55K variant of PD1B750	PD1H402	PD1L470
PD1B849	N55E variant of PD1B750	PD1H403	PD1L470
PD1B850	G56I variant of PD1B750	PD1H404	PD1L470
PD1B512	Parental	PD1H81	PD1L43
PD1B756	Humanized PD1B512	PD1H389	PD1L472
PD1B757	Humanized PD1B512	PD1H389	PD1L473

Table 4.

mAb	VH name	VL name	Amino acid sequence SEQ ID NOs:			
			VH	VL	HC	LC
PD1B505	PD1H93	PD1L30	8	14	20	26
PD1B742	PD1H384	PD1L468	9	15	21	27
PD1B743	PD1H384	PD1L469	9	16	21	28
PD1B878	PD1H405	PD1L469	10	16	22	28
PD1B506	PD1H90	PD1L28	44	60	66	82
PD1B750	PD1H388	PD1L470	45	61	67	83
PD1B751	PD1H388	PD1L471	45	62	67	84
PD1B845	PD1H399	PD1L470	46	61	68	83
PD1B846	PD1H400	PD1L470	47	61	69	83
PD1B847	PD1H401	PD1L470	48	61	70	83
PD1B848	PD1H402	PD1L470	49	61	71	83
PD1B849	PD1H403	PD1L470	50	61	72	83
PD1B850	PD1H404	PD1L470	51	61	73	83
PD1B512	PD1H81	PD1L43	94	98	104	108
PD1B756	PD1H389	PD1L472	95	99	105	109
PD1B757	PD1H389	PD1L473	95	100	105	110

Table 5.

mAb	VH name	VL name	Polynucleotide sequence SEQ ID NOs:			
			VH	VL	HC	LC
PD1B505	PD1H93	PD1L30	11	17	23	29
PD1B742	PD1H384	PD1L468	12	18	24	30
PD1B743	PD1H384	PD1L469	12	19	24	31
PD1B878	PD1H405	PD1L469	13	19	25	31
PD1B506	PD1H90	PD1L28	52	63	74	85
PD1B750	PD1H388	PD1L470	53	64	75	86
PD1B751	PD1H388	PD1L471	53	65	75	87
PD1B845	PD1H399	PD1L470	54	64	76	86
PD1B846	PD1H400	PD1L470	55	64	77	86
PD1B847	PD1H401	PD1L470	56	64	78	86
PD1B848	PD1H402	PD1L470	57	64	79	86
PD1B849	PD1H403	PD1L470	58	64	80	86
PD1B850	PD1H404	PD1L470	59	64	81	86
PD1B512	PD1H81	PD1L43	96	101	106	111
PD1B756	PD1H389	PD1L472	97	102	107	112
PD1B757	PD1H389	PD1L473	97	103	107	113

Table 6.

Antibody	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
PD1B505	2	3	4	5	6	7
PD1B742	2	3	4	5	6	7
PD1B743	2	3	4	5	6	7
PD1B878	2	3	4	5	6	7
PD1B506	32	33	40	41	42	43
PD1B750	32	33	40	41	42	43
PD1B751	32	33	40	41	42	43
PD1B845	32	34	40	41	42	43
PD1B846	32	35	40	41	42	43
PD1B847	32	36	40	41	42	43
PD1B848	32	37	40	41	42	43
PD1B849	32	38	40	41	42	43
PD1B850	32	39	40	41	42	43
PD1B512	88	89	90	91	92	93
PD1B756	88	89	90	91	92	93
PD1B757	88	89	90	91	92	93

Table 7.

Antibody	HCDR1 (SEQ ID NO:)	HCDR2 (SEQ ID NO:)	HCDR3 (SEQ ID NO:)
PD1B505	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGEPT (SEQ ID NO: 3)	DYYGTYFYAMDY (SEQ ID NO: 4)
PD1B742	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGEPT (SEQ ID NO: 3)	DYYGTYFYAMDY (SEQ ID NO: 4)
PD1B743	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGEPT (SEQ ID NO: 3)	DYYGTYFYAMDY (SEQ ID NO: 4)
PD1B878	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGEPT (SEQ ID NO: 3)	DYYGTYFYAMDY (SEQ ID NO: 4)
PD1B506	GYTFTTYWMH (SEQ ID NO: 32)	EINPNNGGIN (SEQ ID NO: 33)	DYYDYGGY (SEQ ID NO: 40)
PD1B750	GYTFTTYWMH (SEQ ID NO: 32)	EINPNNGGIN (SEQ ID NO: 33)	DYYDYGGY (SEQ ID NO: 40)
PD1B751	GYTFTTYWMH	EINPNNGGIN	DYYDYGGY

	(SEQ ID NO: 32)	(SEQ ID NO: 33)	(SEQ ID NO: 40)
PD1B845	GYTFTTYWMH (SEQ ID NO: 32)	EINPNNAGIN (SEQ ID NO: 34)	DYYDYGGY (SEQ ID NO: 40)
PD1B846	GYTFTTYWMH (SEQ ID NO: 32)	EINPNDAGIN (SEQ ID NO: 35)	DYYDYGGY (SEQ ID NO: 40)
PD1B847	GYTFTTYWMH (SEQ ID NO: 32)	EINPNQGGIN (SEQ ID NO: 36)	DYYDYGGY (SEQ ID NO: 40)
PD1B848	GYTFTTYWMH (SEQ ID NO: 32)	EINPNKGGIN (SEQ ID NO: 37)	DYYDYGGY (SEQ ID NO: 40)
PD1B849	GYTFTTYWMH (SEQ ID NO: 32)	EINPNEGGIN (SEQ ID NO: 38)	DYYDYGGY (SEQ ID NO: 40)
PD1B850	GYTFTTYWMH (SEQ ID NO: 32)	EINPNNIGIN (SEQ ID NO: 39)	DYYDYGGY (SEQ ID NO: 40)
PD1B512	GFSLSSTSGMGVS (SEQ ID NO: 88)	HIYWDDDKR (SEQ ID NO: 89)	KGYDYGYVMDY (SEQ ID NO: 90)
PD1B756	GFSLSSTSGMGVS (SEQ ID NO: 88)	HIYWDDDKR (SEQ ID NO: 89)	KGYDYGYVMDY (SEQ ID NO: 90)
PD1B757	GFSLSSTSGMGVS (SEQ ID NO: 88)	HIYWDDDKR (SEQ ID NO: 89)	KGYDYGYVMDY (SEQ ID NO: 90)

Table 8.

Antibody	LCDR1 (SEQ ID NO:)	LCDR2 (SEQ ID NO:)	LCDR3 (SEQ ID NO:)
PD1B505	TASSSVSSSYLH (SEQ ID NO: 5)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)
PD1B742	TASSSVSSSYLH (SEQ ID NO: 5)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)
PD1B743	TASSSVSSSYLH (SEQ ID NO: 5)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)
PD1B878	TASSSVSSSYLH (SEQ ID NO: 5)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)

PD1B506	KASQNVGTNVA (SEQ ID NO: 41)	SASYRYS (SEQ ID NO: 42)	QQYNIYPYT (SEQ ID NO: 43)
PD1B750	KASQNVGTNVA (SEQ ID NO: 41)	SASYRYS (SEQ ID NO: 42)	QQYNIYPYT (SEQ ID NO: 43)
PD1B751	KASQNVGTNVA (SEQ ID NO: 41)	SASYRYS (SEQ ID NO: 42)	QQYNIYPYT (SEQ ID NO: 43)
PD1B845	KASQNVGTNVA (SEQ ID NO: 41)	SASYRYS (SEQ ID NO: 42)	QQYNIYPYT (SEQ ID NO: 43)
PD1B846	KASQNVGTNVA (SEQ ID NO: 41)	SASYRYS (SEQ ID NO: 42)	QQYNIYPYT (SEQ ID NO: 43)
PD1B847	KASQNVGTNVA (SEQ ID NO: 41)	SASYRYS (SEQ ID NO: 42)	QQYNIYPYT (SEQ ID NO: 43)
PD1B848	KASQNVGTNVA (SEQ ID NO: 41)	SASYRYS (SEQ ID NO: 42)	QQYNIYPYT (SEQ ID NO: 43)
PD1B849	KASQNVGTNVA (SEQ ID NO: 41)	SASYRYS (SEQ ID NO: 42)	QQYNIYPYT (SEQ ID NO: 43)
PD1B850	KASQNVGTNVA (SEQ ID NO: 41)	SASYRYS (SEQ ID NO: 42)	QQYNIYPYT (SEQ ID NO: 43)
PD1B512	RSSKSLLHSNGITYLN (SEQ ID NO: 91)	QMSNLAS (SEQ ID NO: 92)	AQNLELPLT (SEQ ID NO: 93)
PD1B756	RSSKSLLHSNGITYLN (SEQ ID NO: 91)	QMSNLAS (SEQ ID NO: 92)	AQNLELPLT (SEQ ID NO: 93)
PD1B757	RSSKSLLHSNGITYLN (SEQ ID NO: 91)	QMSNLAS (SEQ ID NO: 92)	AQNLELPLT (SEQ ID NO: 93)

Table 9.

VH or VL chain name (SEQ ID NO)	Amino acid sequence
PD1H93 (SEQ ID NO: 8)	DVQLQESGPELKKPGETVKISCKASGYTFTDYSMHWVKQAP GKGLKWMGWINIETGEPTYADDFKGRFAFSLETSASTAYLQ INNLNEDTATYFCARDYYGTYFYAMDYWGQGTTTLTVSS
PD1H384 (SEQ ID NO: 9)	QVQLVQSGSELKKPGASVKVSCCKASGYTFTDYSMHWVRQA PGQGLEWMGWINIETGEPTYAQGFTGRFVFSLDTSVSTAYL QICSLKAEDTAVYFCARDYYGTYFYAMDYWGQGTTLTVSS
PD1H405 (SEQ ID NO: 10)	QVQLVQSGSELKKPGASVKVSCCKASGYTFTDYSMHWVRQA PGQGLEWMGWINIETGEPTYAQGFTGRFVFSLDTSVSTAYL QISSLKAEDTAVYFCARDYYGTYFYAMDYWGQGTTLTVSS
PD1H90 (SEQ ID NO: 44)	QVQLQQPGAELVKPGASVKLSCKASGYTFTTYWMHWVKQ RPGQGLEWIGEINPNNGGINYNEKFKKKATLTVDKSSSTAY MQLSSLTSEDSAVYYCTIDYYDYGGYWGQGTTTLTVSS
PD1H388 (SEQ ID NO: 45)	QVQLVQSGAEVKKPGASVKVSCCKASGYTFTTYWMHWVRQ APGQGLEWMGEINPNNGGINYAQKFQGRVTLTVDKSISTAY MELSRLRSDDTAVYYCTIDYYDYGGYWGQGTTLTVSS
PD1H399 (SEQ ID NO: 46)	QVQLVQSGAEVKKPGASVKVSCCKASGYTFTTYWMHWVRQ APGQGLEWMGEINPNNAGINYAQKFQGRVTLTVDKSISTAY MELSRLRSDDTAVYYCTIDYYDYGGYWGQGTTLTVSS
PD1H400 (SEQ ID NO: 47)	QVQLVQSGAEVKKPGASVKVSCCKASGYTFTTYWMHWVRQ APGQGLEWMGEINPDAGINYAQKFQGRVTLTVDKSISTAY MELSRLRSDDTAVYYCTIDYYDYGGYWGQGTTLTVSS
PD1H401 (SEQ ID NO: 48)	QVQLVQSGAEVKKPGASVKVSCCKASGYTFTTYWMHWVRQ APGQGLEWMGEINPNQGGINYAQKFQGRVTLTVDKSISTAY MELSRLRSDDTAVYYCTIDYYDYGGYWGQGTTLTVSS
PD1H402 (SEQ ID NO: 49)	QVQLVQSGAEVKKPGASVKVSCCKASGYTFTTYWMHWVRQ APGQGLEWMGEINPNKGGINYAQKFQGRVTLTVDKSISTAY MELSRLRSDDTAVYYCTIDYYDYGGYWGQGTTLTVSS
PD1H403 (SEQ ID NO: 50)	QVQLVQSGAEVKKPGASVKVSCCKASGYTFTTYWMHWVRQ APGQGLEWMGEINPNEGGINYAQKFQGRVTLTVDKSISTAY MELSRLRSDDTAVYYCTIDYYDYGGYWGQGTTLTVSS
PD1H404 (SEQ ID NO: 51)	QVQLVQSGAEVKKPGASVKVSCCKASGYTFTTYWMHWVRQ APGQGLEWMGEINPNNIGINYAQKFQGRVTLTVDKSISTAY MELSRLRSDDTAVYYCTIDYYDYGGYWGQGTTLTVSS
PD1H81 (SEQ ID NO: 94)	QVTLKESGPGLLQPSQTLSTCSFSGFSLSTSGMGVSWIRQPS GKGLEWLAHIYWDDDKRYNPSTLSRLTISKDTSSNQVFLKIT SVDTADTGTYCYCRKGYDYGYVMDYWGQGTTLTVSS

PD1H389 (SEQ ID NO: 95)	QITLKESGPTLVKPTQTLTLCTFSGFSLSTSGMGVSWIRQPP GKALEWLAHIYWDDDKRYSPSLKSRLTITKDTSKNQVVL MTNMDPVDGTGYTCVRKGYDYGYVMDYWGQGT LTVTS
PD1L30 (SEQ ID NO: 14)	QIVLTQSPAISASLGERVTMTCTASSSVSSSYLHWYQQKPG SSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAA TYYCHQYHRSPLTFGAGTKLELK
PD1L468 (SEQ ID NO: 15)	EIVLTQSPATLSLSPGERATLSCTASSSVSSSYLHWYQQKPG APRLLIYSTSNLASGIPDRFSGSGSGTDFTLTISRLEPEDFAVY YCHQYHRSPLTFGQGTKLEIK
PD1L469 (SEQ ID NO: 16)	EIVLTQSPATLSLSPGERATLSCTASSSVSSSYLHWYQQKPG APRLLIYSTSNLASGIPDRFSGSGSGTDYTLTISRLEPEDFAVY YCHQYHRSPLTFGQGTKLEIK
PD1L28 (SEQ ID NO: 60)	DIVMTQSQKFMSTSVRDRVSVTCKASQNVGTNVAWYQQKP GQSPKALIYSASYRSGVPDRFTGSGSGTDFTLTITNVQSEDL AEYFCQQYNIYPYTFGSGGTKLEMK
PD1L470 (SEQ ID NO: 61)	DIQMTQSPSSLSASVGDRTITCKASQNVGTNVAWYQQKPE KAPKSLIYSASYRSGVPSRFSGSGSGTDFTLTISLQPEDFAT YYCQQYNIYPYTFGQGTKLEIK
PD1L471 (SEQ ID NO: 62)	DIQMTQSPSSLSASVGDRTITCKASQNVGTNVAWYQQKPE KAPKALIYSASYRSGVPSRFSGSGSGTDFTLTISLQPEDFA TYFCQQYNIYPYTFGQGTKLEIK
PD1L43 (SEQ ID NO: 98)	DIVMTQAALSNPVTLGTSASISCRSSKSLLSHNGITYLNWYL QKPGQSPQLLIYQMSNLASGVDPDRFSSSGSGTDFTLRISRVEA EDVGVYYCAQNLELPLTFGSGGTKLEMK
PD1L472 (SEQ ID NO: 99)	DIVMTQSPLSLPVTGPGEPAISCRSSKSLLSHNGITYLNWYLQ KPGQSPQLLIYQMSNLASGVDPDRFSGSGSGTDFTLKISRVEA EDVGVYYCAQNLELPLTFGGGTKVEIK
PD1L473 (SEQ ID NO: 100)	DIVMTQSPLSLPVTGPGEPAISCRSSKSLLSHNGITYLNWYLQ KPGQSPQLLIYQMSNLASGVDPDRFSSSGSGTDFTLKISRVEAE DVGYYCAQNLELPLTFGGGTKVEIK

Table 10.

VH chain (polynucleotide SEQ ID NO:)	Polynucleotide sequence
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PD1H93 (SEQ ID NO: 11)	GATGTACAGCTTCAGGAGTCAGGACCTGAGCTGAAGAAGC CTGGAGAGACAGTCAAGATCTCCTGCAAGGCTTCTGGTTAT ACCTTCACAGACTATTCAATGCACTGGGTGAAGCAGGCTCC AGGAAAGGGTTTAAAGTGGATGGGCTGGATAAACATTGAG ACTGGTGAGCCAACATATGCAGATGACTTCAAGGGACGGTT TGCCTTCTCTTTGGAAACCTCTGCCAGCACTGCCTATTTGCA GATCAACAACCTCAAAAATGAGGACACGGCTACATATTTCT GTGCTAGAGATTACTACGGTACTTACTTCTATGCTATGGACT ACTGGGGTCAAGGCACCACTCTCACAGTCTCCTCA
PD1H384 (SEQ ID NO: 12)	CAGGTGCAGCTGGTGCAGTCTGGAAGCGAACTGAAGAAAC CTGGAGCCTCTGTGAAAGTGTCTTGTAAGGCCAGCGGCTAC ACCTTCACCGACTACAGCATGCACTGGGTGCGGCAGGCCCC TGGACAGGGCCTGGAATGGATGGGCTGGATCAACATCGAGA CCGGCGAGCCCACCTACGCCCAGGGCTTTACCGGACGGTTC GTGTTACGCCTGGATAACATCTGTGTCTACAGCCTATCTGCAG ATCTGCTCTCTGAAGGCCGAAGATACAGCCGTGTACTTCTGC GCCCCGGGACTACTACGGCACCTACTTCTACGCCATGGACTA CTGGGGCCAGGGAACACTGGTGACAGTGTCTTCT
PD1H405 (SEQ ID NO: 13)	CAAGTGCAGCTGGTGCAGTCTGGCAGCGAGCTGAAAAAACC TGGCGCCTCCGTGAAGGTGTCCTGCAAGGCTAGCGGCTACA CCTTTACCGACTACAGCATGCACTGGGTCCGACAGGCTCCA GGACAAGGCTTGGAATGGATGGGCTGGATCAACATCGAGAC AGGCGAGCCACATACGCCCAGGGCTTTACCGGCAGATTCTG TGTTACGCCTGGACACCTCTGTGTCCACCGCCTACCTGCAGA TCAGCTCTCTGAAGGCCGAGGATAACGCGGTGTACTTCTGC GCCAGAGACTACTACGGCACCTACTTCTACGCCATGGATTA CTGGGGCCAGGGCACCCCTGGTTACCGTTTCTTCT
PD1H90 (SEQ ID NO: 52)	CAGGTCCAACCTGCAGCAGCCTGGGGCTGAACTTGTGAAGCC TGGGGCTTCAGTGAAGTTGTCCTGCAAGGCTTCTGGCTACAC CTTCACCACCTACTGGATGCACTGGGTGAAGCAGAGGCCTG GACAAGGCCTTGAGTGGATTGGAGAGATTAATCCTAACAA GGTGGTATTAATTACAATGAGAAAGTTCAAGAAGAAGGCCAC ACTGACTGTAGACAAATCCTCCAGCACAGCCTACATGCAGC TCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATTACTGTA CAATAGACTACTATGATTACGGGGGCTACTGGGGCCAAGGC ACCACTCTCACAGTCTCCTCA

PD1H388 (SEQ ID NO: 53)	CAGGTGCAGCTGGTGCAGTCTGGAGCCGAAGTGAAGAAAC CTGGAGCCTCTGTGAAAGTGTCTTGTAAGGCCAGCGGCTAC ACCTTCACCACCTACTGGATGCACTGGGTGCGGCAGGCCCC TGGACAGGGCCTGGAATGGATGGGCGAGATCAACCCCAAC AACGGCGGCATCAACTACGCCCAGAAATTTACAGGGACGGGT GACCCTGACAGTGGATAAGAGCATCTCTACAGCCTACATGG AACTGTCTCGGCTGCGGAGCGATGACACAGCCGTGTACTAC TGCACCATCGACTACTACGACTACGGCGGCTACTGGGGCCA GGGAACACTGGTGACAGTGTCTTCT
PD1H399 (SEQ ID NO: 54)	CAGGTGCAGCTGGTGCAGTCTGGAGCCGAAGTGAAGAAAC CTGGAGCCTCTGTGAAAGTGTCTTGTAAGGCCAGCGGCTAC ACCTTCACCACCTACTGGATGCACTGGGTGCGGCAGGCCCC TGGACAGGGCCTGGAATGGATGGGCGAGATCAACCCCAAC AACGCCGGCATCAACTACGCCCAGAAATTTACAGGGACGGGT GACCCTGACAGTGGATAAGAGCATCTCTACAGCCTACATGG AACTGTCTCGGCTGCGGAGCGATGACACAGCCGTGTACTAC TGCACCATCGACTACTACGACTACGGCGGCTACTGGGGCCA GGGAACACTGGTGACAGTGTCTTCT
PD1H400 (SEQ ID NO: 55)	CAGGTGCAGCTGGTGCAGTCTGGAGCCGAAGTGAAGAAAC CTGGAGCCTCTGTGAAAGTGTCTTGTAAGGCCAGCGGCTAC ACCTTCACCACCTACTGGATGCACTGGGTGCGGCAGGCCCC TGGACAGGGCCTGGAATGGATGGGCGAGATCAACCCCAAC GACGCCGGCATCAACTACGCCCAGAAATTTACAGGGACGGGT GACCCTGACAGTGGATAAGAGCATCTCTACAGCCTACATGG AACTGTCTCGGCTGCGGAGCGATGACACAGCCGTGTACTAC TGCACCATCGACTACTACGACTACGGCGGCTACTGGGGCCA GGGAACACTGGTGACAGTGTCTTCT
PD1H401 (SEQ ID NO: 56)	CAGGTGCAGCTGGTGCAGTCTGGAGCCGAAGTGAAGAAAC CTGGAGCCTCTGTGAAAGTGTCTTGTAAGGCCAGCGGCTAC ACCTTCACCACCTACTGGATGCACTGGGTGCGGCAGGCCCC TGGACAGGGCCTGGAATGGATGGGCGAGATCAACCCCAACC AGGGCGGCATCAACTACGCCCAGAAATTTACAGGGACGGGTG ACCCTGACAGTGGATAAGAGCATCTCTACAGCCTACATGGA ACTGTCTCGGCTGCGGAGCGATGACACAGCCGTGTACTACT GCACCATCGACTACTACGACTACGGCGGCTACTGGGGCCAG GGAACACTGGTGACAGTGTCTTCT
PD1H402 (SEQ ID NO: 57)	CAGGTGCAGCTGGTGCAGTCTGGAGCCGAAGTGAAGAAAC CTGGAGCCTCTGTGAAAGTGTCTTGTAAGGCCAGCGGCTAC ACCTTCACCACCTACTGGATGCACTGGGTGCGGCAGGCCCC TGGACAGGGCCTGGAATGGATGGGCGAGATCAACCCCAAC AAGGGCGGCATCAACTACGCCCAGAAATTTACAGGGACGGGT GACCCTGACAGTGGATAAGAGCATCTCTACAGCCTACATGG AACTGTCTCGGCTGCGGAGCGATGACACAGCCGTGTACTAC TGCACCATCGACTACTACGACTACGGCGGCTACTGGGGCCA GGGAACACTGGTGACAGTGTCTTCT

PD1H403 (SEQ ID NO: 58)	CAGGTGCAGCTGGTGCAGTCTGGAGCCGAAGTGAAGAAAC CTGGAGCCTCTGTGAAAGTGTCTTGTAAGGCCAGCGGCTAC ACCTTCACCACCTACTGGATGCACTGGGTGCGGCAGGCCCC TGGACAGGGCCTGGAATGGATGGGCGAGATCAACCCCAAC GAGGGCGGCATCAACTACGCCCAGAAATTTACGGGACGGGT GACCCTGACAGTGGATAAGAGCATCTCTACAGCCTACATGG AACTGTCTCGGCTGCGGAGCGATGACACAGCCGTGTACTAC TGCACCATCGACTACTACGACTACGGCGGCTACTGGGGCCA GGGAACACTGGTGACAGTGTCTTCT
PD1H404 (SEQ ID NO: 59)	CAGGTGCAGCTGGTGCAGTCTGGAGCCGAAGTGAAGAAAC CTGGAGCCTCTGTGAAAGTGTCTTGTAAGGCCAGCGGCTAC ACCTTCACCACCTACTGGATGCACTGGGTGCGGCAGGCCCC TGGACAGGGCCTGGAATGGATGGGCGAGATCAACCCCAAC AACATCGGCATCAACTACGCCCAGAAATTTACGGGACGGGT GACCCTGACAGTGGATAAGAGCATCTCTACAGCCTACATGG AACTGTCTCGGCTGCGGAGCGATGACACAGCCGTGTACTAC TGCACCATCGACTACTACGACTACGGCGGCTACTGGGGCCA GGGAACACTGGTGACAGTGTCTTCT
PD1H81 (SEQ ID NO: 96)	CAGGTACTCTGAAAGAGTCTGGCCCTGGGTTATTGCAGCC CTCCCAGACCCTCAGTCTGACTTGTTCTTTCTCTGGGTTTTCA CTGAGCACTTCTGGTATGGGTGTGAGCTGGATTCTGTCAGCCT TCAGGAAAGGGTCTGGAGTGGCTGGCACACATTTACTGGGA TGATGACAAGCGCTATAACCCATCCCTGAAGAGCCGGCTCA CAATCTCCAAAGATACCTCCAGCAACCAGGTATTCCTCAAG ATCACCAGTGTGGACACTGCAGATACTGGCACATACTACTG TGTTGAAAGGGCTACTATGATTACGGCTATGTAATGGACT ACTGGGGTCAAGGGACCACGGTCACCGTCTCCTCA
PD1H389 (SEQ ID NO: 97)	CAGATCACACTGAAAGAATCTGGACCTACACTGGTGAAACC TACACAGACCCTGACACTGACCTGTACCTTCAGCGGCTTCA GCCTGAGCACCAGCGGCATGGGCGTGAGCTGGATTTCGGCAG CCTCCTGGAAAGGCCCTGGAATGGCTGGCCCACATCTACTG GGACGACGACAAGCGGTACAGCCCTAGCCTGAAGTCTCGGC TGACAATCACCAAGGATACCTCTAAGAACCAGGTGGTGCTG ACAATGACCAACATGGACCCTGTGGACACAGGCACCTACTA CTGCGTGCGGAAGGGCTACTACGACTACGGCTACGTGATGG ACTACTGGGGCCAGGGAACACTGGTGACAGTGTCTTCT

Table 11.

VL name (polynucleo tide SEQ ID NO:)	VL cDNA
PD1L30 (SEQ ID NO: 17)	CAAATTGTTCTCACCCAGTCTCCAGCAATCATGTCTGCATC TCTAGGGGAACGGGTCACCATGACCTGCACTGCCAGCTCA AGTGTAAGTTCCAGTTACTTGCCTGGTACCAGCAGAAAGCC AGGATCCTCCCCCAAACCTCTGGATTTATAGCACATCCAACC TGGCTTCTGGAGTCCCAGCTCGCTTCAGTGGCAGTGGGTCT GGGACCTCTTACTCTCTCACAATCAGCAGCATGGAGGCTGA AGATGCTGCCACTTATTACTGCCACCAGTATCATCGTTCCC CGCTCACGTTTCGGTGCTGGGACCAAGCTGGAGCTGAAA
PD1L468 (SEQ ID NO: 18)	GAGATCGTGCTGACACAGTCTCCTGCCACACTGTCTCTGTC TCCTGGAGAACGGGCCACACTGAGCTGCACCGCCAGCAGC AGCGTGAGCAGCAGCTACCTGCACTGGTACCAGCAGAAAC CTGGACTGGCCCCCTCGGCTGCTGATCTACAGCACCAGCAAC CTGGCCAGCGGCATCCCTGATCGGTTTTCTGGCAGCGGATC TGGCACAGATTTTACACTGACCATCAGCCGGCTGGAACCTG AGGATTTTGCCGTGTACTACTGCCACCAGTACCACCGGAGC CCCCTGACCTTCGGCCAGGGAACAAAGCTGGAAATCAAG
PD1L469 (SEQ ID NO: 19)	GAGATCGTGCTGACACAGTCTCCTGCCACACTGTCTCTGTC TCCTGGAGAACGGGCCACACTGAGCTGCACCGCCAGCAGC AGCGTGAGCAGCAGCTACCTGCACTGGTACCAGCAGAAAC CTGGACTGGCCCCCTCGGCTGCTGATCTACAGCACCAGCAAC CTGGCCAGCGGCATCCCTGATCGGTTTTCTGGCAGCGGATC TGGCACAGATTACACACTGACCATCAGCCGGCTGGAACCT GAGGATTTTGCCGTGTACTACTGCCACCAGTACCACCGGAG CCCCCTGACCTTCGGCCAGGGAACAAAGCTGGAAATCAAG
PD1L28 (SEQ ID NO: 63)	GACATTGTGATGACCCAGTCTCAAAAATTCATGTCCACATC AGTAAGAGACAGGGTCAGCGTCACCTGCAAGGCCAGTCAG AATGTGGGCACTAATGTAGCCTGGTATCAACAGAAACCAG GGCAATCTCCTAAAGCACTGATTTACTCGGCATCCTACCGG TACAGTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGG GACAGATTTCACTCTCACCATCACCAATGTGCAGTCTGAAG ACTTGGCAGAATATTTCTGTGTCAGCAATATAACATCTATCCG TACACGTTTCGGATCGGGACCAAGCTGGAAATGAAA

PD1L470 (SEQ ID NO: 64)	GACATCCAGATGACACAGTCTCCTAGCTCTCTGAGCGCCTC TGTGGGAGATCGGGTGACAATCACCTGCAAGGCCAGCCAG AACGTGGGCACCAACGTGGCCTGGTACCAGCAGAAACCTG AAAAAGCCCCTAAGAGCCTGATCTACAGCGCCAGCTACCG GTACAGCGGCGTGCCTTCTCGGTTTAGCGGCTCTGGAAGCG GAACAGATTTACACTGACCATCTCTAGCCTGCAGCCTGAA GATTTTGCCACATACTACTGCCAGCAGTACAACATCTACCC CTACACCTTCGGCCAGGGAACAAAGCTGGAAATCAAG
PD1L471 (SEQ ID NO: 65)	GACATCCAGATGACACAGTCTCCTAGCTCTCTGAGCGCCTC TGTGGGAGATCGGGTGACAATCACCTGCAAGGCCAGCCAG AACGTGGGCACCAACGTGGCCTGGTACCAGCAGAAACCTG AAAAAGCCCCTAAGAGCCTGATCTACAGCGCCAGCTACCG GTACAGCGGCGTGCCTTCTCGGTTTAGCGGCTCTGGAAGCG GAACAGATTTACACTGACCATCTCTAGCCTGCAGCCTGAA GATTTTGCCACATACTTTTGCCAGCAGTACAACATCTACCC CTACACCTTCGGCCAGGGAACAAAGCTGGAAATCAAG
PD1L43 (SEQ ID NO: 101)	GATATTGTGATGACTCAGGCTGCACTCTCCAATCCAGTCAC TCTTGGAACATCAGCTTCCATCTCCTGCAGGTCTAGTAAGA GTCTCCTACATAGTAATGGCATCACTTATTTGAATTGGTAT CTGCAGAAAGCCAGGCCAGTCTCCTCAGCTCCTGATTTATCA GATGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTTCACTA GCAGTGGGTCAGGAACTGATTTACACTGAGAATCAGCAG AGTGGAGGCTGAGGATGTGGGTGTTTATTACTGTGCTCAA ATCTAGAACTTCCGCTCACGTTCCGATCGGGGACCAAGCTG CAAAATCAAA
PD1L472 (SEQ ID NO: 102)	GACATCGTGATGACACAGTCTCCTCTGTCTCTGCCTGTGAC ACCTGGCGAACCTGCCTCTATCAGCTGCCGGAGCAGCAAG AGCCTGCTGCACAGCAACGGCATCACCTACCTGAACTGGTA CCTGCAGAAACCTGGACAGTCTCCTCAGCTGCTGATCTACC AGATGAGCAACCTGGCCAGCGGCGTGCCTGATCGGTTTAG CGGCTCTGGAAGCGGCACAGACTTCACACTGAAGATCTCTC GGGTGGAAGCCGAGGACGTGGGAGTGTACTACTGCGCCCA GAACCTGGAGCTGCCCCTGACCTTCGGAGGCGGAACAAAG CTCCAGATCAAG
PD1L473 (SEQ ID NO: 103)	GACATCGTGATGACACAGTCTCCTCTGTCTCTGCCTGTGAC ACCTGGCGAACCTGCCTCTATCAGCTGCCGGAGCAGCAAG AGCCTGCTGCACAGCAACGGCATCACCTACCTGAACTGGTA CCTGCAGAAACCTGGACAGTCTCCTCAGCTGCTGATCTACC AGATGAGCAACCTGGCCAGCGGCGTGCCTGATCGGTTTAG CAGCTCTGGAAGCGGCACAGACTTCACACTGAAGATCTCTC GGGTGGAAGCCGAGGACGTGGGAGTGTACTACTGCGCCCA GAACCTGGAGCTGCCCCTGACCTTCGGAGGCGGAACAAAG CTCCAGATCAAG

Table 12.

mAb	HC
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PD1B505 HC (SEQ ID NO: 20)	DVQLQESGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPG KGLKWMGWINIETGEPTYADDFKGRFAFSLETSASTAYLQINN LKNEDTATYFCARDYYGTYFYAMDYWGQGTTLTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
PD1B742, PD1B743 HC (SEQ ID NO: 21)	QVQLVQSGSELKKPGASVKVSCASGYTFTDYSMHWVRQAP GQGLEWMGWINIETGEPTYAQGFTGRFVFSLDTSVSTAYLQIC SLKAEDTAVYFCARDYYGTYFYAMDYWGQGTTLTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
PD1B878 HC (SEQ ID NO: 22)	QVQLVQSGSELKKPGASVKVSCASGYTFTDYSMHWVRQAP GQGLEWMGWINIETGEPTYAQGFTGRFVFSLDTSVSTAYLQISS LKAEDTAVYFCARDYYGTYFYAMDYWGQGTTLTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
PD1B506 HC (SEQ ID NO: 66)	QVQLQQPGAELVKPGASVKLSCKASGYTFTTYWMHWVKQRP GQGLEWIGEINPNNGGINYNEKFKKKATLTVDKSSSTAYMQLS SLTSEDSAVYYCTIDYYDYGGYWGQGTTLTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
PD1B750, PD1B751 HC	QVQLVQSGAEVKKPGASVKVSCASGYTFTTYWMHWVRQAP GQGLEWMGEINPNNGGINYAQKFQGRVTLTVDKSISTAYMEL SRLRSDDTAVYYCTIDYYDYGGYWGQGTTLTVSSASTKGPSV

(SEQ ID NO: 67)	FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
PD1B845 HC (SEQ ID NO: 68)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWMHWVRQAP GQGLEWMGEINPNAGINYAQKFQGRVTLTVDKSISTAYMEL SRLRSDDTAVYYCTIDYYDYGGYWGQGTLLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
PD1B846 HC (SEQ ID NO: 69)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWMHWVRQAP GQGLEWMGEINPDAGINYAQKFQGRVTLTVDKSISTAYMEL SRLRSDDTAVYYCTIDYYDYGGYWGQGTLLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
PD1B847 HC (SEQ ID NO: 70)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWMHWVRQAP GQGLEWMGEINPNQGGINYAQKFQGRVTLTVDKSISTAYMEL SRLRSDDTAVYYCTIDYYDYGGYWGQGTLLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
PD1B848 HC (SEQ ID NO: 71)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWMHWVRQAP GQGLEWMGEINPNKGGINYAQKFQGRVTLTVDKSISTAYMEL SRLRSDDTAVYYCTIDYYDYGGYWGQGTLLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK

	EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSS VMHEALHNHYTQKSLSLSPGK
PD1B849 HC (SEQ ID NO: 72)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWMHWVRQAP GQGLEWMGEINPNEGGINYAQKFQGRVTLTVDKSISTAYMELS RLRSDDTAVYYCTIDYYDYGGYWGQGLTVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSV MHEALHNHYTQKSLSLSPGK
PD1B850 HC (SEQ ID NO: 73)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYWMHWVRQAP GQGLEWMGEINPNNIGINYAQKFQGRVTLTVDKSISTAYMELS RLRSDDTAVYYCTIDYYDYGGYWGQGLTVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSV MHEALHNHYTQKSLSLSPGK
PD1B512 HC (SEQ ID NO: 104)	QVTLKESGPGLLQPSQTLSTCSFSGFSLSTSGMGVSWIRQPSG KGLEWLAHIYWDDDKRYNPSLKSRLTISKDTSSNQVFLKITSV DTADTGTYTCVRKGYDYGYVMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
PD1B756, PD1B757 HC (SEQ ID NO: 105)	QITLKESGPTLVKPTQTLTLCTFSGFSLSTSGMGVSWIRQPPGK ALEWLAHIYWDDDKRYSPSLKSRLTITKDTSKNQVVLMTNM DPVDTGTYYCVRKGYDYGYVMDYWGQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW

	ESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
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Table 13.

mAb	LC (SEQ ID NO:)
PD1B505 LC (SEQ ID NO: 26)	QIVLTQSPAIMASASLGERVTMTCTASSSVSSSYLHWYQQKPGS SPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAAT YYCHQYHRSPLTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC
PD1B742 LC (SEQ ID NO: 27)	EIVLTQSPATLSLSPGERATLSCTASSSVSSSYLHWYQQKPG APRLLIYSTSNLASGIPDRFSGSGSGTDFTLTISRLEPEDFAVY CHQYHRSPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS YSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
PD1B743, PD1B878 LC (SEQ ID NO: 28)	EIVLTQSPATLSLSPGERATLSCTASSSVSSSYLHWYQQKPG APRLLIYSTSNLASGIPDRFSGSGSGTDYTLISRLEPEDFAVY CHQYHRSPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS YSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
PD1B506 LC (SEQ ID NO: 82)	DIVMTQSQKFMSTSVRDRVSVTCKASQNVGTNVAWYQQKP GQSPKALIYSASYRYSQVPSRFGSGSGTDFTLTITNVQSEDL AEYFCQQYNIYPYTFGSGTKLEMKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC
PD1B750, PD1B845, PD1B846, PD1B847, PD1B848, PD1B849, PD1B850 LC (SEQ ID NO: 83)	DIQMTQSPSSLSASVGDRVTITCKASQNVGTNVAWYQQKPEK APKSLIYSASYRYSQVPSRFGSGSGTDFTLTISLQPEDFATY YCQQYNIYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C
PD1B751 LC (SEQ ID NO: 84)	DIQMTQSPSSLSASVGDRVTITCKASQNVGTNVAWYQQKPEK APKALIYSASYRYSQVPSRFGSGSGTDFTLTISLQPEDFATY FCQQYNIYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C

PD1B512 LC (SEQ ID NO: 108)	DIVMTQAALSNPVTLGTSASISCRSSKSLHLSNGITYLNWYLQ KPGQSPQLLIYQMSNLASGVDPDRFSSSGSGTDFTLRISRVEAED VGYYCAQNLELPLTFGSGTKLEMKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC
PD1B756 LC (SEQ ID NO: 109)	DIVMTQSPLSLPVTGPGEPAISCRSSKSLHLSNGITYLNWYLQK PGQSPQLLIYQMSNLASGVDPDRFSSSGSGTDFTLKISRVEAED VGYYCAQNLELPLTFGGGKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC
PD1B757 LC (SEQ ID NO: 110)	DIVMTQSPLSLPVTGPGEPAISCRSSKSLHLSNGITYLNWYLQK PGQSPQLLIYQMSNLASGVDPDRFSSSGSGTDFTLKISRVEAED VGYYCAQNLELPLTFGGGKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC

Table 14.

HC cDNA (SEQ ID NO:)
PD1B505 HC (SEQ ID NO: 23) GATGTACAGCTTCAGGAGTCAGGACCTGAGCTGAAGAAGCCTGGAGAGAC AGTCAAGATCTCCTGCAAGGCTTCTGGTTATACCTTCACAGACTATTCAAT GCACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGGCTGGA TAAACATTGAGACTGGTGAGCCAACATATGCAGATGACTTCAAGGGACGG TTTGCCTTCTCTTTGGAAACCTCTGCCAGCACTGCCTATTTGCAGATCAACA ACCTCAAAAATGAGGACACGGCTACATATTTCTGTGCTAGAGATTACTACG GTACTTACTTCTATGCTATGGACTACTGGGGTCAAGGCACCACTCTCACAG TCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTC CAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT ACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCG GCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCA GCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG CCCAAATCTTGAGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAA CTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCCAAGGACACC CTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGC CACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGT GCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACC GTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGG AGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAA ACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGACCTGACCTGCC TGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT

GGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGGACTCCGA
CGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCA
GCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCA
CTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

PD1B742, PD1B743 HC (SEQ ID NO: 24)

CAGGTGCAGCTGGTGCAGTCTGGAAGCGAACTGAAGAAACCTGGAGCCTC
TGTGAAAGTGTCTTGTAAGGCCAGCGGCTACACCTTCACCGACTACAGCAT
GCACTGGGTGCGGCAGGCCCCCTGGACAGGGCCTGGAATGGATGGGCTGGA
TCAACATCGAGACCGGCGAGCCACCTACGCCAGGGCTTTACCGGACGG
TTCGTGTTTACGCTGGATACATCTGTGTCTACAGCCTATCTGCAGATCTGCT
CTCTGAAGGCCGAAGATACAGCCGTGTACTTCTGCGCCCCGGGACTACTACG
GCACCTACTTCTACGCCATGGACTACTGGGGCCAGGGAACACTGGTGACA
GTGTCTTCTGCCTCCACCAAGGGGCCATCGGTCTTCCCCCTGGCACCCCTCCT
CCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC
TACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGC
GGCGTGCACACCTTCCCGGCTGTCTTACAGTCCTCAGGACTCTACTCCCTC
AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATC
TGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGA
GCCCCAAATCTTGTGACAAAACCTCACACATGCCACCCGTGCCCAGCACCTGA
ACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCCTAAACCCAAGGACAC
CCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAG
CCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGG
TGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTAC
CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAG
GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAA
AACCATCTCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACC
TGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGC
CTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAA
TGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGGACTCCG
ACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGC
AGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACC
ACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

PD1B878 HC (SEQ ID NO: 25)

CAAGTGCAGCTGGTGCAGTCTGGCAGCGAGCTGAAAAAACCTGGCGCCTC
CGTGAAGGTGTCCTGCAAGGCTAGCGGCTACACCTTTACCGACTACAGCAT
GCACTGGGTCCGACAGGCTCCAGGACAAGGCTTGGATGGATGGGCTGGA
TCAACATCGAGACAGGCGAGCCACATACGCCAGGGCTTTACCGGCAGA
TTCGTGTTTACGCTGGACACCTCTGTGTCCACCGCCTACCTGCAGATCAGCT
CTCTGAAGGCCGAGGATACCGCCGTGTACTTCTGCGCCAGAGACTACTACG
GCACCTACTTCTACGCCATGGATTACTGGGGCCAGGGCACCCCTGGTTACCG
TTTCTTCTGCCTCCACCAAGGGGCCATCGGTCTTCCCCCTGGCACCCCTCCTC
CAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT
ACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCG
GCGTGCACACCTTCCCGGCTGTCTTACAGTCCTCAGGACTCTACTCCCTCA
GCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT
GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG
CCCAAATCTTGTGACAAAACCTCACACATGCCACCCGTGCCCAGCACCTGAA

CTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAACCCAAGGACACC
 CTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGC
 CACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGT
 GCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACC
 GTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGG
 AGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAA
 ACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT
 GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCC
 TGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT
 GGGCAGCCGGAGAACTACAAGACCACGCCTCCCGTGCTGGACTCCGA
 CGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCA
 GCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCA
 CTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

PD1B506 HC (SEQ ID NO: 74)

CAGGTCCAAGTGCAGCAGCCTGGGGCTGAACTTGTGAAGCCTGGGGCTTCA
 GTGAAGTTGTCTTCAAGGCTTCTGGCTACACCTTCACCACCTACTGGATG
 CACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGAGAGAT
 TAATCCTAACAATGGTGGTATTAATTACAATGAGAAGTTCAAGAAGAAGG
 CCACACTGACTGTAGACAAATCCTCCAGCACAGCCTACATGCAGCTCAGCA
 GCCTGACATCTGAGGACTCTGCGGTCTATTACTGTACAATAGACTACTATG
 ATTACGGGGGCTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGCCT
 CCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCTCCTCCAAGAGCACCT
 CTGGGGGCACAGCGGCCCTGGGTGCCTGGTCAAGGACTACTTCCCCGAAC
 CGGTGACGGTGTCTGGAAGTCAAGGCGCCCTGACCAGCGGCGTGCACACC
 TTCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTG
 ACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT
 CACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTG
 TGACAAAACCTACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGG
 GACCGTCAGTCTTCTCTTCCCCCAAACCCAAGGACACCCTCATGATCT
 CCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGAC
 CCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC
 CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCA
 GCGTCTCACCCTGCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAG
 TGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC
 AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATC
 CCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAG
 GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCG
 GAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTC
 TTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAA
 CGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCA
 GAAGAGCCTCTCCCTGTCTCCGGGTAAA

PD1B750, PD1B751 HC (SEQ ID NO: 75)

CAGGTGCAGCTGGTGCAGTCTGGAGCCGAAGTGAAGAAACCTGGAGCCTC
 TGTGAAAGTGTCTTGTAAGGCCAGCGGCTACACCTTCACCACCTACTGGAT
 GCACTGGGTGCGGCAGGCCCCCTGGACAGGGCCTGGAATGGATGGGCGAGA
 TCAACCCCAACAACGGCGGCATCAACTACGCCAGAAATTTAGGGGACGG
 GTGACCCTGACAGTGGATAAGAGCATCTCTACAGCCTACATGGAAGTGTCT

CGGCTGCGGAGCGATGACACAGCCGTGTACTACTGCACCATCGACTACTAC
GACTACGGGCGGCTACTGGGGCCAGGGAACACTGGTGACAGTGTCTTCTGCC
TCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCTCCTCCAAGAGCACC
TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAA
CCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACAC
CTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT
GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA
TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAAGTTGAGCCCCAAATCTT
GTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGG
GGACCGTCAGTCTTCCTCTTCCCCCCTCAAGGACACCCTCATGATC
TCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGA
CCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG
CCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA
GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTC
CAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT
CCCGGGAGGAGATGACCAAGAACCAGGTACGCTGACCTGCCTGGTCAA
GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCC
GGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTT
CTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGA
ACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGC
AGAAGAGCCTCTCCCTGTCTCCGGGTAAA

PD1B845 HC (SEQ ID NO: 76)

CAGGTGCAGCTGGTGCAGTCTGGAGCCGAAGTGAAGAAACCTGGAGCCTC
TGTGAAAGTGTCTTGTAAGGCCAGCGGCTACACCTTCACCACCTACTGGAT
GCACTGGGTGCGGCAGGCCCCCTGGACAGGGCCTGGAATGGATGGGCGAGA
TCAACCCCAACAACGCCGGCATCAACTACGCCCAGAAATTTTCAGGGACGG
GTGACCCTGACAGTGGATAAGAGCATCTCTACAGCCTACATGGAACGTCT
CGGCTGCGGAGCGATGACACAGCCGTGTACTACTGCACCATCGACTACTAC
GACTACGGGCGGCTACTGGGGCCAGGGAACACTGGTGACAGTGTCTTCTGCC
TCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCTCCTCCAAGAGCACC
TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAA
CCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACAC
CTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT
GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA
TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAAGTTGAGCCCCAAATCTT
GTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGG
GGACCGTCAGTCTTCCTCTTCCCCCCTCAAGGACACCCTCATGATC
TCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGA
CCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG
CCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA
GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTC
CAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT
CCCGGGAGGAGATGACCAAGAACCAGGTACGCTGACCTGCCTGGTCAA
GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCC
GGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTT

CTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGA
ACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGC
AGAAGAGCCTCTCCCTGTCTCCGGGTAAA

PD1B846 HC (SEQ ID NO: 77)

CAGGTGCAGCTGGTGCAGTCTGGAGCCGAAGTGAAGAAACCTGGAGCCTC
TGTGAAAGTGTCTTGTAAGGCCAGCGGCTACACCTTCACCACCTACTGGAT
GCACTGGGTGCGGCAGGCCCCCTGGACAGGGCCTGGAATGGATGGGCGAGA
TCAACCCCAACGACGCCGGCATCAACTACGCCCAGAAATTTTCAGGGACGG
GTGACCCTGACAGTGGATAAGAGCATCTCTACAGCCTACATGGAACGTGTCT
CGGCTGCGGAGCGATGACACAGCCGTGTACTACTGCACCATCGACTACTAC
GACTACGGCGGCTACTGGGGCCAGGGAACACTGGTGACAGTGTCTTCTGCC
TCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCTCCTCCAAGAGCACC
TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAA
CCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACAC
CTTCCCGGCTGTCTTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT
GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA
TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTT
GTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGG
GGACCGTCAGTCTTCTCTTCCCCCAAAAACCCAAGGACACCCTCATGATC
TCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGA
CCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG
CCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
AGCGTCCTCACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA
GTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTC
CAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT
CCCGGGAGGAGATGACCAAGAACCAGGTGACCTGACCTGCCTGGTCAAA
GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCC
GGAGAACAACCTACAAGACCACGCTCCCGTGTCTGGACTCCGACGGCTCCTT
CTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGA
ACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGC
AGAAGAGCCTCTCCCTGTCTCCGGGTAAA

PD1B847 HC (SEQ ID NO: 78)

CAGGTGCAGCTGGTGCAGTCTGGAGCCGAAGTGAAGAAACCTGGAGCCTC
TGTGAAAGTGTCTTGTAAGGCCAGCGGCTACACCTTCACCACCTACTGGAT
GCACTGGGTGCGGCAGGCCCCCTGGACAGGGCCTGGAATGGATGGGCGAGA
TCAACCCCAACCAGGGCGGCATCAACTACGCCCAGAAATTTTCAGGGACGG
GTGACCCTGACAGTGGATAAGAGCATCTCTACAGCCTACATGGAACGTGTCT
CGGCTGCGGAGCGATGACACAGCCGTGTACTACTGCACCATCGACTACTAC
GACTACGGCGGCTACTGGGGCCAGGGAACACTGGTGACAGTGTCTTCTGCC
TCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCTCCTCCAAGAGCACC
TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAA
CCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACAC
CTTCCCGGCTGTCTTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT
GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA
TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTT
GTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGG
GGACCGTCAGTCTTCTCTTCCCCCAAAAACCCAAGGACACCCTCATGATC

TCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGA
CCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG
CCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA
GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTC
CAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT
CCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAA
GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCC
GGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTT
CTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGA
ACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGC
AGAAGAGCCTCTCCCTGTCTCCGGGTAAA

PD1B848 HC (SEQ ID NO: 79)

CAGGTGCAGCTGGTGCAGTCTGGAGCCGAAGTGAAGAAACCTGGAGCCTC
TGTGAAAGTGTCTTGTAAGGCCAGCGGCTACACCTTCACCACCTACTGGAT
GCACTGGGTGCGGCAGGCCCCCTGGACAGGGCCTGGAATGGATGGGCGAGA
TCAACCCCAACAAGGGCGGCATCAACTACGCCCAGAAATTTAGGGACGG
GTGACCTTGACAGTGGATAAGAGCATCTCTACAGCCTACATGGAAGTGTCT
CGGCTGCGGAGCGATGACACAGCCGTGTACTACTGCACCATCGACTACTAC
GACTACGGGCGGCTACTGGGGCCAGGGAACACTGGTGACAGTGTCTTCTGCC
TCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCTCCTCCAAGAGCACC
TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAA
CCGGTGACGGTGTCTGGAAGTCAAGGCGCCCTGACCAGCGGCGTGCACAC
CTTCCCGGTGTCTTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGT
GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA
TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTT
GTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGG
GGACCGTCAAGTCTTCTTCTTCCCCCAAAACCCAAGGACACCCTCATGATC
TCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGA
CCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG
CCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA
GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTC
CAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT
CCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAA
GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCC
GGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTT
CTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGA
ACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGC
AGAAGAGCCTCTCCCTGTCTCCGGGTAAA

PD1B849 (SEQ ID NO: 80)

CAGGTGCAGCTGGTGCAGTCTGGAGCCGAAGTGAAGAAACCTGGAGCCTC
TGTGAAAGTGTCTTGTAAGGCCAGCGGCTACACCTTCACCACCTACTGGAT
GCACTGGGTGCGGCAGGCCCCCTGGACAGGGCCTGGAATGGATGGGCGAGA
TCAACCCCAACGAGGGCGGCATCAACTACGCCCAGAAATTTAGGGACGG
GTGACCTTGACAGTGGATAAGAGCATCTCTACAGCCTACATGGAAGTGTCT
CGGCTGCGGAGCGATGACACAGCCGTGTACTACTGCACCATCGACTACTAC

GACTACGGCGGCTACTGGGGCCAGGGAACACTGGTGACAGTGTCTTCTGCC
 TCCACCAAGGGGCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACC
 TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAA
 CCGGTGACGGTGTCTGGAAGTCAAGGCGCCCTGACCAGCGGCGTGCACAC
 CTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT
 GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA
 TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTT
 GTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGG
 GGACCGTCAGTCTTCTCTTCCCCCCTAAACCAAGGACACCCTCATGATC
 TCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGA
 CCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG
 CCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
 AGCGTCCTCACCCTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA
 GTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTC
 CAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT
 CCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAA
 GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCC
 GGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTT
 CTTCTCTACAGCAAGCTCACCCTGGACAAGAGCAGGTGGCAGCAGGGGA
 ACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGC
 AGAAGAGCCTCTCCCTGTCTCCGGGTAA

PD1B850 HC (SEQ ID NO: 81)

CAGGTGCAGCTGGTGCAGTCTGGAGCCGAAGTGAAGAAACCTGGAGCCTC
 TGTGAAAGTGTCTTGTAAGGCCAGCGGCTACACCTTCACCACCTACTGGAT
 GCACTGGGTGCGGCAGGCCCCCTGGACAGGGCCTGGAATGGATGGGCGAGA
 TCAACCCCAACAACATCGGCATCAACTACGCCCAGAAATTTTCAGGGACGG
 GTGACCCTGACAGTGGATAAGAGCATCTCTACAGCCTACATGGAAGTGTCT
 CGGCTGCGGAGCGATGACACAGCCGTGTACTACTGCACCATCGACTACTAC
 GACTACGGCGGCTACTGGGGCCAGGGAACACTGGTGACAGTGTCTTCTGCC
 TCCACCAAGGGGCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACC
 TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAA
 CCGGTGACGGTGTCTGGAAGTCAAGGCGCCCTGACCAGCGGCGTGCACAC
 CTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT
 GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA
 TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTT
 GTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGG
 GGACCGTCAGTCTTCTCTTCCCCCCTAAACCAAGGACACCCTCATGATC
 TCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGA
 CCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG
 CCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
 AGCGTCCTCACCCTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA
 GTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTC
 CAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT
 CCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAA
 GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCC
 GGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTT
 CTTCTCTACAGCAAGCTCACCCTGGACAAGAGCAGGTGGCAGCAGGGGA

ACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGC
AGAAGAGCCTCTCCCTGTCTCCGGGTAAA

PD1B512 HC (SEQ ID NO: 106)

CAGGTTACTCTGAAAGAGTCTGGCCCTGGGTATTGCAGCCCTCCCAGACC
CTCAGTCTGACTTGTTCTTTCTCTGGGTTTTCACTGAGCACTTCTGGTATGG
GTGTGAGCTGGATTTCGTCAGCCTTCAGGAAAGGGTCTGGAGTGGCTGGCAC
ACATTTACTGGGATGATGACAAGCGCTATAACCCATCCCTGAAGAGCCGGC
TCACAATCTCCAAAGATACCTCCAGCAACCAGGTATTCCTCAAGATCACCA
GTGTGGACACTGCAGATACTGGCACATACTACTGTGTTTCGAAAGGGCTACT
ATGATTACGGCTATGTAATGGACTACTGGGGTCAAGGGACCACGGTCACC
GTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCT
CCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC
TACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGGCGCCCTGACCAGC
GGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTC
AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATC
TGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGA
GCCCCAAATCTTGTGACAAAACCTCACACATGCCACCCGTGCCCAGCACCTGA
ACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCCTTCCCCCCTTCCCCCCTT
CCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAG
CCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGG
TGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTAC
CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAG
GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAA
AACCATCTCCAAAGGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCC
TGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGACCTGACCTGC
CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAA
TGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCG
ACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGC
AGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACC
ACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

PD1B756, PD1B757 HC (SEQ ID NO: 107)

CAGATCACACTGAAAGAATCTGGACCTACACTGGTGAAACCTACACAGAC
CCTGACACTGACCTGTACCTTCAGCGGCTTCAGCCTGAGCACCAGCGGCAT
GGGCGTGAGCTGGATTTCGGCAGCCTCCTGGAAAGGCCCTGGAATGGCTGG
CCCACATCTACTGGGACGACGACAAGCGGTACAGCCCTAGCCTGAAGTCTC
GGCTGACAATCACCAAGGATACCTCTAAGAACCAGGTGGTGTGACAATG
ACCAACATGGACCCTGTGGACACAGGCACCTACTACTGCGTGCGGAAGGG
CTACTACGACTACGGCTACGTGATGGACTACTGGGGCCAGGGAACACTGG
TGACAGTGTCTTCTGCCTCCACCAAGGGCCCCTCGGTCTTCCCCCTGGCAC
CCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCA
AGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGGCGCCCTGA
CCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACT
CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCT
ACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAA
GTTGAGCCCCAAATCTTGTGACAAAACCTCACACATGCCACCCGTGCCCAGCA
CCTGAATCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCCTTCCCCCCTT
GACACCCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGAC

GTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
 GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGC
 ACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAAT
 GGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCAT
 CGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGT
 ACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTG
 ACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAG
 AGCAATGGGCAGCCGGAGAACAATAAGACCACGCCTCCCGTGCTGGA
 CTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAG
 GTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCA
 CAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

Table 15.

LC cDNA (SEQ ID NO:)
PD1B505 LC (SEQ ID NO: 29) CAAATTGTTCTCACCCAGTCTCCAGCAATCATGTCTGCATCTCTAGGGGAA CGGGTCACCATGACCTGCACTGCCAGCTCAAGTGTAAGTTCCAGTTACTTG CACTGGTACCAGCAGAAGCCAGGATCTCCCCCAAACCTCTGGATTTATAGC ACATCCAACCTGGCTTCTGGAGTCCCAGCTCGCTTCAGTGGCAGTGGGTCT GGGACCTCTTACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATGCTGCC ACTTATTACTGCCACCAGTATCATCGTTCCCGCTCACGTTCCGGTGCTGGGA CCAAGCTGGAGCTGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCC CGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGC TGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAAC GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAA GGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACT ACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGC TCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
PD1B742 LC (SEQ ID NO: 30) GAGATCGTGCTGACACAGTCTCCTGCCACACTGTCTCTGTCTCCTGGAGAA CGGGCCACACTGAGCTGCACCGCCAGCAGCAGCGTGAGCAGCAGCTACCT GCACTGGTACCAGCAGAAACCTGGACTGGCCCCCTCGGCTGCTGATCTACAG CACCAGCAACCTGGCCAGCGGCATCCCTGATCGGTTTTTCTGGCAGCGGATC TGGCACAGATTTTACACTGACCATCAGCCGGCTGGAACCTGAGGATTTTGC CGTGTACTACTGCCACCAGTACCACCGGAGCCCCCTGACCTTCGGCCAGGG AACAAAGCTGGAAATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTT CCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCT GCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATA ACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGC AAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGA CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGA GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
PD1B743, PD1B878 LC (SEQ ID NO: 31) GAGATCGTGCTGACACAGTCTCCCGCCACACTGTCACTGTCTCCAGGCGAA AGAGCCACACTGAGCTGTACCGCCAGCAGCTCTGTGTCCAGCAGCTACCTG CACTGGTATCAGCAGAAGCCTGGACTGGCCCCCTCGGCTGCTGATCTACAGC

ACAAGCAATCTGGCCAGCGGCATCCCCGATAGATTTTCCGGCTCTGGAAGC
GGCACCGACTACACCCTGACAATCAGCAGACTGGAACCCGAGGACTTCGC
CGTGTAATACTGACCACTGACCAAGAGCCCTCTGACCTTTGGCCAGGG
CAGCAAGCTGGAAATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTT
CCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCT
GCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATA
ACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGC
AAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGA
CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGA
GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

PD1B506 LC (SEQ ID NO: 85)

GACATTGTGATGACCCAGTCTCAAAAATTCATGTCCACATCAGTAAGAGAC
AGGGTCAGCGTCACCTGCAAGGCCAGTCAGAATGTGGGCACTAATGTAGC
CTGGTATCAACAGAAACCAGGGCAATCTCCTAAAGCACTGATTTACTCGGC
ATCCTACCGGTACAGTGGAGTCCCTGATCGCTTACAGGCAGTGGATCTGG
GACAGATTTCACTCTCACCATCACCATGTGCAGTCTGAAGACTTGGCAGA
ATATTTCTGTGACCAATATAACATCTATCCGTACACGTTCCGGATCGGGGAC
CAAGCTGGAAATGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCC
GCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCT
GAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACG
CCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAG
GACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTA
CGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCT
CGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

PD1B750, PD1B845, PD1B846, PD1B847, PD1B848, PD1B849, PD1B850 LC (SEQ ID NO: 86)

GACATCCAGATGACACAGTCTCCTAGCTCTCTGAGCGCCTCTGTGGGAGAT
CGGGTGACAATCACCTGCAAGGCCAGCCAGAACGTGGGCACCAACGTGGC
CTGGTACCAGCAGAAACCTGAAAAAGCCCCTAAGAGCCTGATCTACAGCG
CCAGCTACCGGTACAGCGGCGTGCCTTCTCGGTTTAGCGGCTCTGGAAGCG
GAACAGATTTTCACTGACCATCTCTAGCCTGCAGCCTGAAGATTTTGCCA
CATACTACTGCCAGCAGTACAACATCTACCCCTACACCTTCGGCCAGGGAA
CAAAGCTGGAAATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCC
CGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGC
TGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAAC
GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAA
GGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACT
ACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGC
TCGCCCCGTACAAAGAGCTTCAACAGGGGAGAGTGT

PD1B751 LC (SEQ ID NO: 87)

GACATCCAGATGACACAGTCTCCTAGCTCTCTGAGCGCCTCTGTGGGAGAT
CGGGTGACAATCACCTGCAAGGCCAGCCAGAACGTGGGCACCAACGTGGC
CTGGTACCAGCAGAAACCTGAAAAAGCCCCTAAGGCCCTGATCTACAGCG
CCAGCTACCGGTACAGCGGCGTGCCTTCTCGGTTTAGCGGCTCTGGAAGCG
GAACAGATTTTCACTGACCATCTCTAGCCTGCAGCCTGAAGATTTTGCCA
CATACTTTTGCCAGCAGTACAACATCTACCCCTACACCTTCGGCCAGGGAA
CAAAGCTGGAAATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCC

CGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGC
TGAATAACTTCTATCCCAGAGAGGGCCAAAGTACAGTGGAAGGTGGATAAC
GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAA
GGACAGCACCTACAGCCTCAGCAGCACCTTGACGCTGAGCAAAGCAGACT
ACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGC
TCGCCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

PD1B512 LC (SEQ ID NO: 111)

GATATTGTGATGACTCAGGCTGCACTCTCCAATCCAGTCACTCTTGGAACA
TCAGCTTCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTACATAGTAATGGC
ATCACTTATTTGAATTGGTATCTGCAGAAGCCAGGCCAGTCTCCTCAGCTC
CTGATTTATCAGATGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTTCACT
AGCAGTGGGTGAGGAAGTATTTCACTGAGAATCAGCAGAGTGGAGGC
TGAGGATGTGGGTGTTTATTACTGTGCTCAAAATCTAGAACTTCCGCTCAC
GTTTCGGATCGGGGACCAAGCTGGAAATGAAACGTACGGTGGCTGCACCAT
CTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTC
TGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGGCCAAAGTACAGTG
GAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAG
AGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTTGACGCTG
AGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCA
TCAGGGCCTGAGCTCGCCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

PD1B756 LC (SEQ ID NO: 112)

GACATCGTGATGACACAGTCTCCTCTGTCTCTGCCTGTGACACCTGGCGAA
CCTGCCTCTATCAGCTGCCGGAGCAGCAAGAGCCTGCTGCACAGCAACGG
CATCACCTACCTGAACTGGTACCTGCAGAAACCTGGACAGTCTCCTCAGCT
GCTGATCTACCAGATGAGCAACCTGGCCAGCGGCGTGCCTGATCGGTTTAG
CGGCTCTGGAAGCGGCACAGACTTCACACTGAAGATCTCTCGGGTGGAAAG
CCGAGGACGTGGGAGTGTACTACTGCGCCCAGAACCTGGAGCTGCCCCCTG
ACCTTCGGAGGCGGAACAAAGGTGGAGATCAAGCGTACGGTGGCTGCACC
ATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGC
TCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGGCCAAAGTACAG
TGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTAC
AGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTTGACGC
TGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACC
CATCAGGGCCTGAGCTCGCCCCGTCACAAAGAGCTTCAACAGGGGAGAGTG
T

PD1B757 LC (SEQ ID NO: 113)

GACATCGTGATGACACAGTCTCCTCTGTCTCTGCCTGTGACACCTGGCGAA
CCTGCCTCTATCAGCTGCCGGAGCAGCAAGAGCCTGCTGCACAGCAACGG
CATCACCTACCTGAACTGGTACCTGCAGAAACCTGGACAGTCTCCTCAGCT
GCTGATCTACCAGATGAGCAACCTGGCCAGCGGCGTGCCTGATCGGTTTAG
CAGCTCTGGAAGCGGCACAGACTTCACACTGAAGATCTCTCGGGTGGAAAG
CCGAGGACGTGGGAGTGTACTACTGCGCCCAGAACCTGGAGCTGCCCCCTG
ACCTTCGGAGGCGGAACAAAGGTGGAGATCAAGCGTACGGTGGCTGCACC
ATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGC
TCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGGCCAAAGTACAG
TGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTAC
AGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTTGACGC

TGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACC
CATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTG
T

The cDNA sequences encoding antibody VH, VL, HC and LC provided herein can be codon optimized for expression in various cells, for example for expression in HEK or CHO cells. Additional cDNA sequences that may be used to encode and express PD1B878 and PD1B849 VH, VL, HC and LC are shown below.

SEQ ID NO: 132 PD1B878 VH cDNA

CAAGTGCAGCTGGTGCAGTCTGGCAGCGAGCTGAAAAACCTGGCGCCTCCGT
GAAGGTGTCCTGCAAGGCTAGCGGCTACACCTTTACCGACTACAGCATGCACT
GGGTCCGACAGGCTCCAGGACAAGGCTTGGAATGGATGGGCTGGATCAACATC
GAGACAGGCGAGCCACATACGCCCAGGGCTTTACCGGCAGATTTCGTGTTCAG
CCTGGACACCTCTGTGTCCACCGCCTACCTGCAGATCAGCTCTCTGAAGGCCGA
GGATACCGCCGTGTACTTCTGCGCCAGAGACTACTACGGCACCTACTTCTACGC
CATGGATTACTGGGGCCAGGGCACCCCTGGTTACCGTTTCTTCT

SEQ ID NO: 133 PD1B878 VL cDNA

GAGATCGTGCTGACACAGTCTCCCGCCACACTGTCACTGTCTCCAGGCGAAAG
AGCCACACTGAGCTGTACCGCCAGCAGCTCTGTGTCCAGCAGCTACCTGCACTG
GTATCAGCAGAAGCCTGGACTGGCCCCTCGGCTGCTGATCTACAGCACAAGCA
ATCTGGCCAGCGGCATCCCCGATAGATTTTCCGGCTCTGGAAGCGGCACCGACT
ACACCCTGACAATCAGCAGACTGGAACCCGAGGACTTCGCCGTGTACTACTGC
CACCAGTACCACAGAAGCCCTCTGACCTTTGGCCAGGGCACCAAGCTGGAAAT
CAAG

SEQ ID NO: 134 PD1B878 HC cDNA

CAAGTGCAGCTGGTGCAGTCTGGCAGCGAGCTGAAAAACCTGGCGCCTCCGT
GAAGGTGTCCTGCAAGGCTAGCGGCTACACCTTTACCGACTACAGCATGCACT
GGGTCCGACAGGCTCCAGGACAAGGCTTGGAATGGATGGGCTGGATCAACATC
GAGACAGGCGAGCCACATACGCCCAGGGCTTTACCGGCAGATTTCGTGTTCAG
CCTGGACACCTCTGTGTCCACCGCCTACCTGCAGATCAGCTCTCTGAAGGCCGA
GGATACCGCCGTGTACTTCTGCGCCAGAGACTACTACGGCACCTACTTCTACGC
CATGGATTACTGGGGCCAGGGCACCCCTGGTTACCGTTTCTTCTGCCTCCACCAA
GGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCAC
AGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGT
CGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTAC
AGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCT
TGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAG
GTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACC

GTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCC AAA
ACCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGG
TGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC
GTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA
CGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA
AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAA
ACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCC
CCCATCCCCGGGAGGAGATGACCAAGAACCAGGTGACCTGACCTGCCTGGTCA
AAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCG
GAGAACAAC TACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT
CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCC
TCTCCCTGTCTCCGGGTAAA

SEQ ID NO: 135 PD1B878 LC cDNA

GAGATCGTGCTGACACAGTCTCCCGCCACACTGTCACTGTCTCCAGGCGAAAG
AGCCACACTGAGCTGTACCGCCAGCAGCTCTGTGTCCAGCAGCTACCTGCACTG
GTATCAGCAGAAGCCTGGACTGGCCCCCTCGGCTGCTGATCTACAGCACAAGCA
ATCTGGCCAGCGGCATCCCCGATAGATTTTCCGGCTCTGGAAGCGGCACCGACT
ACACCCTGACAATCAGCAGACTGGAACCCGAGGACTTCGCCGTGTACTACTGC
CACCAGTACCACAGAAGCCCTCTGACCTTTGGCCAGGGCACCAAGCTGGAAAT
CAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCA
GTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAG
AGAGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCTCCAATCGGGTAACTCCC
AGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAG
CACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCG
AAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGA
GAGTGT

SEQ ID NO: 136 PD1B849 VH cDNA

CAAGTGCAGCTGGTGCAATCTGGCGCCGAAGTGAAAAAGCCTGGCGCCTCTGT
GAAGGTGTCCTGCAAGGCCAGCGGCTACACCTTTACCACCTACTGGATGCACT
GGGTCCGACAGGCTCCAGGACAAGGCTTGGAGTGGATGGGCGAGATCAACCCC
AATGAAGGCGGCATCAACTACGCCCAGAAATTCCAGGGCAGAGTGACCCTGAC
CGTGGACAAGAGCATCAGCACCGCCTACATGGAAGTGGAGCCGGCTGAGATCCG
ATGACACCGCCGTGTACTACTGCACCATCGACTACTACGACTACGGCGGCTATT
GGGGCCAGGGCACACTGGTTACAGTGTCTCT

SEQ ID NO: 137 PD1B849 VL cDNA

GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGCCTCTGTGGGCGATAG
AGTGACCATCACATGCAAGGCCAGCCAGAACGTGGGCACCAATGTGGCCTGGT
ATCAGCAGAAGCCTGAGAAGGCCCTAAGAGCCTGATCTACAGCGCCAGCTAC
AGATACAGCGGCGTGCCAAGCAGATTTTCTGGAAGCGGCAGCGGCACCGACTT
CACCCTGACAATTAGTAGCCTGCAGCCTGAGGACTTCGCCACCTACTACTGCCA

GCAGTACAACATCTACCCCTACACCTTCGGCCAGGGCACCAAGCTGGAAATCA
AG

SEQ ID NO: 138 PD1B849 HC cDNA

CAAGTGCAGCTGGTGCAATCTGGCGCCGAAGTGAAAAAGCCTGGCGCCTCTGT
GAAGGTGTCCTGCAAGGCCAGCGGCTACACCTTTACCACCTACTGGATGCACT
GGGTCCGACAGGCTCCAGGACAAGGCTTGGAGTGGATGGGCGAGATCAACCCC
AATGAAGGCGGCATCAACTACGCCCAGAAATTCAGGGCAGAGTGACCCTGAC
CGTGGAACAAGAGCATCAGCACCGCCTACATGGAAGTGAAGCCGGCTGAGATCCG
ATGACACCGCCGTGTACTACTGCACCATCGACTACTACGACTACGGCGGCTATT
GGGGCCAGGGCACACTGGTTACAGTGTCTCTGCCTCCACCAAGGGCCCATCG
GTCTTCCCCCTGGCACCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTG
GGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAAGTC
AGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCTCAGG
ACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCA
GACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGA
AAGTTGAGCCCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGCCAGCA
CCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCCTAAACCAAGGAC
ACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAG
CCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGC
ATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGT
GGTCAGCGTCTCTACCGTCTCTGCACAGGACTGGCTGAATGGCAAGGAGTACA
AGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCC
AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCG
GGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCT
ATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACA
CTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAG
CAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT
CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
TCTCCGGGTAA

SEQ ID NO: 139 PD1B849 LC cDNA

GACATCCAGATGACACAGAGCCCTAGCAGCCTGTCTGCCTCTGTGGGCGATAG
AGTGACCATCACATGCAAGGCCAGCCAGAACGTGGGCACCAATGTGGCCTGGT
ATCAGCAGAAGCCTGAGAAGGCCCTAAGAGCCTGATCTACAGCGCCAGCTAC
AGATACAGCGGCGTGCCAAGCAGATTTTCTGGAAGCGGCAGCGGCACCGACTT
CACCTGACAATTAGTAGCCTGCAGCCTGAGGACTTCGCCACCTACTACTGCCA
GCAGTACAACATCTACCCCTACACCTTCGGCCAGGGCACCAAGCTGGAAATCA
AGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGT
TGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCAGAG
AGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCAG
GAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCA
CCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAA
GTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGA
GTGT

Figure 2A and Figure 2B shows the alignment of the VH and the VL amino acid sequences of PD1B505 lineage mAbs, respectively.

Figure 3A and Figure 3B shows the alignment of the VL and the VL amino acid sequences of PD1B506 lineage mAbs, respectively.

Figure 4A and Figure 4B shows the alignment of the VH and the VL amino acid sequences of PD1B512 lineage mAbs, respectively.

Example 4. Humanized anti-PD-1 antibodies inhibit antigen specific T cells

Select humanized antibodies were characterized for their ability to inhibit activated T cells in a CMV-specific recall assay (CMV-PBMC assay described in Example 1). The parental antibodies PD1B505 and PD1B506 demonstrated robust inhibition of activated T cells assessed across three different donors in several separate experiments as shown in **Table 16**, in which the results are shown as percentage (%) inhibition of T cell proliferation at 10 µg/ml of mAb. **Figure 5A** shows the mean % inhibition and STDEV for PD1B505 ($57.8\% \pm 9.5\%$) and PD1B506 ($77.0\% \pm 7.8\%$) when compared to the isotype control (human IgG1) ($-9.5\% \pm 28.8\%$). Humanized antibodies PD1B743, PD1B750 and PD1B756 also inhibited T cell activation as shown in **Table 17** as % inhibition at 10 µg/ml. **Figure 5B** shows the mean % inhibition and STDEV for PD1B743 ($58.0\% \pm 11.3\%$) PD1B750 ($65.9\% \pm 13.2\%$), PD1B756 ($36.7\% \pm 15.5\%$), when compared to the isotype control (human IgG1) ($3.5\% \pm 25.6\%$). Assays demonstrating over 25% STDEV were excluded from the analyses. Engineered antibodies PD1B878 and PD1B849 similarly inhibited activated T cells as shown in **Table 18**. **Figure 5C** shows the mean % inhibition and STDEV for PD1B878 ($78.3\% \pm 18.1\%$) and PD1B849 ($69.0\% \pm 4.0\%$) when compared to the isotype control (human IgG1) ($4.1\% \pm 28.7\%$).

Table 16.

	PD1B505		PD1B506		Isotype	
Donor	Mean	STDEV	Mean	STDEV	Mean	STDEV
D1	86.5	9.0	95.2	1.4	4.7	13.3
D2	74.8	0.8	100.1	3.3	13.2	12.9
D1	77	23.0	98	9.8	4.2	33.6
D2	87.7	3.7	100.0	5.8	-16.6	30.6
D3	0.7	8.3	49.5	11.4	6.1	5.7

D1	77.1	4.1	87.8	4.0	-33.1	59.1
D1	69.7	5.4	85.2	2.7	3.1	24.5
D3	63.4	4.9	101.4	8.7	-23.5	44.3
D1	84.5	7.2	86.4	6.5	-35.6	60.4
D1	76.6	2.5	103.1	5.2	-17.7	28.1
D1	49.8	12.7	64.2	11.5	-14.9	19.5
D1	-7.0	17.1	3.8	12.1	-20.0	29.3
D2	49.1	16.6	76.6	6.8	-0.9	28.1
D1	20.1	17.0	26.2	20.7	-2.0	14.2

Table 17.

	PD1B743		PD1B750		PD1B756		Isotype	
Donor	Mean	STDEV	Mean	STDEV	Mean	STDEV	Mean	STDEV
D1	90.1	3.1	97.2	10.0	59.3	18.2	-17.7	28.1
D3	21.1	13.8	74.9	16.7	27.4	14.2	-6.6	17.7
D4	30.5	23.6	24.2	15.5	0.3	17.1	2.7	7.7
D5	64.0	5.9	75.0	9.9	69.3	12.8	14.1	35.6
D1	66.7	14.8	58.1	19.8	27.4	15.4	-13.1	45.2
D3	35.1	8.2	55.6	5.8			0.5	20.8
D1	84.2	2.2	72.1	18.8			43.1	17.5
D1	58.4	8.4	64.7	7.3			5.8	31.6
D1	72.1	21.4	71.6	14.8			2.5	25.9

Table 18.

	PD1B878		PD1B849		Isotype	
Donor	Mean	STDEV	Mean	STDEV	Mean	STDEV
D1			64.3	2.5	5.8	31.6
D1	78.3	18.1	73.7	5.6	2.5	25.9

Example 5. PD-1 antibodies bind human PD-1 with high affinity

Affinity of the parental and humanized antibodies to PD-1 was measured using SPR as described in Example 1.

Table 19 shows the results of the affinity measurements. The antibodies bound PD-1 with a K_D ranging between about 5.2×10^{-8} M and 2.1×10^{-8} M.

Table 19.

mAb	ka (1/Ms)	kd (1/s)	K _D (M)
PD1B505	8.11E+04	2.36E-03	2.91E-08
PD1B742			
PD1B743	9.15E+04	3.00E-03	3.28E-08
PD1B878	5.98E+04	3.51E-03	5.89E-08
PD1B506	1.95E+05	1.02E-02	5.24E-08
PD1B750	3.59E+05	8.32E-03	2.32E-08
PD1B751			
PD1B845*	3.51E+05	8.43E-03	2.40E-08
PD1B846*	3.49E+05	7.53E-03	2.16E-08
PD1B847*	3.03E+05	7.44E-03	2.46E-08
PD1B848*	2.53E+05	9.91E-03	3.92E-08
PD1B849	1.88E+05	8.83E-03	4.71E-08
PD1B850*	3.13E+05	7.19E-03	2.30E-08
PD1B512			
PD1B756	1.18E+05	2.14E-03	1.81E-08
PD1B757			
*Binding was assessed as crude supernatants			

Example 6. PD-1 antibodies differentially block PD-1/ ligand interaction

Ligand blocking was assessed by evaluating the effect of the antibodies on clustering of cells overexpressing either PD-1 or PD-1 ligand (PD-L1 or PD-L2) using the protocol described in Example 1. Lower percentage (%) of recorded double positive events indicated the tested mAb blocked PD-1 binding to the tested ligand.

Figure 6A shows the % PD-1-HEK and PD-L1-HEK cell clusters that remained after treating cells with PD1B743, PD1B750 or PD1B756. PD1B743 and PD1B756 did not block PD-L1 binding to PD-1 whereas PD1B750 did. Similarly, PD1B743 and PD1B756 did not block PD-L2 binding to PD-1 whereas PD1B750 did (**Figure 6B**). Known anti-PD-1 ligand blocking mAb was used as a positive control in the experiments.

Example 7. Epitope binning of antibodies

Five distinct epitope bins were identified in initial matrix assays using chimeric antibodies following the protocol described in Example 1. Bins 4 and 5 did not cross-compete with any other bins. Bins 1, 2 and 3 were partially overlapping; Bin 1 competed with Bin 2 and 3, and Bin 2 and 3 competed with Bin 1. Bins 1, 2, 3 and 4 did not block

PD-L1 binding to PD-1, whereas Bin 5 did block PD-L1/PD-1 interaction. **Figure 7** shows the distinct epitope bins and antibodies within each bin.

A control experiment with isotype control as the first antibody added was done to demonstrate how the second antibody would bind alone to human PD-1. Then in the competition experiment, the signal (nm) of the association of the second antibody was taken at 180 seconds after the start of this step. The signal of each potentially competing antibody was compared to an average of the signal from 3 runs of the same antibody added after isotype control. A ratio of these signals was determined. If the ratio was below 0.7, then it was determined that the second antibody could not bind to human PD-1 and the two antibodies share the same epitope. If the ratio was greater than 0.7, it was determined the second antibody could bind in the presence of the first antibody, and therefore the two antibodies bound to noncompeting epitopes.

Humanization and PTM engineering is not expected to result in a shift in epitope, therefore PD1B743, PD1B742 and PD1B878 is expected to bind the same epitope as the parental chimeric Bin 1 PD1B505. Similarly, PD1B750, PD1B751 and PD1B849 are expected to bind the same epitope as the parental chimeric Bin 5 PD1B506, and PD1B756 is expected to bind the same epitope as the parental chimeric Bin 2 PD1B512.

PD1B503 comprises VH and VL of **SEQ ID NOs: 114 and 115**, respectively.

PD1B517 comprises VH and VL of **SEQ ID NOs: 116 and 117**, respectively.

SEQ ID NO: 114 PD1B503 VH (PD1H96)

QVQLQQSGAELVKPGASVKLSCKASGYTFTSYDINWVRQRPEQGLEWIGWIFPGD
GSTKYNEKFKGKATLTDDKSSSTAYMQFSRLTSEDSAVYFCARGGMRQLGRFVY
WGQGTTTLTVSS

SEQ ID NO: 115 PD1B503 VL (PD1L32)

DIVLTQSPSSLSASLGERVSLTCRASQEISGYLSWLQQKPDGTIKRLIYAASTLDSGV
PKRFSGSRSGSDYSLTISSESEDFADYYCLQYASNPYTFGGGGTKLEIK

SEQ ID NO: 116 PD1B517 VH (PD1H73)

EVQLQQSGAELVKPGASVKLSCTASGFNVKDTYFHWVKQRPDQGLEWIGRIVSAN
 GDTKYAPKLQDKATITTTDTSSNTAYLQLSRLTSEDNAVYYCVLIYYGFEEGDFWG
 QGTTLTVSS

SEQ ID NO: 117 PD1B517 VL (PD1L34)

DIVMTQSPSSLSASLGDTITITCHASQNINWLSWYQQKPGNVPKLLIYKASNLHTG
 VPSRFSGSGSGTGFTLNISLQPEDATYYCQQGQSFPLTFGAGTKLELK

Example 8. Affinity maturation of PD1B878

To improve binding affinity of PD1B878, CDR scans were performed on both heavy and light chains. Non-combinatorial libraries were designed to diversify each position of all six CDRs (HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3). Briefly, Fab libraries were constructed in a pIX phage Fab display system as described in WO2009/085462, Shi *et al.*, *J Mol Biol* 397: 385-396 (2010), and Tornetta *et al.* *J Immunol Methods* 360: 39-46 (2010) with minor modifications to restriction enzyme sites. These libraries were panned against biotinylated Human PD-1/PDCD1 (Acro Biosystems, Cat# PD1-H82E4) according to panning schemes known in the art, such as described in WO2009/085462 and in Shi *et al.*, *J Mol Biol* 397: 385-396 (2010). Phage was produced by helper phage infection. Binders were retrieved by addition of beads to form a bead/antigen/phage complex. After the final wash, phage was rescued by infection of exponentially growing TG-1 *Escherichia coli* cells.

For follow-up screening, plasmid DNA was prepared from overnight culture of the TG-1 *Escherichia coli* cells and the pIX gene was excised by NheI/SpeI digestion. After ligation, the DNA was transformed into TG-1 cells and grown on LB/Agar plates overnight. The next day, colonies were picked, grown overnight, and the cultures used for (i) colony sequencing of the V-regions, and (ii) induction of Fab production. For Fab production, the overnight culture was diluted 100 folds in new media and grown for 5-6 hours at 37°C. Fab production was induced by the addition of fresh media containing IPTG and the cultures were grown overnight at 30°C. The following day, the cultures were spun down and the supernatants, containing the soluble Fab proteins, were used for Fab ELISA. For the ELISA, the soluble Fab proteins were captured onto plates by a polyclonal anti-

Fd(CH1) antibody. After washing and blocking, biotinylated Human PD-1/PDCD1 was added at 5 nM concentration. This concentration enabled ranking of the Fab variants, defined as fold change versus the parent, in which the parental Fab, present as a control in all plates, is defined as 100% binding. The biotinylated human PD-1 was detected by HRP-conjugated streptavidin with chemiluminescence measured in a plate reader. By this criterion, 3 heavy and 2 light chains binding human PD-1 at 10-fold or higher relative to parental Fab were selected.

Table 20 shows the variant with substitutions at position 57 in the HCDR2 of VH and position 29 or 30 in the LCDR1 of VL. **Table 21** shows the SEQ ID NOs: of the CDRs of the antibodies. **Table 22** shows the SEQ ID NOs: of the VH, the VL, the HC and the LC amino acid sequences. **Table 23** shows the SEQ ID NOs: of the polynucleotides encoding the VH, the VL, the HC and the LC of the antibodies. **Table 24** shows the HCDR1, the HCDR2 and the HCDR3 amino acid sequences. **Table 25** shows the LCDR1, the LCDR2 and the LCDR3 amino acid sequences. **Table 26** shows the VH amino acid sequences. **Table 27** shows the VL amino acid sequences. The affinity matured variants are expected to bind the same epitope on PD-1 as the parental antibody PD1B878.

Table 20.

mAb ID	VH peptide ID	VH mutation compared to parental	VL peptide ID	VL mutation compared to parental
PD1B1085	PD1H585	E57Y	PD1L469	
PD1B1086	PD1H586	E57H	PD1L469	
PD1B1087	PD1H587	E57W	PD1L469	
PD1B1088	PD1H405		PD1L651	V29F
PD1B1089	PD1H405		PD1L652	S30P
PD1B1090	PD1H585	E57Y	PD1L651	V29F
PD1B1091	PD1H586	E57H	PD1L651	V29F
PD1B1092	PD1H587	E57W	PD1L651	V29F
PD1B1093	PD1H585	E57Y	PD1L652	S30P
PD1B1094	PD1H586	E57H	PD1L652	S30P
PD1B1095	PD1H587	E57W	PD1L652	S30P

Table 21.

Antibody	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
PD1B1085	2	145	4	5	6	7
PD1B1086	2	146	4	5	6	7
PD1B1087	2	147	4	5	6	7
PD1B1088	2	3	4	148	6	7
PD1B1089	2	3	4	149	6	7
PD1B1090	2	145	4	148	6	7
PD1B1091	2	146	4	148	6	7
PD1B1092	2	147	4	148	6	7
PD1B1093	2	145	4	149	6	7
PD1B1094	2	146	4	149	6	7
PD1B1095	2	147	4	149	6	7

Table 22.

mAb ID	VH peptide ID	VL peptide ID	VH SEQ ID NO:	VL SEQ ID NO:	HC SEQ ID NO:	LC SEQ ID NO:
PD1B1085	PD1H585	PD1L469	140	16	150	28
PD1B1086	PD1H586	PD1L469	141	16	151	28
PD1B1087	PD1H587	PD1L469	142	16	152	28
PD1B1088	PD1H405	PD1L651	10	143	22	153
PD1B1089	PD1H405	PD1L652	10	144	22	154
PD1B1090	PD1H585	PD1L651	140	143	150	153
PD1B1091	PD1H586	PD1L651	141	143	151	153
PD1B1092	PD1H587	PD1L651	142	143	152	153
PD1B1093	PD1H585	PD1L652	140	144	150	154
PD1B1094	PD1H586	PD1L652	141	144	151	154
PD1B1095	PD1H587	PD1L652	142	144	152	154

Table 23.

mAb ID	VH peptide name	VL peptide name	VH cDNA SEQ ID NO:	VL cDNA SEQ ID NO:	HC cDNA SEQ ID NO:	LC cDNA SEQ ID NO:
PD1B1085	PD1H585	PD1L469	155	19	160	31
PD1B1086	PD1H586	PD1L469	156	19	161	31
PD1B1087	PD1H587	PD1L469	157	19	162	31
PD1B1088	PD1H405	PD1L651	13	158	25	163

PD1B1089	PD1H405	PD1L652	13	159	25	164
PD1B1090	PD1H585	PD1L651	155	158	160	163
PD1B1091	PD1H586	PD1L651	156	158	161	163
PD1B1092	PD1H587	PD1L651	157	158	162	163
PD1B1093	PD1H585	PD1L652	155	159	160	164
PD1B1094	PD1H586	PD1L652	156	159	161	164
PD1B1095	PD1H587	PD1L652	157	159	162	164

Table 24.

Antibody	HCDR1 (SEQ ID NO:)	HCDR2 (SEQ ID NO:)	HCDR3 (SEQ ID NO:)
PD1B1085	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGYPT (SEQ ID NO: 145)	DYYGTYFYAMDY (SEQ ID NO: 4)
PD1B1086	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGHPT (SEQ ID NO: 146)	DYYGTYFYAMDY (SEQ ID NO: 4)
PD1B1087	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGWPT (SEQ ID NO: 147)	DYYGTYFYAMDY (SEQ ID NO: 4)
PD1B1088	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGEPT (SEQ ID NO: 3)	DYYGTYFYAMDY (SEQ ID NO: 4)
PD1B1089	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGEPT (SEQ ID NO: 3)	DYYGTYFYAMDY (SEQ ID NO: 4)
PD1B1090	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGYPT (SEQ ID NO: 145)	DYYGTYFYAMDY (SEQ ID NO: 4)
PD1B1091	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGHPT (SEQ ID NO: 146)	DYYGTYFYAMDY (SEQ ID NO: 4)
PD1B1092	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGWPT (SEQ ID NO: 147)	DYYGTYFYAMDY (SEQ ID NO: 4)
PD1B1093	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGYPT (SEQ ID NO: 145)	DYYGTYFYAMDY (SEQ ID NO: 4)
PD1B1094	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGHPT (SEQ ID NO: 146)	DYYGTYFYAMDY (SEQ ID NO: 4)
PD1B1095	GYTFTDYSMH (SEQ ID NO: 2)	WINIETGWPT (SEQ ID NO: 147)	DYYGTYFYAMDY (SEQ ID NO: 4)

Table 25.

Antibody	LCDR1 (SEQ ID NO:)	LCDR2 (SEQ ID NO:)	LCDR3 (SEQ ID NO:)
PD1B1085	TASSSVSSSYLH (SEQ ID NO: 5)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)
PD1B1086	TASSSVSSSYLH (SEQ ID NO: 5)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)
PD1B1087	TASSSVSSSYLH (SEQ ID NO: 5)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)
PD1B1088	TASSSFSSSYLH (SEQ ID NO: 148)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)
PD1B1089	TASSVPSSYLH (SEQ ID NO: 149)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)
PD1B1090	TASSSFSSSYLH (SEQ ID NO: 148)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)
PD1B1091	TASSSFSSSYLH (SEQ ID NO: 148)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)
PD1B1092	TASSSFSSSYLH (SEQ ID NO: 148)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)
PD1B1093	TASSVPSSYLH (SEQ ID NO: 149)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)
PD1B1094	TASSVPSSYLH (SEQ ID NO: 149)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)
PD1B1095	TASSVPSSYLH (SEQ ID NO: 149)	STSNLAS (SEQ ID NO: 6)	HQYHRSPLT (SEQ ID NO: 7)

Table 26.

VH peptide name	VH amino acid sequence	SEQ ID NO:
PD1H585	QVQLVQSGSELKKPGASVKVSCKASGYTFTDYSMHWVR QAPGQGLEWMGWINIETGYPTYAQGFTGRFVFSLDTSVS TAYLQISLKAEDTAVYFCARDYYGTYFYAMDYWGQGT LTVSS	140
PD1H586	QVQLVQSGSELKKPGASVKVSCKASGYTFTDYSMHWVR QAPGQGLEWMGWINIETGHPTYAQGFTGRFVFSLDTSVS TAYLQISLKAEDTAVYFCARDYYGTYFYAMDYWGQGT LTVSS	141
PD1H587	QVQLVQSGSELKKPGASVKVSCKASGYTFTDYSMHWVR QAPGQGLEWMGWINIETGWPTYAQGFTGRFVFSLDTSVS	142

	TAYLQISSLKAEDTAVYFCARDYYGTYFYAMDYWGQGT LVTVSS	
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Table 27.

VL peptide name	VL amino acid sequence	SEQ ID NO:
PD1L651	EIVLTQSPATLSLSPGERATLSCTASSSFSSSYLHWYQQKP GLAPRLLIYSTSNLASGIPDRFSGSGSGTDYTLTISRLEPEDF AVYYCHQYHRSPLTFGQGTKLEIK	143
PD1L652	EIVLTQSPATLSLSPGERATLSCTASSSVPSYHLHWYQQKP GLAPRLLIYSTSNLASGIPDRFSGSGSGTDYTLTISRLEPEDF AVYYCHQYHRSPLTFGQGTKLEIK	144

SEQ ID NO: 150 PD1B1085, PD1B1090, PD1B1093 HC

QVQLVQSGSELKKPGASVKVSCASGYTFTDYSMHWVRQAPGQGLEWMGWINIE
TGYPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCARDYYGTYFYAMD
YWGQGTSLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

SEQ ID NO: 151 PD1B1086, PD1B1091, PD1B1094 HC

QVQLVQSGSELKKPGASVKVSCASGYTFTDYSMHWVRQAPGQGLEWMGWINIE
TGHTPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCARDYYGTYFYAMD
YWGQGTSLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

SEQ ID NO: 152 PD1B1087, PD1B1092, PD1B1095 HC

QVQLVQSGSELKKPGASVKVSCASGYTFTDYSMHWVRQAPGQGLEWMGWINIE
TGWPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCARDYYGTYFYAMD
YWGQGTSLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP

KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVK
 FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
 LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
 GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
 KSLSLSPGK

SEQ ID NO: 153 PD1B1088, PD1B1090, PD1B1091, PD1B1092 LC

EIVLTQSPATLSLSPGERATLSCTASSSFSSSYLHWYQQKPGAPRLLIYSTSNLASGI
 PDRFSGSGSGTDYTLTISRLEPEDFAVYYCHQYHRSPLTFGQGTKLEIKRTVAAPSV
 FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
 TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 154 PD1B1089, PD1B1093, PD1B1094, PD1B1095 LC

EIVLTQSPATLSLSPGERATLSCTASSSVPSYHLHWYQQKPGAPRLLIYSTSNLASG
 IPDRFSGSGSGTDYTLTISRLEPEDFAVYYCHQYHRSPLTFGQGTKLEIKRTVAAPS
 VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
 DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 155 PD1H585 cDNA

CAGGTGCAGCTGGTGCAGTCTGGAAGCGAACTGAAGAAACCTGGAGCCTCTGT
 GAAAGTGTCTTGTAAGGCCAGCGGCTACACCTTCACCGACTACAGCATGCACT
 GGGTGCGGCAGGCCCTGGACAGGGCCTGGAATGGATGGGCTGGATCAACATC
 GAGACCGGCTATCCACCTACGCCAGGGCTTTACCGGACGGTTCGTGTTCAGC
 CTGGATACATCTGTGTCTACAGCCTATCTGCAGATCAGCTCTCTGAAGGCCGAA
 GATACAGCCGTGTACTTCTGCGCCCGGGACTACTACGGCACCTACTTCTACGCC
 ATGGACTACTGGGGCCAGGGAACACTGGTGACAGTGTCTTCT

SEQ ID NO: 156 PD1H586 cDNA

CAGGTGCAGCTGGTGCAGTCTGGAAGCGAACTGAAGAAACCTGGAGCCTCTGT
 GAAAGTGTCTTGTAAGGCCAGCGGCTACACCTTCACCGACTACAGCATGCACT
 GGGTGCGGCAGGCCCTGGACAGGGCCTGGAATGGATGGGCTGGATCAACATC
 GAGACCGGCCATCCACCTACGCCAGGGCTTTACCGGACGGTTCGTGTTCAGC
 CTGGATACATCTGTGTCTACAGCCTATCTGCAGATCAGCTCTCTGAAGGCCGAA
 GATACAGCCGTGTACTTCTGCGCCCGGGACTACTACGGCACCTACTTCTACGCC
 ATGGACTACTGGGGCCAGGGAACACTGGTGACAGTGTCTTCT

SEQ ID NO: 157 PD1H587 cDNA

CAGGTGCAGCTGGTGCAGTCTGGAAGCGAACTGAAGAAACCTGGAGCCTCTGT
 GAAAGTGTCTTGTAAGGCCAGCGGCTACACCTTCACCGACTACAGCATGCACT
 GGGTGCGGCAGGCCCTGGACAGGGCCTGGAATGGATGGGCTGGATCAACATC
 GAGACCGGCTGGCCACCTACGCCAGGGCTTTACCGGACGGTTCGTGTTCAG
 CCTGGATACATCTGTGTCTACAGCCTATCTGCAGATCAGCTCTCTGAAGGCCGA

AGATACAGCCGTGTACTTCTGCGCCCGGGACTACTACGGCACCTACTTCTACGC
CATGGACTACTGGGGCCAGGGAACACTGGTGACAGTGTCTTCT

SEQ ID NO: 158 PD1L651 cDNA

GAGATCGTGCTGACACAGTCTCCTGCCACACTGTCTCTGTCTCCTGGAGAACGG
GCCACACTGAGCTGCACCGCCAGCAGCAGCTTCAGCAGCAGCTACCTGCACTG
GTACCAGCAGAAACCTGGACTGGCCCCCTCGGCTGCTGATCTACAGCACCAGCA
ACCTGGCCAGCGGCATCCCTGATCGGTTTTCTGGCAGCGGATCTGGCACAGATT
ACACACTGACCATCAGCCGGCTGGAACCTGAGGATTTTGCCGTGTACTACTGCC
ACCAGTACCACCGGAGCCCCCTGACCTTCGGCCAGGGAACAAAGCTGGAAATC
AAG

SEQ ID NO: 159 PD1L652 cDNA

GAGATCGTGCTGACACAGTCTCCTGCCACACTGTCTCTGTCTCCTGGAGAACGG
GCCACACTGAGCTGCACCGCCAGCAGCAGCGTGCCAAGCAGCTACCTGCACTG
GTACCAGCAGAAACCTGGACTGGCCCCCTCGGCTGCTGATCTACAGCACCAGCA
ACCTGGCCAGCGGCATCCCTGATCGGTTTTCTGGCAGCGGATCTGGCACAGATT
ACACACTGACCATCAGCCGGCTGGAACCTGAGGATTTTGCCGTGTACTACTGCC
ACCAGTACCACCGGAGCCCCCTGACCTTCGGCCAGGGAACAAAGCTGGAAATC
AAG

SEQ ID NO: 160 PD1B1085, PD1B1090, PD1B1093 HC cDNA

TCTGATAAGAGTCAGAGGTAAC TCCCGTTGCGGTGCTGTTAACGGTGGAGGGC
AGTGTAGTCTGAGCAGTACTCGTTGCTGCCGCGCGGCCACCAGACATAATAG
CTGACAGACTAACAGACTGTTCCCTTTCCATGGGTCTTTTCTGCAGTCACCGTCCT
TAGATCCACTAGTCCAGTGTGGTGAAGCTTGCCGCCACCATGGCTTGGGTGTGG
ACCTTGCTATTCCCTGATGGCAGCTGCCCAAAGTATACAGGCCCAGGTGCAGCTG
GTGCAGTCTGGAAGCGAACTGAAGAAACCTGGAGCCTCTGTGAAAGTGTCTTG
TAAGGCCAGCGGCTACACCTTCACCGACTACAGCATGCACTGGGTGCGGCAGG
CCCCTGGACAGGGCCTGGAATGGATGGGCTGGATCAACATCGAGACCGGCTAT
CCCACCTACGCCCAGGGCTTTACCGGACGGTTCGTGTTACGCCTGGATACATCT
GTGTCTACAGCCTATCTGCAGATCAGCTCTCTGAAGGCCGAAGATACAGCCGT
GTACTTCTGCGCCCGGGACTACTACGGCACCTACTTCTACGCCATGGACTACTG
GGGCCAGGGAACACTGGTGACAGTGTCTTCTGCCTCCACCAAGGGCCCATCGG
TCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGG
GCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCA
GGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGA
CTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG
ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAA
AGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGCCAGCAC
CTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAACCCAAGGACA
CCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGACGTGAGCC

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SEQ ID NO: 161 PD1B1086, PD1B1091, PD1B1094 HC cDNA

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SEQ ID NO: 162 PD1B1087, PD1B1092, PD1B1095 HC cDNA

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SEQ ID NO: 163 PD1B1088, PD1B1090, PD1B1091, PD1B1092 LC cDNA

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SEQ ID NO: 164 PD1B1089, PD1B1093, PD1B1094, PD1B1095 LC cDNA

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 TCGAATTCCTAGAAGACATGATAAGATACATTGATGAGTTTGGACAAACCACA
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 TTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATT
 CATTTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGA

Affinity of the antibodies to human and cyno PD-1 was measured using SPR as described in Example 1. The antibodies bound human PD-1 with a K_D ranging between 1×10^{-8} M to 10×10^{-10} M (**Table 28**) and to cyno PD-1 with a K_D ranging between 7×10^{-8} M

to 1×10^{-9} M (Table 26). The affinities of majority of the affinity matured PD1B878 variants improved about 100-fold when compared to the parental antibody.

Table 28.

mAb	k_a (1/Ms)	k_d (1/s)	KD (M)
PD1B1085	1.56E+05	1.42E-04	9.11E-10
PD1B1086	1.24E+05	3.94E-04	3.19E-09
PD1B1087	1.66E+05	2.39E-04	1.44E-09
PD1B1088	3.13E+04	3.41E-04	1.09E-08
PD1B1089	5.86E+04	4.74E-04	8.09E-09
PD1B1090	1.06E+05	2.37E-05	2.23E-10
PD1B1091	7.77E+04	6.44E-05	8.30E-10
PD1B1092	1.40E+05	3.00E-05	2.14E-10
PD1B1093	1.25E+05	4.36E-05	3.49E-10
PD1B1094	1.05E+05	9.16E-05	8.71E-10
PD1B1095	1.51E+05	7.12E-05	4.71E-10

Table 29.

mAb	k_a (1/Ms)	k_d (1/s)	KD (M)
PD1B1085	2.00E+05	1.29E-03	6.45E-09
PD1B1086	1.71E+05	5.44E-03	3.18E-08
PD1B1087	2.12E+05	2.47E-03	1.16E-08
PD1B1088	6.61E+04	4.79E-03	7.24E-08
PD1B1089	9.32E+04	8.39E-03	9.00E-08
PD1B1090	1.30E+05	1.85E-04	1.43E-09
PD1B1091	9.91E+04	9.24E-04	9.32E-09
PD1B1092	1.29E+05	4.11E-04	3.18E-09
PD1B1093	1.69E+05	3.68E-04	2.18E-09
PD1B1094	1.35E+05	1.62E-03	1.20E-08
PD1B1095	1.85E+05	7.81E-04	4.23E-09

PD1B1086, PD1B1090 and PD1B1094 were characterized for their ability to inhibit activated T cells in the CMV-specific recall assay (CMV-PBMC assay described in Example 1). Table 30 shows the mean percentage inhibition across two donors in one experiment. All tested antibodies inhibited CMV-specific recall assay by a degree of 70% or more.

Table 30.

	PD1B1086	PD1B1090	PD1B1094	IgG1
Mean %	72.7	74.8	77.9	6.0

Example 9. PD-1 agonistic antibodies selectively target chronically activated memory T cells

PD-1 was found mainly expressed on memory T cells (CD45RO⁺ cells) and not on naïve T cells, and the expression was found upregulated upon T cell activation. PD-1 expression increased on memory T cells stimulated with CMV peptides (**Figure 8A**).

Antibodies PD1B849 and PD1B878 were tested for their ability to inhibit activated memory CD4⁺ or CD8⁺ T cell proliferation utilizing CMV activated PBMCs. Both PD1B849 and PD1B878 inhibited proliferation of CMV-specific activated PBMCs in the CMV-PBMC recall assay (**Figure 8B**).

Example 10. PD-1 agonistic antibodies deplete activated but not resting memory T cells by ADCC

PD1B849 and PD1B878 were tested for their ability to mediate ADCC of activated memory T cells or resting memory T cells using NK cells or PBMCs as effector cells. Activated memory T cells were identified to have higher expression of PD-1 when compared to resting memory T cells. The experiment was conducted according to protocol described in Example 1. PD1B849 and PD1B878 were expressed in two separate CHO cell lines, the one producing antibodies with normal CHO antibody glycosylation profile and the other producing antibodies having reduced carbohydrate fucosyl content (e.g. low fucose (LF) cell line). Antibodies expressed in the low fucose cell line had a fucosyl content of about 1-15%.

PD1B849 and PD1B878 elicited ADCC of activated memory T cells both in the presence of NK (**Figure 9A**, left panel) cells and PBMC (**Figure 9A**, right panel) effector cells. Antibodies with low fucose content (PD1B849-LF and PD1B878-LF) demonstrated stronger ADCC activity towards memory T cells. PD1B849 and PD1B878 were unable to elicit detectable ADCC in resting memory T Cells which express low levels of PD-1 either in the presence of NK cells (**Figure 10A**, left panel) or PBMCs (**Figure 10B**, right panel)

effector cells. PD1B849-LF and PD1B878-LF triggered low level ADCC in the presence of either NK cells or PBMCs.

Example 11. PD-1 agonistic antibodies do not elicit CDC

PD1B849 and PD1B878 were tested for their ability to mediate CDC of activated pan T cells using added rabbit complement. Activated T cells have higher expression of PD-1 when compared to resting T cells. The experiment was conducted according to protocol described in Example 1. PD1B849 and PD1B878 did not elicit CDC of activated T cells at the concentrations tested (**Figure 11**). The positive control OKT3 demonstrated CDC activity towards activated T cells in the presence, but not absence of complement.

Example 12. Affinity-matured antibodies do not block PD-L1 binding to PD-1

Select antibodies were tested for their ability to block binding of dextramerized PD-L1-Fc to PD-1 expressing Jurkat cells. The experiment was conducted according to protocol described below. PD1B878, PD1B1090 and PD1B1094 did not block the binding of PD-L1 at the concentrations tested (**Figure 12**). As positive control, a known antagonist antibody was shown to compete with PD-L1 for binding to PD-1 in a dose dependent manner.

Method: Biotinylated PD-L1-Fc (Acro Biosystems) and SA & APC conjugated Dextramers (Immudex) were prepared at 4x concentration and were mixed at a ratio of 100nM:10nM in staining buffer. Biotinylated IgG1-Fc with Dextramers and Dextramers with media alone mixtures were prepared in the same manner for negative or non-specific binding controls. This mixture was covered with foil and incubated on ice for one hour while preparing the rest of the experiment. Serial dilutions of test antibodies were prepared in stain buffer at a 2x concentration of 20nM, 2nM and 0.2nM. Jurkat cells over expressing PD-1 were harvested on the day of the assay and washed once with stain buffer (BD Pharmingen) by spinning the cells at 300g for 5 minutes at 4°C. Cells were counted, checked for viability and were resuspended at 2×10^6 cell/mL in stain buffer. Cells were added at 25uL/well (50000 cells/well) to U-bottom 96-well assay plate followed by the addition of prepared test antibodies at 50 μ L/well. The cells with antibodies were incubated for 15 minutes on ice. Pre-mixed complexes of biotinylated PD-L1-Fc:Dextramer, biotinylated IgG1-Fc:Dextramer and Dextramers: buffer were added at 25

μL/well. This mixture of cells, antibodies and PD-L1 bound to Dextramers was incubated for 1 hour on ice and covered with foil.

Cells were washed two times by adding 150 μL stain buffer, centrifuging at 300g for 5 minutes to pellet cells and flicking plates to remove supernatants. Cell pellet after final wash was resuspended in 40 μL of IntelliCyt running buffer (BD stain buffer supplemented with 1mM EDTA and 0.1% Pluronic acid) containing 1:1000 dilution of Sytox green live/dead cell viability stain (ThermoFisher). Final antibody concentrations in the assay were at 10nM, 1nM and 0.1nM. Final biotinylated PD-L1-Fc ligand protein concentration was at 25nM. Final Dextramer concentration was at 2.5nM.

Plates were run on iQue Screener (IntelliCyt). Briefly cells were gated on FCS v. SCS to eliminate debris. Singlets were gated on SCS-A vs SCS-H and from singlet population, live cells were gated on low BL1 channel for negative with Sytox green viability stain. Percent positive live cells binding to PD-L1-Fc-Dextramers was assessed by Geomeans in RL1/APC channel and compared to negative control, Fc-Dex alone binding. Using advanced metrics in ForeCyt (software program from IntelliCyt), PD-L1 positive population was calculated as % of live population. Percent specific PD-L1 binding was calculated as follows = (%positive with mAb - %positive with IgG1-Fc biotin dextramer) / (%positive with Isotype control - %positive with IgG1-Fc biotin dextramer) * 100. Final results are tabulated in excel and graphed in prism showing % PD-L1 ligand binding in the presence of anti PD-1 mAbs.

Example 13. PD-1 agonistic antibodies are effective in a mouse model of graft vs. host disease (GvHD)

To study the effect of PD-1 agonist mAbs on pathogenic T cells *in vivo*, a xenogeneic Graft-versus-Host Disease (Xeno-GVHD) model was developed by adoptively transferring human peripheral blood mononuclear cells (PBMC) into immunocompromised NOD-scid IL-2Rγ^{null} (NSG) mice. Female NSG mice (7-9 weeks of age) were obtained from Jackson Labs. The mice were quarantined in the vivarium facility for one week before use. Frozen human PBMCs isolated from buffy coats were obtained from AllCells, Alameda, CA. Before injection, frozen cells were quickly thawed at 37°C in water bath and washed 3 times with sterile phosphate buffered saline (PBS) by centrifugation at 500 g

for 5 min at room temperature. The cells were suspended in cold PBS to obtain a final cell concentration of $50 \times 10^6/\text{mL}$.

Animals were randomized by body weight and divided into various treatment groups (N=10/group). Following randomization conscious and freely moving mice received total body irradiation of 100 Rad (1Gy) using Gammacell® 3000 Elan Irradiator (9.72 Gy/minute). Mice were placed back into their home cages.

Each mouse received 25×10^6 human PBMCs in a volume of 500 μL intraperitoneally. Clinical scores and body weight of each animal was recorded daily according to **Table 31**. Mice that lost $\geq 20\%$ of their body weight were sacrificed and their clinical score at end-point was recorded in accordance with institutional IACUC guidelines. All animals were euthanized on day 21. Spleens were collected for FACS analysis. Skin and colon tissue was collected for histological analyses.

Table 31.

Description	Clinical Score
Normal Alert and Reactive	0
Ruffled Haircoat, Decreased activity, Ocular Discharge	1
Hunched posture, Moderate Hypothermia or Hyperthermia, labored breathing during prodding	2
Labored Breathing during rest, Ataxia, tremor, Hypothermia or Hyperthermia	3
Loss of ability to ambulate with gentle prodding, unconscious	4
Death	5

Mice were injected intraperitoneally with 10 mg/kg PD1B505, PD1B506, PD1B849 and PD1B878 mAbs which were cloned as chimeric mIgG2a antibodies, as well as PD-1 antagonist mAb (PD1B786 on mouse effector silent Fc), on a Q4d/Q3d regimen (dosing on days 0, 4, 7, 11, 14, and 18). CTLA-4-Ig, used as a control, was dosed on a Q3d dosing regimen (Day 0, 3, 6, 9, 12, 15 and 18) 10 mg/kg intraperitoneally.

At study termination, spleens were removed to cold RPMI1640 medium. After crushing spleens through a 70 μm filter and pelleting cells by centrifugation at 1200 rpm

for 5 minutes at 4°C, red blood cells were lysed in ACK lysing buffer (Lonza) on ice for 5 minutes, and washed multiple times in in FACS buffer (PBS/0.5% BSA/2 mM EDTA). Viability of splenocytes was assessed by staining cells with ef506 viability dye (eBioscience) according to the manufacturer's instructions. Splenocytes were incubated with human and mouse Fc block (BD Biosciences) for 15 min on ice, then stained with optimal concentrations of fluorochrome conjugated mAbs (1×10^6 cells in 100 μ l Brilliant Stain buffer) in U shaped microtiter plates at 4°C for 30 minutes, and fixed with Fixation buffer (BD Biosciences). Count Brite beads (Thermofisher) were added to each sample. FMO (fluorescence minus one) controls were prepared as negative controls for each fluorochrome. Samples were analyzed on an LSRII instrument (BD Biosciences). The following mAbs were used for regulatory T cell analysis, anti-hCD45 peridinin chlorophyll alpha protein (PCP, clone 2D1), anti-FoxP3 allophycocyanin (APC, clone pCH101), anti-hCD3 allophycocyanin-cyanine 7 (APC-Cy7, clone HIT3a), anti-hCD4 Brilliant Violet 605 (BV605, clone OKT4), and anti-hCD25 Brilliant violet 650 (BV650, clone M-A251). Regulatory T cells were defined as hCD45⁺ hCD3⁺ hCD4⁺, Foxp3⁺ CD25⁺.

Figure 13A shows that treatment with PD1B505-mIgG2a and PD1B506-mIgG2a prevented disease development in the mouse model of GvHD. **Figure 13B** shows that treatment with PD1B505-mIgG2a and PD1B506-mIgG2a prevented weight loss in the mouse model of GvHD. **Figure 14A** shows that treatment with PD1B849-mIgG2a and PD1B878-mIgG2a prevented disease development in the mouse model of GvHD. **Figure 14B** shows that treatment with PD1B849-mIgG2a and PD1B878-mIgG2a prevented weight loss in the mouse model of GvHD. Inhibition of weight loss and clinical score was associated with an increase in regulatory T cells in the spleen. **Figure 15** shows the Treg frequency in spleen of animals treated with PD1B849-mIgG2a or PD1B878-mIgG2a.

Example 14. PD-1 agonistic antibodies deplete T follicular helper (T_{FH}) and T peripheral helper (T_{PH}) cells

Human PBMCs were retrieved from liquid nitrogen cryostorage and thawed rapidly in 37°C water bath until just thawed. Contents of vial were transferred to a sterile 50ml conical tube (separate tubes for each donor used) and complete RPMI media (10% FBS, 1x Penicillin/streptomycin, 1x sodium pyruvate) was added to each tube drop-wise to a total volume of 15ml. Cells were centrifuged at 250xg for 10min at RT then supernatant

was discarded and cells were resuspended in 5-10ml of complete media and counted using trypan blue exclusion. Cells were resuspended at 2.5×10^6 cells/ml and plated at 2.5×10^5 cells/well (=100 μ l/well) in triplicate in a 96-well sterile U-bottom polystyrene plate. PD-1 mAbs or human IgG1 isotype control were diluted to 4x final concentrations and 50 μ l/well were added to appropriate wells. After addition of antibodies, normal human serum was added to a final concentration of 5%. Total volume in each well was 200 μ l and cells were incubated for 96 hours at 37°C, 5% CO₂. In addition to samples, several wells of extra cells were plated, received 5% human serum, and were incubated alongside treated samples to be used as flow cytometry staining controls.

After incubation, plates were centrifuged at 350xg for 5min and supernatants were vacuumed off. Cells were resuspended in 200 μ l PBS and replicate wells were pooled then transferred to a new 96-well U-bottom plate to stain for flow cytometry. Pooled cells were centrifuged at 350xg for 5min, supernatants vacuumed off and fluorescent antibody cocktail was added to each sample well (see table below for antibodies used). Additional cells saved for controls were stained with FMO cocktails for critical markers such as PD-1, CXCR5, ICOS, among others, to be used in analysis for defining gates. Cells were stained for 25min in the dark at RT then centrifuged at 350xg for 5min. Cells were washed twice in 200 μ l PBS then 100 μ l of 4% paraformaldehyde was added to each well to fix the cells. Cells were fixed for 10min in the dark at 4°C then washed once with PBS+1% BSA before resuspending in 200 μ l PBS+1% BSA. Counting beads (Invitrogen) were added at 5 μ l/well (5000 beads) for each sample prior to acquiring samples on the BD LSR II flow cytometer. Flow cytometry data was analyzed using FlowJo software. Sample counts were normalized to bead counts as such: # cells in sample = (# cells counted*5000 beads added)/(# beads counted). Samples were normalized to huIgG1 isotype control as such: (bead-normalized cell counts “sample”/bead-normalized cell counts “isotype”)*100 and represented as a percentage (%). Data was graphed using GraphPad Prism v7.

During analysis, the following cell populations were described:

T follicular helper (Tfh): live, CD19-CD56-/CD4+CD45RO+/HLADR+/CXCR5+/ICOS+PD1+;
 T peripheral helper (Tph): live, CD19-CD56-/CD4+CD45RO+/HLADR+/CXCR5-/ICOS+PD1+;
 Combination Tfh/Tph population: live, CD19-CD56-/CD4+CD45RO+/HLADR+/ICOS+PD1+.

Figure 16 shows the dose response curve of antibody-mediated depletion of the combined T_{HF}/T_{PH} population by PD1B878, PD1B878-FL (low fucose), PD1B1090 and PD1B1094. The data was respresented as mean % fold change in the number of T_{FH}/T_{PH} cells from isotype control using n=8 healthy human donors (n=7 for PD1B1090). PD1B878-FL were most effective in depleting the T_{FH}/T_{PH} population.

We claim

- 1) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, a HCDR3, a light chain complementarity determining region 1 (LCDR1), a LCDR2 and a LCDR3 of SEQ ID NOS: 2, 165, 4, 166, 6 and 7, respectively.
- 2) The antibody or the antigen binding fragment thereof of claim 1, wherein the antibody or the antigen binding fragment thereof has one, two, three, four or five of the following properties:
 - a) does not block PD-L1 binding to PD-1; wherein lack of blocking is measured by inability of the antibody to inhibit clustering of PD-L1 expressing and PD-1 expressing cells as described in Example 1;
 - b) binds PD-1 with an equilibrium dissociation constant (K_D) of about 5×10^{-8} M or less, wherein the K_D is measured using a ProteOn XPR36 system at +25°C;
 - c) binds PD-1 with an association constant (k_a) of about 3×10^4 1/Ms or more, wherein the k_a is measured using a ProteOn XPR36 system at +25°C;
 - d) binds PD-1 with a dissociation constant (k_d) of about 3×10^{-3} 1/s or less, wherein the k_d is measured using a ProteOn XPR36 system at +25°C; or
 - e) inhibits proliferation of antigen specific T cells; wherein proliferation is assessed in a CMV-PBMC assay as described in Example 1.
- 3) The antibody or the antigen binding fragment thereof of claim 1 or 2, comprising
 - a) a heavy chain variable region (VH) framework derived from IGHV7-4-1*1 (SEQ ID NO: 125);
 - b) a light chain variable region (VL) framework derived from IGKV3D-20*1 (SEQ ID NO: 126); or
 - c) the VH framework derived from IGHV7-4-1*1 (SEQ ID NO: 125) and the VL framework derived from IGKV3D-20*1 (SEQ ID NO: 126).
- 4) The antibody or the antigen binding fragment thereof of any one of claims 1-3, comprising the HCDR2 of SEQ ID NOS: 3, 145, 146 or 147 and/or the LCDR1 of SEQ ID NOS: 5, 148 or 149.

- 5) The antibody or the antigen binding fragment thereof of any one of claims 1-4, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of
- a) SEQ ID NOs: 2, 3, 4, 5, 6 and 7, respectively;
 - b) SEQ ID NOs: 2, 145, 4, 5, 6, and 7, respectively;
 - c) SEQ ID NOs: 2, 146, 4, 5, 6, and 7, respectively;
 - d) SEQ ID NOs: 2, 147, 4, 5, 6, and 7, respectively;
 - e) SEQ ID NOs: 2, 3, 4, 148, 6 and 7, respectively;
 - f) SEQ ID NOs: 2, 3, 4, 149, 6 and 7, respectively;
 - g) SEQ ID NOs: 2, 145, 4, 148, 6 and 7, respectively;
 - h) SEQ ID NOs: 2, 146, 4, 148, 6 and 7, respectively;
 - i) SEQ ID NOs: 2, 147, 4, 148, 6 and 7, respectively;
 - j) SEQ ID NOs: 2, 145, 4, 149, 6 and 7, respectively;
 - k) SEQ ID NOs: 2, 146, 4, 149, 6 and 7, respectively; or
 - l) SEQ ID NOs: 2, 147, 4, 149, 6 and 7, respectively.
- 6) The antibody or the antigen binding fragment thereof of any one of claims 1-5, comprising
- a) a heavy chain variable region (VH) of SEQ ID NO: 118;
 - b) a light chain variable region (VL) of SEQ ID NO: 119; or
 - c) the VH and the VL of SEQ ID NOs: 118 and 119, respectively.
- 7) The antibody or the antigen binding fragment thereof of any one of claims 1-6, comprising
- a) the VH of SEQ ID NOs: 8, 9, 10, 140, 141 or 142;
 - b) the VL of SEQ ID NOs: 14, 15, 16, 143 or 144;
 - c) the VH of SEQ ID NO: 8 and the VL of SEQ ID NO: 14;
 - d) the VH of SEQ ID NO: 9 and the VL of SEQ ID NO: 15;
 - e) the VH of SEQ ID NO: 9 and the VL of SEQ ID NO: 16;
 - f) the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 16;
 - g) the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 16;
 - h) the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 16;
 - i) the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 16;
 - j) the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 143;

- k) the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 144;
 - l) the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 143;
 - m) the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 143;
 - n) the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 143;
 - o) the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 144;
 - p) the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 144; or
 - q) the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 144.
- 8) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, wherein the antibody or the antigen binding fragment thereof competes for binding to PD-1 with the antibody or the antigen binding fragment thereof of claim 7.
- 9) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 3, 4, 5, 6 and 7, respectively;
 - b) the VH of SEQ ID NOs: 8, 9 or 10 and the VL of SEQ ID NOs: 14, 15 or 16; and/or
 - c) a heavy chain (HC) of SEQ ID NO: 20, 21 or 22 and a light chain (LC) of SEQ ID NO: 26, 27 or 28.
- 10) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 145, 4, 5, 6, and 7, respectively;
 - b) the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 16; and/or
 - c) the HC of SEQ ID NO: 150 and the LC of SEQ ID NO: 28.
- 11) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 146, 4, 5, 6, and 7, respectively;
 - b) the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 16; and/or
 - c) the HC of SEQ ID NO: 151 and the LC of SEQ ID NO: 28.
- 12) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising

- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 147, 4, 5, 6, and 7, respectively;
 - b) the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 16; and/or
 - c) the HC of SEQ ID NO: 152 and the LC of SEQ ID NO: 28.
- 13) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 3, 4, 148, 6 and 7, respectively;
 - b) the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 143; and/or
 - c) the HC of SEQ ID NO: 22 and the LC of SEQ ID NO: 153.
- 14) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 3, 4, 149, 6 and 7, respectively;
 - b) the VH of SEQ ID NO: 10 and the VL of SEQ ID NO: 144; and/or
 - c) the HC of SEQ ID NO: 22 and the LC of SEQ ID NO: 154.
- 15) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 145, 4, 148, 6 and 7, respectively;
 - b) the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 143; and/or
 - c) the HC of SEQ ID NO: 150 and the LC of SEQ ID NO: 153.
- 16) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 146, 4, 148, 6 and 7, respectively;
 - b) the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 143; and/or
 - c) the HC of SEQ ID NO: 151 and the LC of SEQ ID NO: 153.
- 17) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 147, 4, 148, 6 and 7, respectively;

- b) the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 143; and/or
 - c) the HC of SEQ ID NO: 152 and the LC of SEQ ID NO: 153.
- 18) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 145, 4, 149, 6 and 7, respectively;
 - b) the VH of SEQ ID NO: 140 and the VL of SEQ ID NO: 144; and/or
 - c) the HC of SEQ ID NO: 150 and the LC of SEQ ID NO: 154.
- 19) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 146, 4, 149, 6 and 7, respectively;
 - b) the VH of SEQ ID NO: 141 and the VL of SEQ ID NO: 144; and/or
 - c) the HC of SEQ ID NO: 151 and the LC of SEQ ID NO: 154.
- 20) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 2, 147, 4, 149, 6 and 7, respectively.
 - b) the VH of SEQ ID NO: 142 and the VL of SEQ ID NO: 144; and/or
 - c) the HC of SEQ ID NO: 152 and the LC of SEQ ID NO: 154.
- 21) The antibody or the antigen binding fragment thereof of any one of claims 1-20, wherein the antibody or the antigen binding fragment thereof is an agonist of PD-1 or mediates antibody-dependent cell cytotoxicity (ADCC) of PD-1 expressing cells, or both.
- 22) The antibody or the antigen binding fragment thereof of claim 21, wherein the PD-1 expressing cells are activated memory T cells, T follicular helper cells (T_{FH}) or T peripheral helper cells (T_{PH}), or any combination thereof.
- 23) The antibody or the antigen binding fragment thereof of any one of claims 1-22, wherein the antibody or the antigen binding fragment thereof is an IgG1, an IgG2, and IgG3 or an IgG4 isotype.
- 24) The antibody or the antigen binding fragment thereof of any one of claims 1-23, wherein the antibody or the antigen binding fragment thereof comprises at least one

mutation in the antibody Fc that modulates binding of the antibody to an Fc receptor (FcR).

25) The antibody or the antigen binding fragment thereof of claim 24, wherein the at least one mutation is

- a) a S267E mutation;
- b) a S267D mutation;
- c) a S267E/I332E mutation;
- d) a S267E/L328F mutation;
- e) a G236D/S267E mutation;
- f) a P238D mutation; or
- g) a P238D/E233D/G237D/H268D/P271G/A330R mutation.

26) The antibody or the antigen binding fragment thereof of any one of claims 1-8, wherein the antibody comprises

- a) a heavy chain (HC) of SEQ ID NOs: 20, 21, 22, 150, 151 or 152;
- b) a light chain (LC) of SEQ ID NOs: 26, 27, 28, 153 or 154;
- c) the HC of SEQ ID NOs: 20, 21, 22, 150, 151 or 152 and the LC of SEQ ID NOs: 26, 27, 28, 153 or 154;
- d) the HC of SEQ ID NO: 20 and the LC of SEQ ID NO: 26;
- e) the HC of SEQ ID NO: 21 and the LC of SEQ ID NO: 27;
- f) the HC of SEQ ID NO: 21 and the LC of SEQ ID NO: 28;
- g) the HC of SEQ ID NO: 22 and the LC of SEQ ID NO: 28;
- h) the HC of SEQ ID NO: 150 and the LC of SEQ ID NO: 28;
- i) the HC of SEQ ID NO: 151 and the LC of SEQ ID NO: 28;
- j) the HC of SEQ ID NO: 152 and the LC of SEQ ID NO: 28;
- k) the HC of SEQ ID NO: 22 and the LC of SEQ ID NO: 153;
- l) the HC of SEQ ID NO: 22 and the LC of SEQ ID NO: 154;
- m) the HC of SEQ ID NO: 150 and the LC of SEQ ID NO: 153;
- n) the HC of SEQ ID NO: 151 and the LC of SEQ ID NO: 153;
- o) the HC of SEQ ID NO: 152 and the LC of SEQ ID NO: 153;
- p) the HC of SEQ ID NO: 150 and the LC of SEQ ID NO: 154;
- q) the HC of SEQ ID NO: 151 and the LC of SEQ ID NO: 154; or
- r) the HC of SEQ ID NO: 152 and the LC of SEQ ID NO: 154.

- 27) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, wherein the antibody VH and the antibody VL or the antibody HC and the antibody LC are encoded by a polynucleotide comprising the polynucleotide sequence of
- a) SEQ ID NOs: 11 and 17, respectively;
 - b) SEQ ID NOs: 12 and 18, respectively;
 - c) SEQ ID NOs: 12 and 19, respectively;
 - d) SEQ ID NOs: 13 and 19, respectively;
 - e) SEQ ID NOs: 23 and 29, respectively;
 - f) SEQ ID NOs: 24 and 30, respectively;
 - g) SEQ ID NOs: 24 and 31, respectively;
 - h) SEQ ID NOs: 25 and 31, respectively;
 - i) SEQ ID NOs: 132 and 133, respectively;
 - j) SEQ ID NOs: 134 and 135, respectively;
 - k) SEQ ID NOs: 155 and 19, respectively;
 - l) SEQ ID NOs: 156 and 19, respectively;
 - m) SEQ ID NOs: 157 and 19, respectively;
 - n) SEQ ID NOs: 13 and 158, respectively;
 - o) SEQ ID NOs: 13 and 159, respectively;
 - p) SEQ ID NOs: 155 and 158, respectively;
 - q) SEQ ID NOs: 156 and 158, respectively;
 - r) SEQ ID NOs: 157 and 158, respectively;
 - s) SEQ ID NOs: 155 and 159, respectively;
 - t) SEQ ID NOs: 156 and 159, respectively;
 - u) SEQ ID NOs: 157 and 159, respectively;
 - v) SEQ ID NOs: 160 and 31, respectively;
 - w) SEQ ID NOs: 161 and 31, respectively;
 - x) SEQ ID NOs: 162 and 31, respectively;
 - y) SEQ ID NOs: 25 and 163, respectively;
 - z) SEQ ID NOs: 25 and 164, respectively;
 - aa) SEQ ID NOs: 160 and 163, respectively;
 - bb) SEQ ID NOs: 161 and 163, respectively;

- cc) SEQ ID NOs: 162 and 163, respectively;
 - dd) SEQ ID NOs: 160 and 164, respectively;
 - ee) SEQ ID NOs: 161 and 164, respectively; or
 - ff) SEQ ID NOs: 162 and 164, respectively.
- 28) The antibody or the antigen binding fragment thereof of any one of claims 1-27, wherein the antibody or the antigen binding fragment thereof has a biantennary glycan structure with a fucose content of about between 1% to about 15%.
- 29) A polynucleotide
- a) encoding the VH of SEQ ID NOs: 118, 8, 9, 10, 140, 141 or 142;
 - b) encoding the VL of SEQ ID NOs: 119, 14, 15, 16, 143 or 144;
 - c) encoding the VH of SEQ ID NOs: 118, 8, 9, 10, 140, 141 or 142 and the VL of SEQ ID NOs: 119, 14, 15, 16, 143 or 144;
 - d) encoding the HC of SEQ ID NOs: 20, 21, 22, 150, 151 or 152;
 - e) encoding the LC of SEQ ID NOs: 26, 27, 28, 153 or 154;
 - f) encoding the HC of SEQ ID NOs: 20, 21, 22, 150, 151 or 152 and the LC of SEQ ID NOs: 26, 27, 28, 153 or 154; or
 - g) comprising the polynucleotide sequence of SEQ ID NOs: 11, 12, 13, 17, 18, 19, 23, 24, 25, 29, 30, 31, 132, 133, 134, 135, 155, 156, 157, 158, 159, 160, 161, 162, 163 or 164.
- 30) A vector comprising at least one polynucleotide of claim 29.
- 31) A host cell comprising the vector of claim 30.
- 32) The host cell of claim 31, wherein the host cell is a eukaryotic cell, a prokaryotic cell, a CHO cell, a HEK293 cell or a hybridoma.
- 33) A method of making the antibody or the antigen binding fragment thereof of any one of claims 1-28, comprising culturing the host cell of claim 31 in conditions that the antibody or the antigen binding fragment thereof is expressed, and isolating the antibody or the antigen binding fragment thereof.
- 34) A pharmaceutical composition comprising the antibody or the antigen binding fragment thereof of any one of claims 1-28.
- 35) A kit comprising the antibody or the antigen binding fragment thereof of any one of claims 1-28.

- 36) A method of suppressing activation of a PD-1 expressing T cell in a subject, comprising administering to a subject the isolated antibody or the antigen binding fragment thereof of any one of claims 1-28 for a time sufficient to suppress activation of the PD-1 expressing T cell.
- 37) The method of claim 36, wherein the PD-1 expressing T cell is an antigen-specific CD4⁺ T cell and/or an antigen-specific CD8⁺ T cell.
- 38) A method of downmodulating an immune response comprising administering to a subject in need thereof a therapeutically effective amount of the isolated antibody or the antigen binding fragment thereof of any one of claims 1-28 to downmodulate the immune response.
- 39) A method of treating an immune disorder comprising administering to a subject in need thereof a therapeutically effective amount of the isolated antibody or the antigen binding fragment thereof of any one of claims 1-28 to treat the immune disorder.
- 40) The method of claim 39, wherein the immune disorder is lupus, systemic lupus erythematosus, Sjogren's Syndrome, arthritis, rheumatoid arthritis, asthma, COPD, pelvic inflammatory disease, Alzheimer's Disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, Peyronie's Disease, coeliac disease, gallbladder disease, Pilonidal disease, peritonitis, psoriasis, psoriatic arthritis, vasculitis, surgical adhesions, stroke, Type I Diabetes, Lyme disease, meningoencephalitis, autoimmune uveitis, multiple sclerosis, , Guillain-Barr syndrome, Atopic dermatitis, autoimmune hepatitis, fibrosing alveolitis, Grave's disease, IgA nephropathy, idiopathic thrombocytopenic purpura, Meniere's disease, pemphigus, primary biliary cirrhosis, sarcoidosis, scleroderma, Wegener's granulomatosis, other autoimmune disorders, pancreatitis, trauma (surgery), graft-versus-host disease, transplant rejection, heart disease including ischaemic diseases such as myocardial infarction as well as atherosclerosis, intravascular coagulation, bone resorption, osteoporosis, osteoarthritis, periodontitis and hypochlorhydria, infertility related to lack of fetal-maternal tolerance, Sjogren's Syndrome, vitiligo, myasthenia gravis or systemic sclerosis.
- 41) The method of any one of claims 36-40, wherein the antibody or the antigen binding fragment thereof is administered in combination with a second therapeutic agent.
- 42) An anti-idiotypic antibody that specifically binds the antibody or the antigen-binding fragment of claim 7.

- 43) An immunoconjugate comprising the antibody or the antigen binding fragment of any one of claims 1-28 conjugated to a heterologous molecule.
- 44) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, a HCDR3, a light chain complementarity determining region 1 (LCDR1), a LCDR2 and a LCDR3 of SEQ ID NOs: 32, 124, 40, 41, 42 and 43, respectively.
- 45) The antibody or the antigen binding fragment thereof of claim 44, wherein the antibody or the antigen binding fragment thereof has one, two, three, four or five of the following properties:
- a) blocks PD-L1 binding to PD-1, wherein lack of blocking is measured by inability of the antibody to inhibit clustering of PD-L1 expressing and PD-1 expressing cells as described in Example 1;
 - b) binds PD-1 with an equilibrium dissociation constant (K_D) of about 5×10^{-8} M or less, wherein the K_D is measured using a ProteOn XPR36 system at +25°C;
 - c) binds PD-1 with an association constant (k_a) of about 4×10^5 1/Ms or more, wherein the k_a is measured using a ProteOn XPR36 system at +25°C;
 - d) binds PD-1 with a dissociation constant (k_d) of about 1×10^{-2} 1/s or less, wherein the k_d is measured using a ProteOn XPR36 system at +25°C; or
 - e) inhibits proliferation of antigen specific T cells; wherein proliferation is assessed in a CMV-PBMC assay as described in Example 1.
- 46) The antibody or the antigen binding fragment thereof of claim 44 or 45, comprising
- a) a heavy chain variable region (VH) framework derived from IGHV1-2*02 (SEQ ID NO: 127);
 - b) a light chain variable region (VL) framework derived from IGKV1D-16*1 (SEQ ID NO: 128); or
 - c) the VH framework derived from IGHV1-2*02 (SEQ ID NO: 127) and the VL framework derived from IGKV1D-16*1 (SEQ ID NO: 128).
- 47) The antibody or the antigen binding fragment thereof of any one of claims 44-46, comprising the HCDR2 of SEQ ID NOs: 33, 34, 35, 36, 37, 38 or 39.
- 48) The antibody or the antigen binding fragment thereof of any one of claims 44-47, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

- a) SEQ ID NOs: 32, 33, 40, 41, 42 and 43, respectively;
 - b) SEQ ID NOs: 32, 34, 40, 41, 42 and 43, respectively;
 - c) SEQ ID NOs: 32, 35, 40, 41, 42 and 43, respectively;
 - d) SEQ ID NOs: 32, 36, 40, 41, 42 and 43, respectively;
 - e) SEQ ID NOs: 32, 37, 40, 41, 42 and 43, respectively;
 - f) SEQ ID NOs: 32, 38, 40, 41, 42 and 43, respectively; or
 - g) SEQ ID NOs: 32, 39, 40, 41, 42 and 43, respectively.
- 49) The antibody or the antigen binding fragment thereof of any one of claims 44-48, comprising
- a) a heavy chain variable region (VH) of SEQ ID NO: 120;
 - b) a light chain variable region (VL) of SEQ ID NO: 121; or
 - c) the VH and the VL of SEQ ID NOs: 120 and 121, respectively.
- 50) The antibody or the antigen binding fragment thereof of any one of claims 44-49, comprising
- a) the VH of SEQ ID NOs: 44, 45, 46, 47, 48, 49, 50 or 51;
 - b) the VL of SEQ ID NOs: 60, 61 or 62;
 - c) the VH of SEQ ID NOs: 44, 45, 46, 47, 48, 49, 50 or 51 and the VL of SEQ ID NOs: 60, 61 or 62;
 - d) the VH of SEQ ID NO: 44 and the VL of SEQ ID NO: 60;
 - e) the VH of SEQ ID NO: 45 and the VL of SEQ ID NO: 61;
 - f) the VH of SEQ ID NO: 45 and the VL of SEQ ID NO: 62;
 - g) the VH of SEQ ID NO: 46 and the VL of SEQ ID NO: 61;
 - h) the VH of SEQ ID NO: 47 and the VL of SEQ ID NO: 61;
 - i) the VH of SEQ ID NO: 48 and the VL of SEQ ID NO: 61;
 - j) the VH of SEQ ID NO: 49 and the VL of SEQ ID NO: 61;
 - k) the VH of SEQ ID NO: 50 and the VL of SEQ ID NO: 61; or
 - l) the VH of SEQ ID NO: 51 and the VL of SEQ ID NO: 61.
- 51) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, wherein the antibody or the antigen binding fragment thereof competes for binding to PD-1 with the antibody or the antigen binding fragment thereof of claim 50.
- 52) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising

- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 33, 40, 41, 42 and 43, respectively;
 - b) the VH of SEQ ID NOs: 44 or 45 and the VL of SEQ ID NOs: 60, 61 or 62; and/or
 - c) the HC of SEQ ID NOs: 66 or 67 and the LC of SEQ ID NOs: 82, 83 or 84.
- 53) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 34, 40, 41, 42 and 43, respectively;
 - b) the VH of SEQ ID NO: 46 and the VL of SEQ ID NO: 61; and/or
 - c) the HC of SEQ ID NO: 68 and the LC of SEQ ID NO: 83.
- 54) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 35, 40, 41, 42 and 43, respectively;
 - b) the VH of SEQ ID NO: 47 and the VL of SEQ ID NO: 61; and/or
 - c) the HC of SEQ ID NO: 69 and the LC of SEQ ID NO: 83.
- 55) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 36, 40, 41, 42 and 43, respectively;
 - b) the VH of SEQ ID NO: 48 and the VL of SEQ ID NO: 61; and/or
 - c) the HC of SEQ ID NO: 70 and the LC of SEQ ID NO: 83.
- 56) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 37, 40, 41, 42 and 43, respectively;
 - b) the VH of SEQ ID NO: 49 and the VL of SEQ ID NO: 61; and/or
 - c) the HC of SEQ ID NO: 71 and the LC of SEQ ID NO: 83.
- 57) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 38, 40, 41, 42 and 43, respectively;

- b) the VH of SEQ ID NO: 50 and the VL of SEQ ID NO: 61; and/or
 - c) the HC of SEQ ID NO: 72 and the LC of SEQ ID NO: 83.
- 58) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising
- a) the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 32, 39, 40, 41, 42 and 43, respectively;
 - b) the VH of SEQ ID NO: 51 and the VL of SEQ ID NO: 61; and/or
 - c) the HC of SEQ ID NO: 73 and the LC of SEQ ID NO: 83.
- 59) The antibody or the antigen binding fragment thereof of any one of claims 44-58, wherein the antibody or the antigen binding fragment thereof is an agonist of PD-1 or mediates antibody-dependent cell cytotoxicity (ADCC) of PD-1 expressing cells, or both.
- 60) The antibody or the antigen binding fragment thereof of claim 59, wherein the PD-1 expressing cells are activated memory T cells, T follicular helper cells (T_{FH}) or T peripheral helper cells (T_{PH}), or any combination thereof.
- 61) The antibody or the antigen binding fragment thereof of any one of claims 44-60, wherein the antibody or the antigen binding fragment thereof is an IgG1, an IgG2, and IgG3 or an IgG4 isotype.
- 62) The antibody or the antigen binding fragment thereof of any one of claims 44-61, wherein the antibody or the antigen binding fragment thereof comprises at least one mutation in the antibody Fc that modulates binding of the antibody to an Fc receptor (FcR).
- 63) The antibody or the antigen binding fragment thereof of claim 62, wherein the at least one mutation is:
- a) a S267E mutation;
 - b) a S267D mutation;
 - c) a S267E/I332E mutation;
 - d) a S267E/L328F mutation;
 - e) a G236D/S267E mutation;
 - f) a P238D mutation; or
 - g) a P238D/E233D/G237D/H268D/P271G/A330R mutation.

- 64) The antibody or the antigen binding fragment thereof of any one of claims 44-50, wherein the antibody comprises
- a) a heavy chain (HC) of SEQ ID NOs: 66, 67, 68, 69, 70, 71, 72 or 73;
 - b) a light chain (LC) of SEQ ID NOs: 82, 83 or 84;
 - c) the HC of SEQ ID NOs: 66, 67, 68, 69, 70, 71, 72 or 73 and the LC of SEQ ID NOs: 82, 83 or 84;
 - d) the HC of SEQ ID NO: 66 and the LC of SEQ ID NO: 82;
 - i) the HC of SEQ ID NO: 67 and the LC of SEQ ID NO: 83;
 - e) the HC of SEQ ID NO: 67 and the LC of SEQ ID NO: 84;
 - f) the HC of SEQ ID NO: 68 and the LC of SEQ ID NO: 83;
 - g) the HC of SEQ ID NO: 69 and the LC of SEQ ID NO: 83;
 - h) the HC of SEQ ID NO: 70 and the LC of SEQ ID NO: 83;
 - i) the HC of SEQ ID NO: 71 and the LC of SEQ ID NO: 83;
 - j) the HC of SEQ ID NO: 72 and the LC of SEQ ID NO: 83; or
 - k) the HC of SEQ ID NO: 73 and the LC of SEQ ID NO: 83.
- 65) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof that specifically binds PD-1, wherein the antibody VH and the antibody VL or the antibody HC and the antibody LC are encoded by a polynucleotide comprising the polynucleotide sequence of
- a) SEQ ID NOs: 52 and 63, respectively;
 - b) SEQ ID NOs: 53 and 64, respectively;
 - c) SEQ ID NOs: 53 and 65, respectively;
 - d) SEQ ID NOs: 54 and 64, respectively;
 - e) SEQ ID NOs: 55 and 64, respectively;
 - f) SEQ ID NOs: 56 and 64, respectively;
 - g) SEQ ID NOs: 57 and 64, respectively;
 - h) SEQ ID NOs: 58 and 64, respectively;
 - i) SEQ ID NOs: 59 and 64, respectively;
 - j) SEQ ID NOs: 74 and 85, respectively;
 - k) SEQ ID NOs: 75 and 86, respectively;
 - l) SEQ ID NOs: 75 and 87, respectively;
 - m) SEQ ID NOs: 76 and 86, respectively;

- n) SEQ ID NOs: 77 and 86, respectively;
 - o) SEQ ID NOs: 78 and 86, respectively;
 - p) SEQ ID NOs: 79 and 86, respectively;
 - q) SEQ ID NOs: 80 and 86, respectively;
 - r) SEQ ID NOs: 81 and 86, respectively;
 - s) SEQ ID NOs: 136 and 137, respectively; or
 - t) SEQ ID NOs: 138 and 139, respectively.
- 66) The antibody or the antigen binding fragment thereof of any one of claims 44-65, wherein the antibody or the antigen binding fragment thereof has a biantennary glycan structure with a fucose content of about between 1% to about 15%.
- 67) A polynucleotide
- a) encoding the VH of SEQ ID NOs: 120, 44, 45, 46, 47, 48, 49, 50 or 51;
 - b) encoding the VL of SEQ ID NOs: 121, 60, 61 or 62;
 - c) encoding the VH of SEQ ID NOs: 120, 44, 45, 46, 47, 48, 49, 50 or 51 and the VL of SEQ ID NOs: 121, 60, 61 or 62;
 - d) encoding the HC of SEQ ID NOs: 66, 67, 68, 69, 70, 71, 72 or 73;
 - e) encoding the LC of SEQ ID NOs: 82, 83 or 84;
 - f) encoding the HC of SEQ ID NOs: 66, 67, 68, 69, 70, 71, 72 or 73 and the LC of SEQ ID NOs: 82, 83 or 84; or
 - g) comprising the polynucleotide sequence of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, 59, 63, 64, 65, 74, 75, 76, 77, 78, 79, 80, 81, 85, 86, 87, 136, 137, 138 or 139.
- 68) A vector comprising at least one polynucleotide of claim 67.
- 69) A host cell comprising the vector of claim 68.
- 70) The host cell of claim 69, wherein the host cell is a eukaryotic cell, a prokaryotic cell, a CHO cell, a HEK293 cell or a hybridoma.
- 71) A method of making an antibody or the antigen binding fragment thereof of claim 44, comprising culturing the host cell of claim 69 in conditions that the antibody or the antigen binding fragment thereof is expressed, and isolating the antibody or the antigen binding fragment thereof.
- 72) A pharmaceutical composition comprising the antibody or the antigen binding fragment thereof of any one of claims 44-66.

- 73) A kit comprising the antibody or the antigen binding fragment thereof of any one of claims 44-66.
- 74) A method of suppressing activation of a PD-1 expressing T cell in a subject, comprising administering to the subject the isolated antibody or the antigen binding fragment thereof of any one of claims 44-66 for a time sufficient to suppress activation of the PD-1 expressing T cell.
- 75) The method of claim 74, wherein the PD-1 expressing T cell is an antigen-specific CD4⁺ T cell and/or an antigen-specific CD8⁺ T cell.
- 76) A method of downmodulating an immune response comprising administering to a subject in need thereof a therapeutically effective amount of the isolated antibody or the antigen binding fragment thereof of any one of claims 44-66 to downmodulate the immune response.
- 77) A method of treating an immune disorder comprising administering to a subject in need thereof a therapeutically effective amount of the isolated antibody or the antigen binding fragment thereof of any one of claims 44-66 to treat the immune disorder.
- 78) The method of claim 77, wherein the immune disorder is lupus, systemic lupus erythematosus, Sjogren's Syndrome, arthritis, rheumatoid arthritis, asthma, COPD, pelvic inflammatory disease, Alzheimer's Disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, Peyronie's Disease, coeliac disease, gallbladder disease, Pilonidal disease, peritonitis, psoriasis, psoriatic arthritis, vasculitis, surgical adhesions, stroke, Type I Diabetes, Lyme disease, meningoencephalitis, autoimmune uveitis, multiple sclerosis, , Guillain-Barr syndrome, Atopic dermatitis, autoimmune hepatitis, fibrosing alveolitis, Grave's disease, IgA nephropathy, idiopathic thrombocytopenic purpura, Meniere's disease, pemphigus, primary biliary cirrhosis, sarcoidosis, scleroderma, Wegener's granulomatosis, other autoimmune disorders, pancreatitis, trauma (surgery), graft-versus-host disease, transplant rejection, heart disease including ischaemic diseases such as myocardial infarction as well as atherosclerosis, intravascular coagulation, bone resorption, osteoporosis, osteoarthritis, periodontitis and hypochlorhydria, infertility related to lack of fetal-maternal tolerance, Sjogren's Syndrome, vitiligo, myasthenia gravis or systemic sclerosis.
- 79) The method of any one of claims 74-78, wherein the antibody or the antigen binding fragment thereof is administered in combination with a second therapeutic agent.

- 80) An anti-idiotypic antibody that specifically binds the antibody or the antigen-binding fragment of any one of claims 52-58.
- 81) An immunoconjugate comprising the antibody or the antigen binding fragment thereof of any one of claims 44-66 conjugated to an imaging agent or a cytotoxic agent.
- 82) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, a HCDR3, a light chain complementarity determining region 1 (LCDR1), a LCDR2 and a LCDR3 of SEQ ID NOs: 88, 89, 90, 91, 92 and 93, respectively.
- 83) The antibody or the antigen binding fragment thereof of claim 82, wherein the antibody or the antigen binding fragment thereof does not block PD-L1 binding to PD-1, wherein lack of blocking is measured by inability of the antibody to inhibit clustering of PD-L1 expressing and PD-1 expressing cells as described in Example 1.
- 84) The antibody or the antigen binding fragment thereof of claim 82 or 83, comprising
- a) a heavy chain variable region (VH) framework derived from IGHV2-5*04 (SEQ ID NO: 129);
 - b) a light chain variable region (VL) framework derived from IGKV2-28*01 (SEQ ID NO: 130); or
 - c) the VH framework derived from IGHV2-5*04 (SEQ ID NO: 129) and the VL framework derived from IGKV2-28*01 (SEQ ID NO: 130).
- 85) The antibody or the antigen binding fragment thereof of any one of claims 82-84, comprising
- a) a heavy chain variable region (VH) of SEQ ID NO: 122;
 - b) a light chain variable region (VL) of SEQ ID NO: 123; or
 - c) the VH and the VL of SEQ ID NOs: 122 and 123, respectively.
- 86) The antibody or the antigen binding fragment thereof of any one of claims 82-85, comprising
- a) the VH of SEQ ID NOs: 94 or 95;
 - b) the VL of SEQ ID NOs: 98, 99 or 100;
 - c) the VH of SEQ ID NOs: 94 or 95 and the VL of SEQ ID NOs: 98, 99 or 100;
 - d) the VH of SEQ ID NO: 94 and the VL of SEQ ID NO: 98;
 - e) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 99; or
 - f) the VH of SEQ ID NO: 95 and the VL of SEQ ID NO: 100.

- 87) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, wherein the antibody competes for binding to PD-1 with the antibody or the antigen binding fragment thereof of claim 86.
- 88) The antibody or the antigen binding fragment thereof of any one of claims 82-87, wherein the antibody is an agonistic antibody or mediates antibody-dependent cell cytotoxicity (ADCC) of PD-1 expressing cells, or both.
- 89) The antibody or the antigen binding fragment thereof of claim 88, wherein the PD-1 expressing cells are activated memory T cells, T follicular helper cells (T_{FH}) or T peripheral helper cells (T_{PH}), or any combination thereof.
- 90) The antibody or the antigen binding fragment thereof of any one of claims 82-89, wherein the antibody or the antigen binding fragment thereof is an IgG1, an IgG2, and IgG3 or an IgG4 isotype.
- 91) The antibody or the antigen binding fragment thereof of any one of claims 82-90, wherein the antibody or the antigen binding fragment thereof comprises at least one mutation in the antibody Fc that modulates binding of the antibody to an Fc receptor.
- 92) The antibody or the antigen binding fragment thereof of claim 91, wherein the at least one mutation is
- a) a S267E mutation;
 - b) a S267D mutation;
 - c) a S267E/I332E mutation;
 - d) a S267E/L328F mutation;
 - e) a G236D/S267E mutation;
 - f) a P238D mutation; or
 - g) a P238D/E233D/G237D/H268D/P271G/A330R mutation.
- 93) The antibody or the antigen binding fragment thereof of any one of claims 82-92, comprising
- a) a heavy chain (HC) of SEQ ID NOs: 104 or 105;
 - b) a light chain (LC) of SEQ ID NOs: 108, 109 or 110;
 - c) the HC of SEQ ID NOs: 104 or 105 and the LC of SEQ ID NOs: 108, 109 or 110;
 - d) the HC of SEQ ID NO: 104 and the LC of SEQ ID NO: 108;
 - e) the HC of SEQ ID NO: 105 and the LC of SEQ ID NO: 109; or
 - f) the HC of SEQ ID NO: 105 and the LC of SEQ ID NO: 110.

- 94) An isolated antibody that specifically binds PD-1 or an antigen binding fragment thereof, wherein the antibody VH and the antibody VL or the antibody HC and the antibody LC are encoded by a polynucleotide comprising the polynucleotide sequence of
- a) SEQ ID NOs: 96 and 101, respectively;
 - b) SEQ ID NOs: 97 and 102, respectively;
 - c) SEQ ID NOs: 97 and 103, respectively;
 - d) SEQ ID NOs: 106 and 111, respectively;
 - e) SEQ ID NOs: 107 and 112, respectively; or
 - f) SEQ ID NOs: 107 and 113, respectively.
- 95) The antibody or the antigen binding fragment thereof of any one of claims 82-94, wherein the antibody or the antigen binding fragment thereof has a biantennary glycan structure with a fucose content of about between 1% to about 15%.
- 96) A polynucleotide
- a) encoding the VH of SEQ ID NOs: 122, 94 or 95;
 - b) encoding the VL of SEQ ID NOs: 123, 98, 99 or 100;
 - c) encoding the VH of SEQ ID NOs: 122, 94 or 95 and the VL of SEQ ID NOs: 123, 98, 99 or 100;
 - d) encoding the HC of SEQ ID NOs: 104 or 105;
 - e) encoding the LC of SEQ ID NOs: 108, 109 or 110;
 - f) encoding the HC of SEQ ID NOs: 104 or 105 and the LC of SEQ ID NOs: 108, 109 or 110; or
 - g) comprising the polynucleotide sequence of SEQ ID NOs: 96, 97, 101, 102, 103, 106, 107, 111, 112 or 113.
- 97) A vector comprising at least one polynucleotide of claim 96.
- 98) A host cell comprising the vector of claim 97.
- 99) The host cell of claim 98, wherein the host cell is a eukaryotic cell, a prokaryotic cell, a CHO cell, a HEK293 cell or a hybridoma.
- 100) A method of making the antibody or the antigen binding fragment thereof of any one of claims 82-95, comprising culturing the host cell of claim 98 in conditions that the antibody or the antigen binding fragment thereof is expressed, and isolating the antibody or the antigen binding fragment thereof.

- 101) A pharmaceutical composition comprising the antibody or the antigen binding fragment thereof of any one of claims 82-95.
- 102) A kit comprising the antibody or the antigen binding fragment thereof of any one of claims 82-95.
- 103) A method of suppressing activation of a PD-1 expressing T cell in a subject, comprising administering to the subject the isolated antibody or the antigen binding fragment thereof of any one of claims 82-95 for a time sufficient to suppress activation of the PD-1 expressing T cell.
- 104) The method of claim 103, wherein the PD-1 expressing T cell is an antigen-specific CD4⁺ T cell and/or an antigen-specific CD8⁺ T cell.
- 105) A method of downmodulating an immune response comprising administering to a subject in need thereof a therapeutically effective amount of the isolated antibody or the antigen binding fragment thereof of any one of claims 82-95 for downmodulate the immune response.
- 106) A method of treating an immune disorder comprising administering to a subject in need thereof a therapeutically effective amount of the isolated antibody or the antigen binding fragment thereof of any one of claims 82-95 to treat the immune disorder.
- 107) The method of claim 106, wherein the immune disorder is lupus, systemic lupus erythematosus, Sjogren's Syndrome, arthritis, rheumatoid arthritis, asthma, COPD, pelvic inflammatory disease, Alzheimer's Disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, Peyronie's Disease, coeliac disease, gallbladder disease, Pilonidal disease, peritonitis, psoriasis, psoriatic arthritis, vasculitis, surgical adhesions, stroke, Type I Diabetes, Lyme disease, meningoencephalitis, autoimmune uveitis, multiple sclerosis, , Guillain-Barr syndrome, Atopic dermatitis, autoimmune hepatitis, fibrosing alveolitis, Grave's disease, IgA nephropathy, idiopathic thrombocytopenic purpura, Meniere's disease, pemphigus, primary biliary cirrhosis, sarcoidosis, scleroderma, Wegener's granulomatosis, other autoimmune disorders, pancreatitis, trauma (surgery), graft-versus-host disease, transplant rejection, heart disease including ischaemic diseases such as myocardial infarction as well as atherosclerosis, intravascular coagulation, bone resorption, osteoporosis, osteoarthritis, periodontitis and hypochlorhydria, infertility related to lack of fetal-maternal tolerance, Sjogren's Syndrome, vitiligo, myasthenia gravis or systemic sclerosis.

- 108) The method of any one of claims 103-107, wherein the antibody or the antigen binding fragment thereof is administered in combination with a second therapeutic agent.
- 109) An anti-idiotypic antibody that specifically binds the antibody or the antigen-binding fragment of claim 64.
- 110) An immunoconjugate comprising the antibody or the antigen-binding fragment thereof of any one of claims 82-96 conjugated to an imaging agent or a cytotoxic agent.

Figure 1A.

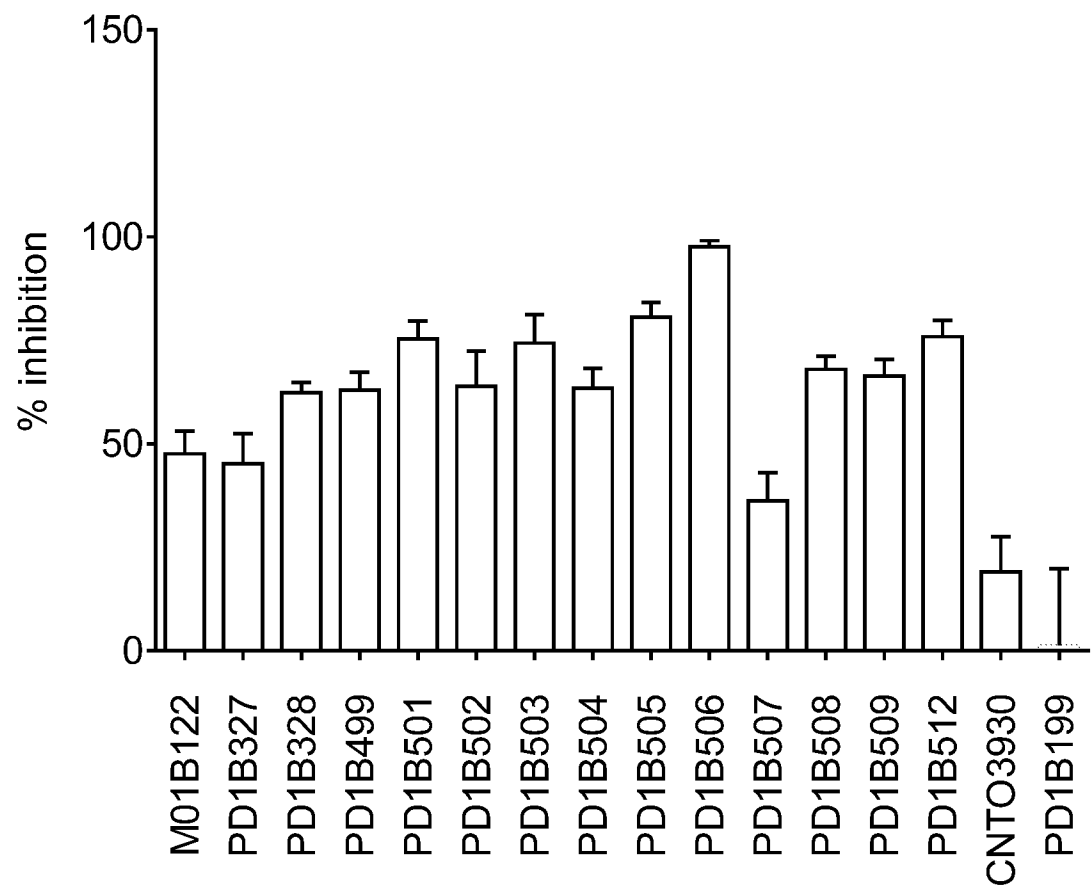
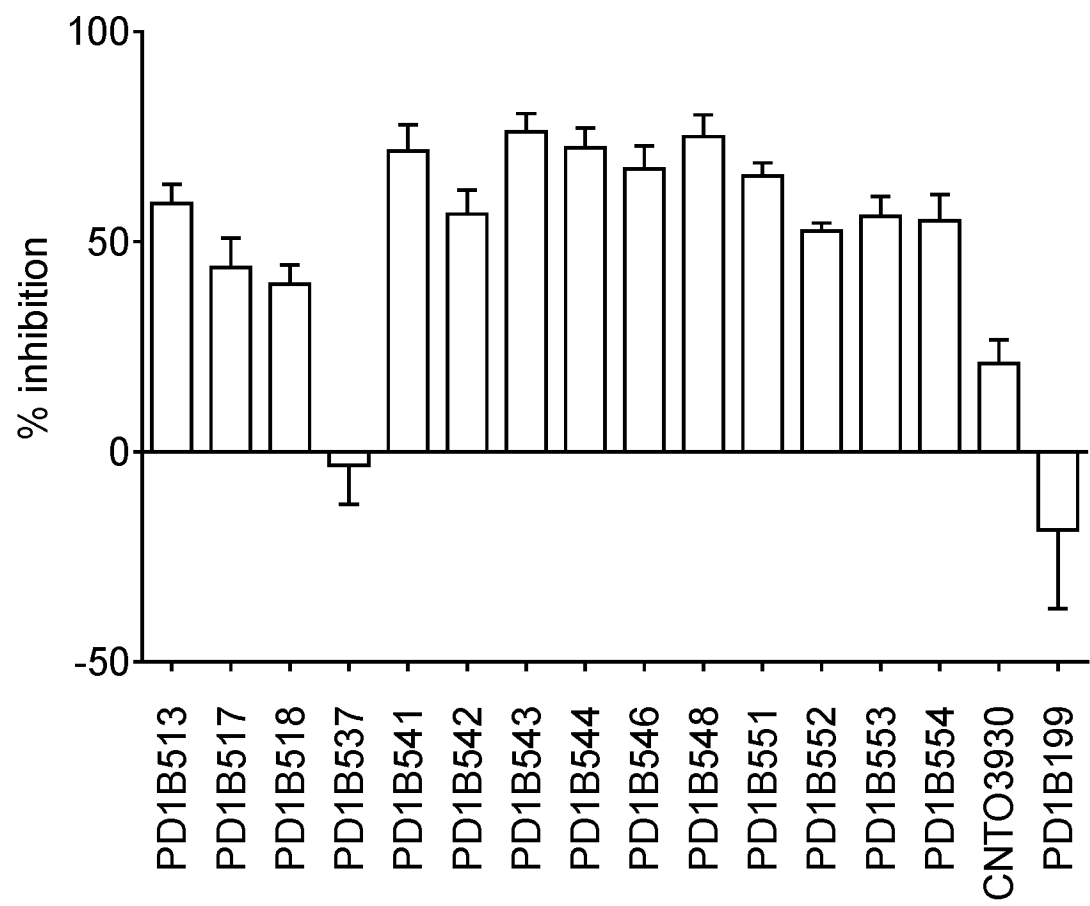


Figure 1B.



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Figure 2A.

	1
PD1H93_8	DVQLQESGPELKKPGETVKISCKASGYTFT
PD1H384_9	QVQLVQSGSELKKPGASVKVSCKASGYTFT
PD1H405_10	QVQLVQSGSELKKPGASVKVSCKASGYTFT
PD1H585_140	QVQLVQSGSELKKPGASVKVSCKASGYTFT
PD1H586_141	QVQLVQSGSELKKPGASVKVSCKASGYTFT
PD1H587_142	QVQLVQSGSELKKPGASVKVSCKASGYTFT
	:*** :*.***** :*:*****
PD1H93_8	DYSMHWVKQAPGKGLKWMGWINIETGEPTY
PD1H384_9	DYSMHWVRQAPGQGLEWMGWINIETGEPTY
PD1H405_10	DYSMHWVRQAPGQGLEWMGWINIETGEPTY
PD1H585_140	DYSMHWVRQAPGQGLEWMGWINIETGYPTY
PD1H586_141	DYSMHWVRQAPGQGLEWMGWINIETGHPTY
PD1H587_142	DYSMHWVRQAPGQGLEWMGWINIETGWPTY
	*****:****:*.***** **
PD1H93_8	ADDFKGRFAFSLETSASTAYLQINNLIKNE
PD1H384_9	AQGFTGRFVFSLDTSVSTAYLQICSLKAED
PD1H405_10	AQGFTGRFVFSLDTSVSTAYLQISSLKAED
PD1H585_140	AQGFTGRFVFSLDTSVSTAYLQISSLKAED
PD1H586_141	AQGFTGRFVFSLDTSVSTAYLQISSLKAED
PD1H587_142	AQGFTGRFVFSLDTSVSTAYLQISSLKAED
	:..***.***:*.***** .** **
	121
PD1H93_8	TATYFCARDYYGTYFYAMDYWGQGTLTVSS
PD1H384_9	TAVYFCARDYYGTYFYAMDYWGQGLTVTVSS
PD1H405_10	TAVYFCARDYYGTYFYAMDYWGQGLTVTVSS
PD1H585_140	TAVYFCARDYYGTYFYAMDYWGQGLTVTVSS
PD1H586_141	TAVYFCARDYYGTYFYAMDYWGQGLTVTVSS
PD1H587_142	TAVYFCARDYYGTYFYAMDYWGQGLTVTVSS
	.****:****

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Figure 2B.

	1
PD1L30_14	<u>QIVLTQSPAIMSASLGERVTMTCTASSSVS</u>
PD1L468_15	<u>EIVLTQSPATLSLSPGERATLSCTASSSVS</u>
PD1L469_16	<u>EIVLTQSPATLSLSPGERATLSCTASSSVS</u>
PD1L651_143	<u>EIVLTQSPATLSLSPGERATLSCTASSSFS</u>
PD1L652_144	<u>EIVLTQSPATLSLSPGERATLSCTASSSVP</u>
	:***** :* * ***.*:;*****..
PD1L30_14	<u>SSYLHWYQQKPGSSPKLWIYSTSNLASGVP</u>
PD1L468_15	<u>SSYLHWYQQKPG LAPRLLIYSTSNLASGIP</u>
PD1L469_16	<u>SSYLHWYQQKPG LAPRLLIYSTSNLASGIP</u>
PD1L651_143	<u>SSYLHWYQQKPG LAPRLLIYSTSNLASGIP</u>
PD1L652_144	<u>SSYLHWYQQKPG LAPRLLIYSTSNLASGIP</u>
	***** :*: *****:*
PD1L30_14	<u>ARFSGSGSGTSYSLTISSEAEADAATYYCH</u>
PD1L468_15	<u>DRFSGSGSGTDFTLTISRLEPEDFAVYYCH</u>
PD1L469_16	<u>DRFSGSGSGTDYTLTISRLEPEDFAVYYCH</u>
PD1L651_143	<u>DRFSGSGSGTDYTLTISRLEPEDFAVYYCH</u>
PD1L652_144	<u>DRFSGSGSGTDYTLTISRLEPEDFAVYYCH</u>
	*****.:;***** :*.** *.****
	108
PD1L30_14	<u>QYHRSPLTFGAGTKLELK</u>
PD1L468_15	<u>QYHRSPLTFGQGTKLEIK</u>
PD1L469_16	<u>QYHRSPLTFGQGTKLEIK</u>
PD1L651_143	<u>QYHRSPLTFGQGTKLEIK</u>
PD1L652_144	<u>QYHRSPLTFGQGTKLEIK</u>
	***** *****:*

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Figure 3A.

1
 PD1H90 QVQLQQPGAELVKPGASVKLSCKASGYTFT
 PD1H388 QVQLVQSGAEVKKPGASVKVSCCKASGYTFT
 PD1H399 QVQLVQSGAEVKKPGASVKVSCCKASGYTFT
 PD1H400 QVQLVQSGAEVKKPGASVKVSCCKASGYTFT
 PD1H401 QVQLVQSGAEVKKPGASVKVSCCKASGYTFT
 PD1H402 QVQLVQSGAEVKKPGASVKVSCCKASGYTFT
 PD1H403 QVQLVQSGAEVKKPGASVKVSCCKASGYTFT
 PD1H404 QVQLVQSGAEVKKPGASVKVSCCKASGYTFT
 **** *.***: *****:*****

PD1H90 TYWMHWVKQRPGQGLEWIGEINPNNGGINY
 PD1H388 TYWMHWVRQAPGQGLEWMGEINPNNGGINY
 PD1H399 TYWMHWVRQAPGQGLEWMGEINPNNAGINY
 PD1H400 TYWMHWVRQAPGQGLEWMGEINPNDAGINY
 PD1H401 TYWMHWVRQAPGQGLEWMGEINPNQGGINY
 PD1H402 TYWMHWVRQAPGQGLEWMGEINPNKGGINY
 PD1H403 TYWMHWVRQAPGQGLEWMGEINPNEGGINY
 PD1H404 TYWMHWVRQAPGQGLEWMGEINPNNIGINY
 *****:*. *****:*****. ****

PD1H90 NEKFKKKATLTVDKSSSTAYMQLSSLTSED
 PD1H388 AQKFQGRVTLTVDKSISTAYMELSRLRSDD
 PD1H399 AQKFQGRVTLTVDKSISTAYMELSRLRSDD
 PD1H400 AQKFQGRVTLTVDKSISTAYMELSRLRSDD
 PD1H401 AQKFQGRVTLTVDKSISTAYMELSRLRSDD
 PD1H402 AQKFQGRVTLTVDKSISTAYMELSRLRSDD
 PD1H403 AQKFQGRVTLTVDKSISTAYMELSRLRSDD
 PD1H404 AQKFQGRVTLTVDKSISTAYMELSRLRSDD
 : :.*** *****:*** * *:*

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PD1H90 SAVYYCTIDYYDYGGYWGQGTTLTVSS
 PD1H388 TAVYYCTIDYYDYGGYWGQGTTLTVSS
 PD1H399 TAVYYCTIDYYDYGGYWGQGTTLTVSS
 PD1H400 TAVYYCTIDYYDYGGYWGQGTTLTVSS
 PD1H401 TAVYYCTIDYYDYGGYWGQGTTLTVSS
 PD1H402 TAVYYCTIDYYDYGGYWGQGTTLTVSS
 PD1H403 TAVYYCTIDYYDYGGYWGQGTTLTVSS
 PD1H404 TAVYYCTIDYYDYGGYWGQGTTLTVSS
 :*****:*****

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Figure 3B.

	1
PD1L28	<u>DIVMTQSQKFMSTSVRDRVSVTCKASQNVG</u>
PD1L470	<u>DIQMTQSPSSLSASVGDRVTITCKASQNVG</u>
PD1L471	<u>DIQMTQSPSSLSASVGDRVTITCKASQNVG</u>
	** ***** . :*:** ***:*****
PD1L28	<u>TNVAWYQQKPGQSPKALIYSASYRYS</u> GVDP
PD1L470	<u>TNVAWYQQKPEKAPKSLIYSASYRYS</u> GVPS
PD1L471	<u>TNVAWYQQKPEKAPKALIYSASYRYS</u> GVPS
	***** :*:*****.
PD1L28	RFTGSGSGTDFTLTITNVQSEDLAEYFC <u>QQ</u>
PD1L470	RFSGSGSGTDFTLTISSLQPEDFATYYC <u>QQ</u>
PD1L471	RFSGSGSGTDFTLTISSLQPEDFATYFC <u>QQ</u>
	*:*****:.*.*:* *:*
	107
PD1L28	<u>YNIYPYTFGSGTKLEMK</u>
PD1L470	<u>YNIYPYTFGQGTKLEIK</u>
PD1L471	<u>YNIYPYTFGQGTKLEIK</u>
	*****.******:

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Figure 4A.

	1
PD1H81	<u>QVTLKESGPGLLQPSQTL</u> SLTCSFSGFSL <u>S</u>
PD1H389	<u>QITLKESGPTLVKPTQTL</u> TLTCTFS <u>G</u> FSL <u>S</u>
	*:***** *:*:*:*:*:*:*:*****
PD1H81	<u>TSGMGVSWIRQPSGKGLEW</u> LAHIYWDDDKR
PD1H389	<u>TSGMGVSWIRQPPGKALEW</u> LAHIYWDDDKR
	*****.***.*****
PD1H81	YNPSLKSRLTISKDTSSNQVFLKITSVDTA
PD1H389	YSPSLKSRLTITKDTSKNQVVLMTNMDPV
	*.*****:****.***.*.:*.:*..
	122
PD1H81	DTGTYYCVRKGYDYGYVMDYWGQGTTVTVSS
PD1H389	DTGTYYCVRKGYDYGYVMDYWGQGLVTVSS

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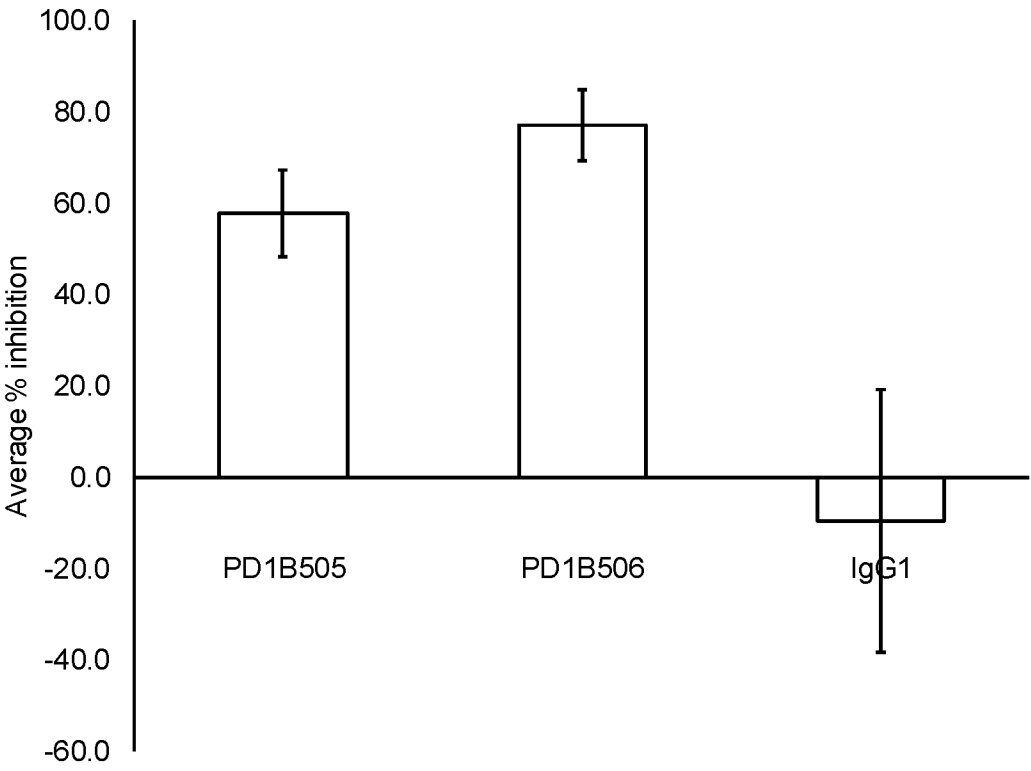
Figure 4B.

	1
PD1L43	<u>DIVMTQAALSNPVT</u> LGTSASISCRSSKSLL
PD1L472	DIVMTQSPLSLPVT <u>PGEPASISCRSSKSLL</u>
PD1L473	DIVMTQSPLSLPVT <u>PGEPASISCRSSKSLL</u>
	*****:.* ** *
PD1L43	<u>HSNGITYLNWYLQKPGQSPQLLIYQMSNLA</u>
PD1L472	<u>HSNGITYLNWYLQKPGQSPQLLIYQMSNLA</u>
PD1L473	<u>HSNGITYLNWYLQKPGQSPQLLIYQMSNLA</u>

PD1L43	<u>SGVPDRFSSSGSGTDFTLRISRVEAEDVGV</u>
PD1L472	<u>SGVPDRFSGSGSGTDFTLKISRVEAEDVGV</u>
PD1L473	<u>SGVPDRFSSSGSGTDFTLKISRVEAEDVGV</u>
	*****.******:*****
	112
PD1L43	<u>YYCAQNLELPLTFGSGTKLEMK</u>
PD1L472	<u>YYCAQNLELPLTFGGGTKVEIK</u>
PD1L473	<u>YYCAQNLELPLTFGGGTKVEIK</u>
	*****.**:*:*

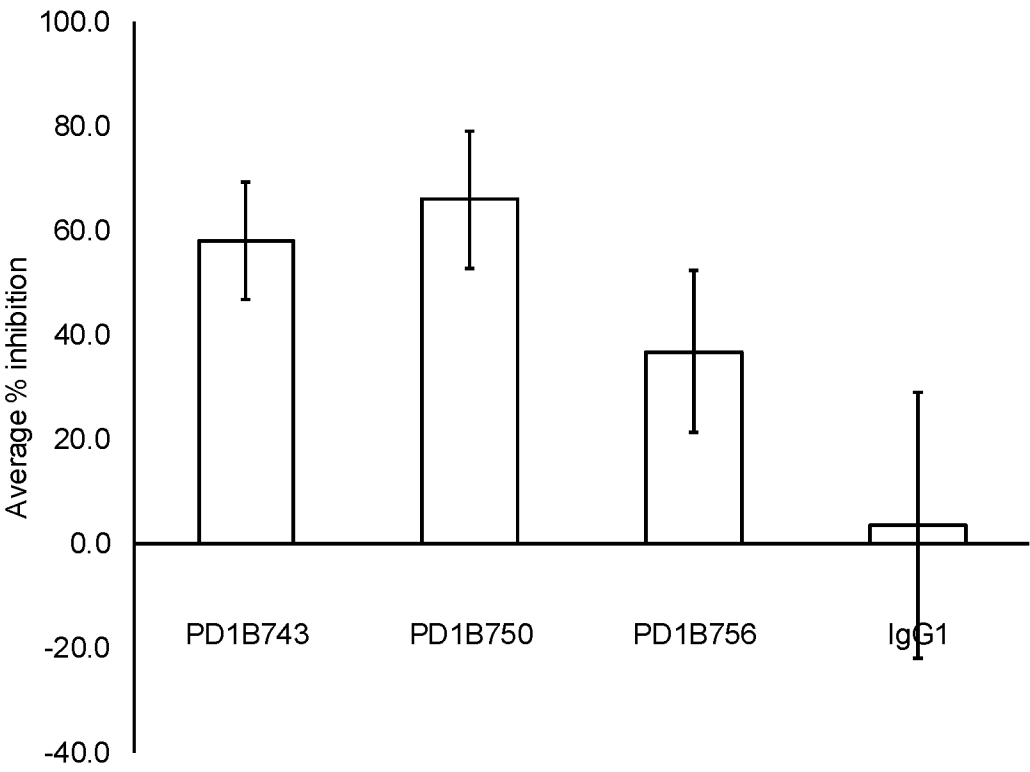
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Figure 5A.



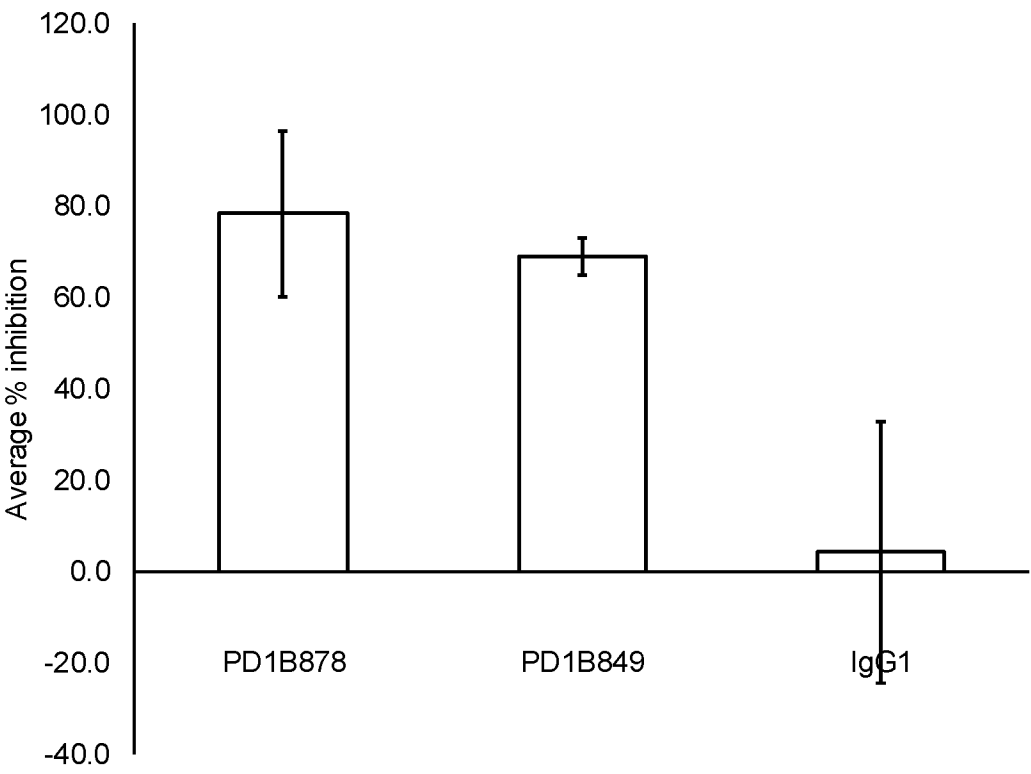
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Figure 5B.



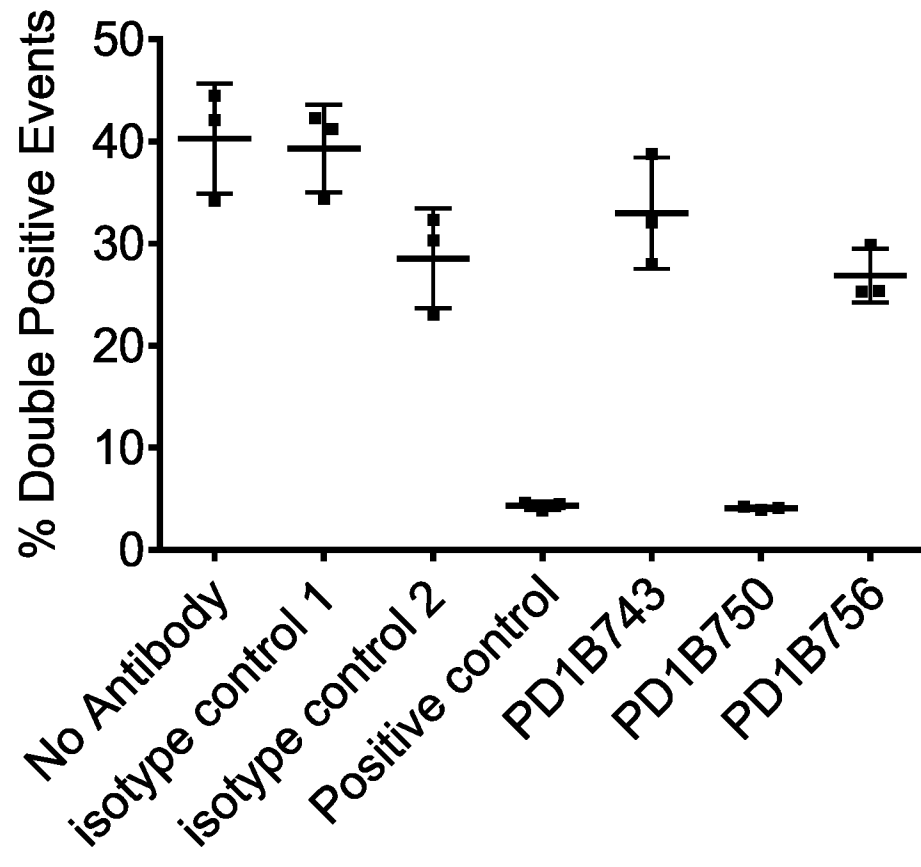
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Figure 5C.



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Figure 6A.



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Figure 6B.

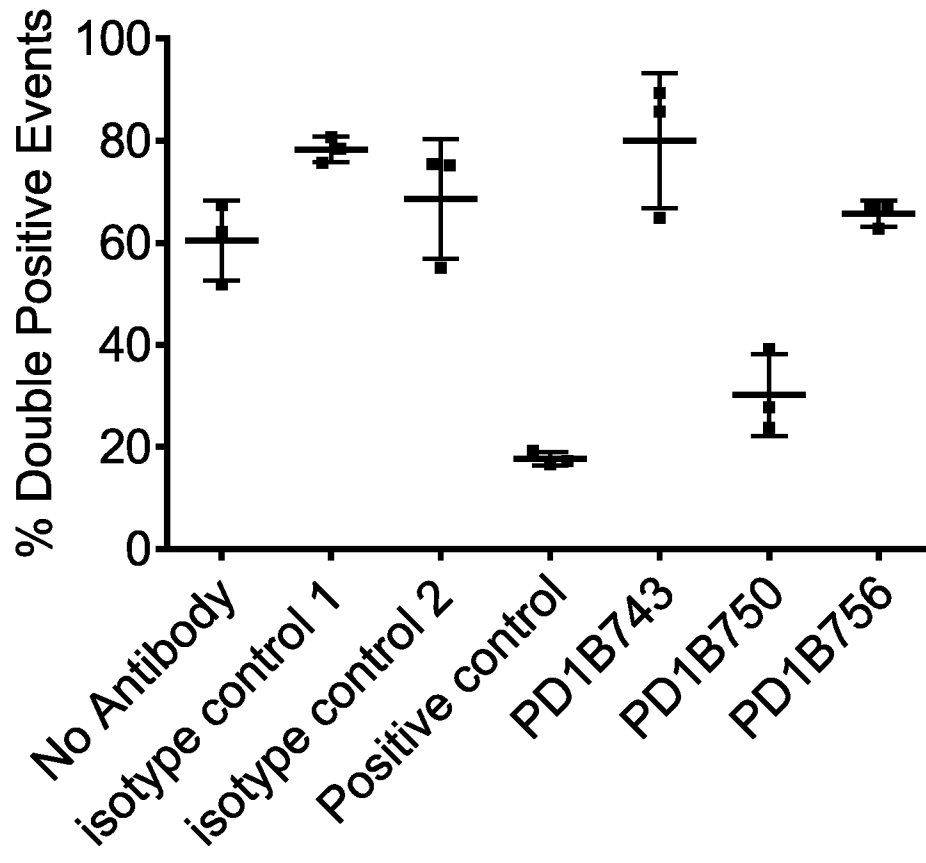


Figure 7.

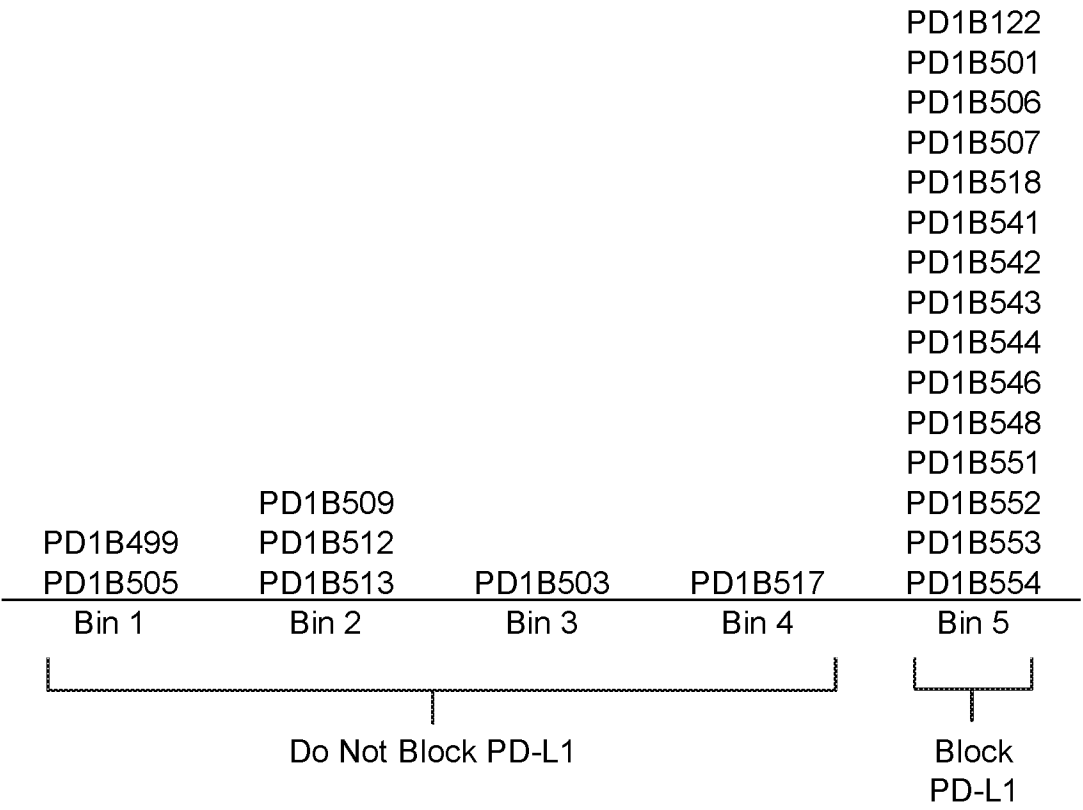
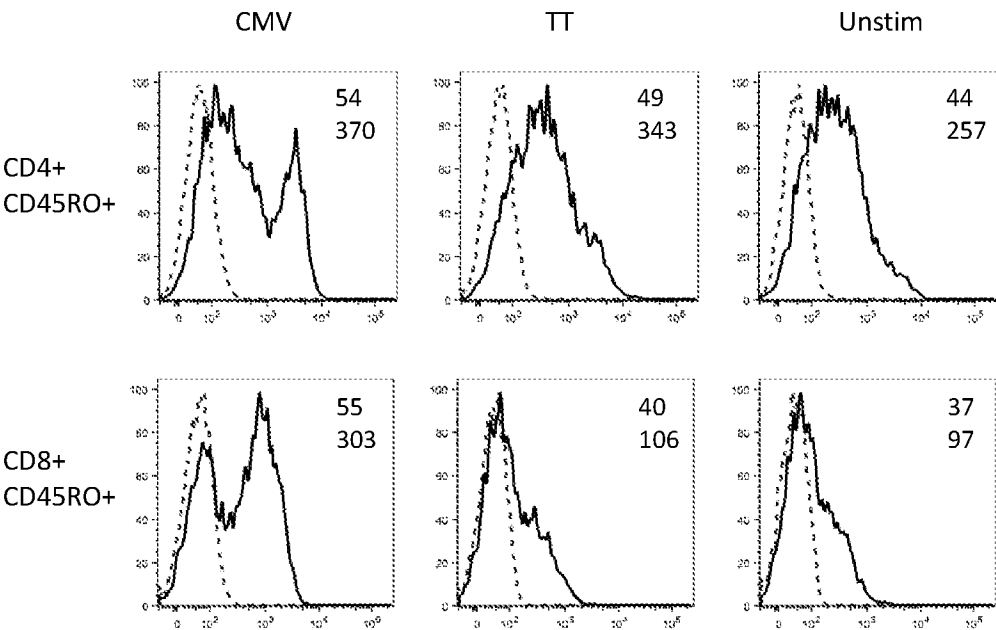


Figure 8A.



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Figure 8B.

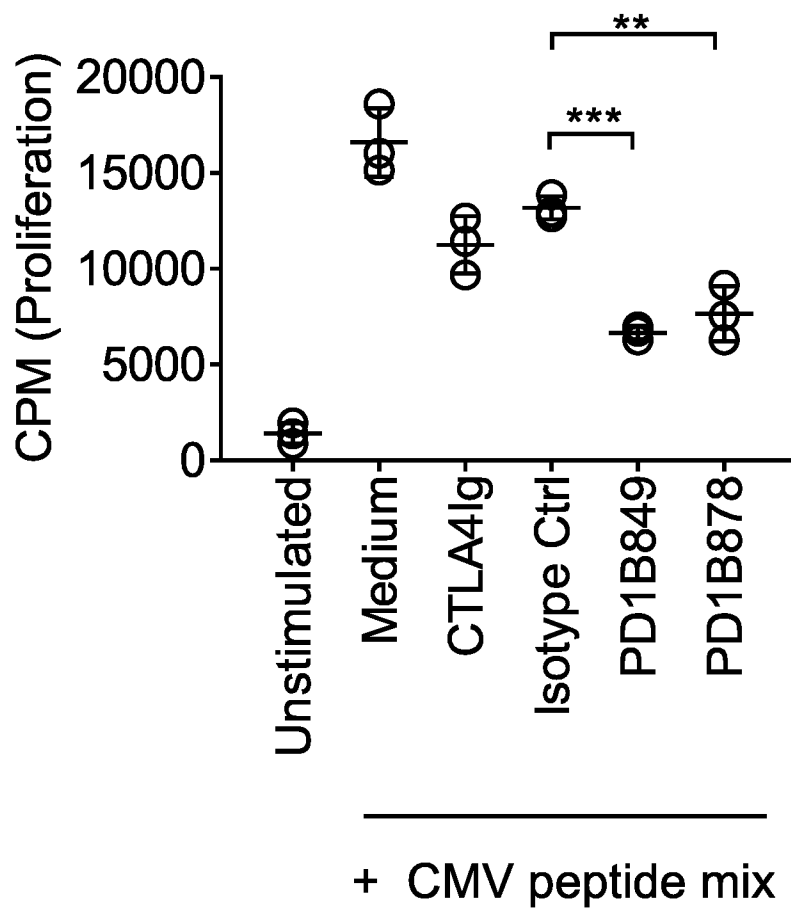


Figure 9A.

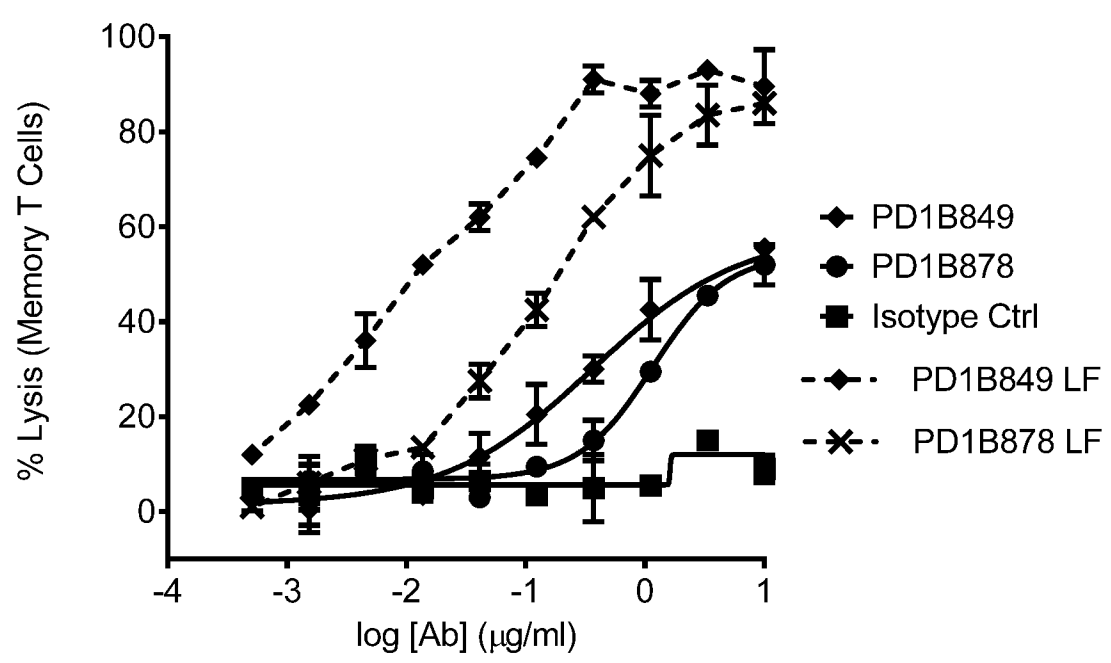


Figure 9B.

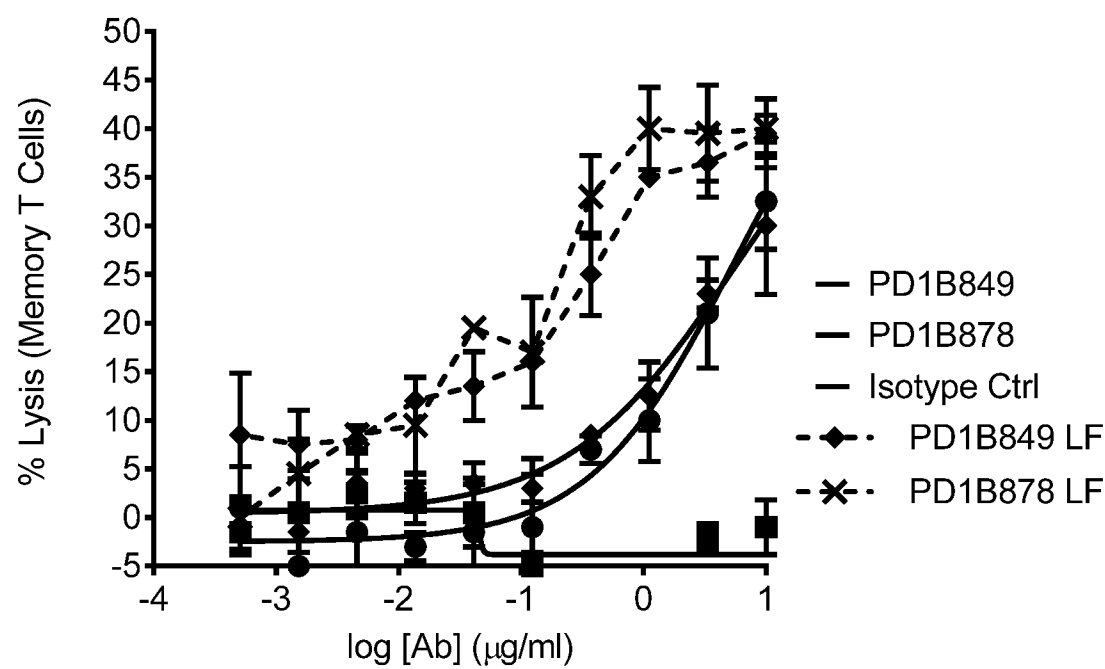


Figure 10A.

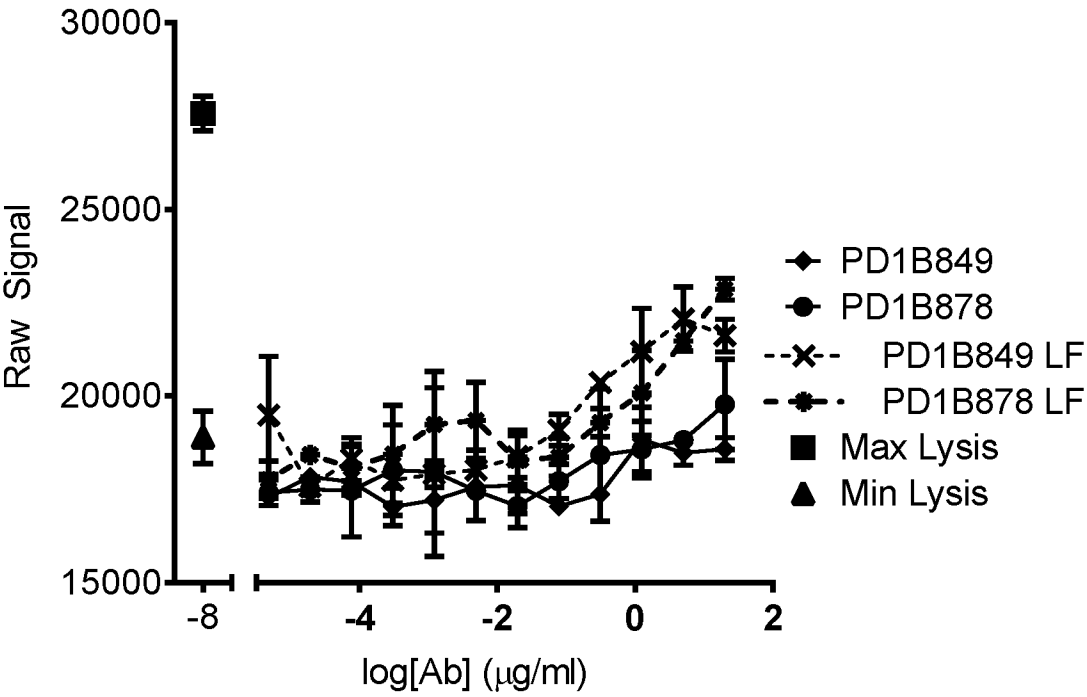


Figure 10B.

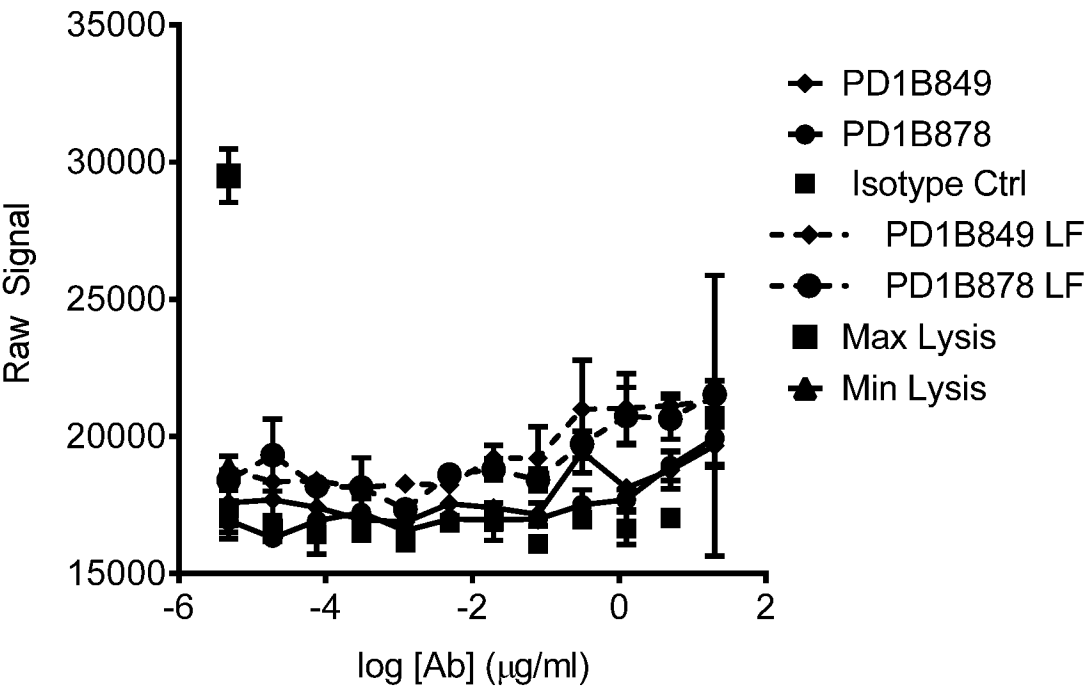
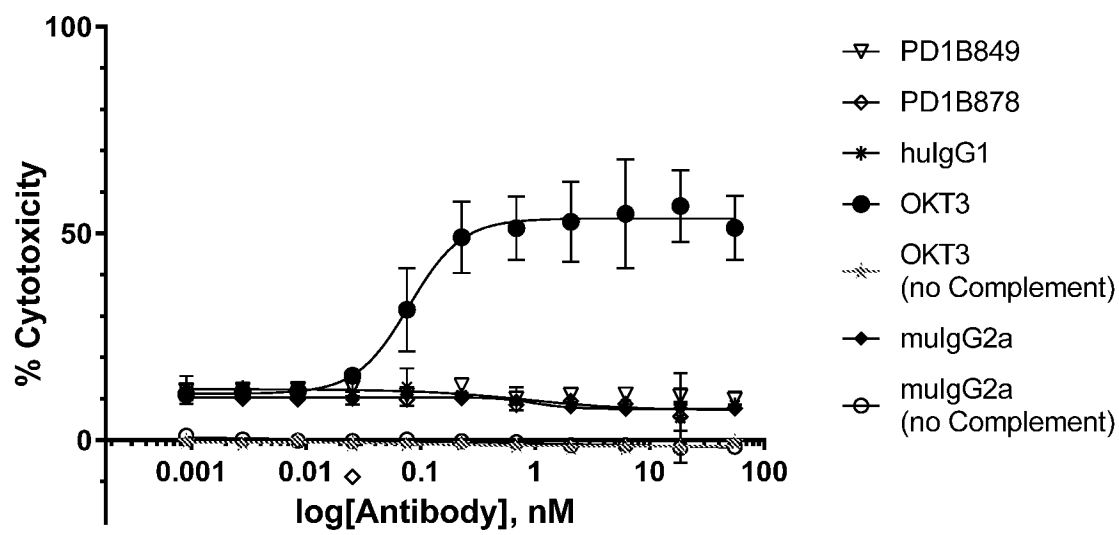


Figure 11.



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Figure 12.

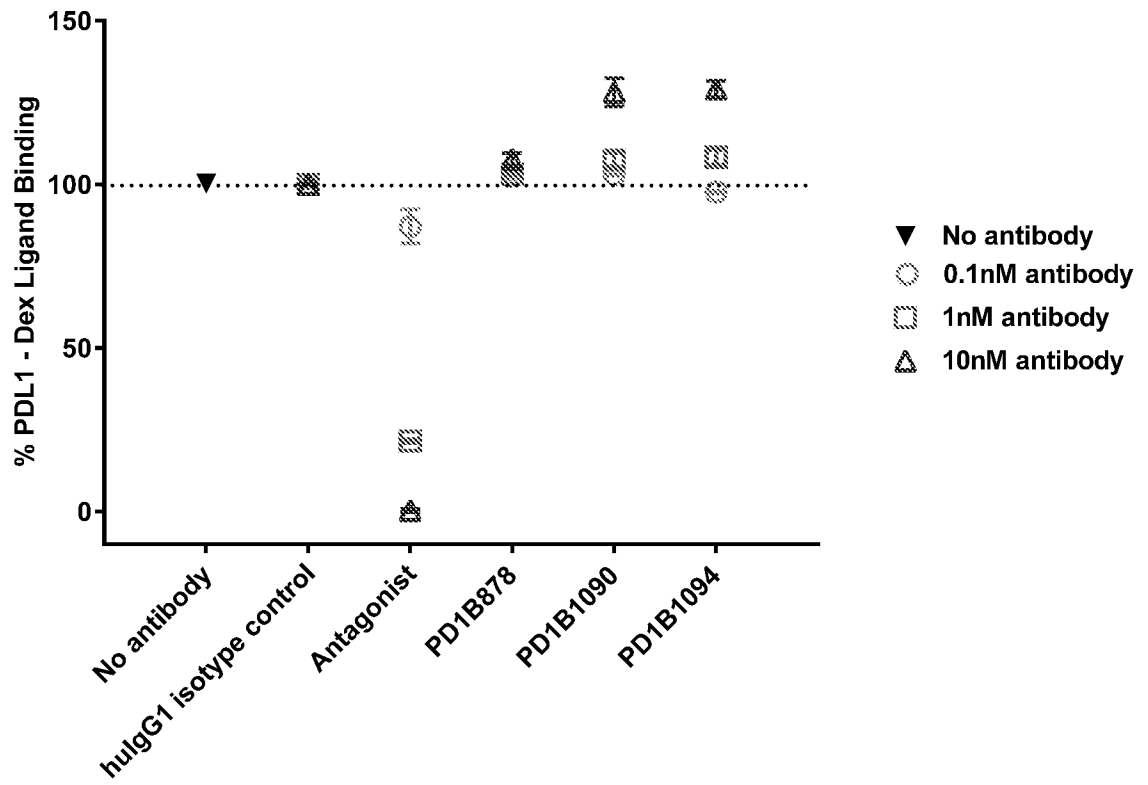


Figure 13A.

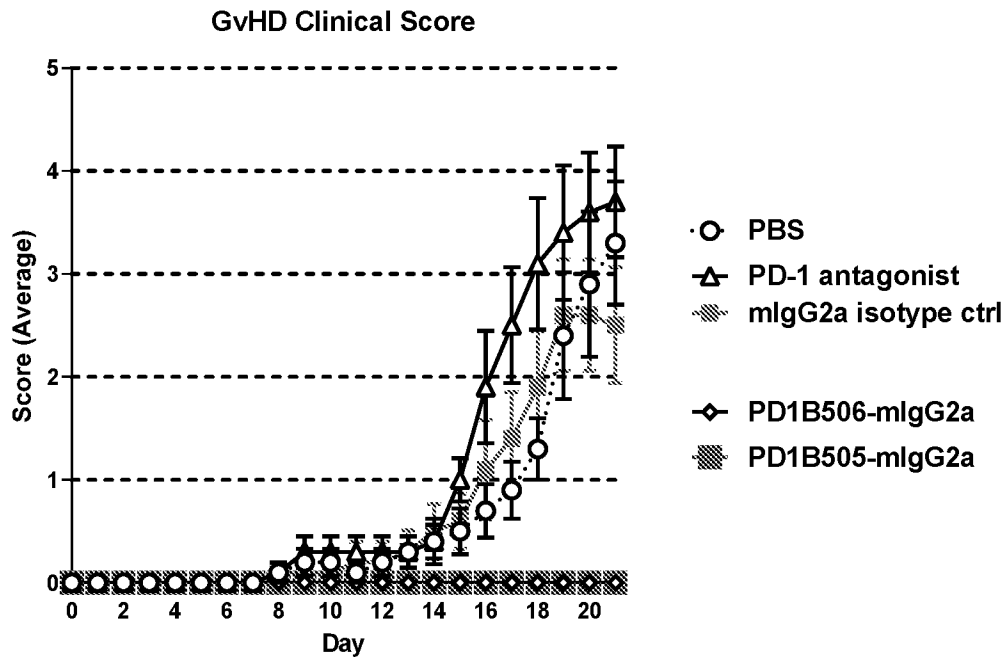


Figure 13B.

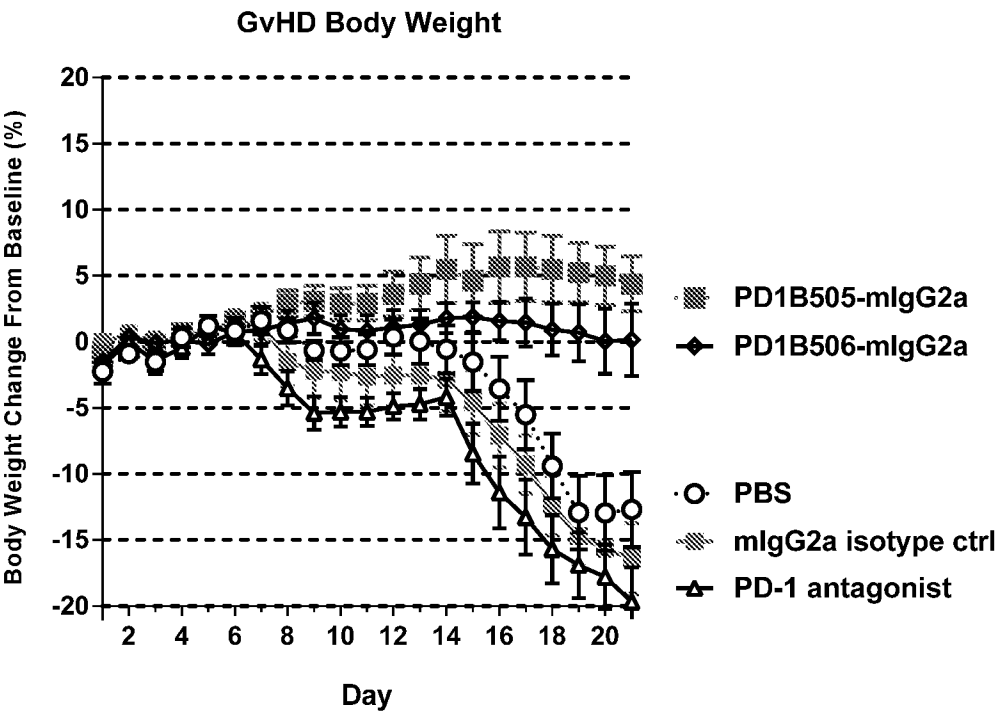
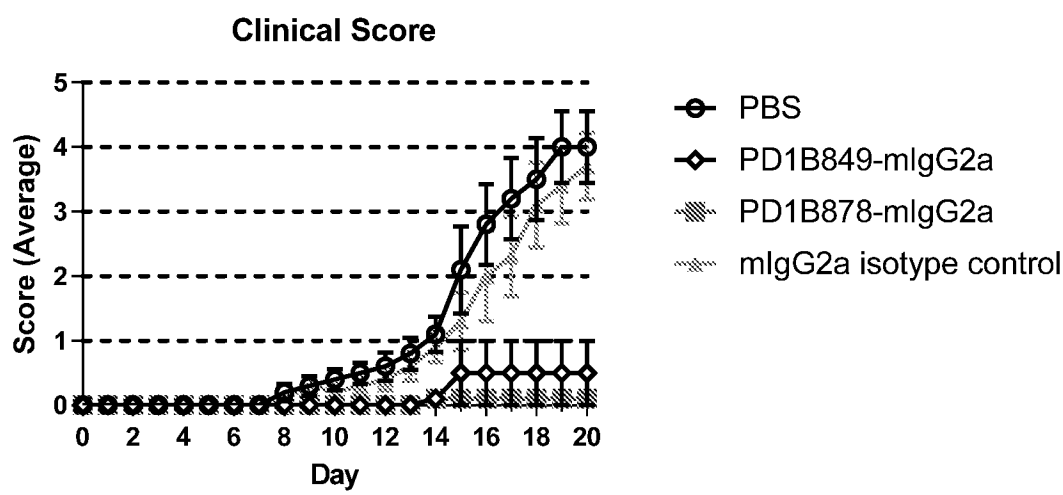


Figure 14A.



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Figure 14B.

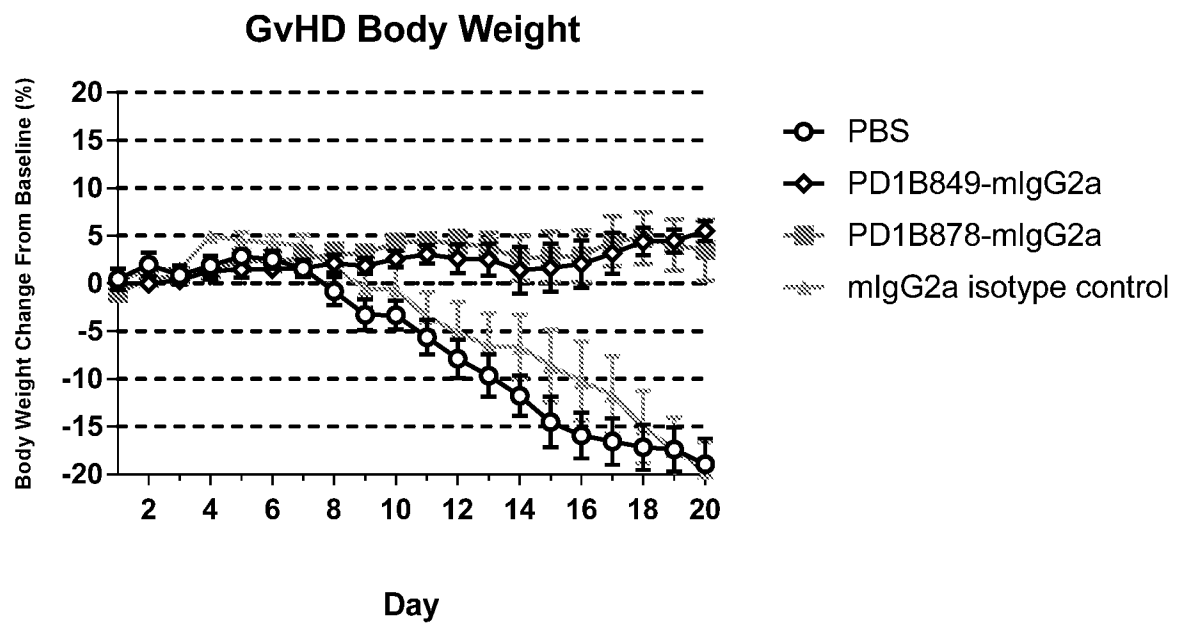


Figure 15.

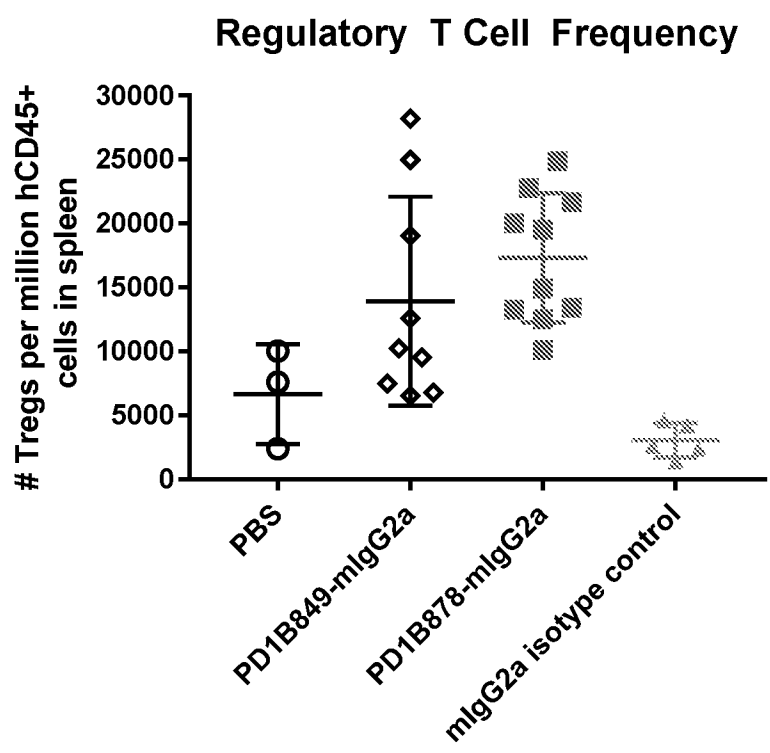


Figure 16.

