

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

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

17 September 2020 (17.09.2020)



(10) International Publication Number

WO 2020/186111 A2

(51) International Patent Classification:

A61K 39/395 (2006.01)

(21) International Application Number:

PCT/US2020/022476

(22) International Filing Date:

12 March 2020 (12.03.2020)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/817,242 12 March 2019 (12.03.2019) US

62/828,259 02 April 2019 (02.04.2019) US

62/869,507 01 July 2019 (01.07.2019) US

62/914,355 11 October 2019 (11.10.2019) US

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(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,

DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,

HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP,

KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME,

MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,

OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,

SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR,

TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH,

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

Published:

— without international search report and to be republished
upon receipt of that report (Rule 48.2(g))

(54) Title: VISTA-BINDING ANTIBODIES AND USES THEREOF

(57) Abstract: The invention provides therapeutic methods of using anti-VISTA antibodies in the treatment of diseases such as cancer.

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VISTA-BINDING ANTIBODIES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] The present application claims the benefit of U.S. Provisional Application No. 62/817,242, filed March 12, 2019, U.S. Provisional Application No. 62/828,259, filed April 2, 2019, U.S. Provisional Application No. 62/869,507 filed July 1, 2019, and U.S. Provisional Application No. 62/914,355 filed October 11, 2019, all of which are expressly incorporated by reference in their entireties for all purposes.

TECHNICAL FIELD

[0002] The present disclosure relates to therapeutic methods of using anti-VISTA antibodies in the treatment of diseases such as cancer.

BACKGROUND OF THE INVENTION

[0003] The treatment of diseases by modulating an immune response is referred to as immunotherapy. Immunotherapy has demonstrated increasing effectiveness in treating cancer. Much immunotherapeutic success in cancer treatment is based on the use of immune-modulating antibodies that target immune checkpoints.

[0004] V-domain Ig suppressor of T cell activation (VISTA) is a type I transmembrane protein that functions as an immune checkpoint and is encoded by the *C10orf54* gene. VISTA is an approximately 50 kDa protein and belongs to the immunoglobulin superfamily and has one IGV domain. VISTA is part of the B7 family and is primarily expressed in white blood cells. The transcription of VISTA is controlled by p53. VISTA can act as both a ligand and a receptor on T-cells to inhibit T cell effector function and maintain peripheral tolerance. VISTA is expressed at high levels in tumor-infiltrating lymphocytes, such as myeloid-derived suppressor cells and regulatory T cells, and its blockade with an antibody results in delayed tumor growth in mouse models of melanoma, and squamous cell carcinoma.

[0005] The present invention provides novel monotherapies and combination therapies for use in treatment of diseases.

SUMMARY OF THE INVENTION

[0006] In one aspect, the present invention relates to novel anti-VISTA antibodies.

[0007] In some embodiments, the anti-VISTA antibodies include a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:5. In some embodiments, the anti-VISTA antibodies include a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:9 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:13. In some embodiments, the anti-VISTA antibodies include a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:17 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:21.

[0008] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:2, a vhCDR2 comprising SEQ ID NO:3, a vhCDR3 comprising SEQ ID NO:4, a vlCDR1 comprising SEQ ID NO:6, a vlCDR2 comprising SEQ ID NO:7, and a vlCDR3 comprising SEQ ID NO:8. In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:10, a vhCDR2 comprising SEQ ID NO:11, a vhCDR3 comprising SEQ ID NO:12, a vlCDR1 comprising SEQ ID NO:14, a vlCDR2 comprising SEQ ID NO:15, and a vlCDR3 comprising SEQ ID NO:16. In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:18, a vhCDR2 comprising SEQ ID NO:19, a vhCDR3 comprising SEQ ID NO:20, a vlCDR1 comprising SEQ ID NO:22, a vlCDR2 comprising SEQ ID NO:23, and a vlCDR3 comprising SEQ ID NO:24.

[0009] In some embodiments, the present invention includes a method of modulating an immune response in a subject, the method comprising administering to the subject an effective amount of an anti-VISTA antibody comprising a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0010] In some embodiments, the present invention includes a method of modulating an immune response in a subject, the method comprising administering to the subject an effective amount of an anti-VISTA antibody comprising a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or and/or Figure 46 and/or Figure 47.

[0011] In some embodiments, the anti-VISTA antibodies described herein include a constant region with an amino acid sequence at least 90% identical to a human IgG. In some embodiments, the IgG is selected from the group consisting of IgG1, IgG2, IgG3 and IgG4. In some embodiments, the IgG is an IgG4.

[0012] In another aspect, the present invention relates to a nucleic acid composition comprising a first nucleic acid encoding any one of the heavy chain variable regions described herein and a second nucleic acid encoding any one of the light chain variable regions described herein.

[0013] Another aspect of the present invention relates to an expression vector composition that includes any one of the nucleic acid compositions described herein. In some embodiments, the first nucleic acid is contained in a first expression vector and the second nucleic acid is contained in a second expression vector. In some other embodiments, the first nucleic acid and the second nucleic acid are contained in a single expression vector.

[0014] Another aspect of the present invention relates to a host cell that includes any one of the expression vectors described herein. Also presented is a method of making anti-VISTA antibodies, and the method includes culturing the host cell under conditions wherein the antibodies expressed, and recovering the antibodies.

[0015] In another aspect, the present invention relates to a composition that includes any one of the anti-VISTA antibodies described herein, and a pharmaceutical acceptable carrier or diluent.

[0016] Also described is a method of modulating an immune response in a subject, and the method includes administering to the subject an effective amount of any one of the anti-VISTA antibodies described herein, or any one of the compositions described herein.

[0017] In some embodiments, the method modulates an immune response in the subject, and the method includes administering to the subject an effective amount of an anti-VISTA antibody, wherein the antibody includes a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:5; and/or a vhCDR1 comprising SEQ ID NO:2, a vhCDR2 comprising SEQ ID NO:3, a vhCDR3 comprising SEQ ID NO:4, a vlCDR1 comprising SEQ ID NO:6, a vlCDR2 comprising SEQ ID NO:7, and a vlCDR3 comprising SEQ ID NO:8.

[0018] In some embodiments, the method modulates an immune response in the subject, and the method includes administering to the subject an effective amount of an anti-VISTA antibody, wherein the antibody includes a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:9 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:13; and/or a vhCDR1 comprising SEQ ID NO:10, a vhCDR2 comprising SEQ ID NO:11, a vhCDR3 comprising SEQ ID NO:12, a vlCDR1 comprising SEQ ID NO:14, a vlCDR2 comprising SEQ ID NO:15, and a vlCDR3 comprising SEQ ID NO:16.

[0019] In some embodiments, the method modulates an immune response in the subject, and the method includes administering to the subject an effective amount of an anti-VISTA antibody, wherein the anti-VISTA antibody includes a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:17 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:21; and/or a vhCDR1 comprising SEQ ID NO:18, a vhCDR2 comprising SEQ ID NO:19, a vhCDR3 comprising SEQ ID NO:20, a vlCDR1 comprising SEQ ID NO:22, a vlCDR2 comprising SEQ ID NO:23, and a vlCDR3 comprising SEQ ID NO:24.

[0020] In another aspect, the present invention relates to a method of treating cancer in a subject, and the method includes administering to the subject an effective amount of an anti-VISTA antibody described herein, or a composition thereof. In some embodiments, the cancer to be treated expresses VISTA. The cancer to be treated can be colorectal cancer, breast cancer, rectal cancer, lung (including non-small cell lung cancer), non-Hodgkin's lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, Kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, melanoma, ovarian cancer, endometrial cancer, cervical cancer, colorectal cancer, mesothelioma, and multiple myeloma. In some embodiments, an anti-VISTA antibody is used in combination with one or more additional therapeutic agents to treat cancer. In some embodiments, the additional therapeutic agents are other immune checkpoint inhibitors, such as a PD-1 inhibitor, PD-L1 inhibitor, CTLA-inhibitor, TIM-3 inhibitor, and a LAG-3 inhibitor. In some embodiments, the additional therapeutic agents are tumor targeting antibodies. In some embodiments the tumor targeting antibodies are anti-CD20, anti-EGFR, and anti-Her2. In some embodiments, the tumor targeting antibodies are trastuzumab, rituximab, and cetuximab. In some embodiments, the additional therapeutic agents are integrin-binding polypeptide-Fc fusions. In some embodiments, the integrin-binding polypeptide-Fc fusion is NOD-201.

[0021] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 2; a heavy chain variable region CDR2 comprising SEQ ID NO: 3; a heavy chain variable region CDR3 comprising SEQ ID NO: 4; a light chain variable region CDR1 comprising SEQ ID NO: 6; a light chain variable region CDR2 comprising SEQ ID NO: 7; and a light chain variable region CDR3 comprising SEQ ID NO: 8. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO: 1. In some embodiments, the antibody further comprises a light chain variable region comprising SEQ ID NO: 5.

[0022] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 10; a heavy chain variable region CDR2 comprising SEQ ID NO: 11; a heavy chain variable region CDR3 comprising SEQ ID NO: 12; a light chain variable region CDR1 comprising SEQ ID NO: 14; a light chain variable region CDR2 comprising SEQ ID NO: 15; and a light chain variable region CDR3 comprising SEQ ID NO: 16. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO: 9. In some embodiments, the antibody further comprises a light chain variable region comprising SEQ ID NO: 13.

[0023] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 18; a heavy chain variable region CDR2 comprising SEQ ID NO: 19; a heavy chain variable region CDR3 comprising SEQ ID NO: 20; a light chain variable region CDR1 comprising SEQ ID NO: 22; a light chain variable region CDR2 comprising SEQ ID NO: 23; and a light chain variable region CDR3 comprising SEQ ID NO: 24. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO: 17. In some embodiments, the antibody further comprises a light chain

variable region comprising SEQ ID NO:21. In another aspect, the present invention relates to a method of inhibiting growth of VISTA expressing cells comprising contacting the cells with a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit growth of VISTA expressing cells, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 2; a heavy chain variable region CDR2 comprising SEQ ID NO: 3; a heavy chain variable region CDR3 comprising SEQ ID NO: 4; a light chain variable region CDR1 comprising SEQ ID NO: 6; a light chain variable region CDR2 comprising SEQ ID NO: 7; and a light chain variable region CDR3 comprising SEQ ID NO: 8. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO:1. In some embodiments, the antibody further comprises a light chain variable region comprising SEQ ID NO:5.

[0024] In another aspect, the present invention relates to a method of inhibiting growth of VISTA expressing cells comprising contacting the cells with a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit growth of VISTA expressing cells, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 10; a heavy chain variable region CDR2 comprising SEQ ID NO: 11; a heavy chain variable region CDR3 comprising SEQ ID NO: 12; a light chain variable region CDR1 comprising SEQ ID NO: 14; a light chain variable region CDR2 comprising SEQ ID NO: 15; and a light chain variable region CDR3 comprising SEQ ID NO: 16. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO:9. In some embodiments, the antibody further comprises a light chain variable region comprising SEQ ID NO:13.

[0025] In another aspect, the present invention relates to a method of inhibiting growth of VISTA expressing cells comprising contacting the cells with a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit growth of VISTA expressing cells, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 18; a heavy chain variable region CDR2 comprising SEQ ID NO: 19; a heavy chain variable region CDR3 comprising SEQ ID NO: 20; a light chain variable region CDR1 comprising SEQ ID NO: 22; a light chain variable region CDR2 comprising SEQ ID NO: 23; and a light chain variable region CDR3 comprising SEQ ID NO: 24. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO:17. In some embodiments, the antibody further comprises a light chain variable region comprising SEQ ID NO:21.

[0026] In another aspect, the present invention relates to a method of inhibiting the binding of VISTA to VSIG3 on cells in a subject having a disorder by administering to the subject a monoclonal antibody which binds to human VISTA, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 2; a heavy chain variable region CDR2 comprising SEQ ID NO: 3; a heavy chain variable region CDR3 comprising SEQ ID NO: 4; a light chain variable region CDR1 comprising SEQ ID NO: 6; a light chain variable region CDR2 comprising SEQ ID NO: 7; and a light chain variable region CDR3 comprising SEQ ID NO: 8. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO:1. In some embodiments, the antibody further comprises a light chain variable region comprising SEQ ID NO:5.

[0027] In another aspect, the present invention relates to a method of inhibiting the binding of VISTA to VSIG3 on cells in a subject having a disorder by administering to the subject a monoclonal antibody which binds to human VISTA, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 10; a heavy chain variable region CDR2 comprising SEQ ID NO: 11; a heavy chain variable region CDR3 comprising SEQ ID NO: 12; a light chain variable region CDR1 comprising SEQ ID NO: 14; a light chain variable region CDR2 comprising SEQ ID NO: 15; and a light chain variable region CDR3 comprising SEQ ID NO: 16. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO:9. In some embodiments, the antibody further comprises a light chain variable region comprising SEQ ID NO:13.

[0028] In another aspect, the present invention relates to a method of inhibiting the binding of VISTA to VSIG3 on cells in a subject having a disorder by administering to the subject a monoclonal antibody which binds to human VISTA, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 18; a heavy chain variable region CDR2 comprising SEQ ID NO: 19; a heavy chain variable region CDR3 comprising SEQ ID NO: 20; a light chain variable region CDR1 comprising SEQ ID NO: 22; a light chain variable region CDR2 comprising SEQ ID NO: 23; and a light chain variable region CDR3 comprising SEQ ID NO: 24. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO:17. In some embodiments, the antibody further comprises a light chain variable region comprising SEQ ID NO:21.

[0029] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a

monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 1 and 5, respectively.

[0030] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 9 and 13, respectively.

[0031] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 17 and 21, respectively.

[0032] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 1 and 5, respectively.

[0033] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 9 and 13, respectively.

[0034] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to

the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 17 and 21, respectively.

[0035] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 1 and 5, respectively.

[0036] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 9 and 13, respectively.

[0037] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 17 and 21, respectively.

[0038] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 1 and 5, respectively.

[0039] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to

the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 9 and 13, respectively.

[0040] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 17 and 21, respectively.

[0041] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises heavy and light chain variable region sequences having at least 95% identity to SEQ ID NO: 1 and 5, respectively.

[0042] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises heavy and light chain variable region sequences having at least 95% identity to SEQ ID NO: 9 and 13, respectively.

[0043] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises heavy and light chain variable region sequences having at least 95% identity to SEQ ID NO: 17 and 21, respectively.

[0044] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody

comprises heavy and light chain variable region sequences having at least 95% identity to SEQ ID NO: 1 and 5, respectively.

[0045] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody comprises heavy and light chain variable region sequences having at least 95% identity to SEQ ID NO: 9 and 13, respectively.

[0046] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody comprises heavy and light chain variable region sequences having at least 95% identity to SEQ ID NO: 17 and 21, respectively.

[0047] In another aspect, the present invention relates to a method for inducing or enhancing an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 2; a heavy chain variable region CDR2 comprising SEQ ID NO: 3; a heavy chain variable region CDR3 comprising SEQ ID NO: 4; a light chain variable region CDR1 comprising SEQ ID NO: 6; a light chain variable region CDR2 comprising SEQ ID NO: 7; and a light chain variable region CDR3 comprising SEQ ID NO: 8. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO: 1. In some embodiments, the antibody further comprises a light chain variable region comprising SEQ ID NO: 5.

[0048] In another aspect, the present invention relates to a method for inducing or enhancing an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 10; a heavy chain variable region CDR2 comprising SEQ ID NO: 11; a heavy chain variable region CDR3 comprising SEQ ID NO: 12; a light chain variable region CDR1 comprising SEQ ID NO: 14; a light chain variable region CDR2 comprising SEQ ID NO: 15; and a light chain

variable region CDR3 comprising SEQ ID NO: 16. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO: 9. In some embodiments, the antibody further comprises a light chain variable region comprising SEQ ID NO: 13.

[0049] In another aspect, the present invention relates to a method for inducing or enhancing an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 18; a heavy chain variable region CDR2 comprising SEQ ID NO: 19; a heavy chain variable region CDR3 comprising SEQ ID NO: 20; a light chain variable region CDR1 comprising SEQ ID NO: 22; a light chain variable region CDR2 comprising SEQ ID NO: 23; and a light chain variable region CDR3 comprising SEQ ID NO: 24. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO: 17. In some embodiments, the antibody further comprises a light chain variable region comprising SEQ ID NO: 21.

[0050] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 2; a heavy chain variable region CDR2 comprising SEQ ID NO: 3; a heavy chain variable region CDR3 comprising SEQ ID NO: 4; a light chain variable region CDR1 comprising SEQ ID NO: 6; a light chain variable region CDR2 comprising SEQ ID NO: 7; and a light chain variable region CDR3 comprising SEQ ID NO: 8. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO: 1. In some embodiments, the antibody further comprises a light chain variable region comprising SEQ ID NO: 5.

[0051] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 10; a heavy chain variable region CDR2 comprising SEQ ID NO: 11; a heavy chain variable region CDR3 comprising SEQ ID NO: 12; a light chain variable region CDR1 comprising SEQ ID NO: 14; a light chain variable region CDR2 comprising SEQ ID NO: 15; and a light chain variable region CDR3 comprising SEQ ID NO:

16. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO: 9. In some embodiments, the antibody further comprises a light chain variable region comprising SEQ ID NO: 13.

[0052] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 18; a heavy chain variable region CDR2 comprising SEQ ID NO: 19; a heavy chain variable region CDR3 comprising SEQ ID NO: 20; a light chain variable region CDR1 comprising SEQ ID NO: 22; a light chain variable region CDR2 comprising SEQ ID NO: 23; and a light chain variable region CDR3 comprising SEQ ID NO: 24. In some embodiments, the antibody further comprises a heavy chain variable region comprising SEQ ID NO: 17. In some embodiments, the antibody further comprises a light chain variable region comprising SEQ ID NO: 21.

[0053] In another aspect, the present invention relates to a method of treating a non-cancerous disease in a subject comprising administering to the subject an effective amount of the antibody according to any one of methods or compositions described herein.

[0054] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0055] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0056] In another aspect, the present invention relates to a method of inhibiting growth of VISTA expressing cells comprising contacting the cells with a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit growth of VISTA expressing cells, wherein the antibody comprises a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0057] In another aspect, the present invention relates to a method of inhibiting growth of VISTA expressing cells comprising contacting the cells with a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit growth of VISTA expressing cells, wherein the antibody comprises a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0058] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0059] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0060] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0061] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 46 and/or Figure 47.

[0062] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0063] In another aspect, the present invention relates to a method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0064] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody comprises a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0065] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody comprises a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 46 and/or Figure 47.

[0066] In another aspect, the present invention relates to a method for inducing or enhancing an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response, wherein the antibody comprises a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0067] In another aspect, the present invention relates to a method for inducing or enhancing an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response, wherein the antibody comprises a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0068] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response, wherein the antibody comprises a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0069] In another aspect, the present invention relates to a method for inhibiting the suppression of an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response, wherein the antibody comprises a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[0070] In another aspect, the present invention relates to a method according to any of the preceding claims, wherein the immune response is antigen-specific T cell response.

BRIEF DESCRIPTION OF THE DRAWINGS

[0071] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings.

[0072] **FIG. 1A** provides the variable heavy and light chains and corresponding vhCDR1, vhCDR2, vhCDR3, vlCDR1, vlCDR2, and vlCDR3 sequences for the VS7 anti-VISTA antibody.

[0073] **FIG. 1B** provides the variable heavy and light chains and corresponding vhCDR1, vhCDR2, vhCDR3, vlCDR1, vlCDR2, and vlCDR3 sequences for the VS147 anti-VISTA antibody.

[0074] **FIG. 2A - FIG. 2B** provide examples of IgG1, IgG2, IgG3, and IgG4 sequences.

[0075] **FIG. 3** provides flow cytometry data demonstrating antigen binding and display (c-myc) with anti-VISTA antibody. Initial rounds of screening yielded 20+ clones, many with sub-nM affinity to human antigen. Subsequent affinity maturation & cross-reactivity selection yielded mouse and human cross-reactive clone VS147.

[0076] **FIG. 4A – FIG. 4B** provides human and murine VISTA binding data and Kd of can anti-VISTA antibody clone. VS147 exhibited sub-nM affinity to human antigen and single nM affinity to mouse antigen. High VISTA expressing macrophage cell line. Co-culture of HIGH cells with T-cells inhibits activation and IL-2 secretion.

[0077] **FIG. 5** provides data showing VS147 abrogates VISTA-mediated T-cell inhibition *in vitro*.

[0078] **FIG. 6** provides data showing that VS147 shows anti-tumor efficacy as a monotherapy in B16 melanoma. VS147 blocks inhibitory activity of VISTA *in vitro* and *in vivo*.

[0079] **FIG. 7A – FIG. 7D** provides the crystallization of th human VISTA. **(a)** Sequence of human VISTA ECD with secondary structure and cysteines forming disulfide bonds indicated. **(b)** Cartoon structure of human VISTA ECD colored by secondary structure with beta strands labeled by Ig-domain nomenclature. **(c)** Omit map of C-C' loop. **(d)** Electrostatic map of human VISTA (red = negative, blue = positive) revealing positively charged face of protein (black square).

[0080] **FIG. 8A – FIG. 8F** provides B7 Family Comparison. **(A)** Cartoon structure of human VISTA ECD (red) aligned with IgV domain of human PD-L1 (gray). **(B)** Unique helix in VISTA in place of beta sheet in PD-L1. **(C)** Unique C-C' loop in VISTA that extends from the beta-sandwich core. **(D)** Disulfide bonds in VISTA, including two unique disulfides in addition to conserved bond (22, 114) between sheets B and F. **(E)** Heatmap of Dali pairwise Z-scores between hVISTA and five other B7 family proteins. **(F)** Omit map of C-C' loop.

[0081] **FIG. 9A– FIG. 9E** provides a comparison of human, murine, and cyno VISTA. **(A)** PyMOL Alignment of human VISTA ECD (red) and homology models of mouse VISTA (cyan) and cyno VISTA (beige). **(B)** Beta sheet that is absent in mouse VISTA. Side chains shown as sticks, highlighting the lack of leucine in the mouse VISTA sequence. **(C)** Portion of the C-C' loop that is not conserved among species of VISTA. Residue differences in the mouse sequence are evident at positions 54 and 62. **(D)** Binding of VSTB to human or mouse VISTA displayed on yeast. **(E)** Protein sequence alignment of human, mouse, and cyno VISTA. Residue differences from human VISTA (bold red), differences within same amino acid category (blue), unique loop+helix (bold underlined, B), and unique beta sheet (bold underlined, C).

[0082] **FIG. 10A – FIG. 10D** provides information regarding mapping the VSTB binding epitope of VISTA, including data showing the mapping the VSTB binding epitope of VISTA. **(A)** Library screening used to isolate yeast-displaying VISTA mutants that retain or reduce Ab binding. FACS gates are shown in gray boxes on dot plots of individual positive or negative sorts. **(B)** Four residues identified from epitope mapping highlighted in red on the human VISTA ECD structure. **(C)** Binding of soluble VSTB antibody to yeast-displayed human VISTA with single amino acid alanine substitutions. **(D)** Binding signal comparison of 250 nM VSTB to yeast-displayed hVISTA alanine mutants.

[0083] **FIG. 11A – FIG. 11E** provides data showing the mapping the VSTB binding epitope of VISTA. VSIG3 interacts with VISTA via VSTB binding epitope. **(A)** Sequence of hVISTA ECD with C-C' loop underlined and mutated residues (54, 62, and 63) in red. **(B)** Binding of WT or VISTA 54A/62A/63A to VS147 or VSTB antibody in ELISA format. VISTA was added to well coated antibody at three different concentrations. **(C)** Binding assay via indirect ELISA with well coated VSIG3 incubated with soluble WT or VISTA 52A/62A/63A. **(D)** Binding of 1 μ M WT VISTA pre-complexed with a titration of VSTB concentrations to well coated VSIG3 in ELISA format. **(e)** VSIG3 binding signal comparison of 1 μ M WT VISTA, 1 μ M VISTA 54A/62A/63A, 1 μ M VISTA precomplexed with 500 nM VSTB, and 1 μ M VISTA precomplexed with an IgG isotype control.

[0084] **FIG. 12** provides data showin deglycosylation of VISTA. SDS -PAGE gel of VISTA ECD at different stages of deglycosylation (deglycosylation of VISTA). **(A)** Wild-type VISTA Wild-type (Met1-Ala194), **(B)** VISTA with three asparagine to glutamine mutations (N59Q, N76Q, N158Q), **(c)** VISTA with 3 N→Q mutations, Kifunensine in culture media, and Endo Hf enzymatic cleavage. The predicted molecular mass of VISTA ECD is 19 kDa. Only the

combination of genetic mutations and enzymatic cleavage produced a distinct band at the estimated molecular mass.

[0085] FIG. 13A – FIG. 13B provides data showing antibody verification for epitope mapping. **(A)** Flow cytometry plot of VS147 antibodyAb binding to yeast-displayed hVISTA with and without heat denaturation of yeast. The decreased binding after heat denaturation confirms conformational (rather than linear) epitope binding. **(B)** Relative binding plot of VS147 and VSTB antibodies to yeast-displayed hVISTA. Antibodies were added sequentially, where the first antibodyAb was allowed to reach equilibrium, then the second antibodyAb was added for 15 min, or together where both antibodiesAbs were allowed to reach equilibrium. Binding of VS147 antibody = blue bar; binding of VSTB antibody = red bar, normalized to binding signal when antibodies are bound individually. The VSTB antibody does not preclude VS147 antibody binding, while VS147 antibody binding blocks the binding of VSTB. As shown in VS147+VSTB, it is possible for both antibodies to bind simultaneously, indicating distinct epitopes. Error bars represent standard deviation of the mean fluorescence intensity for triplicate measurements.

[0086] FIG. 14 provides data showing point mutant binding to VSTB. The five hVISTA mutations identified from screening (red) and alanine variants at the same position (gray) were displayed as individual clones on yeast and measured for binding to VSTB (200 μ M). Binding was normalized to wild-type (WT) hVISTA binding to 200 μ M VSTB. S124A showed binding equivalent to WT VISTA levels, while all other hVISTA mutants and alanine variants showed a strong reduction in VSTB binding, suggesting these residues are involved in VSTB binding. Error bars represent standard deviation of the mean fluorescence intensity for triplicate measurements.

[0087] FIG. 15 shows the B7 Family Sequence Alignment. Multiple sequence alignment of human B7 family extracellular domains generated from Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). Sequences are truncated after the end of the human VISTA ECD sequence. Amino acid matches to VISTA are shown in red and residue similarities to VISTA (same subtype of side chain) are shown in blue.

[0088] FIG. 16A-FIG. 16 shows the structural deviations of VISTA from B7 family. **(a)** Cartoon structure of human VISTA ECD (red) aligned with IgV domain of human PD-L1 (gray). **(b)** Unique helix in VISTA in place of beta strands in PD-L1. **(c)** Unique C-C' loop in

VISTA that extends from the beta-sandwich core. **(d)** Disulfide bonds in VISTA, including two unique disulfides (red spheres) in addition to conserved disulfide bond (C22, C114) between strands B and F (red and gray spheres). **(e)** Heatmap of pairwise Tm-scores (bottom left half) and sequence identities (top right half) between hVISTA and the IgV domains of four other B7 family proteins.

[0089] FIG. 17 shows Hydrogen bonds originating from C-C' loop, related to Figure 7. A single VISTA ECD molecule is shown in green with the extended C-C' loop in pink and two surrounding symmetry molecules in beige and black. Hydrogen bonds from the extended portion of the C-C' loop (residues 42-53) are depicted as dashed lines. Minor hydrogen bonding contacts between loop side chains and symmetry molecules are shown compared to the extensive hydrogen bonding within a single VISTA monomer.

[0090] FIG. 18A-FIG. 18C shows the proximity of N→Q mutations and effects on VSTB Binding, related to Figure 3. (a) The isolated epitope residues (red) and the sites of N→Q mutations (cyan). Three N→Q mutations, N59Q/N76Q/N158Q, were introduced to facilitate crystallization of the hVISTA ECD domain. N158 is part of the C-terminal section that could not be resolved and is therefore not shown in the structure. (b) Yeast displayed WT VISTA or N59Q/N76Q/N158Q triple mutant VISTA was measured for affinity to soluble VSTB. Binding affinities are not significantly different. (c) Hydrophobic packing of the C-C' loop turn region. Gray spheres indicate hydrophobic packing and hydrogen bonds are shown as dashed lined. Side chains of residues 57 and 58 are involved with hydrogen bonding or hydrophobic packing while residue 59 points away and is not involved with intramolecular interactions.

[0091] FIG. 19 shows the solvent accessibility of epitope residues, related to Figure 10. Percent accessible surface area was calculated via the PISA server (<https://www.ebi.ac.uk/pdbe/pisa/>). Heavily buried residues (W40, F97) were used as a comparison to highlight solvent exposure of epitope mapped region. The three residues isolated as the epitope hotspot (54, 62, 63) have solvent accessibilities above 35%.

[0092] FIG. 20 shows hydrogen bonds observed within C-C' loop, related to Figure 7. Intermolecular interactions from side chains of C-C' loop residues are listed (Residues 42-59). Hydrogen bonds acting through secondary interactions are shown in blue. There are 30 unique hydrogen bonds originating from the C-C' loop side chains compared to four between symmetry molecules (bold).

[0093] **FIG. 21** shows the SG7 monotherapy study with B16F10 syngeneic mouse model. 250,000 B16F10 cells were implanted in the right flank of shaved C57Bl/6 mice. Treatment with 15 mg/kg SG7 began on day 2 and proceeded every other day until day 16. Mouse tumors were measured every other day and tumor volume was calculated with formula $(\text{Length} \times \text{Width}^2)/2$. T-tests were performed with tumor volumes of different treatment groups. Significant tumor volume differences were observed on day 14 and 16.

[0094] **FIG. 22** shows the SG7 combination and Fc-dead study with B16F10 syngeneic mouse model (B16F10 Study with 2.5F-Fc combination). 100,000 B16F10 cells were implanted in the right flank of shaved C57Bl/6 mice. Treatment was administered on day 10, 13, 17, and 20. Treatments were 10 mg/kg SG7, 10 mg/kg of SG7 D265A (Fc dead version), and combination of 10 mg/kg SG7 and 20 mg/kg 2.5F-Fc (integrin binding agent fused to mouse IgG2a Fc domain). **(a)** Mouse tumors were measured every other day and tumor volume was calculated with formula $(\text{Length} \times \text{Width}^2)/2$. T-tests were performed with tumor volumes of different treatment groups. Significant tumor volume differences were observed starting on Day 17. **(b)** All treated groups had smaller tumors than untreated mice by day 21. Survival of mice based on a 100 mm² tumor area euthanasia criteria shows survival extension of treated groups.

[0095] **FIG. 23** shows the SG7 competition assay with 13F3 on yeast and that SG7 competes with 13F3 for binding. Mouse VISTA was displayed on the surface of yeast, incubated with SG7 and/or 13F3, and detected by anti-mouse 488 and anti-hamster 647 secondary antibodies, respectively, via flow cytometry. 'SG7 only' and '13F3 only' samples were incubated with either SG7 or 13F3. For 'SG7 then 13F3' and '13F3 and SG7' columns, the first antibody was incubated with mVISTA yeast for 3 hrs at room temperature before the subsequent antibody was added at 4 °C for 15 mins. For the 'SG7 and 13F3' column, both antibodies were added at the same time for 3 hrs at room temperature. All samples were detected with both secondary antibodies. SG7 and 13F3 were added at a saturating concentration of 250 nM. Lower binding of 13F3 after SG7 bound and the lack of binding from both antibodies when added together demonstrates overlapping epitopes.

[0096] **FIG. 24A-FIG. 24E** shows SG7 Affinity Data. **(a)** Binding of soluble full-length SG7-mIgG2a to yeast-displayed human or mouse VISTA. **(b)** Binding of soluble full-length SG7-mIgG2a to RAW 264.7 cells with high levels of mouse VISTA expression (HIGH) or WT RAW cells with low expression of mouse VISTA (WT). **(c)** Binding of soluble SG7-mIgG2a to a constant concentration of soluble 100 pM human VISTA using KinExA. **(d)** Binding of

soluble SG7-mIgG2a to a constant concentration of soluble 1 nM mouse VISTA using KinExA. KinExA curves were fit using standard equilibrium binding on KinExA Pro Software. (e) Table of K_d values determined from multiple binding assay modalities.

[0097] **FIG. 25** shows an antibody competition assay used to assess the ability of XC147 to bind to VISTA simultaneously with other anti-VISTA antibodies. The BMS antibody (clone '767), VSTB antibody (Janssen Therapeutics, VSTB112 clone), and R&D Systems antibody (clone #730804) were coated on an ELISA plate at 5 ug/mL. An anti-EGFR and anti-HIS antibody were used as a negative and positive control, respectively. Human VISTA-HIS was added at 1 nM to all wells and a titration of XC147 concentrations were added on top. The amount of SG7 that remain bound was detected. Only the positive control anti-HIS antibody and the R&D systems antibody were found to bind to VISTA simultaneously with XC147. The BMS and Janssen antibodies could not bind simultaneously with XC147, suggesting overlapping epitopes.

[0098] **FIG. 26** shows a PSGL-1 blocking assay used to assess XC147 blockade of the VISTA/PSGL-1 binding interaction. Human PSGL-1 Fc was coated on an ELISA plate at 10 ug/mL. Human VISTA-Fc at 250 nM was pre-complexed with a titration of either XC147, a positive control BMS antibody (clone '767), or a negative isotype control antibody. The amount of VISTA that remained bound decreased with increasing concentrations of BMS and XC147 but to a much lesser degree with the negative isotype control. Both XC147 and BMS demonstrate robust blockade of the VISTA/PSGL-1 interaction.

[0099] **FIG. 27** shows a functional T cell assay used to demonstrate inhibition of VISTA signaling. Plates were coated with CD3 only, CD3 + hVISTA, or CD3 + precomplexed hVISTA and antibody. Janssen antibody (VSTB112) was used as a positive control for inhibition and the anti-mouse VISTA antibody (13F3) was used as a negative control. Jurkat T cells with an NFAT BFP reporter added to wells and incubated for 24 hrs. The addition of XC147 and VSTB, but not 13F3, rescued levels of activation in the presence of VISTA. Both XC147 and VSTB demonstrate functional blockade of VISTA.

[00100] **FIG. 28A-C** shows sequences for XC147 (an anti-VISTA antibody).

[00101] **FIG. 29** shows the scFv sorting schematic. Library screening progression was used to isolate scFv mutants that bound human VISTA (Round 1) and mouse VISTA (Round 2). FACS gates are shown on dot plots of individual sorts.

[00102] **FIG. 30** shows single clone analysis after sort round 1. Binding intensity to human VISTA (red) and mouse VISTA (gray) of individual scFv clones isolated after Round 1 of screening. All clones (except V5) showed above background binding signal to human VISTA but only the V9 clone displayed above background binding signal to mouse VISTA. Binding intensity was measured by cloning each individual mutant post-sort 1.4 into pCTCON2 and inducing for surface expression. Binding intensity under equilibrium conditions between each clone and 10 nM hVISTA-Fc or 100 nM-mVISTA-HIS was measured on a BD Accuri.

[00103] **FIG. 31** shows Binding Affinity to mVISTA and hVISTA by KinExA. Full kinetic binding curves of top clone (XC147) against human VISTA monomer and mouse VISTA monomer. XC147 binds human VISTA with a K_d of 138 pM and binds mouse VISTA with a K_d of 1.03 nM. Soluble hVISTA-HIS or mVISTA-HIS was incubated at a constant concentration with serially diluted XC147 and then run on the KinExA instrument as an Equilibrium Assay. PMMA beads coated with XC147 was used to detect free hVISTA-HIS or mVISTA-HIS.

[00104] **FIG. 32** shows an antibody competition ELISA. Human VISTA pre-complexed with competitor antibody (BMS767, VSTB112) remains singly bound after incubation with XC147. XC147 must therefore bind an overlapping epitope or competitor antibody changes conformation to prevent XC147 binding. This assay was done by coating ELISA plates with competitor antibody, adding soluble hVISTA-Fc, and then a short incubation with XC147.

[00105] **FIG. 33** shows epitope binning of XC147 with competitor antibodies. Epitope binning experiments with a ForteBio Octet were done to examine overlapping epitopes. The association step of the binding curve is shown after tips loaded with antibody bound to hVISTA. No significant association of second antibody occurs after first antibody is already bound with VISTA antigen. All three antibodies cross-block the other two antibodies.

[00106] **FIG. 34** shows epitope binning of XC147 with competitor antibodies. XC147 competes with VISTA binding to primary T cells in a dose-dependent manner. A) Mouse VISTA-Fc binding to activated mouse T cells at pH 6.0 with increasing concentrations of pre-complexed XC147. B) Human VISTA-Fc binding to activated human T cells at pH 6.0 with

increasing concentrations of pre-complexed XC147. Mouse T cells were activated from fresh splenocytes using α -CD3/ α -CD28 mouse activator Dynabeads for 72 hr and human T cells were activated from frozen CD4/CD8 pure T cells using α -CD3/ α -CD28 human activator Dynabeads for 72 hr before measuring VISTA binding. A serial dilution of XC147 concentrations was incubated with a constant concentration of mVISTA-Fc (100 nM) and hVISTA-Fc (125 nM) and then added to activated T cells. VISTA binding signal was measured on a BD Accuri.

[00107] **FIG. 35** shows pH Dependence of antibody binding to hVISTA. Human VISTA displayed on yeast was incubated with serial dilutions of XC147, VSTB112, or BMS767 in PBS+0.1% BSA pH 6.0 or pH 7.4. Binding signal of each antibody was detected by flow cytometry and curves were fit to a saturation binding curve with GraphPad Prism to obtain K_d values. XC147 binds better than both competitor antibodies at pH 6.0 and pH 7.4. BMS767 is heavily pH-dependent with no binding signal detected at pH 7.4. The binding affinity of XC147 and VSTB112 does not change significantly at different pHs.

[00108] **FIG. 36** shows a Jurkat T Cell activation assay. Rescuing effect of XC147 and VSTB112 on the activation of Jurkat NFAT (BFP) T cells in the presence of human VISTA-Fc. Anti-CD3 (OKT3) was coated on tissue culture plates with or without hVISTA-Fc (in a 1:4 weight ratio, α CD3:hVISTA-Fc). XC147, VSTB112, or an mIgG2a isotype control at 1 μ M concentration were co-coated with anti-CD3 and hVISTA-Fc. The presence of XC147 and VSTB112 antibody blocked the suppressive function of hVISTA-Fc and restored WT levels of T Cell Activation, measured by an NFAT reporter leading to BFP expression. The isotype control antibody that does not bind VISTA did not change rescue activation.

[00109] **FIG. 37** shows epitope mapping sorts. Library screening progression used to isolate human VISTA mutants that lost binding to XC147 but retained binding to VSTB112. A library of human VISTA mutants was created and displayed on yeast as fusions to Aga2p such that only one amino acid mutation was present in each gene. A series of positive and negative sorts against XC147 and VSTB112 isolated mutants that disrupted binding to the target (XC147) but still retained structural integrity. FACS gates are shown on dot plots of individual sorts.

[00110] **FIG. 38** shows VISTA enrichment during XC147 epitope map sorts. Enrichment of residues after each epitope mapping sort round (negative – red, positive – blue). Plasmid DNA was extracted from yeast pools after sort 2, sort 3, and sort 4 and deep sequenced by GeneWiz (Amplicon-EZ). The enrichment of mutations at a residue location was calculated compared to

the base library. Dark blue residues were positively enriched and therefore mutations at this location disrupted XC147 binding to human VISTA.

[00111] **FIG. 39** shows single clone epitope analysis for XC147, BMS767, and VSTB112. Single clone analysis of hVISTA mutants displayed on yeast binding to XC147, BMS767, VSTB112. Antibodies were incubated with yeast-displayed hVISTA mutants near the approximate K_d of each antibody interaction (XC147: 300 pM, BMS767: 3 nM, VSTB112: 3 nM). The panel was designed based on enrichment results and elimination of residues that are buried in the beta sandwich. Binding signal of each mutant was normalized to WT mVISTA. Low signal signifies residues that are important for antibody binding. XC147 and BMS767 are heavily affected by 122G and 125A while VSTB112 is more affected by 37A, 54A, 62A, and 63A. The effect of 36A and 38A is unique to XC147.

[00112] **FIG. 40** shows predicted epitopes for each antibody. Predicted binding epitopes of XC147, BMS767, and VSTB112 on the human VISTA ECD (PDB: 6OIL). The XC147 epitope contains residues in the histidine-rich tip as well as unique F36 and K38 residues that point towards the back of the protein. The BMS767 epitope contains residues that span the entire convex front of the protein. The VSTB112 epitope contains residues that are mostly concentrate in or around the C-C' loop and adjacent helix.

[00113] **FIG. 41** shows single clone analysis of mVISTA mutants binding to XC147. Clonal analysis of individual mouse VISTA mutants displayed on yeast. Selected mutants correspond with aligned human VISTA residues (in parentheses) that make up or are near the predicted XC147 epitope. Binding intensity of each mutant to 4 nM XC147 is shown. Binding signal of each mutant was normalized to WT mVISTA. The two epitope residues in the histidine rich tip (human: H122, E125; mouse: H121, E124) as well as the back-facing residues of F36 (I36 in mouse) and, to a lesser extent, K38 are mediators of XC147 binding to mVISTA. These data suggest that XC147 has a unique antibody binding interface; it shares the histidine rich tip with BMS767 but binding is also dependent on the back side of this region which includes the F36 residue.

[00114] **FIG. 42** shows XC147 competes with native binding interactions between VISTA and two proposed binding partners, PSGL1 and VSIG3. Assays were performed in ELISA format at pH 6.0 by coating hPSGL1-Fc or hVSIG3-Fc on a plate and adding hVISTA-Fc by itself or pre-complexed with a serial dilution of antibody. A) Direct ELISA between coated PSGL1-Fc or

VSIG3-Fc and VISTA-Fc to measure apparent K_d of both interactions. B) A competition binding ELISA with well-coated PSGL1-Fc and increasing concentrations of XC147 or BMS767 in complex with 250 nM human VISTA-Fc. The binding signal of VISTA that was able to bind coated PSGL1 was detected. C) A competition binding ELISA with well-coated VSIG3-Fc and increasing concentrations of XC147 or VSTB767 in complex with 1 uM human VISTA-Fc. The binding signal of VISTA that was able to bind coated VSIG3 was detected.

[00115] **FIG. 43** shows *In vivo* efficacy of XC147 in B16F10. B16F10 tumor-bearing c57bl/6 mice were treated with 10 mg/kg XC147 bi-weekly, starting on day 10 (red arrows). n=5 mice per group; Mean tumor volume of each group (left) and individual tumor growth curves (right). These data are representative of two independent experiments. Means +/- SEM are shown. P-values calculated by two-tailed unpaired Student's t test.

[00116] **FIG. 44** shows *in vivo* efficacy of XC147 in combination with PD-1 in MC38. Combination of XC147 and anti-PD1 (29F.1A12) is a better tumor growth inhibitor than XC147 or anti-PD1 alone. MC38 tumor-bearing c57bl/6 mice were treated with 30 mg/kg XC147 and/or 5 mg/kg anti-PD1, starting on day 9 (black arrows); n=7 mice per group; Mean tumor volume of each group (left) and individual tumor growth curves (right). These data are representative of two independent experiments. Means +/- SEM are shown. P-values calculated by two-way ANOVA with DMCT.

[00117] **FIG. 45** shows *in vivo* efficacy of XC147 in 4T1. Both versions of XC147 slow tumor growth in a 4T1 model of triple negative breast cancer. The active version of XC147 has a potent immune remodeling effect by decreasing PMN-MDSCs and increase CD4 and CD8 T cells. A) 4T1 tumor-bearing Balb/c mice were treated with 30 mg/kg XC147 Active Fc (mIgG2a) or 30 mg/kg XC147 Dead Fc (mIgG2a-LALA/PG) bi-weekly, starting on day 8 (black arrows). n=5-7 mice per group as indicated. Mean tumor volume of each group is shown over time. Means +/- SEM are shown. P-values calculated by two-way ANOVA with DMCT. B) Immune flow analysis of extracted 4T1 tumors on Day 17. The percentage of PMN-MDSCs, CD4+ T cells and CD8+ T cells out of total CD45+ cells in each tumor sample are shown.

[00118] **FIG. 46A-46UU** show sequences for anti-VISTA antibodies.

[00119] **FIG. 47A-47M** show sequences for anti-VISTA antibodies.

DETAILED DESCRIPTION

I. INTRODUCTION

[00120] The present disclosure provides novel anti-VISTA antibodies. The anti-VISTA antibodies described herein bind human VISTA. In some embodiments, the anti-VISTA antibodies bind human VISTA with high affinities. In some embodiments, the anti-VISTA antibodies act as functional VISTA agonists, and upon binding to VISTA they induce or enhance an immune response. In some embodiments, the anti-VISTA antibodies act as functional VISTA antagonists, and upon binding to VISTA they block interaction of VISTA with VSIG3, and inhibit an immune response, or in some instances inhibit the suppression of an immune response. Also provided in the present disclosure are methods of using such antibodies to modulate an immune response in a subject, and, for example, to treat cancer. The ligand for VISTA has been shown to be VSIG3. (*See*, for example WO2018027042 and US20170306020, incorporated by reference herein in their entirety.) In addition, nucleic acids encoding these antibodies, as well as host cells that include such nucleic acids are described in the present disclosure.

II. DEFINITIONS

[00121] To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

[00122] As used herein, each of the following terms has the meaning associated with it in this section.

[00123] Terms used in the claims and specification are defined as set forth below unless otherwise specified. In the case of direct conflict with a term used in a parent provisional patent application, the term used in the instant specification shall control.

[00124] “Amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, meaning one of the 20 naturally occurring amino acids that are coded for by DNA and RNA, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refer to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a

carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that function in a manner similar to a naturally occurring amino acid. Amino acids can be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, can be referred to by their commonly accepted single-letter codes.

[00125] An “amino acid substitution” refers to the replacement of at least one existing amino acid residue in a predetermined amino acid sequence (an amino acid sequence of a starting polypeptide) with a second, different “replacement” amino acid residue. An “amino acid insertion” refers to the incorporation of at least one additional amino acid into a predetermined amino acid sequence. While the insertion will usually consist of the insertion of one or two amino acid residues, the present larger “peptide insertions,” can be made, *e.g.* insertion of about three to about five or even up to about ten, fifteen, or twenty amino acid residues. The inserted residue(s) may be naturally occurring or non- naturally occurring as disclosed above. An “amino acid deletion” refers to the removal of at least one amino acid residue from a predetermined amino acid sequence.

[00126] By “amino acid substitution” or “substitution” herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with a different amino acid. In particular, in some embodiments, the substitution is to an amino acid that is not naturally occurring at the particular position, either not naturally occurring within the organism or in any organism. For example, the substitution M252Y refers to a variant polypeptide, in this case an Fc variant, in which the methionine at position 252 is replaced with tyrosine. For clarity, a protein which has been engineered to change the nucleic acid coding sequence but not change the starting amino acid (for example exchanging CGG (encoding arginine) to CGA (still encoding arginine) to increase host organism expression levels) is not an “amino acid substitution”; that is, despite the creation of a new gene encoding the same protein, if the protein has the same amino acid at the particular position that it started with, it is not an amino acid substitution.

[00127] “Polypeptide,” “peptide”, and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

[00128] As used herein, “protein” herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The peptidyl group may comprise naturally occurring amino acids and peptide bonds.

[00129] “Nucleic acid” refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double- stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions) and complementary sequences and as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions can be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081, 1991; Ohtsuka et al., Biol. Chem. 260:2605-2608, 1985; and Cassol et al, 1992; Rossolini et al, Mol. Cell. Probes 8:91-98, 1994). For arginine and leucine, modifications at the second base can also be conservative. The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene. Polynucleotides used herein can be composed of any polyribonucleotide or polydeoxribonucleotide, which can be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double- stranded DNA, DNA that is a mixture of single- and double- stranded regions, single- and double- stranded RNA, and RNA that is mixture of single- and double- stranded regions, hybrid molecules comprising DNA and RNA that can be single- stranded or, more typically, double- stranded or a mixture of single- and double- stranded regions. In addition, the polynucleotide can be composed of triple- stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide can also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. “Modified” bases include, for example, tritylated bases and unusual bases such as

inosine. A variety of modifications can be made to DNA and RNA; thus, “polynucleotide” embraces chemically, enzymatically, or metabolically modified forms.

[00130] The term “nucleotide sequence” includes the ordering of nucleotides in an oligonucleotide or polynucleotide in a single-stranded form of nucleic acid.

[00131] By “nucleic acid construct” it is meant a nucleic acid sequence that has been constructed to comprise one or more functional units not found together in nature. Examples include circular, linear, double-stranded, extrachromosomal DNA molecules (plasmids), cosmids (plasmids containing COS sequences from lambda phage), viral genomes including non-native nucleic acid sequences, and the like.

[00132] The terms “oligonucleotide,” “polynucleotide,” and “nucleic acid molecule”, used interchangeably herein, refer to a polymeric forms of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. The backbone of the polynucleotide can comprise sugars and phosphate groups (as may typically be found in RNA or DNA), or modified or substituted sugar or phosphate groups.

[00133] The term “antibody” is used in the broadest sense and includes, for example, an intact immunoglobulin or an antigen binding portion. Antigen binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Thus the term antibody includes traditional tetrameric antibodies of two heavy chains and two light chains, as well as antigen binding fragments such as Fv, Fab and scFvs. In some cases, the invention provides bispecific antibodies that include at least one antigen binding domain as outlined herein.

[00134] As used herein, the term “PK” is an acronym for “pharmacokinetic” and encompasses properties of a compound including, by way of example, absorption, distribution, metabolism, and elimination by a subject. As used herein, an “extended-PK group” refers to a protein, peptide, or moiety that increases the circulation half-life of a biologically active molecule when fused to or administered together with the biologically active molecule. Examples of an extended-PK group include PEG, human serum albumin (HSA) binders (as disclosed in U.S.

Publication Nos. 2005/0287153 and 2007/0003549, PCT Publication Nos. WO 2009/083804 and WO 2009/133208, and SABA molecules as described in US Publication No. 2012/094909), human serum albumin, Fc or Fc fragments and variants thereof, and sugars (*e.g.*, sialic acid). Other exemplary extended-PK groups are disclosed in Kontermann et al., *Current Opinion in Biotechnology* 2011;22:868-876, which is herein incorporated by reference in its entirety.

[00135] The term “K_{assoc}” or “K_a”, as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term “K_{dis}” or “K_d,” as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term “K_D”, as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of K_d to K_a (*i.e.*, K_d/K_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. In some embodiments, the method for determining the K_D of an antibody is by using surface plasmon resonance, for example, by using a biosensor system such as a BIACORE® system. In some embodiments, the K_D of an antibody is determined by Bio-Layer Interferometry. In some embodiments, the K_D value is measured with the immobilized. In other embodiments, the K_D value is measured with the antibody (*e.g.*, parent mouse antibody, chimeric antibody, or humanized antibody variants) immobilized. In certain embodiments, the K_D value is measured in a bivalent binding mode. In other embodiments, the K_D value is measured in a monovalent binding mode.

[00136] In certain aspects, the polypeptide described can employ one or more “linker domains,” such as polypeptide linkers. As used herein, the term “linker” or “linker domain” refers to a sequence which connects two or more domains in a linear sequence. As used herein, the term “polypeptide linker” refers to a peptide or polypeptide sequence (*e.g.*, a synthetic peptide or polypeptide sequence) which connects two or more domains in a linear amino acid sequence of a polypeptide chain. For example, polypeptide linkers may be used to connect a polypeptide to an Fc domain or other PK-extender such as HSA. In some embodiments, such polypeptide linkers can provide flexibility to the polypeptide molecule. Exemplary linkers include Gly-Ser linkers, such as but not limited to [Gly4Ser], comprising 4 glycines followed by 1 serine and [Gly4Ser3], comprising 4 glycines followed by 3 serines. The term “linker” herein can also refer to a linker used in scFv and/or other antibody structures. Generally, there are a number of suitable scFv linkers that can be used, including traditional peptide bonds, generated by recombinant techniques. The linker peptide may predominantly include the following amino acid residues: Gly, Ser, Ala, or Thr. The linker peptide should have a length that is adequate to

link two molecules in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. In one embodiment, the linker is from about 1 to 50 amino acids in length, preferably about 1 to 30 amino acids in length. In one embodiment, linkers of 1 to 20 amino acids in length may be used, with from about 5 to about 10 amino acids finding use in some embodiments. Useful linkers include glycine-serine polymers, including for example (GS)_n, (GSGGS)_n, (GGGGS)_n, and (GGGS)_n, where n is an integer of at least one (and generally from 3 to 4), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers. Alternatively, a variety of non-proteinaceous polymers, including but not limited to polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, may find use as linkers, that is may find use as linkers. Other linker sequences may include any sequence of any length of CL/CH1 domain but not all residues of CL/CH1 domain; for example, the first 5-12 amino acid residues of the CL/CH1 domains. Linkers can be derived from immunoglobulin light chain, for example C κ or C λ . Linkers can be derived from immunoglobulin heavy chains of any isotype, including for example C γ 1, C γ 2, C γ 3, C γ 4, C α 1, C α 2, C δ , C ϵ , and C μ . Linker sequences may also be derived from other proteins such as Ig-like proteins (*e.g.*, TCR, FcR, KIR), hinge region-derived sequences, and other natural sequences from other proteins. In some embodiments, the linker is a “domain linker”, used to link any two domains as outlined herein together. While any suitable linker can be used, many embodiments utilize a glycine-serine polymer, including for example (GS)_n, (GSGGS)_n, (GGGGS)_n, and (GGGS)_n, where n is an integer of at least one (and generally from 3 to 4 to 5) as well as any peptide sequence that allows for recombinant attachment of the two domains with sufficient length and flexibility to allow each domain to retain its biological function.

[00137] As used herein, the terms “linked,” “fused”, or “fusion” are used interchangeably. These terms refer to the joining together of two or more elements or components or domains, by whatever means including chemical conjugation or recombinant means. Methods of chemical conjugation (*e.g.*, using heterobifunctional crosslinking agents) are known in the art.

[00138] The term “integrin” means a transmembrane heterodimeric protein important for cell adhesion. Integrins comprise an α and β subunit. These proteins bind to extracellular matrix components (*e.g.*, fibronectin, collagen, laminin, etc.) and respond by inducing signaling cascades. Integrins bind to extracellular matrix components by recognition of an Arg-Gly-Asp (RGD) motif. Certain integrins are found on the surface of tumor cells and therefore make

promising therapeutic targets. In certain embodiments, the integrins being targeted are $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_5\beta_1$, individually or in combination.

[00139] The term “integrin-binding polypeptide” refers to a polypeptide which includes an integrin-binding domain or loop within a knottin polypeptide scaffold. The integrin binding domain or loop includes at least one RGD peptide. In certain embodiments, the RGD peptide is recognized by $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$, and $\alpha_5\beta_1$ integrins. In certain embodiments the RGD peptide binds to a combination of $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$, and $\alpha_5\beta_1$ integrins. These specific integrins are found on tumor cells and their vasculature and are therefore the targets of interest.

[00140] Integrins are a family of extracellular matrix adhesion proteins that noncovalently associate into α and β heterodimers with distinct cellular and adhesive specificities (Hynes, 1992; Luscinskas and Lawler, 1994). Cell adhesion, mediated through integrin-protein interactions, is responsible for cell motility, survival, and differentiation. Each α and β subunit of the integrin receptor contributes to ligand binding and specificity.

[00141] Protein binding to many different cell surface integrins can be mediated through the short peptide motif Arg-Gly-Asp (RGD) (Pierschbacher and Ruoslahti, 1984). These peptides have dual functions: They promote cell adhesion when immobilized onto a surface, and they inhibit cell adhesion when presented to cells in solution. Adhesion proteins that contain the RGD sequence include: fibronectin, vitronectin, osteopontin, fibrinogen, von Willebrand factor, thrombospondin, laminin, entactin, tenascin, and bone sialoprotein (Ruoslahti, 1996). The RGD sequence displays specificity to about half of the 20 known integrins including the $\alpha_5\beta_1$, $\alpha_8\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$, $\alpha_v\beta_8$, and $\alpha_v\beta_3$ integrins, and, to a lesser extent, the $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_4\beta_1$, and $\alpha_7\beta_1$ integrins (Ruoslahti, 1996). In particular, the $\alpha_v\beta_3$ integrin is capable of binding to a large variety of RGD containing proteins including fibronectin, fibrinogen, vitronectin, osteopontin, von Willebrand factor, and thrombospondin (Ruoslahti, 1996; Haubner et al., 1997), while the $\alpha_5\beta_1$ integrin is more specific and has only been shown to bind to fibronectin (D'Souza et al., 1991).

[00142] The linear peptide sequence RGD has a much lower affinity for integrins than the proteins from which it is derived (Hautanen et al., 1989). This due to conformational specificity afforded by folded protein domains not present in linear peptides. Increased functional integrin activity has resulted from preparation of cyclic RGD motifs, alteration of the residues flanking

the RGD sequence, and synthesis of small molecule mimetics (reviewed in (Ruoslahti, 1996; Haubner et al., 1997)).

[00143] The term “loop domain” refers to an amino acid subsequence within a peptide chain that has no ordered secondary structure, and resides generally on the surface of the peptide. The term “loop” is understood in the art as referring to secondary structures that are not ordered as in the form of an alpha helix, beta sheet, etc.

[00144] The term “integrin-binding loop” refers to a primary sequence of about 9-13 amino acids which is typically created *ab initio* through experimental methods such as directed molecular evolution to bind to integrins. In certain embodiments, the integrin-binding loop includes an RGD peptide sequence, or the like, placed between amino acids which are particular to the scaffold and the binding specificity desired. The RGD-containing peptide or similar peptide (such as RYD, etc.) is generally not simply taken from a natural binding sequence of a known protein. The integrin-binding loop is preferably inserted within a knottin polypeptide scaffold between cysteine residues, and the length of the loop adjusted for optimal integrin-binding depending on the three-dimensional spacing between cysteine residues. For example, if the flanking cysteine residues in the knottin scaffold are linked to each other, the optimal loop may be shorter than if the flanking cysteine residues are linked to cysteine residues separated in primary sequence. Otherwise, particular amino acid substitutions can be introduced to constrain a longer RGD-containing loop into an optimal conformation for high affinity integrin binding. The knottin polypeptide scaffolds used herein may contain certain modifications made to truncate the native knottin, or to remove a loop or unnecessary cysteine residue or disulfide bond.

[00145] Incorporation of integrin-binding sequences into a molecular (*e.g.*, knottin polypeptide) scaffold provides a framework for ligand presentation that is more rigid and stable than linear or cyclic peptide loops. In addition, the conformational flexibility of small peptides in solution is high, and results in large entropic penalties upon binding. Such constructs have also been described in detail in International Patent Publication WO 2016/025642, incorporated herein by reference in its entirety.

[00146] Incorporation of an integrin-binding sequence into a knottin polypeptide scaffold provides conformational constraints that are required for high affinity integrin binding.

Furthermore, the scaffold provides a platform to carry out protein engineering studies such as affinity or stability maturation.

[00147] As used herein, the term “knottin protein” refers to a structural family of small proteins, typically 25-40 amino acids, which bind to a range of molecular targets like proteins, sugars and lipids. Their three-dimensional structure is essentially defined by a peculiar arrangement of three to five disulfide bonds. A characteristic knotted topology with one disulfide bridge crossing the macro-cycle limited by the two other intra-chain disulfide bonds, which was found in several different microproteins with the same cystine network, lent its name to this class of biomolecules. Although their secondary structure content is generally low, the knottins share a small triple- stranded antiparallel β -sheet, which is stabilized by the disulfide bond framework. Biochemically well-defined members of the knottin family, also called cystine knot proteins, include the trypsin inhibitor EETI-II from *Ecballium elaterium* seeds, the neuronal N-type Ca^{2+} channel blocker co-conotoxin from the venom of the predatory cone snail *Conus geographus*, agouti- related protein (AgRP, See Millhauser et al., “Loops and Links: Structural Insights into the Remarkable Function of the Agouti-Related Protein,” Ann. N.Y. Acad. ScL, Jun. 1, 2003; 994(1): 27-35), the omega agatoxin family, etc. A suitable agatoxin sequence [SEQ ID NO: 41] is given in US Patent 8,536,301, having a common inventor with the present application. Other agatoxin sequences suitable for use in the methods disclosed herein include, but are not limited to Omega-agatoxin-Aa4b (GenBank Accession number P37045) and Omega-agatoxin-Aa3b (GenBank Accession number P81744). Other knottin sequences suitable for use in the methods disclosed herein include, knottin [*Bemisia tabaci*] (GenBank Accession number FJ601218.1), Omega-lycotoxin (Genbank Accession number P85079), mu-O conotoxin MrVIA=voltage-gated sodium channel blocker (Genbank Accession number AAB34917) and Momordica cochinchinensis Trypsin Inhibitor I (MCoTI-I) or II (MCoTI-II) (Uniprot Accession numbers P82408 and P82409, respectively).

[00148] Knottin proteins have a characteristic disulfide linked structure. This structure is also illustrated in Gelly et al., “The KNOTTIN website and database: a new information system dedicated to the knottin scaffold,” Nucleic Acids Research, 2004, Vol. 32, Database issue D156-D159. A triple-stranded β -sheet is present in many knottins. The spacing between cysteine residues is important, as is the molecular topology and conformation of the integrin-binding loop.

[00149] The term “molecular scaffold” means a polymer having a predefined three-dimensional structure, into which an integrin-binding loop is incorporated, such as an RGD peptide sequence as described herein. The term “molecular scaffold” has an art-recognized meaning (in other contexts), which is also intended here. For example, a review by Skerra, “Engineered protein scaffolds for molecular recognition,” *J. Mol. Recognit.* 2000; 13: 167-187 describes the following scaffolds: single domains of antibodies of the immunoglobulin superfamily, protease inhibitors, helix-bundle proteins, disulfide-knotted peptides and lipocalins. Guidance is given for the selection of an appropriate molecular scaffold.

[00150] The term “knottin polypeptide scaffold” refers to a knottin protein suitable for use as a molecular scaffold, as described herein. Characteristics of a desirable knottin polypeptide scaffold for engineering include 1) high stability in vitro and in vivo, 2) the ability to replace amino acid regions of the scaffold with other sequences without disrupting the overall fold, 3) the ability to create multifunctional or bispecific targeting by engineering separate regions of the molecule, and 4) a small size to allow for chemical synthesis and incorporation of non-natural amino acids if desired. Scaffolds derived from human proteins are favored for therapeutic applications to reduce toxicity or immunogenicity concerns, but are not always a strict requirement. Other scaffolds that have been used for protein design include fibronectin (Koide et al., 1998), lipocalin (Beste et al., 1999), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) (Hufton et al, 2000), and tendamistat (McConnell and Hoess, 1995; Li et al, 2003). While these scaffolds have proved to be useful frameworks for protein engineering, molecular scaffolds such as knottins have distinct advantages: their small size and high stability.

[00151] As used herein, the term “NOD201” refers to an integrin-binding polypeptide-Fc fusion comprising the following sequence:

GCPRPRGDNPLTCSQSDCLAGCVCGPNGFCG (SEQ ID NO:119; 2.5F peptide) and having no linker between the 2.5F peptide and the Fc domain. In some embodiments, the Fc domain is from IgG1, IgG2, IgG3, or IgG4 and can be mouse or human derived.

[00152] As used herein, the term “NOD201modK” refers to an integrin-binding polypeptide-Fc fusion comprising the following sequence:

GCPRPRGDNPLTCKQSDCLAGCVCGPNGFCG (SEQ ID NO:120; 2.5FmodK peptide) and having no linker between the 2.5FmodK peptide and the Fc domain. In some embodiments, the Fc domain is from IgG1, IgG2, IgG3, or IgG4 and can be mouse or human derived.

[00153] As used herein, the term “NOD203” refers to an integrin-binding polypeptide-Fc fusion comprising the following sequence:

GCPRPRGDNPLTCSQSDCLAGCVCGPNGFCGGGGGS (SEQ ID NO:121; 2.5F peptide) and having a Gly₄Ser linker between the 2.5F peptide and the Fc domain. In some embodiments, the Fc domain is from IgG1, IgG2, IgG3, or IgG4 and can be mouse or human derived.

[00154] As used herein, the term “NOD203modK” refers to an integrin-binding polypeptide-Fc fusion comprising the following sequence:

GCPRPRGDNPLTCKQSDCLAGCVCGPNGFCGGGGGS (SEQ ID NO:122; 2.5FmodK peptide) and having a Gly₄Ser linker between the 2.5FmodK peptide and the Fc domain. In some embodiments, the Fc domain is from IgG1, IgG2, IgG3, or IgG4 and can be mouse or human derived.

[00155] As used herein, the term “NOD204” refers to an integrin-binding polypeptide-FC fusion comprising the following sequence:

GCPRPRGDNPLTCSQSDCLAGCVCGPNGFCGGGGGSGGGGSGGGGS (SEQ ID NO:123; 2.5F peptide) and having a Gly₄Ser₃ linker between the 2.5F peptide and the Fc domain. In some embodiments, the Fc domain is from IgG1, IgG2, IgG3, or IgG4 and can be mouse or human derived.

[00156] As used herein, the term “NOD204modK” refers to an integrin-binding polypeptide-FC fusion comprising the following sequence:

CPRPRGDNPLTCKQSDCLAGCVCGPNGFCGGGGGSGGGGSGGGGS (SEQ ID NO:124; 2.5FmodK peptide) and having a Gly₄Ser₃ linker between the 2.5FmodK peptide and the Fc domain. In some embodiments, the Fc domain is from IgG1, IgG2, IgG3, or IgG4 and can be mouse or human derived.

[00157] As used herein, the term “AgRP” means PDB entry 1HYK. Its entry in the Knottin database is SwissProt AGRP_HUMAN, where the full-length sequence of 129 amino acids may be found. It comprises the sequence beginning at amino acid 87. An additional G is added to this construct. It also includes a CI 05 A mutation described in Jackson, et al. 2002 Biochemistry, 41, 7565, as well as International Patent Publication WO 2016/025642, incorporated by reference in its entirety; bold and underlined portion, from loop 4, is replaced by the RGD sequences described herein. Loops 1 and 3 are shown between brackets.

[00158] As used herein, “integrin-binding polypeptide-Fc fusion” is used interchangeably with “knottin-Fc” and refers to an integrin-binding polypeptide that includes an integrin-binding amino acid sequence within a knottin polypeptide scaffold and is operably linked to an Fc domain. In some embodiments, the Fc domain is fused to the N-terminus of the integrin-binding polypeptide. In some embodiments, the Fc domain is fused to the C-terminus of the integrin-binding polypeptide. In some embodiments, the Fc domain is operably linked to the integrin-binding polypeptide via a linker.

[00159] As used herein, the term “Fc region” refers to the portion of a native immunoglobulin formed by the respective Fc domains (or Fc moieties) of its two heavy chains. As used herein, the term “Fc domain” refers to a portion of a single immunoglobulin (Ig) heavy chain wherein the Fc domain does not comprise an Fv domain. As such, an Fc domain can also be referred to as “Ig” or “IgG.” In certain embodiments, an Fc domain begins in the hinge region just upstream of the papain cleavage site and ends at the C-terminus of the antibody. Accordingly, a complete Fc domain comprises at least a hinge domain, a CH₂ domain, and a CH₃ domain. In certain embodiments, an Fc domain comprises at least one of: a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH₂ domain, a CH₃ domain, a CH₄ domain, or a variant, portion, or fragment thereof. In other embodiments, an Fc domain comprises a complete Fc domain (*i.e.*, a hinge domain, a CH₂ domain, and a CH₃ domain). In one embodiment, an Fc domain comprises a hinge domain (or portion thereof) fused to a CH₃ domain (or portion thereof). In another embodiment, an Fc domain comprises a CH₂ domain (or portion thereof) fused to a CH₃ domain (or portion thereof). In another embodiment, an Fc domain consists of a CH₃ domain or portion thereof. In another embodiment, an Fc domain consists of a hinge domain (or portion thereof) and a CH₃ domain (or portion thereof). In another embodiment, an Fc domain consists of a CH₂ domain (or portion thereof) and a CH₃ domain. In another embodiment, an Fc domain consists of a hinge domain (or portion thereof) and a CH₂ domain (or portion thereof). In one embodiment, an Fc domain lacks at least a portion of a CH₂ domain (*e.g.*, all or part of a CH₂ domain). An Fc domain herein generally refers to a polypeptide comprising all or part of the Fc domain of an immunoglobulin heavy-chain. This includes, but is not limited to, polypeptides comprising the entire CH₁, hinge, CH₂, and/or CH₃ domains as well as fragments of such peptides comprising only, *e.g.*, the hinge, CH₂, and CH₃ domain. The Fc domain may be derived from an immunoglobulin of any species and/or any subtype, including, but not limited to, a human IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM antibody. A human IgG1 constant region can be found at Uniprot P01857 and in Figure 2 (FIG.2). The Fc domain of human IgG1 with a

deletion of the upper hinge region can be found in Table 2, SEQ ID NO: 3 from International Patent Publication No. WO 2016/025642. The Fc domain encompasses native Fc and Fc variant molecules. As with Fc variants and native Fc's, the term Fc domain includes molecules in monomeric or multimeric (*e.g.*, dimeric) form, whether digested from whole antibody or produced by other means. The assignment of amino acid residue numbers to an Fc domain is in accordance with the definitions of Kabat. See, *e.g.*, Sequences of Proteins of Immunological Interest (Table of Contents, Introduction and Constant Region Sequences sections), 5th edition, Bethesda, MD:NIH vol. 1:647-723 (1991); Kabat et al., "Introduction" Sequences of Proteins of Immunological Interest, US Dept of Health and Human Services, NIH, 5th edition, Bethesda, MD vol. 1 :xiii-xcvi (1991); Chothia & Lesk, J. Mol. Biol. 196:901-917 (1987); Chothia et al, Nature 342:878-883 (1989), each of which is herein incorporated by reference for all purposes. With regard to the integrin-binding polypeptide-Fc fusions described herein, any Fc domain from any IgG as described herein or known can be employed as part of the Fc fusion, including mouse, human and variants thereof, such as hinge deleted (EPKSC deleted; see, SEQ ID NO: 3 from International Patent Publication No. WO 2016/025642).

[00160] As set forth herein, it will be understood by one of ordinary skill in the art that any Fc domain may be modified such that it varies in amino acid sequence from the native Fc domain of a naturally occurring immunoglobulin molecule. In certain exemplary embodiments, the Fc domain has increased effector function (*e.g.*, FcγR binding).

[00161] The Fc domains of a polypeptide of the invention may be derived from different immunoglobulin molecules. For example, an Fc domain of a polypeptide may comprise a CH₂ and/or CH₃ domain derived from an IgG1 molecule and a hinge region derived from an IgG3 molecule. In another example, an Fc domain can comprise a chimeric hinge region derived, in part, from an IgG1 molecule and, in part, from an IgG3 molecule. In another example, an Fc domain can comprise a chimeric hinge derived, in part, from an IgG1 molecule and, in part, from an IgG4 molecule.

[00162] A polypeptide or amino acid sequence "derived from" a designated polypeptide or protein refers to the origin of the polypeptide. Preferably, the polypeptide or amino acid sequence which is derived from a particular sequence has an amino acid sequence that is essentially identical to that sequence or a portion thereof, wherein the portion consists of at least 10-20 amino acids, preferably at least 20-30 amino acids, more preferably at least 30-50 amino acids, or which is otherwise identifiable to one of ordinary skill in the art as having its origin in

the sequence. Polypeptides derived from another peptide may have one or more mutations relative to the starting polypeptide, *e.g.*, one or more amino acid residues which have been substituted with another amino acid residue or which has one or more amino acid residue insertions or deletions.

[00163] A polypeptide can comprise an amino acid sequence which is not naturally occurring. Such variants necessarily have less than 100% sequence identity or similarity with the starting IL-2 or knottin protein. In some embodiments, the variant will have an amino acid sequence from about 75% to less than 100% amino acid sequence identity or similarity with the amino acid sequence of the starting polypeptide, more preferably from about 80% to less than 100%, more preferably from about 85% to less than 100%, more preferably from about 90% to less than 100% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) and in some embodiments from about 95% to less than 100%, *e.g.*, over the length of the variant molecule.

[00164] In one embodiment, there is one amino acid difference between a starting polypeptide sequence and the sequence derived therefrom. Identity or similarity with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical (*i.e.*, same residue) with the starting amino acid residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity.

Table 1: Sequence Summary

SEQ ID NO	Description	Sequence
25	Human IgG1 constant region (amino acid sequence)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
26	Human IgG1 Fc domain (amino acid sequence)	EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
27	Human IgG1 Fc domain (amino acid sequence)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

	Deletion (ΔEPKSC) Upper Hinge	
28	Human serum albumin (amino acid sequence)	MDMRVPAQLLGLLLWLPGARCADAHKSEVAHRFKDLGEENFKALVLIAFAQYLOQC PFED HVKL VNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERN ECFLQHKDDNP NL PRLVRPEVDV MCTAFHDNEETFLKKYLYEIAARRHPYFYAPELLFFAKR YKAAFTTECCQAADKAACLLPKLDEL RDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQ RFPKAEFAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSI SSKLKECCEKP LLEKSHCIAEVENDEMPADLP SLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDY SV VLLRLAKTYET TLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQ NALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHE KTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQ TALVELVKHKPKATKEQLKAVMD DFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGG GSAPTSSSTKKTQLQLEHLLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKH LQCL EEELKPLEEVLNLAQSKNFHLRPDLI SNINIVIVLELKGSETTFMCEYADETATIVEF LNR WITFCQSIISTLTGGGS
29	Mature HSA (amino acid sequence)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLOQC PFEDHVKL VNEVTEFAKTCVADESAEN CDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECF LQHKDDNP NL PRLVRPEVDV MCTAFHDNEETFLKKYLYEIAARRHPYFYAPELLFFAKRYKAAFTTECCQAADKAACLLPKL ELRDEGKASSAKQRLK CASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTE CCHGDLLECADDRADLAKYICENQDSI SSKLKECCEKP LLEKSHCIAEVENDEMPADLP SL AADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDY SVVLLRLAKTYET TLEKCCAAADP HECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQ NALLVRYTKKVPQVSTPTLVEVS RNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCF SALEVDETYVPKEFNAETFTFHADI CTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMD DFAAFVEKCKADDKETCFAEEGKKLVAASQAALGLGGGSAPTSSSTKKTQLQLEHLLLDL QMLNGINNYKNPKLTRMLTFKFYMPKKATELKH LQCLEELKPLEEVLNLAQSKNFHLRP RDLI SNINIVIVLELKGSETTFMCEYADETATIVEF LNRWITFCQSIISTLTGGGS
30	Human serum albumin (nucleic acid sequence)	ATGGATATGCGGGTGCTGCTCAGCTGCTGGGACTGCTGCTGCTGTGGCTGCCTGGGGCTA GATGCGCCGATGCTCACAAAAGCGAAGTCGCACACAGGTTCAAAGATCTGGGGGAGGAAAA CTTTAAGGCTCTGGTGCTGATTGCATTGCCCCAGTACCTGCAGCAGTGCCCCCTTTGAGGAC CACGTGAAACTGGTCAACGAAGTGACTGAGTTCGCCAAGACCTGCGTGGCCGACGAATCTG CTGAGAATTGTGATAAAAGTCTGCATACTCTGTTTGGGGATAAGCTGTGTACAGTGGCCAC TCTGCGAGAAACCTATGGAGAGATGGCAGACTGCTGTGCCAAACAGGAACCCGAGCGGAAC GAATGCTTCCTGCAGCATAAGGACGATAACCCCAATCTGCCTCGCCTGGTGCGACCTGAGG TGGACGT CATGTGTACAGCCTTCCACGATAATGAGGAAACTTTTCTGAAGAAATACCTGTA CGAAATCGCTCGGAGACATCCTTACTTTTATGCACCAGAGCTGCTGTTCTTTGCCAAACGC TACAAGGCCGCTTTCACCGAGTGCTGT CAGGCAGCCGATAAAGCTGCATGCCTGCTGCCTA AGCTGGACGAAC TGAGGGATGAGGGCAAGGCCAGCTCCGCTAAACAGCGCCTGAAGTGTGC TAGCCTGCAGAAATT CGGGGAGCGAGCCTTCAAGGCTTGGGCAGTGGCACGGCTGAGTCAG AGATTCCCAAAGGCAGAATTTGCCGAGGTCTCAAACTGGTGACCGACCTGACAAAAGGTGC ACACCGAATGCTGT CATGGCGAECTGCTGGAGTGCGCCGACGATCGAGCTGATCTGGCAAA GTATATTTGTGAGAACCAGGACTCCATCTCTAGTAAGCTGAAAGAATGCTGTGAGAAACCA CTGCTGGAAGTCTCACTGCATTGCCGAAGTGGAGAACGACGAGATGCCAGCTGATCTGC CCTCACTGGCCGCTGACTTCGTGCAAAGCAAAGATGTGTGTAAGAATTACGCTGAGGCAAA GGATGTGTTCTGGGAATGTTTCTGTACGAGTATGCCAGGCGCCACCCAGACTACTCCGTG GTCCTGCTGCTGAGGCTGGCTAAAACATATGAAACCACACTGGAGAAGTGCTGTGCAGCCG CTGATCCCCATGAATGCTATGCCAAAGTCTTCGACGAGTTTAAGCCCCCTGGTGGAGGAACC TCAGAACCTGATCAAACAGAATTGTGAAGTGTTCGACGAGCTGGGCGAGTACAAGTTCAG AACGCCCTGCTGGTGCGCTATACCAAGAAAGTCCACAGGTGTCCACACCCACTCTGGTGG AGGTGAGCCGGAATCTGGGCAAGTGGGAGTAAATGCTGTAAGCACCCCTGAAGCCAAGAG GATGCCATGCGCTGAGGATTACCTGAGTGTGGTCTGAATCAGCTGTGTGCTCTGCATGAA AAAACACCTGT CAGCGACCGGGTGACAAAGTGTGTACTGAGTCACTGGTGAACCGACGGC CCTGCTTTAGCGCCCTGGAAGTCGATGAGACTTATGTGCCTAAAGAGTTCAACGCTGAGAC CTTCACATTTACGCAGACATTTGTACCCTGAGCGAAAAGGAGAGACAGATCAAGAAACAG ACAGCCCTGGT CGAACTGGTGAAGCATAAACCCAAAGGCCACAAAAGAGCAGCTGAAGGCTG TCATGGACGATTTTCGACGCTTTGTGAAAAATGCTGTAAGGCAGACGATAAGGAGACTTG CTTTGCCGAGGAAGGAAAGAACTGGTGGCTGCATCCAGGCAGCTCTGGGACTGGGAGGA

		GGATCTGCCCCCTACCTCAAGCTCCACTAAGAAAACCCAGCTGCAGCTGGAGCACCTGCTGC TGGACCTGCAGATGATTCTGAACGGGATCAACAATTACAAAAATCCAAAGCTGACCCGGAT GCTGACATTCAAGTTTATATGCCCAAGAAAGCCACAGAGCTGAAACACCTGCAGTGCCTG GAGGAAGAGCTGAAGCCTCTGGAAGAGGTGCTGAACCTGGCCCAGAGCAAGAATTCCATC TGAGACCAAGGGATCTGATCTCCAACATTAATGTGATCGTCCTGGAAGTGAAGGGATCTGA GACTACCTTTATGTGCGAATACGCTGACGAGACTGCAACCATTGTGGAGTTCCTGAACAGA TGGATCACCTTCTGCCAGTCCATCATTCTACTCTGACAGGCGGGGGGAGC
31	EETI-II from Knottin Database	GC PRILMR CKQSDCLAGCVCGPNGFCG
32	AgRP from Knottin Database “-” indicates where mini protein can be formed	GCVRLHESCLGQQVPCCDPCATCYCRFFNAFCYCR-KLGTAMNPSRT
33	Omega agatoxin from Knottin Database “-” indicates where mini protein can be formed	EDN--CIAEDYGKCTWGGTKCCRGRPCRC SMIGTN CECTPRLIMEGLSFA
34	EETI-II Library	GCXXXRGDXXXXXCKQSDCLAGCVCGPNGFCG
35	EETI-II K15S Mutation Library	GCXXXRGDXXXXXCSQSDCLAGCVCGPNGFCG
36	2.5F- (K15S) mIgG2aFc Nucleic Acid Sequence	GGTTGTCCAAGACCAAGAGGTGATAATCCACCATTGACTTGTCTCAAGATTCTGATTGTT TGGCTGGTTGTGTTTGTGGTCCAAATGGTTTTTGTGGTGGTTCGACTAGAGCCCAGAGTGCC CATAACACAGAACCCCTGTCTCCACTCAAAGAGTGTCCCCATGCGCAGCTCCAGACCTC TTGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTACTCATGATCTCCC TGAGCCCCATGGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGACGTCCAGAT CAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAGGAT TACAACAGTACTCTCCGGGTGGTCACTGCCCTCCCCATCCAGCACCAGGACTGGATGAGTG GCAAGGAGTTCAAATGCAAGGTCAACAACAGAGCCCTCCCATCCCCATCGAGAAAACCAT CTCAAAACCCAGAGGGCCAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCAGCAGAA GAGATGACTAAGAAAGAGTTCACTGTGACCTGCATGATCACAGGCTTCTTACCTGCCGAAA TTGCTGTGGACTGGACCAGCAATGGGCGTACAGAGCAAACTACAAGAACACCGCAACAGT CCTGGACTCTGATGGTCTTACTTTCATGTACAGCAAGCTCAGAGTACAAAAGAGCACTTGG GAAAGAGGAAGTCTTTTCGCCTGCTCAGTGGTCCACGAGGGTCTGCACAATCACCTTACGA CTAAGACCATCTCCCGGTCTCTGGGTAAA
37	2.5F- (K15S) mIgG2aFc Amino Acid Sequence	GCPRPRGDNPLTCSQSDCLAGCVCGPNGFCGEPRVPITQNPCPPLKECPPCAAPDLLGG PSVFI FPPKIKDVLMI SLSPMVT CVVVDVSEDDPDVQI SWFVNNVEVHTAQ TQTHREDYNS TLRVVSALPIQH QDWM SGEFKCKVNNRALPSPI EKTISKPRGPVRAPQVYVLP PPAEEMT KKEFSLTCMITGFLPAELAVDWT SNGRTEQNYKNTATVLDSDGSYFMY SKLRVQKSTWERG SLFACSVVHEGLHNHLTKTISRSLGK

38	2.5D- (K15S) mIgG2aFc Nucleic Acid Sequence	GGTTGTCCACAAGGCAGAGGTGATTGGGCTCCAACTTCTTGTCTCAAGATTCTGATTGTT TGGCTGGTTGTGTTTGTGGTCCAAATGGTTTTTGTGGTGGTCTGACTAGAGCCCAGAGTGCC CATAACACAGAACCCCTGTCTCCACTCAAAGAGTGTCCCCATGCGCAGCTCCAGACCTC TTGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTACTCATGATCTCCC TGAGCCCCATGGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGACGTCCAGAT CAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAGGAT TACAACAGTACTCTCCGGGTGGTCACTGCCCTCCCCATCCAGCACCAGGACTGGATGAGTG GCAAGGAGTTCAAATGCAAGGTCAACAACAGAGCCCTCCCATCCCCATCGAGAAAACCAT CTCAAAACCCAGAGGGCCAGTAAGAGCTCCACAGGTATATGTCTTGCTCCACCAGCAGAA GAGATGACTAAGAAAGAGTTCAGTCTGACCTGCATGATCACAGGCTTCTTACCTGCCGAAA TTGCTGTGGACTGGACCAGCAATGGGCGTACAGAGCAAACTACAAGAACACCGCAACAGT CCTGGACTCTGATGGTCTTACTTTCATGTACAGCAAGCTCAGAGTACAAAAGAGCACTTGG GAAAGAGGAAGTCTTTTCGCCTGCTCAGTGGTCCACGAGGGTCTGCACAATCACCTTACGA CTAAGACCATCTCCCGGTCTCTGGGTAAA
39	2.5D- (K15S) mIgG2aFc Amino Acid Sequence	GCPQGRGDWAPTSCSQSDCLAGCVCGPNGFCGEPKSCDKTHTCPPCPAPELLGGPSVFLF PSVFI FPPKIKDVLMI SLS PMVT CVVVDVSEDDP DVQI SWFVN NVVHTAQ TQTHREDYNS TLRVVSALPIQH QDWMSGKEFKCKVNNRALPSPI EKTISKPRGPVRAPQVYVLP PPAEEMT KKEFSLTCMITGFLPAEIAVDWTSNGRTEQNYKNTATVLDSDGSYFMYSKLRVQKSTWERG SLFACSVVHEGLHNHLTKTISRSLGK
40	2.5F- (K15S) hIgG1Fc Amino Acid Sequence	GCPRPRGDNPPLTCSQSDCLAGCVCGPNGFCGEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYP S DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CS VMHEALHNHYTQKSLSLSPGK
41	2.5F- (K15S) hIgG1Fc Fc Upper Hinge Deletion (ΔEPKSC) Amino Acid Sequence	GCPRPRGDNPPLTCSQSDCLAGCVCGPNGFCGDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYP S DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CS VMHEALHNHYTQKSLSLSPGK
42	2.5D- (K15S) hIgG1Fc Amino Acid Sequence	GCPQGRGDWAPTSCSQSDCLAGCVCGPNGFCGEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYP S DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CS VMHEALHNHYTQKSLSLSPGK
43	2.5D- (K15S) hIgG1Fc Fc Upper Hinge Deletion (ΔEPKSC) Amino Acid Sequence	GCPQGRGDWAPTSCSQSDCLAGCVCGPNGFCGDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYP S DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CS VMHEALHNHYTQKSLSLSPGK

44	hPD-1 amino acid sequence	MQIPQAPWPVVWAVLQLGWRPGWFLDSPDPWNPPTFFPALLVVTEGDNATFTCSFSNTSES FVLNWYRMSPSNQTDKLAAPFEDRSQPGQDCRFRTQLPNGRDFHMSVVRARRNDSTYLC GAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLVVGVGGLLGSVLVLL VWVLAVICSRARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPPEPPVPCVPEQ TEYATIVFPSPMGTSPPARRGSADGPRSAQPLRPEDGHCSWPL
45	hPD-L1 amino acid sequence	MRIFAVFIFMTYWHLNNAFTVTVPKDLVVEYGSNMTIECKFPVEKQLDLAALIVWEMED KNIIQFVHGEEDLKVQHSSYRQARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGAD YKRITVKVNAPYNKINQIRILVVDVPTSEHELTCQAEQYPAEVIWTSDDHQVLSGKTTTTN SKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVPELPLAHPNERNTHLVIL GAILLC LGVALTFIFR LRKGRMMDVKKCGIQDTNSK QSDTHLEET
46	hCTLA-4 amino acid sequence	MACLGFRHKAQLNLATRTWPCTLLFFLLFIPVFCKAMHVAQPAVVLASS RGIASFVCEYASPGKATEVRVTVLRQADSQVTEVCAATYM MGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPPPPY LGIGNGTQIY VIDPEPCPDS DFLWLAAVSSGLFFYSFL LTAVSLSKML KKRSPLTGTVYVKMPPEPE CEKQFQPYFI PIN
47	hLAG-3 amino acid sequence	MWEAQFLGLLFLQPLWVAPVKLPQGAEVPVWVAQEGAPALPCSPPTIPLQDLSLLRRAGV TWQHQPDSGPPAAAPGHPLAPGPHPAAPSSWGPRPRRYTVLSVGPGLRSGRLPLQPRVQL DERGRQRGDFSLWLRPAR RADAGEYRAAVHLRDRALSCRLRLRLGQASMTASPPGSLR ASDWILNCSFSRDPDPAVHFRNRGQGRVPVRESPHHHLAESFLFLPQVSPMDSGPWGC ILTYRDGFNVSIMYNLTVLGLEPPTPLTVYAGAGSRVGLPCRLPAGVGTRSFRTAKWTPPG GGPDLVTGDNGDFTLRL EDVSAQAGT YTCHIHLEEQ QLNATVTLAI ITVTPKSFGS PGSLGKLLCEVTPVSGQERFVWSSLDTPSQRSFSGPWLEAQEAQLLSQPWQCQLYQGERLL GAAVYFTEL SSPGAQRSGR APGALPAGHL LFLILGVLS LLLLVTGAFG FHLWRRQWRPRRFSALEQGI HPPQAQSKIE ELEQEPEPEP EPEPEPEPEP EPEQL
48	hTIM-3 amino acid sequence	MFSLPFDVLLLLLLLLLTSSEVEYRAEVGQNAYLPCFYTPAAPGNLVPCWGKGACPVF ECGNVVLRTDERDVNYWTSRYWLNQDFRKGDVSLTIENVTLADSGIYCCRIQIPGIMNDEK FNLKLVIKPAKVTAPTR QRDFTAAPFR MLTTRGHGPA ETQTLGSLPD INLTQISTLA NELRDSRLANDLRDSGATIRGIYIGAGICAGLALALIFGALIFKWYSHSKEKIQLSLISL ANLPSSGLANAVAEGIRSEENIYTIENVEVEEPNEYCYVSSRQQPSQPLGCREAMP
49	hB7-H3 amino acid sequence	MLRRRGSPGMGVHVGALGALWFCLTGALVQVPEDPVVALVGTDATLCC SFSPPEGFSLQLNLIWQLT DTKQLVHSFA EGQDQGSAYA NRTALFPDLLAQGNASLRLQRVRVADEGSFCFVSIRDFGSAAVSLQVAA PYSKPSMTLE PNKDLRPGDT VTITCSSYQG YPEAEVFWQD GQGVPLTGNVTTSQMANEQGLFDVHSILRVVLGANGTYSCLVRNPVLQQD AHSSVTITPQRSPTGAVEVQVPEDPVVALVGTDATLRCF SPEPGFSLAQLNLIWQLTDTKQLVHSFTEGRDQGSAYANRTALFPDLLAQ GNASLRLQRV RVADEGSFTC FVSIRDFGSA AVSLQVAAPY SKPSMTLEPNKDLRPGDVTITCSSYRGYPEAEVFWQDQGVPLTGNVTT SQMANEQGLFDVHSVLRVVLGANGTYSCLVRNPVLQQDAH GSVTITGQPMFTFPPEALWVTVGLSVCLIALLLVALAFVCWRKIKQSCEEN AGAEDQDGEGEKSTALQPLKHSKEDDQGEIA
50	hB7-H4 amino acid sequence	MASLGQILFWSIIIIIIILAGAIALLIGFGISAFSMPEVNVDYNASSETLRCEAPRWFPQP TVVWASQVDQGANFSEVSNSTFELNSENVTMKVSVLYN VTINNTYSCM IENDIAKATGDIKVTSEIKRRSHLQLLNS KASLCVSSFFAISWALLPLSPYMLK

[00165] In one embodiment, an integrin-binding polypeptide or a variant thereof, consists of, consists essentially of, or comprises an amino acid sequence selected from SEQ ID NOs: 51-119. In an embodiment, a polypeptide includes an amino acid sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence selected from SEQ ID Nos: 51-119. In an embodiment,

a polypeptide includes a contiguous amino acid sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a contiguous amino acid sequence selected from SEQ ID Nos: 51-119. In an embodiment, a polypeptide includes an amino acid sequence having at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, or 500 (or any integer within these numbers) contiguous amino acids of an amino acid sequence selected from SEQ ID NOs: 51-119.

Table 2: Integrin Binding Knottin Sequences

SEQ ID NO:	Peptide Identifier	Scaffold	Sequence (RGD motif is underlined with flanking residues)
51	1.4A	EETI-II	GC <u>AEPRGDMPTW</u> CKQSDCLAGCVCGPNGFCG
52	1.4B	EETI-II	GC <u>VGGRGDWS</u> PKWCKQSDCPAGCVCGPNGFCG
53	1.4C	EETI-II	GC <u>AELRGDRSYPE</u> CKQSDCLAGCVCGPNGFCG
54	1.4E	EETI-II	GC <u>RLPRGDVPRPH</u> CKQSDCQAGCVCGPNGFCG
55	1.4H	EETI-II	GC <u>YPLRGDNPYAA</u> CKQSDCRAGCVCGPNGFCG
56	1.5B	EETI-II	GC <u>TIGRGDWAPSE</u> CKQSDCLAGCVCGPNGFCG
57	1.5F	EETI-II	GC <u>HPPRGDNPPVT</u> CKQSDCLAGCVCGPNGFCG
58	2.3A	EETI-II	GC <u>PEPRGDNPPPS</u> CKQSDCRAGCVCGPNGFCG
59	2.3B	EETI-II	GC <u>LPPRGDNPPPS</u> CKQSDCQAGCVCGPNGFCG
60	2.3C	EETI-II	GCH <u>LGRGDWAPVG</u> CKQSDCPAGCVCGPNGFCG
61	2.3D	EETI-II	GC <u>NVGRGDWAPSE</u> CKQSDCPAGCVCGPNGFCG
62	2.3E	EETI-II	GC <u>FPGRGDWAPSS</u> CKQSDCRAGCVCGPNGFCG
63	2.3F	EETI-II	GC <u>PLPRGDNPPTE</u> CKQSDCQAGCVCGPNGFCG
64	2.3G	EETI-II	GC <u>SEARGDNPRLS</u> CKQSDCRAGCVCGPNGFCG
65	2.3H	EETI-II	GCL <u>LGRGDWAP</u> EACKQSDCRAGCVCPNGFCG
66	2.3I	EETI-II	GCH <u>VGRGDWAPLK</u> CKQSDCQAGCVCGPNGFCG
67	2.3J	EETI-II	GC <u>VRGRGDWAPPS</u> CKQSDCPAGCVCGPNGFCG
68	2.4A	EETI-II	GCL <u>GGRGDWAPP</u> ACKQSDCRAGCVCGPNGFCG
69	2.4C	EETI-II	GC <u>FVGRGDWAPLT</u> CKQSDCQAGCVCGPNGFCG
70	2.4D	EETI-II	GC <u>PVGRGDWSPAS</u> CKQSDCRAGCVCGPNGFCG
71	2.4E	EETI-II	GC <u>PRPRGDNPLT</u> CKQSDCLAGCVCGPNGFCG
72	2.4F	EETI-II	GC <u>YQGRGDWSPSS</u> CKQSDCPAGCVCGPNGFCG
73	2.4G	EETI-II	GC <u>APGRGDWAPSE</u> CKQSDCQAGCVCGPNGFCG
74	2.4J	EETI-II	GC <u>VQGRGDWSPPS</u> CKQSDCPAGCVCGPNGFCG
75	2.5A	EETI-II	GC <u>HVGRGDWAP</u> EECKQSDCQAGCVCGPNGFCG
76	2.5C	EETI-II	GC <u>DGGRGDWAPP</u> ACKQSDCRAGCVCGPNGFCG

77	2.5D	EETI-II	GC <u>PQGRGDWAPTS</u> CKQSDSDCRAGCVCGPNGFCG
78	2.5F	EETI-II	GC <u>PRPRGDNPLT</u> CKQSDSCLAGCVCGPNGFCG
79	2.5D K15S Mutant	EETI-II	GC <u>PQGRGDWAPTS</u> CSQSDSCLAGCVCGPNGFCG
80	2.5F K15S Mutant	EETI-II	GC <u>PRPRGDNPLT</u> CSQSDSCLAGCVCGPNGFCG
81	2.5H	EETI-II	GC <u>PQGRGDWAPW</u> CKQSDSDCPAGCVCGPNGFCG
82	2.5J	EETI-II	GC <u>PRGRGDWSPPA</u> CKQSDSDCQAGCVCGPNGFCG
83	3A	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>VVRGDWRKRC</u> YCR
84	3B	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>EERGDMLEK</u> CYCR
85	3C	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>ETRGDGKEK</u> CYCR
86	3D	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>QWRGDGDVKC</u> YCR
87	3E	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>SRRGDMRERC</u> YCR
88	3F	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>QYRGDGMKHC</u> YCR
89	3G	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>TGRGDTKVL</u> CYCR
90	3H	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>VERGDMKRRC</u> YCR
91	3I	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>TGRGDVRMN</u> CYCR
92	3J	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>VERGDGMSKC</u> YCR
93	4A	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>RGRGDMRREC</u> YCR
94	4B	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>EGRGDVKVN</u> CYCR
95	4C	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>VVRGDEKMSC</u> YCR
96	4D	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>VSRGDMRKRC</u> YCR
97	4E	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>ERRGDSVKK</u> CYCR
98	4F	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>EGRGDTRRR</u> CYCR
99	4G	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>EGRGDVVR</u> CYCR
100	4H	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>KGRGDNKRKC</u> YCR
101	4I	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>KGRGDVRRV</u> CYCR
102	4J	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>VGRGDNKVK</u> CYCR
103	5A	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>VGRGDNRLK</u> CYCR
104	5B	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>VERGDGMKKC</u> YCR
105	5C	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>EGRGDMRRRC</u> YCR
106	5D	AgRp	GCVRLHESCLGQQVPCCDPAATCYC <u>QGRGDGDVKC</u> YCR

107	5E	AgRp	GCVRLHESCLGQQVPCCDPAATCYC SGRGDNDLVCYCR
108	5F	AgRp	GCVRLHESCLGQQVPCCDPAATCYC VERGDGMIRCYCR
109	5G	AgRp	GCVRLHESCLGQQVPCCDPAATCYC SGRGDNDLVCYCR
110	5H	AgRp	GCVRLHESCLGQQVPCCDPAATCYC EGRGDMKMKC YCR
111	5I	AgRp	GCVRLHESCLGQQVPCCDPAATCYC IGRGDVRRRCYCR
112	5J	AgRp	GCVRLHESCLGQQVPCCDPAATCYC EERGDGRKKCYCR
113	6B	AgRp	GCVRLHESCLGQQVPCCDPAATCYC EGRGDRDMKC YCR
114	6C	AgRp	GCVRLHESCLGQQVPCCDPAATCYC TGRGDEKLRCYCR
115	6E	AgRp	GCVRLHESCLGQQVPCCDPAATCYC VERGDGNRRCYCR
116	6F	AgRp	GCVRLHESCLGQQVPCCDPAATCYC ESRGDVVRKCYCR
117	7C	AgRp	GCVRLHESCLGQQVPCCDPAATCYC YGRGDNDLRC YCR

Table 3: Integrin Binding Polypeptide Sequences, Signal Sequences, Linkers, Fc fusions

SEQ ID NO:	Peptide Identifier Scaffold	Sequence
118	NOD201 - 2.5F	GCPRPRGDNPPLTCSQSDCLAGVCVCPNGFCG
119	NOD201modK - 2.5FmodK	GCPRPRGDNPPLTCKQSDCLAGVCVCPNGFCG
120	NOD203 - 2.5F w/GGGGS	GCPRPRGDNPPLTCSQSDCLAGVCVCPNGFCGGGGGS
121	NOD203modK - 2.5FmodK w/GGGGS	GCPRPRGDNPPLTCKQSDCLAGVCVCPNGFCGGGGGS
122	NOD204 - 2.5F w/GGGSGGGSGGGGS	GCPRPRGDNPPLTCSQSDCLAGVCVCPNGFCGGGGSGGGSGGGGS
123	NOD204modK - 2.5FmodK w/GGGSGGGSGGGGS	GCPRPRGDNPPLTCKQSDCLAGVCVCPNGFCGGGGSGGGSGGGGS
124	Linker (short) (linker for use with any sequences disclosed herein)	GGGGS
125	Linker (long) (linker for use with any sequences disclosed herein)	GGGSGGGSGGGGS
126	Signal sequence (signal peptide A)	MTRLTVLALLAGLLASSR

	(signal peptide for use with any sequences disclosed herein, including SEQ ID Nos: 139, 140, 141, 142, and 143)	
127	NOD201 (human Fc; no linker)	GCPRPRGDNPPLTCSQSDSCLAGCVCGPNGFCGEPKSSDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSV MHEALHNHYTQKSLSLSPG
128	NOD201X (control sequence - NOD201 with scrambled seq, human Fc; no linker) Theoretical pI/Mw: 6.19 / 58065.44	GCVTGRDGSPASSCSQSDSCLAGCVCGPNGFCGEPKSSDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSV MHEALHNHYTQKSLSLSPG
129	NOD201M (NOD201 with murine Fc domain; no linker) Theoretical pI/Mw: 6.34 / 59357.92 Ext. coefficient 60525 Abs 0.1% (=1 g/l) 1.020, assuming all pairs of Cys residues form cystines	GCPRPRGDNPPLTCSQSDSCLAGCVCGPNGFCGEPKSSDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSV MHEALHNHYTQKSLSLSPG
130	NOD203 complete (Gly ₄ Ser linker)	GCPRPRGDNPPLTCSQSDSCLAGCVCGPNGFCGEGGGGSEPKSSDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FCSSVMHEALHNHYTQKSLSLSPG
131	NOD204 complete ([Gly ₄ Ser] ₃ linker)	GCPRPRGDNPPLTCSQSDSCLAGCVCGPNGFCGGGGGSGGGGSGGGGSE PKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPG

Table 4: Exemplary IgG sequences:

SEQ ID NO:	Name	Sequence
132	IgG1	ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS 60 GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG 120 PSVFLFPPKP KDTLMISRTPEVTCVVDVSHEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTFR 180 STYRVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE 240 LTKNQVSLTCLVKGFYPSDI AVEWESNGQP ENNYKTTTPV LDSGGSFFLY SKLTVDKSRW 300 QQGNVFSCSV MHEALHNHYT QKSLSLSPGK 330
133	IgG2	ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS 60 GLYSLSSVVT VPSSNFGTQT YTCNVNHHKPS NTKVDKTVR KCCVECPPCP APPVAGPSVF 120 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTFR 180 VVSVLTVVHQ DWLNGKEYK KVSNGKLPAP IEKTISKTKG QPREPQVYTL PPSREEMTKN 240 QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPMLDSD GSFFLYSKLT VDKSRWQQGN 300 VFSCSVMHEA LHNHYTQKSL SLSPGK 326
134	IgG3	ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS 60 GLYSLSSVVT VPSSSLGTQT YTCNVNHHKPS NTKVDKRVEL KTPLGDTTHT CPRCPPEPKSC 120 DTPPPCPRCP EPKSCDTPPP CPRCPPEPKSC DTPPPCPRCP APELLGGPSV FLFPPKPKDT 180 LMISRTPEVT CVVVDVSHED PEVQFKWYVD GVEVHNAKTK PREEQYNSTF RVVSVLTVLH 240 QDWLNGKEYK CKVSNKALPA PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK 300 GFYPSDIAVE WESSGQPENN YNTTPMLDSD DGSFFLYSKL TVDKSRWQQG NIFSCSVMHE 360 ALHNRFTQKS LSLSPGK 377
135	IgG4	ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS 60 GLYSLSSVVT VPSSSLGTQT YTCNVNHHKPS NTKVDKRVEL KYGPPCPSCP APEFLGGPSV 120 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY 180 RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK 240 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS DGSFFLYSRL TVDKSRWQEG 300 NVFSCSVMHE ALHNNHYTQKS LSLSLGK 327

[00166] It will also be understood by one of ordinary skill in the art that the polypeptides, including the integrin-binding polypeptide-Fc fusions, used herein may be altered such that they vary in sequence from the naturally occurring or native sequences from which they were derived, while retaining the desirable activity of the native sequences. For example, nucleotide or amino acid substitutions leading to conservative substitutions or changes at “non-essential” amino acid residues may be made. Mutations may be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis.

[00167] The polypeptides described herein (*e.g.*, knottin, Fc, knottin-Fc, integrin-binding polypeptide-Fc fusion, and the like) may comprise conservative amino acid substitutions at one or more amino acid residues, *e.g.*, at essential or non-essential amino acid residues. A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having

similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagines, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue in a binding polypeptide is preferably replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members. Alternatively, in another embodiment, mutations may be introduced randomly along all or part of a coding sequence, such as by saturation mutagenesis, and the resultant mutants can be incorporated into binding polypeptides of the invention and screened for their ability to bind to the desired target.

[00168] The “Programmed Death-1 (PD-1)” receptor refers to an immuno-inhibitory receptor belonging to the CD28 family. PD-1 is expressed predominantly on previously activated T-cells *in vivo*, and binds to two ligands, PD-L1 and PD-L2. The term “PD-1” as used herein includes human PD-1 (hPD-1), variants, isoforms, and species homologs of hPD-1, and analogs having at least one common epitope with hPD-1. The complete hPD-1 sequence can be found under GenBank Accession No. AAC51773 (SEQ ID NO: 52 from International Publication No. WO 2016/025642).

[00169] “Programmed Death Ligand-1 (PD-L1)” is one of two cell surface glycoprotein ligands for PD-1 (the other being PD-L2) that downregulates T cell activation and cytokine secretion upon binding to PD-1. The term “PD-L1” as used herein includes human PD-L1 (hPD-L1), variants, isoforms, and species homologs of hPD-L1, and analogs having at least one common epitope with hPD-L1. The complete hPD-L1 sequence can be found under GenBank Accession No. Q9NZQ7 (SEQ ID NO: 53 from International Publication No. WO 2016/025642).

[00170] “Cytotoxic T Lymphocyte Associated Antigen-4 (CTLA-4)” is a T cell surface molecule and is a member of the immunoglobulin superfamily. This protein downregulates the immune system by binding to CD80 and CD86. The term “CTLA-4” as used herein includes human CTLA-4 (hCTLA-4), variants, isoforms, and species homologs of hCTLA-4, and analogs having at least one common epitope with hCTLA-4. The complete hCTLA-4 sequence can be

found under GenBank Accession No. P16410 (SEQ ID NO: 54 from International Publication No. WO 2016/025642):

[00171] “Lymphocyte Activation Gene-3 (LAG-3)” is an inhibitory receptor associated with inhibition of lymphocyte activity by binding to MHC class II molecules. This receptor enhances the function of Treg cells and inhibits CD8⁺ effector T cell function. The term “LAG-3” as used herein includes human LAG-3 (hLAG-3), variants, isoforms, and species homologs of hLAG-3, and analogs having at least one common epitope. The complete hLAG-3 sequence can be found under GenBank Accession No. P18627 (SEQ ID NO: 55 from International Publication No. WO 2016/025642).

[00172] “T-Cell Membrane Protein-3 (TIM-3)” is an inhibitory receptor involved in the inhibition of lymphocyte activity by inhibition of T-cell and B-cell responses. Its ligand is galectin 9, which is upregulated in various types of cancers. The term “TIM-3” as used herein includes human TIM-3 (hTIM-3), variants, isoforms, and species homologs of hTIM-3, and analogs having at least one common epitope. The complete hTIM-3 sequence can be found under GenBank Accession No. Q8TDQ0 (SEQ ID NO: 56 from International Publication No. WO 2016/025642).

[00173] The “B7 family” refers to inhibitory ligands with undefined receptors. The B7 family encompasses B7-H3 and B7-H4, both upregulated on tumor cells and tumor infiltrating cells. The complete hB7-H3 and hB7-H4 sequence can be found under GenBank Accession Nos. Q5ZPR3 and AAZ17406 (SEQ ID NOs: 57 and 58 from International Publication No. WO 2016/025642) respectively.

[00174] “Vascular Endothelial Growth Factor (VEGF)” is a secreted disulfide-linked homodimer that selectively stimulates endothelial cells to proliferate, migrate, and produce matrix-degrading enzymes, all of which are processes required for the formation of new vessels. In addition to being the only known endothelial cell specific mitogen, VEGF is unique among angiogenic growth factors in its ability to induce a transient increase in blood vessel permeability to macromolecules. The term “VEGF” or “VEGF-A” is used to refer to the 165-amino acid human vascular endothelial cell growth factor and related 121-, 145-, 189-, and 206-amino acid human vascular endothelial cell growth factors, as described by, *e.g.*, Leung et al. Science, 246: 1306 (1989), and Houck et al. Mol. Endocrin., 5: 1806 (1991), together with the naturally occurring allelic and processed forms thereof. VEGF-A is part of a gene family

including VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and PlGF. VEGF-A primarily binds to two high affinity receptor tyrosine kinases, VEGFR- 1 (Fit- 1) and VEGFR-2 (Flk-1 KDR), the latter being the major transmitter of vascular endothelial cell mitogenic signals of VEGF-A.

[00175] “T-cell immunoreceptor with Ig and ITIM domains (TIGIT)”, is an immune receptor found on T-cells and natural killer cells (NK cells), as described by Yu X, et al., Nat Immunol. 10 (1): 48–57 (2009). It is also referred to as WUCAM and Vstm3. TIGIT binds to CD155(PVR) with high affinity on, for example, dendritic cells (DCs) and macrophages. TIGIT also binds to CD112(PVRL2), but with lower affinity. See, also, Anderson, A., et al., Immunity, 44(5):989-1004 (2016). The human TIGIT sequence can be found on UniProtKB under accession number Q495A1.

[00176] As used herein, “immune checkpoint” refers to stimulatory and inhibitory signals that regulate the amplitude and quality of T cell receptor recognition of an antigen. In certain embodiments, the immune checkpoint is an inhibitory signal. In certain embodiments, the inhibitory signal is the interaction between PD-1 and PD-L1. In certain embodiments, the inhibitory signal is the interaction between CTLA-4 and CD80 or CD86 to displace CD28 binding. In certain embodiments the inhibitory signal is the interaction between LAG-3 and MHC class II molecules. In certain embodiments, the inhibitory signal is the interaction between TIM-3 and galectin 9. In certain embodiments, the inhibitory signal is the interaction between TIGIT and CD155.

[00177] As used herein, “immune checkpoint blocker” or “immune checkpoint inhibitor” or “immune checkpoint modulator” refers to a molecule that reduces, inhibits, interferes with or modulates one or more checkpoint proteins or other proteins in the immune system pathways. In certain embodiments, the immune checkpoint inhibitor prevents inhibitory signals associated with the immune checkpoint. In certain embodiments, the immune checkpoint inhibitor is an antibody, or fragment thereof, that disrupts inhibitory signaling associated with the immune checkpoint. In certain embodiments, the immune checkpoint inhibitor is a small molecule that disrupts inhibitory signaling. In certain embodiments, the immune checkpoint inhibitor is an antibody, fragment thereof, or antibody mimic, that prevents the interaction between checkpoint blocker proteins, *e.g.*, an antibody, or fragment thereof, that prevents the interaction between PD-1 and PD-L1. In certain embodiments, the immune checkpoint inhibitor is an antibody, or fragment thereof, that prevents the interaction between CTLA-4 and CD80 or CD86. In certain

embodiments, the immune checkpoint inhibitor is an antibody, or fragment thereof, that prevents the interaction between LAG-3 and MHC class II molecules. In certain embodiments the, the immune checkpoint inhibitor is an antibody, or fragment thereof, that prevents the interaction between TIM-3 and galectin9. The checkpoint blocker may also be in the form of the soluble form of the molecules (or mutation thereof) themselves, *e.g.*, a soluble PD-L1 or PD-L1 fusion, as well as a soluble TIGIT or TIGIT fusion.

[00178] The term “ameliorating” refers to any therapeutically beneficial result in the treatment of a disease state, *e.g.*, cancer, including prophylaxis, lessening in the severity or progression, remission, or cure thereof.

[00179] The term “*in vivo*” refers to processes that occur in a living organism.

[00180] The term “mammal” or “subject” or “patient” as used herein includes both humans and non-humans and include but is not limited to humans, non-human primates, canines, felines, murines, bovines, equines, and porcines.

[00181] By “individual” or “host” or “subject” or “patient” is meant any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. Other subjects may include cynomolgus monkey, cattle, dogs, cats, guinea pigs, rabbits, rats, mice, horses, and so on.

[00182] As used herein, the term “mammal” refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. In some embodiments, the mammals are from the order Carnivora, including felines (cats) and canines (dogs). In some embodiments, the mammals are from the order Artiodactyla, including bovines (cows) and swines (pigs) or of the order Perssodactyla, including Equines (horses). It is most preferred that the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). In some embodiments, the mammal is a human. In some embodiments, the mammal is cynomolgus monkey.

[00183] The term “percent identity,” in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below

(*e.g.*, BLASTP and BLASTN or other algorithms available to persons of skill) or by visual inspection. Depending on the application, the “percent identity” can exist over a region of the sequence being compared, *e.g.*, over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared.

[00184] For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[00185] Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FAST A, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., *infra*).

[00186] One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al, *J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information website.

[00187] As used herein, the term “gly-ser polypeptide linker” refers to a peptide that consists of glycine and serine residues. An exemplary gly-ser polypeptide linker comprises the amino acid sequence Ser(Gly₄Ser)_n. In one embodiment, n=1. In one embodiment, n=2. In another embodiment, n=3, *i.e.*, Ser(Gly₄Ser)₃. In another embodiment, n=4, *i.e.*, Ser(Gly₄Ser)₄. In another embodiment, n=5. In yet another embodiment, n=6. In another embodiment, n=7. In yet another embodiment, n=8. In another embodiment, n=9. In yet another embodiment, n=10. Another exemplary gly-ser polypeptide linker comprises the amino acid sequence (Gly₄Ser)_n. In one embodiment, n=1. In one embodiment, n=2. In a preferred embodiment, n=3. In another embodiment, n=4. In another embodiment, n=5. In yet another embodiment, n=6. Another exemplary gly-ser polypeptide linker comprises the amino acid sequence (Gly₃Ser)_n. In one

embodiment, n=1. In one embodiment, n=2. In a preferred embodiment, n=3. In another embodiment, n=4. In another embodiment, n=5. In yet another embodiment, n=6.

[00188] As used herein, “half- life” refers to the time taken for the serum or plasma concentration of a polypeptide to reduce by 50%, *in vivo*, for example due to degradation and/or clearance or sequestration by natural mechanisms. The extended-PK IL-2 used herein is stabilized *in vivo* and its half-life increased by, *e.g.*, fusion to HSA, MSA or Fc, through PEGylation, or by binding to serum albumin molecules (*e.g.*, human serum albumin) which resist degradation and/or clearance or sequestration. The half-life can be determined in any manner known *per se*, such as by pharmacokinetic analysis. Suitable techniques will be clear to the person skilled in the art, and may for example generally involve the steps of suitably administering a suitable dose of the amino acid sequence or compound of the invention to a subject; collecting blood samples or other samples from said subject at regular intervals; determining the level or concentration of the amino acid sequence or compound of the invention in said blood sample; and calculating, from (a plot of) the data thus obtained, the time until the level or concentration of the amino acid sequence or compound of the invention has been reduced by 50% compared to the initial level upon dosing. Further details are provided in, *e.g.*, standard handbooks, such as Kenneth, A. et al., *Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists* and in Peters et al., *Pharmacokinetic Analysis: A Practical Approach* (1996). Reference is also made to Gibaldi, M. et al., *Pharmacokinetics*, 2nd Rev. Edition, Marcel Dekker (1982).

[00189] As used herein, a “small molecule” is a molecule with a molecular weight below about 500 Daltons.

[00190] As used herein, “therapeutic protein” refers to any polypeptide, protein, protein variant, fusion protein and/or fragment thereof which may be administered to a subject as a medicament. An exemplary therapeutic protein is an interleukin, *e.g.*, IL-7.

[00191] As used herein, “synergy” or “synergistic effect” with regard to an effect produced by two or more individual components refers to a phenomenon in which the total effect produced by these components, when utilized in combination, is greater than the sum of the individual effects of each component acting alone.

[00192] The term “sufficient amount” or “amount sufficient to” means an amount sufficient to produce a desired effect, *e.g.*, an amount sufficient to reduce the size of a tumor.

[00193] The term “therapeutically effective amount” is an amount that is effective to ameliorate a symptom of a disease. A therapeutically effective amount can be a “prophylactically effective amount” as prophylaxis can be considered therapy.

[00194] An “effective amount” or “therapeutically effective amount” of a composition includes that amount of the composition which is sufficient to provide a beneficial effect to the subject to which the composition is administered. An “effective amount” of a delivery vehicle includes that amount sufficient to effectively bind or deliver a composition.

[00195] As used herein, “combination therapy” embraces administration of each agent or therapy in a sequential manner in a regimen that will provide beneficial effects of the combination and co-administration of these agents or therapies in a substantially simultaneous manner. Combination therapy also includes combinations where individual elements may be administered at different times and/or by different routes but which act in combination to provide a beneficial effect by co- action or pharmacokinetic and pharmacodynamics effect of each agent or tumor treatment approaches of the combination therapy.

[00196] The term “in combination with” as used herein refers to uses where, for example, a first therapy is administered during the entire course of administration of a second therapy; where the first therapy is administered for a period of time that is overlapping with the administration of the second therapy, *e.g.*, where administration of the first therapy begins before the administration of the second therapy and the administration of the first therapy ends before the administration of the second therapy ends; where the administration of the second therapy begins before the administration of the first therapy and the administration of the second therapy ends before the administration of the first therapy ends; where the administration of the first therapy begins before administration of the second therapy begins and the administration of the second therapy ends before the administration of the first therapy ends; where the administration of the second therapy begins before administration of the first therapy begins and the administration of the first therapy ends before the administration of the second therapy ends. As such, “in combination” can also refer to regimen involving administration of two or more therapies. “In combination with” as used herein also refers to administration of two or more

therapies which may be administered in the same or different formulations, by the same or different routes, and in the same or different dosage form type.

[00197] As used herein, “about” will be understood by persons of ordinary skill and will vary to some extent depending on the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill given the context in which it is used, “about” will mean up to plus or minus 10% of the particular value.

[00198] The articles “a”, “an”, and “the” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[00199] “About” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

[00200] By “ADCC” or “antibody dependent cell-mediated cytotoxicity” as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express Fc γ Rs recognize bound antibody on a target cell and subsequently cause lysis of the target cell. ADCC is correlated with binding to Fc γ RIIIa; increased binding to Fc γ RIIIa leads to an increase in ADCC activity. As is discussed herein, many embodiments of the invention ablate ADCC activity entirely.

[00201] By “ADCP” or antibody dependent cell-mediated phagocytosis as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express Fc γ Rs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell.

[00202] By “antigen binding domain” or “ABD” herein is meant a set of six Complementary Determining Regions (CDRs) that, when present as part of a polypeptide sequence, specifically binds a target antigen as discussed herein. Thus, an “antigen binding domain” binds a target antigen as outlined herein. As is known in the art, these CDRs are generally present as a first set of variable heavy CDRs (vhCDRs or VHCDRs or CDR-HC) and a second set of variable light CDRs (vlCDRs or VLCDRs or CDR-LC), each comprising three CDRs: vhCDR1, vhCDR2, vhCDR3 for the heavy chain and vlCDR1, vlCDR2 and vlCDR3 for the light chain. The CDRs are present in the variable heavy and variable light domains, respectively, and together form an

Fv region. Thus, in some cases, the six CDRs of the antigen binding domain are contributed by a variable heavy and variable light chain. In a “Fab” format, the set of 6 CDRs are contributed by two different polypeptide sequences, the variable heavy domain (vh or VH; containing the vhCDR1, vhCDR2 and vhCDR3) and the variable light domain (vl or VL; containing the vlCDR1, vlCDR2 and vlCDR3), with the C-terminus of the vh domain being attached to the N-terminus of the CH1 domain of the heavy chain and the C-terminus of the vl domain being attached to the N-terminus of the constant light domain (and thus forming the light chain). In a scFv format, the VH and VL domains are covalently attached, generally through the use of a linker as outlined herein, into a single polypeptide sequence, which can be either (starting from the N-terminus) vh-linker-vl or vl-linker-vh, with the former being generally preferred (including optional domain linkers on each side, depending on the format used. As is understood in the art, the CDRs are separated by framework regions in each of the variable heavy and variable light domains: for the light variable region, these are FR1-vlCDR1-FR2-vlCDR2-FR3-vlCDR3-FR4, and for the heavy variable region, these are FR1-vhCDR1-FR2-vhCDR2-FR3-vhCDR3-FR4, with the framework regions showing high identity to human germline sequences. Antigen binding domains of the invention include, Fab, Fv and scFv.

[00203] By “modification” herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence or an alteration to a moiety chemically linked to a protein. For example, a modification may be an altered carbohydrate or PEG structure attached to a protein. By “amino acid modification” herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. For clarity, unless otherwise noted, the amino acid modification is always to an amino acid coded for by DNA, *e.g.*, the 20 amino acids that have codons in DNA and RNA.

[00204] By “variant protein” or “protein variant”, or “variant” as used herein is meant a protein that differs from that of a parent protein by virtue of at least one amino acid modification. Protein variant may refer to the protein itself, a composition comprising the protein, or the amino sequence that encodes it. Preferably, the protein variant has at least one amino acid modification compared to the parent protein, *e.g.*, from about one to about seventy amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. As described below, in some embodiments the parent polypeptide, for example an Fc parent polypeptide, is a human wild type sequence, such as the Fc region from IgG1, IgG2, IgG3 or IgG4. The protein variant sequence herein will preferably possess at least

about 80% identity with a parent protein sequence, and most preferably at least about 90% identity, more preferably at least about 95%-98%-99% identity. Variant protein can refer to the variant protein itself, compositions comprising the protein variant, or the DNA sequence that encodes it.

[00205] Accordingly, by “antibody variant” or “variant antibody” as used herein is meant an antibody that differs from a parent antibody by virtue of at least one amino acid modification, “IgG variant” or “variant IgG” as used herein is meant an antibody that differs from a parent IgG (again, in many cases, from a human IgG sequence) by virtue of at least one amino acid modification, and “immunoglobulin variant” or “variant immunoglobulin” as used herein is meant an immunoglobulin sequence that differs from that of a parent immunoglobulin sequence by virtue of at least one amino acid modification. “Fc variant” or “variant Fc” as used herein is meant a protein comprising an amino acid modification in an Fc domain. The Fc variants of the present invention are defined according to the amino acid modifications that compose them. Thus, for example M252Y or 252Y is an Fc variant with the substitution tyrosine at position 252 relative to the parent Fc polypeptide, wherein the numbering is according to the EU index. Likewise, M252Y/S254T/T256E defines an Fc variant with the substitutions M252Y, S254T and T256E relative to the parent Fc polypeptide. The identity of the wild type amino acid may be unspecified, in which case the aforementioned variant is referred to as 252Y/254T/256E. It is noted that the order in which substitutions are provided is arbitrary, that is to say that, for example, 252Y/254T/256E is the same Fc variant as 254T/252Y/256E, and so on. For all positions discussed in the present invention that relate to antibodies, unless otherwise noted, amino acid position numbering is according to Kabat for the variable region numbering and is according to the EU index for the constant regions, including the Fc region. The EU index or EU index as in Kabat or EU numbering scheme refers to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85, hereby entirely incorporated by reference.) The modification can be an addition, deletion, or substitution. Substitutions can include naturally occurring amino acids and, in some cases, synthetic amino acids.

[00206] By “Fv” or “Fv fragment” or “Fv region” as used herein is meant a polypeptide that comprises the VL and VH domains of a single antigen binding domain (ABD). As will be appreciated by those in the art, these generally are made up of two chains, or can be combined (generally with a linker as discussed herein) to form a scFv.

[00207] By “Fab” or “Fab region” as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody, antibody fragment or Fab fusion protein.

[00208] By “effector function” as used herein is meant a biochemical event that results from the interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions include but are not limited to ADCC, ADCP, and CDC.

[00209] By “Fc gamma receptor”, “FcγR” or “FcγgammaR” as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an FcγR gene. In humans this family includes but is not limited to FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIb-NA1 and FcγRIIb-NA2) (Jefferis et al., 2002, Immunol Lett 82:57-65, entirely incorporated by reference), as well as any undiscovered human FcγRs or FcγR isoforms or allotypes. In some cases, as outlined herein, binding to one or more of the FcγR receptors is reduced or ablated. For example, reducing binding to FcγRIIIa reduces ADCC, and in some cases, reducing binding to FcγRIIIa and FcγRIIb is desired.

[00210] By “FcRn” or “neonatal Fc Receptor” as used herein is meant a protein that binds the IgG antibody Fc region and is encoded at least in part by an FcRn gene. The FcRn may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. As is known in the art, the functional FcRn protein comprises two polypeptides, often referred to as the heavy chain and light chain. The light chain is beta-2-microglobulin and the heavy chain is encoded by the FcRn gene. Unless otherwise noted herein, FcRn or an FcRn protein refers to the complex of FcRn heavy chain with beta-2-microglobulin.

[00211] By “parent polypeptide” as used herein is meant a starting polypeptide that is subsequently modified to generate a variant. The parent polypeptide may be a naturally occurring polypeptide, or a variant or engineered version of a naturally occurring polypeptide. Parent polypeptide may refer to the polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it. Accordingly, by “parent immunoglobulin” as used herein is meant an unmodified immunoglobulin polypeptide that is modified to generate a variant, and by “parent antibody” as used herein is meant an unmodified

antibody that is modified to generate a variant antibody. It should be noted that “parent antibody” includes known commercial, recombinantly produced antibodies as outlined below.

[00212] By “heavy constant region” herein is meant the CH1-hinge-CH2-CH3 portion of an antibody, generally from human IgG1, IgG2 or IgG4.

[00213] By “target antigen” as used herein is meant the molecule that is bound specifically by the variable region of a given antibody. In the present case, the target antigen is a VISTA protein.

[00214] By “target cell” as used herein is meant a cell that expresses a target antigen.

[00215] By “variable region” as used herein is meant the region of an immunoglobulin that comprises one or more Ig domains substantially encoded by any of the V.kappa., V.lamda., and/or VH genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively.

[00216] By “wild type or WT” herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

[00217] By “position” as used herein is meant a location in the sequence of a protein. Positions may be numbered sequentially, or according to an established format, for example the EU index for antibody numbering.

[00218] By “residue” as used herein is meant a position in a protein and its associated amino acid identity. For example, Asparagine 297 (also referred to as Asn297 or N297) is a residue at position 297 in the human antibody IgG1.

[00219] The antibodies of the present invention are generally recombinant. “Recombinant” means the antibodies are generated using recombinant nucleic acid techniques in exogenous host cells.

[00220] The term “recombinant,” as applied to a polynucleotide means the polynucleotide is the product of various combinations of cloning, restriction or ligation steps, and other procedures resulting in a construct distinct and/or different from a polynucleotide found in

nature. The terms respectively include replicates of the original polynucleotide construct and progeny of the original virus construct.

[00221] “Percent (%) amino acid sequence identity” with respect to a protein sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific (parental) sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. One particular program is the ALIGN-2 program outlined at paragraphs [0279] to [0280] of US Pub. No. 20160244525, hereby incorporated by reference. Another approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics*, 2:482-489 (1981). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff, *Atlas of Protein Sequences and Structure*, M.O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov, *Nucl. Acids Res.* 14(6):6745-6763 (1986).

[00222] An example of an implementation of this algorithm to determine percent identity of a sequence is provided by the Genetics Computer Group (Madison, WI) in the “BestFit” utility application. The default parameters for this method are described in the Wisconsin Sequence Analysis Package Program Manual, Version 8 (1995) (available from Genetics Computer Group, Madison, WI). Another method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages, the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated the “Match” value reflects “sequence identity.” Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example,

another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the internet address located by placing [http://](http://blast.ncbi.nlm.nih.gov/Blast.cgi) in front of blast.ncbi.nlm.nih.gov/Blast.cgi.

[00223] The degree of identity between an amino acid sequence of the present invention (“invention sequence”) and the parental amino acid sequence is calculated as the number of exact matches in an alignment of the two sequences, divided by the length of the “invention sequence,” or the length of the parental sequence, whichever is the shortest. The result is expressed in percent identity.

[00224] In some embodiments, two or more amino acid sequences are at least 50%, 60%, 70%, 80%, or 90% identical. In some embodiments, two or more amino acid sequences are at least 95%, 97%, 98%, 99%, or even 100% identical.

[00225] “Specific binding” or “specifically binds to” or is “specific for” a particular antigen or an epitope means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target.

[00226] A “disease” includes a state of health of an animal, including a human, wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal’s health continues to deteriorate.

[00227] In contrast, a “disorder” in an animal, including a human, includes a state of health in which the animal is able to maintain homeostasis, but in which the animal’s state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal’s state of health.

[00228] The terms “treatment”, “treating”, “treat”, and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely

or partially preventing a disease or symptom thereof or reducing the likelihood of a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. “Treatment”, as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *i.e.*, arresting its development or progression; and (c) relieving the disease, *i.e.*, causing regression of the disease and/or relieving one or more disease symptoms. “Treatment” is also meant to encompass delivery of an agent in order to provide for a pharmacologic effect, even in the absence of a disease or condition. For example, “treatment” encompasses delivery of a composition that can elicit an immune response or confer immunity in the absence of a disease condition, *e.g.*, in the case of a vaccine.

[00229] The term “regression,” as well as words stemming therefrom, as used herein, does not necessarily imply 100% or complete regression. Rather, there are varying degrees of regression of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the disclosed methods can provide any amount of any level of regression of a cancer in a mammal. Furthermore, the regression provided by the inventive method can include regression of one or more conditions or symptoms of the disease, *e.g.*, a cancer. Also, for purposes herein, “regression” can encompass delaying the onset of the disease, delaying the onset of a symptom, and/or delaying the onset of a condition thereof. With respect to progressive diseases and disorders, “regression” can encompass slowing the progression of the disease or disorder, slowing the progression of a symptom of the disease or disorder, and/or slowing the progression of a condition thereof.

[00230] “Encoding” includes the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*i.e.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if, for example, transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

[00231] The term “operably linked” as used herein includes a polynucleotide in functional relationship with a second polynucleotide, *e.g.*, a single-stranded or double-stranded nucleic acid moiety comprising the two polynucleotides arranged within the nucleic acid moiety in such a manner that at least one of the two polynucleotides is able to exert a physiological effect by which it is characterized, upon the other. By way of example, a promoter operably linked to the coding region of a gene is able to promote transcription of the coding region. The order specified when indicating operably linkage is not important. For example, the phrases: “the promoter is operably linked to the nucleotide sequence” and “the nucleotide sequence is operably linked to the promoter” are used interchangeably herein and are considered equivalent. In some cases, when the nucleic acid encoding the desired protein further comprises a promoter/regulatory sequence, the promoter/regulatory sequence is positioned at the 5' end of the desired protein coding sequence such that it drives expression of the desired protein in a cell.

[00232] The term “promoter” as used herein includes a DNA sequence operably linked to a nucleic acid sequence to be transcribed such as a nucleic acid sequence encoding a desired molecule. A promoter is generally positioned upstream of a nucleic acid sequence to be transcribed and provides a site for specific binding by RNA polymerase and other transcription factors.

[00233] A “vector” is capable of transferring gene sequences to target-cells. Typically, “vector construct,” “expression vector,” and “gene transfer vector,” mean any nucleic acid construct capable of directing the expression of a gene of interest and which can transfer gene sequences to target-cells, which can be accomplished by genomic integration of all or a portion of the vector, or transient or inheritable maintenance of the vector as an extrachromosomal element. Thus, the term includes cloning, and expression vehicles, as well as integrating vectors.

[00234] The term “regulatory element” as used herein includes a nucleotide sequence which controls some aspect of the expression of nucleic acid sequences. Examples of regulatory elements illustratively include an enhancer, an internal ribosome entry site (IRES), an intron, an origin of replication, a polyadenylation signal (pA), a promoter, an enhancer, a transcription termination sequence, and an upstream regulatory domain, which contribute to the replication, transcription, and/or post-transcriptional processing of a nucleic acid sequence. In cases, regulatory elements can also include cis-regulatory DNA elements as well as transposable elements (TEs). Those of ordinary skill in the art are capable of selecting and using these and other regulatory elements in an expression construct with no more than routine experimentation.

Expression constructs can be generated using a genetic recombinant approach or synthetically using well-known methodology.

[00235] A “control element” or “control sequence” is a nucleotide sequence involved in an interaction of molecules contributing to the functional regulation of a polynucleotide, including replication, duplication, transcription, splicing, translation, or degradation of the polynucleotide. The regulation may affect the frequency, speed, or specificity of the process, and may be enhancing or inhibitory in nature. Control elements known in the art include, for example, transcriptional regulatory sequences such as promoters and enhancers. A promoter is a DNA region capable under certain conditions of binding RNA polymerase and initiating transcription of a coding region usually located downstream (in the 3' direction) from the promoter.

[00236] The statement that an amino acid residue is “phosphorylated” used herein means that a phosphate group is ester-linked to the side chain of the amino acid residue. Typical amino acid residues that may be phosphorylated include serine (Ser), threonine (Thr), and tyrosine (Tyr).

[00237] As used herein, the term “pharmaceutical composition” refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use in vivo or ex vivo.

[00238] As used herein, the term “pharmaceutically acceptable carrier” refers to any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see *e.g.*, Martin, Remington's Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, PA [1975].

[00239] As used herein, the term “enhancing an immune response” and “inducing an immune response” are used interchangeably and refer to the stimulation of an immune response.

[00240] As used herein, the term “inhibiting an immune response” means blocking the stimulation of an immune response. The blockade can be partial or complete.

[00241] Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are

compositions of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps.

[00242] As a general matter, compositions specifying a percentage are by weight unless otherwise specified. Further, if a variable is not accompanied by a definition, then the previous definition of the variable controls.

[00243] Various aspects of the invention are set forth below in sections; however, aspects of the invention described in one particular section are not to be limited to any particular section.

[00244]

III. Anti-VISTA Antibodies

[00245] The present disclosure provides novel anti-VISTA antibodies. Such antibodies bind human VISTA. Figure 1 (FIG. 1) lists peptide sequences of heavy chain variable regions and light chain variable regions that, in combination as designated in Figure 1, can bind to human VISTA. In some embodiments, the heavy chain variable region and the light chain variable region are arranged in a Fab format. In some embodiments, the heavy chain variable region and the light chain variable region are fused together to form an scFv.

[00246] In some embodiments, the anti-VISTA antibodies in the present disclosure comprise a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:1 a heavy chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

[00247] In some embodiments, the anti-VISTA antibodies in the present disclosure comprise vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1, Figure 5, Figure 6 and/or Figure 7. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00248] In some embodiments, the anti-VISTA antibodies in the present disclosure is selected from the group consisting of VS7, VS143, VISTA 0.5.9, VISTA1.4.1, VISTA1.4.2, VISTA1.4.3, VISTA1.4.4, VISTA1.4.5, VISTA1.4.6, VISTA1.4.7 (VS147), VISTA1.4.8, V1, V2, V3, V4, V5, V6, V7, V8, V9, V10, V11, V12, V13, V14, V15, V16, V17, V18, XC147 HC, XC147 LC, and V9.7 (scFv version of XC147).

[00249] In some embodiments, the anti-VISTA antibodies in the present disclosure comprise vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in VS7, VS143, VISTA 0.5.9, VISTA1.4.1, VISTA1.4.2, VISTA1.4.3, VISTA1.4.4, VISTA1.4.5, VISTA1.4.6, VISTA1.4.7 (VS147), VISTA1.4.8, V1, V2, V3, V4, V5, V6, V7, V8, V9, V10, V11, V12, V13, V14, V15, V16, V17, V18, XC147 HC, XC147 LC, and V9.7 (scFv version of XC147).. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00250] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:1 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:5.

[00251] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:1, a vhCDR2 comprising SEQ ID NO:3, a vhCDR3 comprising SEQ ID NO:4, a vlCDR1 comprising SEQ ID NO:6, a vlCDR2 comprising SEQ ID NO:7, and a vlCDR3 comprising SEQ ID NO:8. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00252] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:9 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:13.

[00253] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:10, a vhCDR2 comprising SEQ ID NO:11, a vhCDR3 comprising SEQ ID NO:12, a vlCDR1 comprising SEQ ID NO:14, a vlCDR2 comprising SEQ ID NO:15, and a vlCDR3 comprising SEQ ID NO:16. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00254] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:145.

[00255] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:146, a vhCDR2 comprising SEQ ID NO:147, a vhCDR3 comprising SEQ ID NO:148, a vlCDR1 comprising SEQ ID NO:149, a vlCDR2 comprising SEQ ID NO:150, and a vlCDR3 comprising SEQ ID NO:151. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00256] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:152.

[00257] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:153, a vhCDR2 comprising SEQ ID NO:154, a vhCDR3 comprising SEQ ID NO:155, a vlCDR1 comprising SEQ ID NO:156, a vlCDR2 comprising SEQ ID NO:157, and a vlCDR3

comprising SEQ ID NO:158. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00258] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:159.

[00259] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:160, a vhCDR2 comprising SEQ ID NO:161, a vhCDR3 comprising SEQ ID NO:162, a vlCDR1 comprising SEQ ID NO:163, a vlCDR2 comprising SEQ ID NO:164, and a vlCDR3 comprising SEQ ID NO:165. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00260] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:166.

[00261] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:167, a vhCDR2 comprising SEQ ID NO:168, a vhCDR3 comprising SEQ ID NO:169, a vlCDR1 comprising SEQ ID NO:170, a vlCDR2 comprising SEQ ID NO:171, and a vlCDR3 comprising SEQ ID NO:172. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00262] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:173.

[00263] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:174, a vhCDR2 comprising SEQ ID NO:175, a vhCDR3 comprising SEQ ID NO:176, a vlCDR1 comprising SEQ ID NO:177, a vlCDR2 comprising SEQ ID NO:178, and a vlCDR3 comprising SEQ ID NO:179. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00264] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:180.

[00265] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:181, a vhCDR2 comprising SEQ ID NO:182, a vhCDR3 comprising SEQ ID NO:183, a vlCDR1 comprising SEQ ID NO:184, a vlCDR2 comprising SEQ ID NO:185, and a vlCDR3 comprising SEQ ID NO:186. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00266] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:187.

[00267] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:188, a vhCDR2 comprising SEQ ID NO:189, a vhCDR3 comprising SEQ ID NO:190, a vlCDR1 comprising SEQ ID NO:191, a vlCDR2 comprising SEQ ID NO:192, and a vlCDR3 comprising SEQ ID NO:193. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00268] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:194.

[00269] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:195, a vhCDR2 comprising SEQ ID NO:196, a vhCDR3 comprising SEQ ID NO:197, a vlCDR1 comprising SEQ ID NO:198, a vlCDR2 comprising SEQ ID NO:199, and a vlCDR3 comprising SEQ ID NO:200. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00270] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:201.

[00271] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:202, a vhCDR2 comprising SEQ ID NO:203, a vhCDR3 comprising SEQ ID NO:204, a vlCDR1 comprising SEQ ID NO:205, a vlCDR2 comprising SEQ ID NO:206, and a vlCDR3 comprising SEQ ID NO:207. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00272] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:208.

[00273] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:209, a vhCDR2 comprising SEQ ID NO:210, a vhCDR3 comprising SEQ ID NO:211, a vlCDR1 comprising SEQ ID NO:212, a vlCDR2 comprising SEQ ID NO:213, and a vlCDR3 comprising SEQ ID NO:214. In some embodiments, one or more of such 6 CDRs have from 1,

2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00274] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:215.

[00275] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:216, a vhCDR2 comprising SEQ ID NO:217, a vhCDR3 comprising SEQ ID NO:218, a vlCDR1 comprising SEQ ID NO:219, a vlCDR2 comprising SEQ ID NO:220, and a vlCDR3 comprising SEQ ID NO:221. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00276] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:222.

[00277] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:223, a vhCDR2 comprising SEQ ID NO:224, a vhCDR3 comprising SEQ ID NO:225, a vlCDR1 comprising SEQ ID NO:226, a vlCDR2 comprising SEQ ID NO:227, and a vlCDR3 comprising SEQ ID NO:228. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00278] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:229.

[00279] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:230, a vhCDR2 comprising SEQ ID NO:231, a vhCDR3 comprising SEQ ID NO:232, a vlCDR1 comprising SEQ ID NO:233, a vlCDR2 comprising SEQ ID NO:234, and a vlCDR3 comprising SEQ ID NO:235. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00280] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:236.

[00281] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:237, a vhCDR2 comprising SEQ ID NO:238, a vhCDR3 comprising SEQ ID NO:239, a vlCDR1 comprising SEQ ID NO:240, a vlCDR2 comprising SEQ ID NO:241, and a vlCDR3 comprising SEQ ID NO:242. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00282] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:243.

[00283] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:244, a vhCDR2 comprising SEQ ID NO:245, a vhCDR3 comprising SEQ ID NO:246, a vlCDR1 comprising SEQ ID NO:247, a vlCDR2 comprising SEQ ID NO:248, and a vlCDR3 comprising SEQ ID NO:249. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00284] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:250.

[00285] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:251, a vhCDR2 comprising SEQ ID NO:252, a vhCDR3 comprising SEQ ID NO:253, a vlCDR1 comprising SEQ ID NO:254, a vlCDR2 comprising SEQ ID NO:255, and a vlCDR3 comprising SEQ ID NO:256. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00286] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:257.

[00287] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:258, a vhCDR2 comprising SEQ ID NO:259, a vhCDR3 comprising SEQ ID NO:260, a vlCDR1 comprising SEQ ID NO:261, a vlCDR2 comprising SEQ ID NO:262, and a vlCDR3 comprising SEQ ID NO:263. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00288] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:264.

[00289] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:265, a vhCDR2 comprising SEQ ID NO:266, a vhCDR3 comprising SEQ ID NO:267, a vlCDR1 comprising SEQ ID NO:268, a vlCDR2 comprising SEQ ID NO:269, and a vlCDR3 comprising SEQ ID NO:270. In some embodiments, one or more of such 6 CDRs have from 1,

2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00290] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:271 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:275. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:279.

[00291] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:272, a vhCDR2 comprising SEQ ID NO:273, a vhCDR3 comprising SEQ ID NO:274, a vlCDR1 comprising SEQ ID NO:276, a vlCDR2 comprising SEQ ID NO:277, and a vlCDR3 comprising SEQ ID NO:278. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00292] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:280 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:284. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:288.

[00293] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:281, a vhCDR2 comprising SEQ ID NO:282, a vhCDR3 comprising SEQ ID NO:283, a vlCDR1 comprising SEQ ID NO:285, a vlCDR2 comprising SEQ ID NO:286, and a vlCDR3 comprising SEQ ID NO:287. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00294] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:289 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:293. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:297.

[00295] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:290, a vhCDR2 comprising SEQ ID NO:291, a vhCDR3 comprising SEQ ID NO:292, a vlCDR1 comprising SEQ ID NO:294, a vlCDR2 comprising SEQ ID NO:295, and a vlCDR3 comprising SEQ ID NO:296. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00296] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:298 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:302. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%,

83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:306.

[00297] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:299, a vhCDR2 comprising SEQ ID NO:300, a vhCDR3 comprising SEQ ID NO:301, a vlCDR1 comprising SEQ ID NO:303, a vlCDR2 comprising SEQ ID NO:304, and a vlCDR3 comprising SEQ ID NO:305. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00298] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:307 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:311. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:315.

[00299] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:308, a vhCDR2 comprising SEQ ID NO:309, a vhCDR3 comprising SEQ ID NO:310, a vlCDR1 comprising SEQ ID NO:312, a vlCDR2 comprising SEQ ID NO:313, and a vlCDR3 comprising SEQ ID NO:314. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00300] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:316 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:320. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:324.

[00301] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:317, a vhCDR2 comprising SEQ ID NO:318, a vhCDR3 comprising SEQ ID NO:319, a vlCDR1 comprising SEQ ID NO:321, a vlCDR2 comprising SEQ ID NO:322, and a vlCDR3 comprising SEQ ID NO:323. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00302] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:325 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:329. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:333.

[00303] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:326, a vhCDR2 comprising SEQ ID NO:327, a vhCDR3 comprising SEQ ID NO:328, a vlCDR1 comprising SEQ ID NO:330, a vlCDR2 comprising SEQ ID NO:331, and a vlCDR3 comprising SEQ ID NO:332. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00304] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%,

83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:334 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:338. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:342.

[00305] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:335, a vhCDR2 comprising SEQ ID NO:336, a vhCDR3 comprising SEQ ID NO:337, a vlCDR1 comprising SEQ ID NO:339, a vlCDR2 comprising SEQ ID NO:340, and a vlCDR3 comprising SEQ ID NO:341. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00306] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:343 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:347. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:351.

[00307] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:344, a vhCDR2 comprising SEQ ID NO:345, a vhCDR3 comprising SEQ ID NO:346, a vlCDR1 comprising SEQ ID NO:348, a vlCDR2 comprising SEQ ID NO:349, and a vlCDR3 comprising SEQ ID NO:350. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00308] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:352 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:356. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:360.

[00309] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:353, a vhCDR2 comprising SEQ ID NO:354, a vhCDR3 comprising SEQ ID NO:355, a vlCDR1 comprising SEQ ID NO:357, a vlCDR2 comprising SEQ ID NO:358, and a vlCDR3 comprising SEQ ID NO:359. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00310] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:361 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:365. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:369.

[00311] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:362, a vhCDR2 comprising SEQ ID NO:363, a vhCDR3 comprising SEQ ID NO:364, a vlCDR1 comprising SEQ ID NO:366, a vlCDR2 comprising SEQ ID NO:367, and a vlCDR3 comprising SEQ ID NO:368. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2

amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00312] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:370 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:374. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:378.

[00313] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:371, a vhCDR2 comprising SEQ ID NO:372, a vhCDR3 comprising SEQ ID NO:373, a vlCDR1 comprising SEQ ID NO:375, a vlCDR2 comprising SEQ ID NO:376, and a vlCDR3 comprising SEQ ID NO:377. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00314] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:379 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:383. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:387.

[00315] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:380, a vhCDR2 comprising SEQ ID NO:381, a vhCDR3 comprising SEQ ID NO:382, a

vlCDR1 comprising SEQ ID NO:384, a vlCDR2 comprising SEQ ID NO:385, and a vlCDR3 comprising SEQ ID NO:386. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00316] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:388 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:392. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:396.

[00317] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:389, a vhCDR2 comprising SEQ ID NO:390, a vhCDR3 comprising SEQ ID NO:391, a vlCDR1 comprising SEQ ID NO:393, a vlCDR2 comprising SEQ ID NO:394, and a vlCDR3 comprising SEQ ID NO:395. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00318] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:397 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:401. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:405.

[00319] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:398, a vhCDR2 comprising SEQ ID NO:399, a vhCDR3 comprising SEQ ID NO:400, a vlCDR1 comprising SEQ ID NO:401, a vlCDR2 comprising SEQ ID NO:402, and a vlCDR3 comprising SEQ ID NO:403. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00320] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:406 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:410. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:414.

[00321] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:407, a vhCDR2 comprising SEQ ID NO:408, a vhCDR3 comprising SEQ ID NO:409, a vlCDR1 comprising SEQ ID NO:411, a vlCDR2 comprising SEQ ID NO:412, and a vlCDR3 comprising SEQ ID NO:413. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00322] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:415 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:419. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%,

83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:423.

[00323] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:416, a vhCDR2 comprising SEQ ID NO:417, a vhCDR3 comprising SEQ ID NO:418, a vlCDR1 comprising SEQ ID NO:420, a vlCDR2 comprising SEQ ID NO:421, and a vlCDR3 comprising SEQ ID NO:422. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00324] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:424 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:428. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:432.

[00325] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:425, a vhCDR2 comprising SEQ ID NO:426, a vhCDR3 comprising SEQ ID NO:427, a vlCDR1 comprising SEQ ID NO:429, a vlCDR2 comprising SEQ ID NO:430, and a vlCDR3 comprising SEQ ID NO:431. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00326] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:433 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:437. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:441.

[00327] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:434, a vhCDR2 comprising SEQ ID NO:435, a vhCDR3 comprising SEQ ID NO:436, a vlCDR1 comprising SEQ ID NO:438, a vlCDR2 comprising SEQ ID NO:439, and a vlCDR3 comprising SEQ ID NO:440. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00328] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:442 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:446. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:450.

[00329] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:443, a vhCDR2 comprising SEQ ID NO:444, a vhCDR3 comprising SEQ ID NO:445, a vlCDR1 comprising SEQ ID NO:447, a vlCDR2 comprising SEQ ID NO:448, and a vlCDR3 comprising SEQ ID NO:449. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00330] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%,

83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:451 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:455. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:459.

[00331] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:452, a vhCDR2 comprising SEQ ID NO:453, a vhCDR3 comprising SEQ ID NO:454, a vlCDR1 comprising SEQ ID NO:456, a vlCDR2 comprising SEQ ID NO:457, and a vlCDR3 comprising SEQ ID NO:458. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00332] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:460 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:464. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:468.

[00333] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:461, a vhCDR2 comprising SEQ ID NO:462, a vhCDR3 comprising SEQ ID NO:463, a vlCDR1 comprising SEQ ID NO:465, a vlCDR2 comprising SEQ ID NO:466, and a vlCDR3 comprising SEQ ID NO:467. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00334] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:469 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:473. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:477.

[00335] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:470, a vhCDR2 comprising SEQ ID NO:471, a vhCDR3 comprising SEQ ID NO:472, a vlCDR1 comprising SEQ ID NO:474, a vlCDR2 comprising SEQ ID NO:475, and a vlCDR3 comprising SEQ ID NO:476. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00336] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:478 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:482. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:486.

[00337] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:479, a vhCDR2 comprising SEQ ID NO:480, a vhCDR3 comprising SEQ ID NO:481, a vlCDR1 comprising SEQ ID NO:483, a vlCDR2 comprising SEQ ID NO:484, and a vlCDR3 comprising SEQ ID NO:485. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2

amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00338] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:487 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:491. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:495.

[00339] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:488, a vhCDR2 comprising SEQ ID NO:489, a vhCDR3 comprising SEQ ID NO:490, a vlCDR1 comprising SEQ ID NO:492, a vlCDR2 comprising SEQ ID NO:493, and a vlCDR3 comprising SEQ ID NO:494. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00340] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:496 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:500. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:504.

[00341] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:497, a vhCDR2 comprising SEQ ID NO:498, a vhCDR3 comprising SEQ ID NO:499, a

vlCDR1 comprising SEQ ID NO:501, a vlCDR2 comprising SEQ ID NO:502, and a vlCDR3 comprising SEQ ID NO:503. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00342] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:505 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:509. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:513.

[00343] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:506, a vhCDR2 comprising SEQ ID NO:507, a vhCDR3 comprising SEQ ID NO:508, a vlCDR1 comprising SEQ ID NO:510, a vlCDR2 comprising SEQ ID NO:511, and a vlCDR3 comprising SEQ ID NO:512. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00344] In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:514 and a light chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:518. In some embodiments, the anti-VISTA antibodies in the present disclosure include a heavy chain variable region having an amino acid sequence at least 80% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to SEQ ID NO:522.

[00345] In some embodiments, the anti-VISTA antibodies include a vhCDR1 comprising SEQ ID NO:515, a vhCDR2 comprising SEQ ID NO:516, a vhCDR3 comprising SEQ ID NO:517, a vlCDR1 comprising SEQ ID NO:519, a vlCDR2 comprising SEQ ID NO:520, and a vlCDR3 comprising SEQ ID NO:521. In some embodiments, one or more of such 6 CDRs have from 1, 2, 3, 4 or 5 amino acid modifications. In further embodiments, a single CDR contains 1 or 2 amino acid substitutions, and the modified anti-VISTA antibodies retain binding to human VISTA.

[00346]

[00347] In addition to the sequence variants described herein in the heavy chain and light chain variable regions and/or CDRs, changes in the framework region(s) of the heavy and/or light variable region(s) can be made. In some embodiment, variants in the framework regions (*e.g.*, excluding the CDRs) retain at least about 80, 85, 90 or 95% identity to a germline sequence. Variants can be made to retain at least about 80, 85, 90 or 95% identity to any one of the light chain V-GENE, light chain J-GENE, heavy chain V-GENE, heavy chain J-GENE, and heavy chain D-GENE alleles.

[00348] In some embodiments, variations are made in the framework regions that retain at least 80, 85, 90 or 95% identity to the germline gene sequences, while keeping 6 CDRs unchanged.

[00349] In some embodiments, variations are made in both the framework regions that retain at least 80, 85, 90 or 95% identity to the germline gene sequences, and the 6 CDRs. The CDRs can have amino acid modifications (*e.g.*, from 1, 2, 3, 4 or 5 amino acid modifications in the set of CDRs (that is, the CDRs can be modified as long as the total number of changes in the set of 6 CDRs is less than 6 amino acid modifications, with any combination of CDRs being changed; *e.g.*, there may be one change in vlCDR1, two in vhCDR2, none in vhCDR3, etc.).

[00350] By selecting amino acid sequences of CDRs and/or variable regions of a heavy chain and a light chain from those described herein and combining them with amino acid sequences of framework regions and/or constant regions of a heavy chain and a light chain of an antibody as appropriate, a person skilled in the art will be able to design an anti-VISTA antibody according to the present invention. The antibody framework regions and/or constant region (Fc domain)

described in the current invention can derive from an antibody of any species, such as from human, rabbit, dog, cat, mouse, horse or monkey.

[00351] In some embodiments, the constant region is derived from human, and includes a heavy chain constant region derived from those of IgG, IgA, IgM, IgE, and IgD subtypes or variants thereof, and a light chain constant region derived from kappa or lambda subtypes or variants thereof. In some embodiments, the heavy chain constant region is derived from a human IgG, including IgG1, IgG2, IgG3, and IgG4. In some embodiments, the amino acid sequence of the heavy chain constant region is at least 80%, 85%, 90%, or 95% identical to a human IgG1, IgG2, IgG3, or IgG4 constant region. In some other embodiments, the amino acid sequence of the constant region is at least 80%, 85%, 90%, or 95% identical to an antibody constant region from another mammal, such as rabbit, dog, cat, mouse, horse or monkey. In some embodiments, the antibody constant region includes a hinge, a CH2 domain, a CH3 domain and optionally a CH1 domain.

[00352] In some embodiments, the antibodies described herein can be derived from a mixture from different species, *e.g.*, forming a chimeric antibody and/or a humanized antibody. In general, both “chimeric antibodies” and “humanized antibodies” refer to antibodies that combine regions from more than one species. For example, “chimeric antibodies” traditionally comprise variable region(s) from a mouse (or rat, in some cases) and the constant region(s) from a human. “Humanized antibodies” generally refer to non-human antibodies that have had the variable-domain framework regions swapped for sequences found in human antibodies. Generally, in a humanized antibody, the entire antibody, except the CDRs, is encoded by a polynucleotide of human origin or is identical to such an antibody except within its CDRs. The CDRs, some or all of which are encoded by nucleic acids originating in a non-human organism, are grafted into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, *e.g.*, WO 92/11018, Jones, 1986, Nature 321:522-525, Verhoeyen et al., 1988, Science 239:1534-1536, all entirely incorporated by reference. “Backmutation” of selected acceptor framework residues to the corresponding donor residues is often required to regain affinity that is lost in the initial grafted construct (US 5530101; US 5585089; US 5693761; US 5693762; US 6180370; US 5859205; US 5821337; US 6054297; US 6407213, all entirely incorporated by reference). The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin, and thus will

typically comprise a human Fc region. Humanized antibodies can also be generated using mice with a genetically engineered immune system, as described for example in Roque et al., 2004, *Biotechnol. Prog.* 20:639-654, entirely incorporated by reference. A variety of techniques and methods for humanizing and reshaping non-human antibodies are well known in the art (See Tsurushita & Vasquez, 2004, *Humanization of Monoclonal Antibodies*, *Molecular Biology of B Cells*, 533-545, Elsevier Science (USA), and references cited therein, all entirely incorporated by reference). Humanization methods include but are not limited to methods described in Jones et al., 1986, *Nature* 321:522-525; Riechmann et al., 1988, *Nature* 332:323-329; Verhoeyen et al., 1988, *Science*, 239:1534-1536; Queen et al., 1989, *Proc Natl Acad Sci, USA* 86:10029-33; He et al., 1998, *J. Immunol.* 160: 1029-1035; Carter et al., 1992, *Proc Natl Acad Sci, USA* 89:4285-9; Presta et al., 1997, *Cancer Res.* 57(20):4593-9; Gorman et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:4181-4185; O'Connor et al., 1998, *Protein Eng* 11:321-8, all entirely incorporated by reference. Humanization or other methods of reducing the immunogenicity of nonhuman antibody variable regions may include resurfacing methods, as described for example in Roguska et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:969-973, entirely incorporated by reference. Other humanization methods may involve the grafting of only parts of the CDRs, including but not limited to methods described in Tan et al., 2002, *J. Immunol.* 169:1119-1125; De Pascalis et al., 2002, *J. Immunol.* 169:3076-3084, all entirely incorporated by reference.

[00353] In some embodiments, the antibodies of the current invention comprise a heavy chain variable region derived from a particular human germline heavy chain immunoglobulin gene and/or a light chain variable region derived from a particular human germline light chain immunoglobulin gene. Such antibodies may contain amino acid differences as compared to the human germline sequences, due to, for example, naturally-occurring somatic mutations or intentional introduction of site-directed mutation. However, a humanized antibody typically is at least 80% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the antibody as being derived from human sequences when compared to the germline immunoglobulin amino acid sequences of other species (*e.g.*, murine germline sequences). In certain cases, a humanized antibody may be at least 95, 96, 97, 98 or 99%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the human germline immunoglobulin gene. Typically, a humanized antibody derived from a particular human germline sequence will display no more than 10-20 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the humanized

antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

[00354] In some embodiments, the antibodies of the current disclosure are humanized and affinity matured, as is known in the art. Structure-based methods may be employed for humanization and affinity maturation, for example as described in US Patent No 7,657,380. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu et al., 1999, J. Mol. Biol. 294:151-162; Baca et al., 1997, J. Biol. Chem. 272(16):10678-10684; Rosok et al., 1996, J. Biol. Chem. 271(37): 22611-22618; Rader et al., 1998, Proc. Natl. Acad. Sci. USA 95: 8910-8915; Krauss et al., 2003, Protein Engineering 16(10):753-759, all entirely incorporated by reference.

IV. Characteristics of the Anti-VISTA antibodies

[00355] In some embodiments, the anti-VISTA antibodies described herein bind to human VISTA. In some embodiments, binding of the anti-VISTA antibodies to human VISTA is measured by ELISA or any other method known to a person skilled in the art.

[00356] In some embodiments, the anti-VISTA antibodies described herein bind human VISTA with high affinities. The K_D value can be measured with the antigen immobilized or with the antibody immobilized. The K_D value can also be measured in a monovalent or a bivalent binding mode.

[00357] In some embodiments, the anti-VISTA antibodies display low immunogenicity when administered into human subjects. These antibodies can contain an Fc domain derived from human IgG1, human IgG2, human IgG3, or human IgG4. In some embodiments, these antibodies are humanized using the framework regions derived from human immunoglobulins.

[00358] Effects of the anti-VISTA antibodies on T cell function can be assayed using a variety of methods known in the art and described herein. Accordingly, the anti-VISTA antibodies can serve as VISTA agonists or VISTA antagonists.

[00359] In some embodiments anti-VISTA antibodies described act as VISTA agonists, and as a result, such anti-VISTA antibodies induce or enhance an immune response as well as to potentiate or enhance the suppressive effects of the VISTA/VSIG3 pathway. In some

embodiments, inducing or enhancing an immune response means activating immune cells. In some embodiments, inducing or enhancing an immune response means activating immune cells.

[00360] In some embodiments, anti-VISTA antibodies described act by inducing or enhancing an immune response against an antigen. In some embodiments, anti-VISTA antibodies described act by suppressing the immune suppression from the VISTA/VSIG3 response.

[00361] In some embodiments, anti-VISTA antibodies described act as VISTA/VSIG3 pathway agonists, and as a result, such anti-VISTA antibodies potentiate or enhance the VISTA/VSIG3 suppressive effects on T cell immunity, effectively suppressing T-cell immunity. In some embodiments, antagonization can include, for example, inhibition of signaling of VSIG3 and/or VISTA. In some embodiments, the anti-VISTA antibody agonizes the VSIG3/VISTA interaction. In some embodiments, the anti-VISTA antibody that agonizes results in enhancing the signaling of VSIG3 and/or VISTA.

[00362] In some embodiments, anti-VISTA antibodies described act as VISTA/VSIG3 pathway antagonists, and as a result, such anti-VISTA antibodies suppress the VISTA/VSIG3 suppressive effects on T cell immunity, effectively increasing T-cell immunity by reducing the suppression from the VISTA/VSIG3 pathway. In some embodiments, the anti-VISTA antibody antagonizes the VSIG3/VISTA interaction. In some embodiments, antagonism of VISTA signaling can include antagonism of CD3-induced cytokine signals. In some embodiments, antagonism of VISTA signaling can include abrogation of at least one of CD3-induced IL-2 production, CD3-induced IFN- γ production, CD3-induced RANTES production, CD3-induced MIP-1 alpha production, CD3-induced IL-17 production, and CD3-induced CXCL11 production.

[00363] In some embodiments, the anti-VISTA antibodies compete with VSIG3 for binding to VISTA. In some embodiments, inhibition of VISTA/VSIG3 by anti-VISTA antibodies may be partial inhibition. In some embodiment, inhibition of VISTA/VSIG3 by anti-VISTA antibodies may be full inhibition. In some embodiments, anti-VISTA antibodies inhibit binding by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%. In some embodiments, inhibiting an immune response means stopping VISTA⁺ cancer cell growth. In some embodiments, inhibiting an immune response means stopping cell growth in order to treat the cancer. In some embodiments, the anti-VISTA antibodies inhibit cell growth by inhibiting the immune suppression from the VISTA/VSIG3 pathway.

[00364] In some embodiments, inducing or enhancing an immune response means activating immune cells to a particular antigen. In some embodiments, inducing or enhancing an immune response means providing a co-stimulatory signal. In some embodiments, inducing or enhancing an immune response means activating T cells. In some embodiments, inducing or enhancing an immune response means activating B cells. In some embodiments, inducing or enhancing an immune response means increasing the cytotoxic T lymphocyte response. In some embodiments, inducing or enhancing an immune response means increasing CD4⁺ T effector cell function. In some embodiments, inducing or enhancing an immune response means decreasing the suppression of CD4⁺ T effector cell function. In some embodiments, inducing or enhancing an immune response means increasing CD8⁺ T effector cell function. In some embodiments, inducing or enhancing an immune response means decreasing the suppression of CD8⁺ T effector cell function. In some embodiments, inducing or enhancing an immune response means increasing antigen-specific T cell function, proliferation, and/or activation. In some embodiments, inducing or enhancing an immune response means decreasing the suppression of antigen-specific T cell function, proliferation, and/or activation. In some embodiments, inducing or enhancing an immune response means increasing an antigen-specific Th1 response. In some embodiments, inducing or enhancing an immune response means decreasing the suppression of an antigen-specific Th1 response. In some embodiments, inducing or enhancing an immune response means increasing or supporting memory cell formation. In some embodiments, inducing or enhancing an immune response means decreasing the suppression of memory cell formation. In some embodiments, the anti-VISTA antibodies of the present disclosure promotes or enhances at least one effect of human VISTA on immunity, including for example, but not limited to the suppressive effect on any one or more of: T cell immunity; activation of monocytes; induction of T-cell proliferation; induction or suppression of cytokine expression; increased survival of monocytes; induction of antibody-dependent cell-mediated cytotoxicity (ADCC) in cells-expressing VISTA; and/or induction of antibody-dependent cellular phagocytosis (ADCP) in cells-expressing VISTA. In some embodiments, inducing or enhancing an immune response means decreasing the inhibition of ADCC. In some embodiments, inducing or enhancing an immune response means initiating ADCP. In some embodiments, ADCC can be modulated to cause at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% lysis of VISTA expressing cells.

[00365] In some embodiments, inhibiting cell growth means tumor inhibition or a reduction in tumor size.

[00366] Efficacy readouts can include monitoring for changes in $\alpha\beta$ and/or $\gamma\delta$ T cells, cytotoxic T cell activity, changes in markers such as CD137, CD107a, changes in NK and/or NK/T activity, interferon- γ production, changes in regulatory T-cell (including changes in Treg number), changes in macrophage number, changes in neutrophil pro-tumorigenic activity, T-cell activation, CTL activation, changes in activation markers such as CD45RA or CCR7, as well as cancer cell cytotoxicity assays. Efficacy readouts can also include antagonism of CD3-induced cytokine signals. Efficacy readouts can also include abrogation of at least one of CD3-induced IL-2 production, CD3-induced IFN- γ production, CD3-induced RANTES production, CD3-induced MIP-1 alpha production, CD3-induced IL-17 production, and CD3-induced CXCL11 production. Efficacy readouts can also include tumor size reduction, tumor number reduction, reduction in the number of metastases, and decreased disease state (or increased life expectancy). In some embodiments, inhibiting cell growth means tumor inhibition or a reduction in tumor size. In some embodiments, a reduction in tumor size by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% is indicative of therapeutic efficacy. In some embodiments, a reduction in tumor number by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% is indicative of therapeutic efficacy. In some embodiments, a reduction in tumor burden by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% is indicative of therapeutic efficacy. In some embodiments, a reduction in the number of metastases by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% is indicative of therapeutic efficacy.

V. *Nucleic Acids of the Invention*

[00367] Nucleic acids encoding the anti-VISTA antibodies described herein also encompass the invention, as well as expression vectors containing such nucleic acids and host cells transformed with such nucleic acids and/or expression vectors. As will be appreciated by those in the art, the protein sequences depicted herein can be encoded by any number of possible nucleic acid sequences due to the degeneracy of the genetic code.

[00368] Nucleic acid compositions encoding the anti-VISTA antibodies and/or VISTA-binding domains also encompass the invention. As will be appreciated by those in the art, in the case of antigen binding domains, the nucleic acid compositions generally include a first nucleic acid encoding the heavy chain variable region and a second nucleic acid encoding the light chain

variable region. In the case of scFvs, a single nucleic acid encoding the heavy chain variable region and light chain variable region, separated by a linker described herein, can be made. In the case of traditional antibodies, the nucleic acid compositions generally include a first nucleic acid encoding the heavy chain and a second nucleic acid encoding the light chain, which will, upon expression in a cell, spontaneously assemble into the “traditional” tetrameric format of two heavy chains and two light chains.

[00369] As is known in the art, the nucleic acids encoding the components of the invention can be incorporated into expression vectors, and depending on the host cells, used to produce the antibodies of the invention. These two nucleic acids can be incorporated into a single expression vector or into two different expression vectors. Generally, the nucleic acids can be operably linked to any number of regulatory elements (promoters, origin of replication, selectable markers, ribosomal binding sites, inducers, etc.) in an expression vector. The expression vectors can be extra-chromosomal or integrating vectors.

[00370] The nucleic acids and/or expression vectors of the current invention can be introduced into any type of host cells, which are well known in the art, including mammalian, bacterial, yeast, insect and fungal cells. After transfection, single cell clones can be isolated for cell bank generation using methods known in the art, such as limited dilution, ELISA, FACS, microscopy, or Clonepix. Clones can be cultured under conditions suitable for bio-reactor scale-up and maintained expression of the antibodies. The antibodies can be isolated and purified using methods known in the art including centrifugation, depth filtration, cell lysis, homogenization, freeze-thawing, affinity purification, gel filtration, ion exchange chromatography, hydrophobic interaction exchange chromatography, and mixed-mode chromatography.

VI. Therapeutic Applications

[00371] The current disclosure provides a method of modulating an immune response in a subject, and the method includes administering to the subject an effective amount of an anti-VISTA antibody described herein, or a pharmaceutical composition containing an anti-VISTA antibody.

[00372] In some embodiments, the methods of modulating an immune response encompassed by the present disclosure comprises inhibiting an immune response in a subject, and in further embodiments, such methods comprise administering to the subject an effective amount of an

anti-VISTA antibody that acts as a VISTA antagonist, or by administering a pharmaceutical composition containing an antagonistic anti-VISTA antibody.

[00373] In some embodiments, the present disclosure provides methods for inducing or enhancing an immune response in a subject, for example, by administering to the subject an effective amount of an anti-VISTA antibody that acts as a VISTA agonist, or by administering to the subject a pharmaceutical composition containing such an agonistic anti-VISTA antibody.

[00374] The present disclosure also provides methods of treating cancer in a subject, and such methods include administering to the subject an effective amount of an anti-VISTA antibody that acts as a VISTA antagonist, or a pharmaceutical composition containing such anti-VISTA antibody. In some embodiments, the cancer to be treated expresses VISTA on the cancer cell surface. In some embodiments, the cancer to be treated upregulates VISTA compared to the corresponding non-cancerous tissue. In some embodiments, the subject to be treated expresses VISTA on T cells, such as on CD8⁺ and/or CD4⁺ T cells. In some embodiments, the subject to be treated expresses a high level of VISTA on one or more types of immune cells including CD4⁺ T cells, CD8⁺ T cells, B cells, natural killer T cells, natural killer cells, macrophages, and dendritic cells. In some embodiments, the cancer to be treated uses the VISTA/VSIG3 pathway to promote tumor growth. In some embodiments, the cancer to be treated is non-responsive to existing immune-modulating antibodies targeting other immune checkpoints, such as CTLA-4, PD-1 or PD-L1.

[00375] “Cancer therapy” herein refers to any method which prevents or treats cancer or ameliorates one or more of the symptoms of cancer. Typically, such therapies will comprise administration of anti-VISTA alone or in combination (including for example, in combination with integrin-binding polypeptide-Fc fusions), as well as potentially in combination with chemotherapy or radiotherapy or other biologics and for enhancing the activity thereof. In some embodiments, cancer therapy can include or be measured by increased survival. In some embodiments, cancer therapy results in a reduction in tumor volume.

[00376] “Cancer,” as used herein, refers broadly to any neoplastic disease (whether invasive non-invasive or metastatic) characterized by abnormal and uncontrolled cell division causing malignant growth or tumor (*e.g.*, unregulated cell growth). As used herein, we may use the terms “cancer” (or “cancerous”), “hyperproliferative,” and “neoplastic” to refer to cells having the capacity for autonomous growth (*i.e.*, an abnormal state or condition characterized by rapidly

proliferating cell growth). Non-limiting examples of which are described herein. This includes any physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer are exemplified in the working examples and also are described within the specification. The terms “cancer” or “neoplasm” are used to refer to malignancies of the various organ systems, including those affecting the lung, breast, thyroid, lymph glands and lymphoid tissue, gastrointestinal organs, and the genitourinary tract, as well as to adenocarcinomas which are generally considered to include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, melanoma, prostate, ovarian, endometrial, non-small cell lung cancer, lung, pancreas, cervical, colorectal, and head and neck.

[00377] Non-limiting examples of cancers that can be treated using the present disclosure include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, lung cancer (including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenström's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; multiple myeloma and post-transplant lymphoproliferative disorder (PTLD). In some embodiments, other cancers amenable for treatment by the present invention include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include colorectal, bladder, ovarian, melanoma, squamous cell cancer, lung cancer (including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer (including gastrointestinal cancer), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver

cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenström's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome. Preferably, the cancer is selected from the group consisting of colorectal cancer, breast cancer, rectal cancer, non-small cell lung cancer, non-Hodgkin's lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, Kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, melanoma, ovarian cancer, mesothelioma, and multiple myeloma. In an exemplary embodiment the cancer is an early or advanced (including metastatic) bladder, ovarian or melanoma. In another embodiment the cancer is colorectal cancer. In some embodiments, the methods of the present invention are useful for the treatment of vascularized tumors.

[00378] Hyperproliferative and neoplastic disease states may be categorized as pathologic (*i.e.*, characterizing or constituting a disease state), or they may be categorized as non-pathologic (*i.e.*, as a deviation from normal but not associated with a disease state). The terms are meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. "Pathologic hyperproliferative" cells occur in disease states characterized by malignant tumor growth. Examples of non-pathologic hyperproliferative cells include proliferation of cells associated with wound repair.

[00379] Examples of cellular proliferative and/or differentiative disorders include cancer (*e.g.*, carcinoma, sarcoma, metastatic disorders or hematopoietic neoplastic disorders, *e.g.*, leukemias). A metastatic tumor can arise from a multitude of primary tumor types, including but not limited to those of prostate, colon, lung, breast and liver. Accordingly, the compositions used herein and

optionally at least one additional therapeutic agent to treat cancer, can be administered to a patient who has cancer.

[00380] Additional examples of proliferative disorders include hematopoietic neoplastic disorders. As used herein, the term “hematopoietic neoplastic disorders” includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin, *e.g.*, arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. In some embodiments, the diseases arise from poorly differentiated acute leukemias (*e.g.*, erythroblastic leukemia and acute megakaryoblastic leukemia). Additional exemplary myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus, L. (1991) Crit. Rev. in Oncol./Hematol. 11 :267-97); lymphoid malignancies include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's macro globulinemia (WM). Additional forms of malignant lymphomas include, but are not limited to non-Hodgkin lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T cell lymphoma (CTCL), large granular lymphocytic leukemia (LGF), Hodgkin's disease and Reed-Sternberg disease.

[00381] The term “carcinoma” is art recognized and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. The mutant IL-2 polypeptides can be used to treat patients who have, who are suspected of having, or who may be at high risk for developing any type of cancer, including renal carcinoma or melanoma, or any viral disease. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term also includes carcinosarcomas, which include malignant tumors composed of carcinomatous and sarcomatous tissues. An “adenocarcinoma” refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures.

[00382] In some embodiments, the cancer to be treated is melanoma, prostate, ovarian, endometrial, non-small cell lung cancer, lung, pancreas, cervical, colorectal, and head and neck.

VII. Combination therapy

[00383] Anti-VISTA antibodies described herein can be used in combination with additional therapeutic agents to treat cancer.

[00384] It will be appreciated by those skilled in the art that amounts for each of the anti-VISTA antibodies, and optionally at least one or more additional therapeutic agents used to treat cancer, that are sufficient to reduce tumor growth and size, or a therapeutically effective amount, will vary not only on the particular compounds or compositions selected, but also with the route of administration, the nature of the condition being treated, and the age and condition of the patient, and will ultimately be at the discretion of the patient's physician or pharmacist. The length of time during which the compounds used in the instant method will be given varies on an individual basis.

[00385] In some embodiments, the one or more additional therapeutic agents used to treat cancer are immune checkpoint inhibitors. As described herein, immune checkpoint inhibitors include anti-PD-1 inhibitors, anti-PD-L1 inhibitors, anti-CTLA-4 inhibitors, anti-TIM-3 inhibitors, and anti-LAG-3 inhibitors. Examples of types of immune checkpoint inhibitors include antibodies. In some embodiments, the anti-PD-1 antibody is selected from the group consisting of nivolumab, pembrolizumab, and ipilimumab. In some embodiments, the anti-CTLA-4 antibody is selected from the group consisting of ipilimumab and tremelimumab. In some embodiments, the anti-PD-L1 antibody is atezolizumab. In some embodiments, the anti-LAG-3 is IMP-321.

[00386] In some embodiments, the one or more additional therapeutic agents used to treat cancer are tumor targeting agents. As described herein, tumor targeting agents can also include integrin-binding Fc-fusion polypeptides (including, for example, NOD-201. In some embodiments, the one or more additional therapeutic agents used to treat cancer is radiation.

[00387] In some embodiments, the anti-VISTA antibodies and at least one or more additional therapeutic agents used to treat cancer inhibit growth and/or proliferation of tumor cells. In some embodiments, the anti-VISTA antibodies and at least one or more additional therapeutic agents used to treat cancer reduce tumor size. In certain embodiments, the anti-VISTA antibodies and at least one or more additional therapeutic agents used to treat cancer inhibit metastases of a primary tumor.

[00388] In some embodiments, the anti-VISTA antibodies and at least one or more checkpoint inhibitors inhibit growth and/or proliferation of tumor cells. In some embodiments, the anti-VISTA antibodies and at least one or more checkpoint inhibitors reduce tumor size. In certain embodiments, the anti-VISTA antibodies and at least one or more checkpoint inhibitors inhibit metastases of a primary tumor.

[00389] In some embodiments, the anti-VISTA antibodies can be combined with an adjuvant to treat advanced breast carcinoma. In some embodiments, the anti-VISTA antibodies can be combined with an adjuvant to treat advanced ovarian carcinoma.

[00390] In some embodiments, the anti-VISTA antibodies are used in conjunction with a surgical method to treat cancer.

[00391] In some embodiments, the anti-VISTA antibodies are used in conjunction with tumor targeting antibodies. In some embodiments, the tumor targeting antibodies are selected from the group consisting of anti-CD20, anti-EGFR, and anti-Her2. In some embodiments, the tumor targeting antibodies are selected from the group consisting of trastuzumab, rituximab, cetuximab, and anti-Her2.

[00392] In some embodiments, the anti-VISTA antibodies can be combined with the integrin-binding polypeptide-Fc fusions described herein to treat cancer. In some embodiments, the anti-VISTA antibodies can be combined with the integrin-binding polypeptide-Fc fusions described herein along with at least one additional therapeutic agent used to treat cancer discussed herein to treat cancer. In some embodiments, the integrin-binding polypeptide-Fc fusions is one as described herein. In some embodiments, the integrin-binding polypeptide-Fc fusions is one as described herein in Table 2. In some embodiments, the integrin-binding polypeptide-Fc fusion comprises a sequence selected from the group consisting of SEQ ID NOs: 51-119. In some embodiments, the integrin-binding polypeptide-Fc fusions is NOD-201. In some embodiments, the integrin-binding polypeptide-Fc fusions is SEQ ID NO: 118. In some embodiments, the integrin-binding polypeptide-Fc fusions is SEQ ID NO: 119.

[00393] Efficacy readouts can include monitoring for changes in $\alpha\beta$ and/or $\gamma\delta$ T cells, cytotoxic T cell activity, changes in markers such as CD137, CD107a, changes in NK and/or NK/T activity, interferon- γ production, changes in regulatory T-cell (including changes in Treg number), changes in macrophage number, changes in neutrophil pro-tumorigenic activity, T-cell

activation, CTL activation, changes in activation markers such as CD45RA or CCR7, as well as cancer cell cytotoxicity assays. Efficacy readouts can also include antagonism of CD3-induced cytokine signals. Efficacy readouts can also include abrogation of at least one of CD3-induced IL-2 production, CD3-induced IFN- γ production, CD3-induced RANTES production, CD3-induced MIP-1 α production, CD3-induced IL-17 production, and CD3-induced CXCL1 I production. Efficacy readouts can also include tumor size reduction, tumor number reduction, reduction in the number of metastases, and decreased disease state (or increased life expectancy). In some embodiments, inhibiting cell growth means tumor inhibition or a reduction in tumor size. In some embodiments, a reduction in tumor size by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% is indicative of therapeutic efficacy. In some embodiments, a reduction in tumor number by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% is indicative of therapeutic efficacy. In some embodiments, a reduction in tumor burden by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% is indicative of therapeutic efficacy. In some embodiments, a reduction in the number of metastases by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% is indicative of therapeutic efficacy.

[00394] The amount of the antibodies and additional therapeutic agents and the relative timing of administration may be selected in order to achieve a desired combined therapeutic effect. For example, when administering a combination therapy to a patient in need of such administration, the therapeutic agents in the combination, or a pharmaceutical composition or compositions comprising the therapeutic agents, may be administered in any order such as, for example, sequentially, concurrently, together, simultaneously and the like. Further, for example, a multi-specific binding protein may be administered during a time when the additional therapeutic agent(s) exerts its prophylactic or therapeutic effect, or vice versa.

VIII. PHARMACEUTICAL COMPOSITION AND ADMINISTRATION

[00395] The present disclosure also features pharmaceutical compositions/formulations that contain a therapeutically effective amount of an anti-VISTA antibody described herein. The composition can be formulated for use in a variety of drug delivery systems. One or more physiologically acceptable excipients or carriers can also be included in the composition for proper formulation. Suitable formulations for use in the present disclosure are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, Pa., 17th ed.,

1985. For a brief review of methods for drug delivery, see, *e.g.*, Langer (Science 249:1527-1533, 1990).

[00396] The antibodies of the present disclosure can exist in a lyophilized formulation or liquid aqueous pharmaceutical formulation. The aqueous carrier of interest herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a liquid formulation. Illustrative carriers include sterile water for injection (SWFI), bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.*, phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

[00397] The antibodies of the present disclosure could exist in a lyophilized formulation including the proteins and a lyoprotectant. The lyoprotectant may be sugar, *e.g.*, disaccharides. In certain embodiments, the lyoprotectant is sucrose or maltose. The lyophilized formulation may also include one or more of a buffering agent, a surfactant, a bulking agent, and/or a preservative.

[00398] Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. It may be administered in the range of 0.1 mg to 1 g and preferably in the range of 0.5 mg to 500 mg of active antibody per administration for adults. Alternatively, a patient's dose can be tailored to the approximate body weight or surface area of the patient. Other factors in determining the appropriate dosage can include the disease or condition to be treated or prevented, the severity of the disease, the route of administration, and the age, sex and medical condition of the patient. Further refinement of the calculations necessary to determine the appropriate dosage for treatment is routinely made by those skilled in the art, especially in light of the dosage information and assays disclosed herein. The dosage can also be determined through the use of known assays for determining dosages used in conjunction with appropriate dose-response data. An individual patient's dosage can be adjusted as the progress of the disease is monitored. Blood levels of the targetable construct or complex in a patient can be measured to see if the dosage needs to be adjusted to reach or maintain an effective concentration. Pharmacogenomics may be used to determine which targetable constructs and/or complexes, and dosages thereof, are most likely to be effective for a given individual (Schmitz et al., Clinica Chimica Acta 308: 43-53, 2001; Steimer et al., Clinica Chimica Acta 308: 33-41, 2001).

[00399] Doses may be given once or more times daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the targetable construct or complex in bodily fluids or tissues. Administration of the present invention could be intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, intracavitary, by perfusion through a catheter or by direct intralesional injection. This may be administered once or more times daily, once or more times weekly, once or more times monthly, and once or more times annually.

EXAMPLES

EXAMPLE 1: ANTI-VISTA ANTIBODIES

[00400] Rounds of screening were performed to yield 20+ clones of anti-VISTA antibodies, many with sub-nM affinity to human antigen (FIG. 3). Subsequent affinity maturation and cross reactivity selection yielded mouse and human cross-reactive clone VS147. VS147 has sub-nM affinity to human antigen and single nM affinity to mouse antigen (FIG. 4A and FIG. 4B). Co-culture of HIGH cells, a high VISTA expressing macrophage cell line, with T cells inhibits activation and IL-2 secretion. VS147 abrogates VISTA-mediated T-cell inhibition in vitro (FIG. 5). In a mouse model of B16 melanoma, VS147 shows inhibitory activity of VISTA *in vivo* and anti-tumor efficacy as a monotherapy (FIG. 6).

EXAMPLE 2: STRUCTURE AND FUNCTIONAL BINDING EPIOTOPE OF V-DOMAIN IG SUPPRESSOR OF T-CELL ACTIVATION (VISTA)

Abstract

[00401] V-domain Ig Suppressor of T cell Activation (VISTA) is an immune checkpoint protein that inhibits the T-cell response against cancer. Similar to PD-1 and CTLA-4, antibodies that block VISTA signaling can release the brakes of the immune system and promote tumor clearance. VISTA has an Ig-like fold, but little is known about its structure and mechanism of action. Features that make the VISTA IgV-like fold unique among B7 family members are highlighted, including two additional disulfide bonds and an extended

loop region with attached helix that we show forms a contiguous binding epitope for a clinically relevant anti-VISTA antibody. Overlap of this antibody-binding region with the binding epitope for V-set and Ig domain-containing 3 (VSIG3), a purported functional binding partner of VISTA, is proposed. Here, it is described that a 1.85 Å crystal structure of the human VISTA extracellular domain and highlight structural features that make VISTA unique among B7 family members. Through fine-epitope mapping, it was also identified that solvent-exposed residues that underlie binding to a clinically relevant anti-VISTA antibody. This antibody-binding region is also shown to interact with V-set and Ig domain-containing 3 (VSIG3), the recently proposed functional binding partner of VISTA. The structure and functional epitope determined here will help guide future drug development efforts against this important checkpoint target.

Introduction

[00402] V-domain Ig Suppressor of T-cell Activation (VISTA) is an immune checkpoint protein involved in the regulation of T cell activity. VISTA is highly expressed on myeloid-derived cells such as CD11b⁺ monocytes, CD11c⁺ dendritic cells, and to a lesser extent on CD4⁺ and CD8⁺ T cells¹. Similar to the well-studied PD-1, PD-L1, and CTLA-4 checkpoint proteins, the presence of VISTA results in reduced T cell activation and proliferation. The mechanism of action for this effect, however, is unclear as VISTA has been thought to function as both a ligand and a receptor. As a ligand, VISTA is expressed on antigen-presenting cells and binds an unknown receptor on T cells to inhibit downstream T cell activation^{1,2}. As a receptor, VISTA is expressed on T cells and transduces intracellular inhibitory signals after ligand binding to curtail T cell activity^{3,4}. A proposed ligand for VISTA has recently been identified as V-Set and Immunoglobulin domain containing 3 (VSIG3)⁵.

[00403] Checkpoint proteins have been found to be overexpressed by cancer cells or their surrounding immune cells and prevent anti-tumor activity by co-opting natural regulation mechanisms to escape immune clearance. In particular, compared to healthy tissues, VISTA is upregulated on tumor infiltrating leukocytes, including high expression on myeloid-derived suppressor cells (MDSCs)^{6,7}. Through VISTA signaling, these inhibitory immune cells prevent effective antigen presentation and indirectly promote tumor growth. VISTA is implicated in a number of human cancers including skin (melanoma)⁸, prostate⁹, colon¹⁰, pancreatic¹¹, ovarian¹², endometrial¹², and lung (NSCLC)¹³. Additionally, VISTA levels have been found to increase after anti-CTLA-4 treatment (ipilimumab) in prostate cancer⁹ and after anti-PD-1 treatment in

metastatic melanoma⁸, highlighting VISTA expression as a method of acquired resistance to currently available checkpoint inhibitors. For these reasons, VISTA is an important cancer immunotherapy target for drug development efforts.

[00404] The human VISTA protein is 279 amino acids in length, comprising a 162 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 96 amino acid cytoplasmic domain. The cytoplasmic domain lacks any immunoreceptor tyrosine-based signaling motifs, but does contain multiple casein kinase 2 and phosphokinase C phosphorylation sites that could play a role in signal transduction. Protein sequence analysis has clustered VISTA with the B7 family group of ligands (CD80, CD86, PD-L1, PD-L2, ICOSL, and CD276), all of which contain a conserved IgV-like fold. Among these proteins, VISTA is an outlier with relatively low sequence homology to other family members. The closest homolog within the B7 family is PD-L1, which shares only 22% sequence identity with VISTA. The VISTA extracellular domain contains two canonical cysteines that are conserved in Ig-like proteins, and also has four unique cysteine residues that are not present in other B7 family members. The low sequence homology and additional cysteine residues have hindered accurate structural modeling of VISTA based on sequence alone and present a clear need for a high resolution crystal structure.

[00405] Antibodies against VISTA have shown anti-tumor efficacy in multiple syngeneic mouse models^{1,6,14}. Therapeutic development has progressed to human clinical trials with the development of anti-human VISTA antibodies led by Janssen Therapeutics. A purported lead anti-VISTA antibody (called ‘VSTB’ here) inhibits VISTA signaling *in vitro* and shows tumor regression in a MB49 syngeneic mouse model of bladder cancer¹⁵; however, little is known about its mechanism of inhibition. Putative regions of interaction between VSTB and VISTA have been proposed, but a specific binding epitope has not been identified. It is also unknown if the anti-VISTA activity of VSTB is derived from the blockade of VISTA/VSIG3 interaction. Moreover, although murine and human VISTA share 70% sequence homology, VSTB is not cross-reactive between species, which introduces challenges with testing in murine tumor models.

[00406] Antibodies against VISTA have shown anti-tumor efficacy in multiple syngeneic mouse models (Liu et al., 2015; Le Mercier et al., 2014; Wang et al., 2011). Therapeutic development has progressed to human clinical trials with the assessment of a small molecule antagonist targeting VISTA and PD-L1 (NCT02812875) and the recently terminated trial of an anti-VISTA antibody in patients with advanced cancer (NCT02671955). The purported lead

anti-VISTA antibody used in the Phase I trial (called ‘VSTB’ here, based on VSTB112) inhibited VISTA signaling *in vitro* and showed tumor regression in a bladder cancer model using human VISTA knock-in mice (Snyder et al., 2014); however, little is known about its mechanism of inhibition. Putative regions of interaction between VSTB and VISTA have been proposed, but a specific binding epitope has not been identified. It is also unknown if the anti-VISTA activity of VSTB is derived from the blockade of VISTA/VSIG3 interaction.

[00407] A high resolution crystal structure of the human VISTA protein is present and characteristics of the unique IgV-like fold that distinguishes VISTA from other B7 family proteins are highlighted. Combinatorial methods were used to map the VSTB/VISTA binding epitope, and further examine this region for potential VSIG3 interactions. Structural comparisons and epitope analyses performed here provide a blueprint for further VISTA mechanistic research and the development of next generation anti-VISTA therapeutics.

Results

Crystallization of the extracellular domain of human VISTA

[00408] The extracellular domain (ECD) of human VISTA (VISTA ECD), containing a C-terminal hexahistidine tag, was recombinantly expressed in human embryonic kidney cells and purified from the supernatant using immobilized metal affinity chromatography (*see*, for example, FIG. 15). The VISTA ECD was found to be hyper-glycosylated, producing a diffuse protein band that appeared to be ~15 kDa larger than its predicted molecular mass upon analysis by gel electrophoresis (SDS-PAGE). To generate well-formed crystals, minimizing glycosylation was attempted by introducing mutations to known N-linked glycosylation sites and also through enzymatic cleavage of sugars using a glycosidase. Analysis of the VISTA sequence highlighted five potential locations for N-glycan modification via a NXT/S motif. We mutated three of these asparagine residues (N59, N76, and N158) to glutamine. These selected positions were found to be three or more amino acid residues away from predicted secondary structure and therefore changes at these positions were less likely to affect native folding of the protein. In order to further reduce N-linked glycosylation, Kifunensine was added, a mannosidase I inhibitor, to the mammalian cell culture media prior to plasmid transfection. In addition, the purified VISTA protein was treated with the glycosidase Endo Hf prior to crystallization trials. These efforts resulted in improved discreteness and decreased apparent mass of the purified protein as compared to wild-type VISTA, indicating decreased glycosylation (FIG. 12).

[00409] Analysis of the VISTA sequence highlighted five potential locations for N-glycan modification via a NXT/S motif. We mutated three of these asparagine residues (N59, N76, and N158) to glutamine, added Kifunensine, a mannosidase I inhibitor, to the mammalian cell culture media, and treated purified protein with Endo Hf glycosidase prior to crystallization trials. These efforts resulted in improved discreteness and decreased apparent mass of the purified protein as compared to wild-type VISTA (Figure 12) and facilitated crystal formation.

[00410] Crystal trays were established in sitting drop format and placed at 12° C overnight. An optimized condition using seeds from prior, smaller crystal hits produced diffraction quality crystals. A complete dataset to 1.85 Å was collected by x-ray diffraction at the Stanford Synchrotron Radiation Lightsource (SSRL). VISTA does not have a suitable template for molecular replacement as no VISTA homologs are deposited in the PDB and the closest templates have sequence identity under 25%. The crystal structure was therefore solved using a combination of molecular replacement (MR), Rosetta modeling, and native sulfur single-wavelength anomalous diffraction (SAD) methods. Briefly, an iterative MR-Rosetta pipeline was used to find MR solutions, which were further rebuilt and refined with Rosetta. The model from the automated Rosetta procedures was then manually refined with Phenix to obtain the final structure.

[00411] The VISTA ECD contains three disulfide bonds comprising all six cysteine residues found in the VISTA sequence (FIG. 7A). The structure consists of ten beta strands and three alpha helices arranged in a canonical beta-sandwich formation (FIG. 7B). The protein is divided into two faces: six beta strands forming one coplanar surface and four beta strands comprising the other. The protein fragment between strands C and C' is comprised of 21 residues forming an extended loop and four residues in a predicted alpha helix. Of the 25 residues in this region, six are predicted to be positively charged while only three are negatively charged, creating a net positive charge on this face of the protein. This positive plane is reflected in blue using the APBS electrostatic prediction tool (FIG. 7C). The C51/C113 disulfide bond connects the distinct C-C' loop to the internal beta sandwich and likely plays a role minimizing flexibility in this region. The extended loop region was further examined for structural validity and uniqueness. An omit map was generated to verify loop density (Figure 7C). The C-C' loop is stabilized by intramolecular interactions within the loop and with residues from the core of the protein. There are 30 intramolecular hydrogen bonding interactions compared to only four hydrogen bonding interactions with symmetry related molecules (Figure 17 and Figure 20). The structure of the C-

C' loop is therefore most likely positioned by internal interactions instead of an artifact of crystal lattice formation. Of the 25 residues in this region, six are positively charged while only three are negatively charged, creating a net positive charge on this face of the protein. This positive plane is reflected as a blue surface using APBS electrostatic prediction (Figure 7D). The structure presented here represents a common Ig-like fold, but as described below, closer examination reveals important differences that make VISTA unique among B7 family proteins.

Comparison of VISTA with other B7 family proteins

[00412] The canonical fold of the B7 family is comprised of two distinct domains, an IgV domain with nine beta strands and an IgC domain with seven beta strands²⁹. Typically, the IgC domain is proximal to the membrane while the IgV domain is distal and interacts directly with its cognate receptor. Of the seven B7 family proteins that have been crystallized, VISTA is the only family member that lacks an IgC domain. To highlight the structural distinctions of VISTA, the VISTA ECD was aligned with the IgV domain of human PD-L1 (PDB: 4Z18), its closest homolog in the B7 family (22% sequence identity). The overall beta sandwich fold is evident in both proteins with seven beta strands in VISTA aligning to corresponding strands in the PD-L1 structure (FIG. 8A). There are however, four key differences between VISTA and the classic B7 family fold. First, VISTA contains ten beta strands, instead of the nine that typically make up an IgV fold. Second, VISTA contains an extra helix (sequence FQDL) in place of a longer beta strand C' (FIG. 8B). This helix is located in the predicted positively charged patch and may constitute a unique epitope that distinguishes VISTA binding interactions from its B7 homologs. Third, VISTA contains a 21-residue extended loop (C-C' loop) that does not align with any B7 family structure (FIG. 8C). This region contains seven charged, surface exposed residues. PD-L1 and other B7 family proteins have a significantly smaller four residue loop at this location that directly connects two beta strands but does not protrude from the classic beta sandwich fold. Finally, VISTA also contains two additional disulfide bonds that are not present in any other B7 family protein but are conserved in VISTA orthologs, including murine and cyno (FIG. 8D). These two disulfide bonds connect residues C12/C146 and C51/C113, respectively, while the conserved disulfide bond connects beta strands B and F (C22/C114). In particular, the C51/C113 disulfide bond connects the unique extended loop to the internal beta sandwich and likely plays a role minimizing flexibility in this region. The uniqueness of the VISTA structure was further corroborated by structural similarity comparisons with other B7 family proteins. The Tm-align server was used to calculate similarities based on pairwise structural comparisons of known B7

family proteins (Figure 16E). A Tm-score above 0.5 predicts proteins of the same fold family with scores closer to 1.0 indicative of increasingly similar protein structures (Xu and Zhang, 2010). VISTA is most similar to PD-L1 (0.60) and displays greater structural differences with PD-L2 (0.56), CD80 (0.55), and CD86 (0.56). Notably, all pairwise values for VISTA and other B7 family members are 0.60 or below, whereas pairwise comparisons among all other B7 family members have scores of 0.65 or above. This deviation from the B7 family is not observable by sequence differences alone, as pairwise sequence identities among VISTA and the B7 family members are between 15 and 25%, similar to the bulk of other alignment values (Figure 16E). The DALI server was used to calculate z-score similarities based on pairwise structural comparisons of known B7 family proteins (FIG. 8E). VISTA is most similar to PD-L1 (11.2) and PD-L2 (10.2), but displays larger structural differences with CD276 (7.1), CD80 (9.4), and CD86 (9.5), exemplifying its structural individuality among the B7 family. The average pairwise z-score (mean of row in FIG. 8E) of each protein was compared against all other B7 family members. VISTA has an average pairwise z-score below 10 while other B7 family member averages are all 12 or higher.

[00413] The extended C-C' loop was further examined for structural validity and uniqueness. An omit map was generated to verify loop density (FIG. 8F). Additionally, a DALI search of the C-C' loop region uncovered homology with a protein known as immune receptor expressed on myeloid cells-1 (IREM-1). Analogous to VISTA, IREM-1 has an extended C-C' loop held in place by a disulfide bond and also functions as a single domain inhibitory receptor on the surface of myeloid cells³⁰. Even though the C-C' loops differ in size and IREM-1 is not in the same predicted protein family as VISTA, a DALI z-score of 12.4 was calculated between VISTA and IREM-1, higher than any pairwise comparison of VISTA and B7 family proteins.

Comparison of VISTA among species

[00414] When developing drug candidates, researchers often verify functionality by examining therapeutic efficacy and mechanism of action in preclinical murine tumor models, followed by toxicity studies in rodents and cynomolgus monkeys. Generating a species cross-reactive drug that binds its targets across multiple animal models allows for more facile transition between stages of development. Here, the amino acid sequences and structural models of human, mouse, and cyno VISTA were compared to highlight features helpful for directing development of cross-reactive drugs.

[00415] Using the crystal structure of human VISTA as a template, homology models of mouse and cyno VISTA were built using Rosetta. Three and nine-mer fragments of mouse and cyno VISTA were generated through the Robetta server, protein alignments were generated using Clustal Omega, and the Rosetta hybridize protocol was used to generate 10,000 potential structures of each target. These decoy structures were clustered and the lowest free energy structure from the largest cluster was used for structural comparison. Mouse and cyno VISTA homology models were aligned with the human VISTA crystal structure using PyMOL (FIG. 9A). Due to high sequence identity of human to mouse (70.4%) and human to cyno (96.4%), and the fact that human VISTA was used as a singular template for Rosetta hybridization, it is unsurprising that the proposed structures align very well to each other (RMSD of 0.592 and 0.430, respectively).

[00416] There are, however, several important structural distinctions between VISTA from different species. First, beta strand C' (sequence LHHG) is only present in human and cyno VISTA (FIG. 9B). Mouse VISTA contains a H66Q mutation and a deletion of L67 that prevents formation of a beta strand in this position. As a result, mouse VISTA contains a total of 9 beta strands compared to the 10 found in human and cyno VISTA. Perhaps more importantly, the unique extended loop and helix region that comprise a positively charged face is modified in mouse VISTA (FIG. 9C). Within this charged region, mouse VISTA has four residues that differ from human VISTA: R54H, L60F, F62L, and D64H. The lack of two charged residues (R54 and D64) and the presence of two extra aromatic rings (H54 and H64) at these positions could play a role in altering charged electrostatic or pi-stacking interactions in this region. To confirm the importance of these structural differences for the molecular recognition of the VSTB antibody, equilibrium binding constants (K_d) to mouse or human VISTA were measured (FIG. 9D). The K_d of the hVISTA/VSTB interaction is ~250 pM while the K_d of mVISTA/VSTB is undetectable due to lack of significant binding signal. The high degree of similarity between human and cyno VISTA and the increased divergence of mouse VISTA is exemplified in the multiple sequence alignment (FIG. 9E, bold/red). Highlighted differences between mouse and human VISTA in the extended loop and helix region likely underlie the lack of species cross-reactivity for the VSTB antibody.

Mapping the VSTB/VISTA binding epitope - Four residues in the C-C' loop comprise the VSTB/VISTA binding epitope

[00417] Next, a putative binding epitope of an anti-VISTA antibody VSTB, which is a known inhibitor of VISTA signaling and prevents tumor growth in an MB49 mouse model of bladder cancer¹⁵, was determined. Fine-epitope mapping of VSTB was performed by screening a large library of VISTA mutants displayed on the surface of individual yeast cells to isolate variants that exhibited loss of antibody binding. Using this method³¹, a set of VISTA residues that mediate VSTB binding was elucidated. A separate VISTA-binding antibody (referred to as 'VS147') was tested for conformational and distinct epitope binding to validate proper folding of VISTA mutants. Heat denaturation of yeast-displayed VISTA followed by incubation with VS147 antibody showed a lack of binding, confirming a conformational epitope that depends on VISTA structural integrity (Figure 13A). Additionally, the VS147 and VSTB antibodies were found to have distinct epitopes through the detection of simultaneous binding of both antibodies (Figure 13B).

[00418] A library of VISTA mutants was created via error prone-PCR using a low mutagenic rate to achieve, on average, a single amino acid mutation per gene. Restricting the library to single amino acid mutations allows for confident attribution of binding changes to a particular residue. A library with estimated diversity of 3.6×10^8 yeast transformants was generated in a strain of *S. cerevisiae* engineered for surface protein display^{32,33}. In addition to the yeast-displayed VISTA library and VSTB antibody, fine-epitope mapping of the VISTA/VSTB interaction required a control antibody to validate proper folding of VISTA mutants. The control antibody (referred to as 'VS147') was tested for conformational and distinct epitope binding. Heat denaturation of yeast-displayed VISTA followed by incubation with VS147 antibody showed a lack of binding, confirming a conformational epitope that depends on VISTA structural integrity (FIG. 13A). Additionally, the VS147 and VSTB antibodies were found to have distinct epitopes through the detection of simultaneous binding of both antibodies (FIG. 13B) Screening for yeast-displayed VISTA mutants that decreased binding to VSTB but still bound the VS147 antibody allowed the isolation of residues that directly altered VSTB binding without disrupting the structural integrity of VISTA.

[00419] The library was induced for VISTA expression on the cell surface, resulting in each yeast displaying thousands of copies of an individual VISTA variant. Iterative rounds of fluorescence-activated cell sorting (FACS) were used to select yeast-displaying VISTA mutants that either lost binding to VSTB ("negative" sort) or retained binding to the VS147 antibody ("positive" sort) (FIG. 10A). Following each sort round, collected yeast were cultured, and cell

surface display of VISTA was again induced prior to the next round of FACS. In Sort 1, the library was incubated with 10 nM VSTB and screened to isolate VISTA mutants that displayed moderate to negligible binding to VSTB. To increase stringency, yeast collected from Sort 1 were subject to a higher concentration of VSTB in Sort 2 to isolate VISTA mutants demonstrating even weaker binding to VSTB. A VSTB-negative binding population was clearly enriched in Sort 2 (25.2% in gate) compared to the small number of negative clones observed in Sort 1 (3.4% in gate). In Sort 3, 50 nM of VS147 antibody (about 200x the estimated K_d) was used to isolate yeast-displayed VISTA mutants that retained structural integrity to bind VS147 antibody. The screening stringency was again increased in Sort 4 by using an even higher concentration of VSTB (200 nM) to select for mutations that almost completely decreased antibody binding. By Sort 4, close to 70% of the yeast-displayed VISTA clones showed weak to negative binding to VSTB. Remaining yeast clones were subject to a final positive sort using a lower concentration of VS147 (about 20x the estimated K_d) to further confirm retention of VISTA structural integrity.

[00420] Following library screening, 50 yeast clones were randomly selected for sequencing analysis to help identify a subset of residues directly involved in VSTB binding. Five mutations appeared in multiple (>4) sequenced clones: F62L, R54C, S124P, Q63R, and R58G (Table 5).

Table 5. Epitope mapping sequencing results

Mutation	# of Samples
F62L	10
R54C	9
S124P	9
Q63R	5
R58G	4
L67P	3
F1L	2
S8P	2
C51R	2
F62S	2
D64G	2
E118G	2
C113R	2

Table of repeat mutations found from sequencing DNA isolated from the yeast population after Sort 5 of epitope mapping. The top five mutations by frequency are highlighted in yellow. Mutations that appeared four times or more were selected for single clone analysis and site-directed mutagenesis.

[00421] All identified residues, with the exception of S124P, were localized to the alpha helix and extended loop fragment that is unique to the VISTA structure (FIG. 10B). Each residue was then individually mutated to alanine via site-directed mutagenesis to confirm that the residue locations, as opposed to the specific amino acid mutations, were drivers of VSTB binding. VISTA mutants were individually displayed on the surface of yeast and binding to VSTB was measured by flow cytometry. The binding of S124A to VSTB appeared to be equivalent to that of wild-type VISTA binding to VSTB (FIG. 14), suggesting that the proline mutation in S124P indirectly affected VSTB binding by modifying the local fold of the VISTA protein. This mutant was therefore excluded from the proposed epitope and was not subject to further analysis. In contrast, the remaining four mutants (R54A, R58A, F62A, or Q63A) were each found to individually affect binding to VSTB (FIG. 10C). Binding signal generated from 250 nM VSTB clearly shows weak binding of F62A and Q63A mutants and almost no detectable binding of R54A and R58A mutants, demonstrating that these four residues are critical for VISTA binding to VSTB (FIG. 10D).

[00422] Due to the proximity of these mapped residues to the N59 genetically modified glycan site (Figure 18A), binding of VSTB to WT VISTA or the triple N59Q/N76Q/N158Q mutant used for crystallization (Figure 18B) was measured and showed no significant differences (apparent K_d values of ~0.9 nM). Additionally, the N59 residue appears to be solvent exposed and points away from the internal hydrophobic core and therefore glycosylation at this location is unlikely to affect loop structure (Figure 18C). A valid epitope must also be surface exposed to allow for unhindered interactions with antibodies and other binding proteins. Accessible surface area for the purported epitope residues was calculated using the PISA server (Figure 19). Residues R54, F62, and Q63 have exposures above 35% while the R58 residue has a lower exposure of 23%, consistent with its internal facing side chain. All four residues have significantly greater accessible area than fully buried residues (*e.g.*, solvent accessibility for the buried W40 and F97 residues are <1%). Further analysis of intramolecular interactions revealed R58 as integral to the local C-C' loop structure due to its extensive involvement with hydrophobic packing and intramolecular interactions (Figure 18C). We thus hypothesize that

R58 stabilizes a local loop turn and that mutations at this location likely disrupt surrounding residues. Based on this analysis, R58 appears to have been highlighted as an artifact of the screening process and therefore was excluded from further analysis as an epitope residue.

Mapping the VSIG3/VISTA interaction - VISTA interacts with VSIG3 via binding epitope of VSTB antibody

[00423] The VSTB antibody has been shown to inhibit VISTA signaling, thus the residues identified above suggest a functional epitope through which to guide future drug discovery efforts. Since the epitope for the recently-proposed VISTA ligand VSIG3 is unknown, it was tested whether VSTB operates through direct ligand competition of VSIG3 at the residues mapped above and demonstrate overlap of the purported VSTB binding epitope with the VISTA/VSIG3 interaction.

[00424] Wild-type (WT) VISTA and a VISTA triple mutant containing the R54A, F62A, and Q63A mutations (FIG. 11A) were solubly expressed in mammalian cells. The R58 residue was not mutated in this analysis because structural examination revealed its importance to the stability of the entire loop due to its interior direction and proximity to other side chains. To confirm structural integrity of the triple mutant, the VS147 antibody was first tested for binding to WT VISTA or the 54A/62A/63A triple mutant using an ELISA-based assay. Binding to the VS147 Ab was retained for the 54A/62A/63A triple mutant with no significant difference in binding to WT VISTA in any concentration tested (FIG. 1B). In contrast, the three mutations significantly diminish binding to VSTB at every concentration tested (>95% decrease compared to WT VISTA). The R54A, F62A, and Q63A mutations therefore abrogate binding to VSTB but do not alter the VISTA structure significantly to abolish binding to the VS147 antibody. A binding assay was then performed between VSIG3 ligand and the triple mutant or WT VISTA (FIG. 11C). The triple mutant showed a significant decrease in affinity for VSIG3, indicating that VISTA binding to VSIG3 is highly dependent on three of the same mutations that comprise the VSTB binding epitope. To further confirm the shared epitope, WT VISTA was pre-complexed with varying concentrations of VSTB and measured binding to VSIG3 (FIG. 11D). Dose-response disruption of VSIG3 binding is evident, with concentrations above 500 nM VSTB completely abolishing the VISTA/VSIG3 interaction. The VSIG3 binding signal of the 54A/62A/63A mutant and pre-complexed VISTA/VSTB is significantly lower than WT VISTA and pre-complexed VISTA/isotype control (FIG. 11E). This analysis suggests that the mapped VISTA epitope is not only important for interaction with an antibody that has been shown to

inhibit VISTA signaling, but also drives binding to VSIG3, a known functional partner of VISTA.

[00425] Wild-type (WT) VISTA and a VISTA triple mutant containing the R54A, F62A, and Q63A mutations were solubly expressed in mammalian cells. To confirm structural integrity of the triple mutant, the VS147 antibody was first tested for binding to WT VISTA or the 54A/62A/63A triple mutant using an ELISA based assay. Binding to the VS147 Ab was retained for the 54A/62A/63A triple mutant with no significant difference in binding from WT VISTA in any concentration tested (Figure 11A). In contrast, the three mutations significantly diminish binding to VSTB at every concentration tested (>95% decrease compared to WT VISTA). The R54A, F62A, and Q63A mutations therefore abrogate binding to VSTB but do not alter the VISTA structure significantly to abolish binding to the VS147 antibody. A binding assay was then performed between VSIG3 ligand and the triple mutant or WT VISTA (Figure 11B). WT VISTA bound to VSIG3 with an apparent K_d of $\sim 2 \mu\text{M}$ while the triple mutant bound with a significantly weaker apparent K_d of $>20 \mu\text{M}$, indicating that VISTA binding to VSIG3 is highly dependent on three of the same mutations that comprise the VSTB binding epitope. To further confirm the shared epitope, WT VISTA was precomplexed with varying concentrations of VSTB and measured binding to VSIG3 (Figure 11C). Dose-response disruption of VSIG3 binding is evident, with concentrations above 500 nM VSTB completely abolishing the VISTA/VSIG3 interaction. The 54A/62A/63A VISTA mutant and precomplexed VISTA/VSTB have significantly lower VSIG3 binding than WT VISTA or pre-complexed VISTA/isotype control (Figure 4D). This analysis suggests that the mapped VISTA epitope is not only important for interaction with an antibody that has been shown to inhibit VISTA signaling, but also drives binding to VSIG3, a known functional partner of VISTA.

Discussion

[00426] In this example, the structure of VISTA was determined at a high resolution using multiple protein deglycosylation strategies and a combinatorial MR-Rosetta pipeline to solve the final structure. A combination of genetic mutations (N $\rightarrow$ Q) in the VISTA sequence, a glycosylation inhibitor added to the mammalian cell culture media, and enzymatic deglycosylation post-purification was required to produce VISTA at its expected molecular mass. Previous crystallization attempts from our group using wild-type VISTA or Endo Hf-treated VISTA without genetic mutations were not successful, highlighting the importance of these steps for crystal growth. Additionally, solving the structure required the structural

modeling efforts via Rosetta and an iterative pipeline of structure refinement and molecular replacement. In particular, the loop and helix regions that do not fit the classic Ig-like fold could not be solved with molecular replacement alone and required a Rosetta-based design approach. We foresee the strategy described here and the deposited VISTA coordinates assisting future efforts in solving difficult Ig-like protein structures.

[00427] A combinatorial strategy for fine-epitope mapping was used to isolate a shared epitope important for binding to VSTB, an anti-VISTA antibody of therapeutic interest, and this information in turn was used to determine a proposed overlapping epitope for the VSIG3 ligand. Previously, hydrogen-deuterium exchange was used to highlight a number of potential binding hotspots of VSTB to VISTA¹⁵. In this example, detailed information is provided on this important region of interaction and evidence corroborating the relevance of this epitope for VISTA function is also provided. We recognize that the yeast display-based epitope mapping approach used here may not elucidate every region involved in binding, and can be limited or confounded by residues that drive allosteric changes, residues whose glycosylation patterns differ among yeast and mammals, residues that affect VISTA expression but are part of the VSTB binding interface, or residues that are shared between the VSTB and VS147 epitopes. In this work, several factors provide confidence that the described approach has identified the critical binding epitope driving the VISTA/VSTB interaction. WT VISTA and the N->Q VISTA variant bind to VSTB with similar affinities which minimizes the impact of differential glycosylation of yeast-surface displayed proteins. In addition, the three epitope residues identified form a solvent exposed, contiguous surface. Each isolated residue when mutated to alanine disrupts VSTB binding on yeast while the three mutations together completely abrogate VSTB binding as measured by ELISA. However, because these mutations appeared with the highest frequency in our combinatorial library screen and the fact that each isolated residue mutated to alanine disrupts binding, the likelihood is high that this is the critical binding epitope that drives VISTA/VSTB interaction.

[00428] VSIG3 was only recently discovered by ELISA-based binding screens as a cognate binding partner for VISTA expressed on T cells⁵. Here, the ELISA-based binding of VSIG3 to VISTA was confirmed and it was shown that the antagonist antibody VSTB blocks this specific interaction. Although there may be other VISTA binding partners and inhibitory antibodies may partially function through induced allosteric change of VISTA, it was hypothesized that the inhibitory function of the VSTB antibody is at least partly due to its blocking of the

VSIG3/VISTA binding, analogous to anti-PD-1 antibodies blocking the PD-1/PD-L1 signaling axis³⁴. Future experiments using antibodies that bind VISTA but do not block VISTA/VSIG3 binding will be needed to confirm the importance of this signaling axis for anti-VISTA immunotherapy. Here it was demonstrated that the three isolated VISTA residues (R54A, F62A, and Q63A) at least partially drive VSIG3 binding but recognize that additional information would be necessary to completely define this interaction. Fine epitope mapping of the VSIG3/VISTA interaction using a yeast-display method could not be performed due to the lack of a robust binding signal detected on yeast, likely due to its weak affinity. In addition, attempts to crystallize the VSIG3/VISTA co-complex were unsuccessful.

[00429] Comparing the structure of VISTA to PD-L1 reveals important qualities that diverge from the B7 protein family. Previously, sequence alignment of VISTA and PD-L1 highlighted a set of two conserved cysteines (C22/C114) that form the characteristic disulfide bond found in Ig-like proteins¹. Examining the crystal structure of VISTA reveals two extra disulfide bonds that could not be confirmed with sequence analysis alone. Sequence analysis also isolated an unaligned region between strands C and C' (Figure 15), but structural data was needed to represent this as a solvent-exposed, flexible loop and helix. This extended C-C' loop is unique among the B7 family, but shares homology with the inhibitory receptor IREM-1. The non-canonical and conserved C51/C113 disulfide bond is unique to the VISTA structure and likely stabilizes the extension of this C-C' loop outward from the beta sandwich core. The protrusion of this loop could play a role in promoting dimerization with another VISTA molecule, as in the case of growth factor receptor dimerization such as that observed with EGFR (Ogiso et al., 2002). The loop extends outwards from the beta-sandwich core and could play a role in promoting dimerization with another VISTA molecule, as in the case of growth factor receptor dimerization such as that observed with EGFR³⁵. Alternatively, the loop could disrupt VISTA dimerization by preventing intermolecular interactions between Ig-like domains such as those found in PD-L1 dimers^{36,37}. Further work via targeted deletion of the region and downstream functional analysis is needed to elucidate the role of the C-C' loop in VISTA signaling. Additionally, VISTA contains a singular IgV-like domain while all other B7 family members contain both an IgV and an IgC domain. The B7 family members B7-1 (CD80), B7-2 (CD86), B7-DC (PD-L2), B7-H1 (PD-L1), and B7-H3 (CD276) are all dual-domain proteins and all function primarily as ligands. In contrast, the cognate receptors of these proteins including CD28, CTLA-4, ICOS, and PD-1 all have single IgV domain structures. Based on domain composition, VISTA appears to be more similar in architecture with the receptors rather than the

B7 family ligands. Even though VISTA has shown functionality as a ligand in T cell proliferation assays² and as a soluble Fc-fusion drug for autoimmune disease³⁸, its structural composition as a single IgV domain and its binding interaction with VSIG3 point to its functionality as a receptor. Corroborating its functionality as a receptor on T cells, VISTA knockout T cells (Vsig3^{-/-}) have been shown to proliferate more than wild-type cells in response to antigen-presenting cells *in vitro*³, and an agonistic anti-VISTA antibody (MH5A) reduced the allogeneic T cell response in a murine model of graft-versus-host disease⁴.

[00430] Mouse and human VISTA share 70.4% sequence identity but have important structural differences. Through sequence analysis alone, the two proteins were predicted to have very similar folds due to conserved cysteines as well as a lack of significant gaps in the alignment¹. Structural comparisons between human VISTA and a Rosetta-based homology model of mouse VISTA reveal critical structural differences in the fragments surrounding the VISTA epitope. We propose that the lack of a beta strand at residues 67-70 and the differences in the epitope helix (FQDL → LQHL) cause side chain orientation changes that directly prevent VSTB from being cross-reactive with murine VISTA. The differences in this critical region suggest that inhibitor drugs binding to the mapped epitope will be cross-reactive between human and cyno VISTA, which exhibit a high degree of similarity, but will likely not bind to mouse VISTA.

[00431] Knowledge of the three-dimensional structure of VISTA and residues that comprise its binding epitope can help guide future drug development by enabling small molecule library screening through computer-aided drug design (CADD)³⁹ and computational antibody screens through antibody-antigen docking^{40,41}. Additionally, the high resolution structure can support future studies of receptor or ligand interactions through computational docking experiments. The coordinates for the VISTA ECD provided here will also expedite co-crystallization efforts of VISTA complexes by providing a well-suited template for molecular replacement. The initial success of checkpoint inhibitors in the clinic has provided a blueprint for new drugs that release the breaks on the immune system. VISTA inhibitors have the potential to provide an orthogonal method of T cell stimulation and anti-tumor activity by directly affecting the APC/T cell signaling axis. the high resolution crystal structure of VISTA presented here will bolster these efforts by encouraging further VISTA-related research and by directly assisting drug development endeavors.

Materials and Methods

Preparation of recombinant VISTA protein

[00432] The human VISTA extracellular domain (ECD) sequence with native signal peptide (Met1-Ala194, UniProt) was ordered as a gblock Gene Fragment (IDT) and cloned into the cytomegalovirus-driven adenoviral shuttle vector pAdd2 using standard Gibson cloning at EcoRI/XhoI vector cut sites. Protein was expressed in Expi293 cells according to the manufacturer's protocol, and proteins were purified from culture supernatant using nickel affinity chromatography. A hVISTA triple mutant (R54A, F62A, Q63A) used for epitope binding verification was produced in a similar manner. For crystallization, an asparagine triple mutant (N59Q, N76Q, N158Q) was cloned into the pAdd2 expression plasmid as described above and expressed in Expi293 cells in the presence of 10 μ M Kifunensine (Cayman Chemical, 109944-15-2). After nickel affinity chromatography, N-linked glycans were removed using endoglycosidase H (Endo Hf, New England BioLabs, P0703). De-glycosylated VISTA protein was separated from Endo Hf via additional nickel affinity chromatography. Residues are numbered starting after the signal peptide (Phe1, Lys2, Val3).

Crystallization and data collection

[00433] VISTA ECD protein was concentrated to 8 mg/mL and buffer exchanged into 50 mM HEPES (pH 8.2), 50 mM NaCl for crystallization trials. Initial crystals were grown at 12° C by mixing the protein solution with equal volume of reservoir solution (0.2 M NaBr and 20% PEG 3350). The diffraction analysis showed poor multiple diffraction spots to around 4Å. Fine tuning attempts using various additives and detergents did not improve the crystal morphology. Since crystal morphology at 12 °C and 20 °C were similar, further optimization attempts were performed at 20 °C. A grid search using various buffers identified HAT (made by mixing equal volumes of 1M Tris (pH 8.0), 1M HEPES (pH 7.5), and 1M ADA (pH 6.5)) as the optimal buffer for crystal formation. Seeding protocols with 1:1000 diluted crystal seeds introduced to the drop after two days gave small single crystals. A grid search by varying concentration of PEG and NaBr and also varying the drop ratio generated the best crystals with a well solution containing 75 mM NaBr, 18% PEG 3350, and 50 mM HAT buffer. The drop ratio for the best crystals was 0.8 μ L of protein and 0.6 μ L of well solution.

[00434] The crystals were flash cooled by dipping in a well solution containing 32% PEG 3350. Diffraction data sets were collected at 100° K via Stanford Synchrotron Radiation Lightsource (SSRL) beamline 12-2 at a wavelength of 0.98 Å using PILATUS 6M detector.

Data were indexed and integrated using the XDS package¹⁶. The crystals belong to space group P2₁ and contain one monomer per asymmetric unit. The best crystals diffracted to around 1.7 Å and the final data (480 degrees) is processed to 1.85 Å resolution. In addition, low dose and highly redundant sulfur SAD data were collected at a wavelength of 1.55 Å by using the 5 degree inverse beam geometry (total 4670 degrees of data). The crystallographic data are summarized in Table 1.

Structure determination and refinement

[00435] The human VISTA ECD sequence was submitted to the GREMLIN server (gremlin.bakerlab.org) for co-evolution contact predictions and a list of top homology models. Ten thousand decoy structures of hVISTA were generated using Rosetta homology modelling (RosettaCM) with the top ten VISTA homologs obtained from the GREMLIN search as templates. The initial screen running Phaser¹⁷ for possible molecular replacement solutions was conducted on the 10,000 structures generated from the RosettaCM run, but failed to produce definitive hits. Model convergence of the top 100 scoring RosettaCM solutions was analyzed to produce a partial consensus model (88 amino acids). An MR solution was obtained using the partial model. The model generated by MR search and Phenix¹⁸ building resulted in a reliable structure with Rwork and Rfree of 0.48 and 0.54, respectively. Various building attempts with Phenix, Phenix-rosetta, RosettaRemodel (for generating templates for VISTA specific disulfides), and Buccaneer¹⁹ programs brought the Rwork and Rfree to 0.37 and 0.41, respectively. Then, Phenix autobuilding by incorporating the sulfur SAD data, Buccaneer building, and extensive manual building resulted in Rwork and Rfree of 0.26 and 0.31, respectively. Further refinements were done by using Phenix and manual model building. The final model includes one protein monomer, two NAG molecules, and 41 water molecules in the asymmetric unit. No electron density was observed for the residues 29 – 31, and for the C-terminal residues starting from residue 152. The refinement converged to a final Rwork and Rfree of 0.18 and 0.22, respectively. There are no Ramachandran outliers and 98.6% of the residues are in the favored region. The refinement statistics are provided in Table 6. All crystal structure figures were created using PyMOL.

Table 6: Data collection and refinement statistics

VISTA	
Data collection	

Space group	P2 ₁
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	29.17, 64.09, 37.69
α , β , γ (°)	90.0, 91.7, 90.0
Resolution (Å)	40-1.85 (1.90-1.85) *
<i>R</i> _{sym} or <i>R</i> _{merge}	10.1 (156.2)
<i>I</i> / σ <i>I</i>	13.0 (1.5)
Completeness (%)	98.4 (96.2)
Redundancy	9.2 (8.4)
Refinement	
Resolution (Å)	40-1.85
No. reflections	11694
<i>R</i> _{work} / <i>R</i> _{free}	0.18/0.22
No. atoms	
Protein	1219
Ligand/ion	0
Water	41
<i>B</i> -factors (Å ²)	
Protein	41.2
Ligand/ion	
Water	43.3
R.m.s. deviations	
Bond lengths (Å)	0.006
Bond angles (°)	1.002

*Values in parentheses are for highest-resolution shell.

Homology modeling, structural comparisons, and docking

[00436] Structural models of murine VISTA ECD (Phe33-Ala191, Uniprot), cynomolgous monkey (cyno) VISTA ECD (NCBI# XP_005565644, Phe33-Ala194), and human VSIG3 ECD (Lys23-Gly241, Uniprot) were made using RosettaCM homology modeling as described previously²⁰. A single template of human VISTA was used for mouse and cyno VISTA modeling and an eight template set was used for VSIG3 modeling (PDB IDs: 1NBQ_B, 3JZ7_A, 3R4D_A, 4Z18_A, 5JHD_A, 5TEZ_I, 6CPH_D, 6EH4_D). 10,000 decoy structures of mouse VISTA, cyno VISTA and human VSIG3 were generated. The top 100 scores (lowest free energy) for each target were sorted using the standard Rosetta ‘cluster’ application and the top score of the largest cluster was used for downstream structural comparisons.

[00437] Structural alignments between VISTA species were performed using both the pymol ‘super’ and ‘align’ commands. Structural comparisons between the B7 family proteins and

human VISTA were performed using the ‘all against all’ option in the online DALI server²¹ (<http://ekhidna2.biocenter.helsinki.fi/dali/>). The PDB IDs for the B7 family structures used in the comparison were CD80: 1DR9, CD86: 5YXK, PD-L2: 3BP5, PD-L1: 4Z18, CD276: 410K.

[00438] Docking of VISTA and VSIG3 was performed using RosettaDock, as described previously²². First, global docking of the top VSIG3 homology model and the VISTA crystal structure was performed to generate 100,000 conformations and the lowest interface score from the broadest cluster was isolated. The isolated conformation was improved using local docking (10,000 decoy conformations). The top 100 structures by interface energy were clustered. The structure with the lowest interface energy of the largest cluster was chosen as the proposed VISTA/VSIG3 conformation. An energy vs. RMSD plot of the proposed conformation versus the 10,000 decoy conformations generated during local docking was generated using python’s Matplotlib package.

[00439] Structural alignments between VISTA species were performed using both the pymol ‘super’ and ‘align’ commands. Structural comparisons between the B7 family proteins and human VISTA were performed using the Tm-align online server (Zhang and Skolnick, 2005) (<https://zhanglab.ccmb.med.umich.edu/TMalign/>). The PDB IDs for the B7 family structures used in the comparison were CD80: 1DR9, CD86: 5YXK, PD-L2: 3BP5, PD-L1: 4Z18, CD276: 410K.

[00440] Electrostatic surfaces were created using the ‘APBS Electrostatics’ plugin in PyMOL (Version 2.2.3). The program uses the pdb2pqr method (Dolinsky et al., 2007) to add hydrogens, missing atoms, and partial charges. The surface potential is then calculated by approximating the solution at each atom using the Poisson-Boltzmann equation (Jurrus et al., 2018). The charges are represented as a color spectrum where the scale is -5.00 to +5.00 kBT/e. For solvent accessibility calculations, each residue in question was manually moved out to the solvent using Coot and then the PISA server (<https://www.ebi.ac.uk/pdbe/pisa/>) was used to calculate the maximum accessible surface area. Then, the surface area of each residue as part of the structure was calculated and divided by the maximum to compute percent accessible.

Epitope mapping via library generation and screening

Creation of a yeast-displayed human VISTA library

[00441] DNA encoding the human VISTA ECD amino acids (Phe33-Ala194, Uniprot), was cloned into the pCT yeast display plasmid^{23,24} using standard Gibson cloning. An error prone library was created using WT hVISTA as a template, and mutations were introduced using low-fidelity Taq polymerase (Invitrogen) and nucleotide analogs 8-oxo-dGTP and dPTP (TriLink Biotech) as described previously^{25,26}. Three different PCR reactions of 15 cycles were performed with 1.25, 1.5, and 1.75 μ M of dNTP analogs. The 1.75 μ M library was found to have the highest percentage of single amino acid mutations. This library was amplified and purified using gel electrophoresis. Empty pCT vector was cut using NheI and BamHI restriction sites. The amplified insert and cut vector were electroporated in a 5:1 DNA weight ratio into EBY100 yeast, where they were assembled *in vivo* through homologous recombination. Library size was determined to be 3.6×10^8 by dilution plating.

VSTB and VS147 antibodies

[00442] The VSTB antibody used for screening was derived from the Janssen VSTB174 sequence¹⁵. The VSTB174 heavy chain variable domain was paired with the hIgG1 constant domain (Ala1-Lys330, Uniprot P01857) and paired the VSTB174 light chain variable domain with the human kappa light chain constant domain (Arg1-Cys107, Uniprot P01834). Heavy chain and light chain were individually cloned into the pAdd2 expression vector using standard Gibson cloning. The positive control ‘VS147’ antibody variable domains were paired with murine IgG2a constant domains (HC: NCBI# AAA37906, kappa LC: NCBI# BAB33404). Both antibodies were expressed in Expi293 cells with a 1:1 weight ratio of heavy chain: light chain DNA using the manufacturer’s protocol. Antibodies were purified from the supernatant using protein A affinity chromatography.

[00443] The VSTB antibody used for screening was derived from the Janssen Pharmaceuticals VSTB112 sequence (Snyder et al., 2014). We paired the VSTB112 heavy chain variable domain with the hIgG1 constant domain (Ala1-Lys330, Uniprot P01857) and paired the VSTB112 light chain variable domain with the human kappa light chain constant domain (Arg1-Cys107, Uniprot P01834). Heavy chain and light chain were individually cloned into the pAdd2 expression vector using standard Gibson cloning. VSTB was expressed in Expi293 cells with a 1:1 weight ratio of heavy chain: light chain DNA using the manufacturer’s protocol. Antibody were purified from the supernatant using protein A affinity chromatography. The positive control (‘VS147’) antibody was provided by xCella Biosciences. The VS147 antibody was tested for linear versus conformational binding to VISTA using heat denaturation on the surface

of yeast. Yeast cells displaying human VISTA were incubated at room temperature or 80 °C (in a thermocycler) for 30 min and then chilled on ice for 20 min. The samples were incubated with 2 nM VS147 antibody in PBS + 0.1% BSA for 5 hrs at 4 °C to reach equilibrium. Yeast were washed and incubated with 1:5000 chicken anti-c-myc for 30 min at RT, followed by washing and staining for 20 min at 4 °C with 1:250 anti-chicken 647 (abcam ab150171) for VISTA expression and 1:250 anti-mouse-488 (ThermoFisher A11059) for antibody binding. Yeast were analyzed by flow cytometry for quantitative measurements of binding and expression.

Yeast library screening

[00444] Yeast displaying hVISTA mutants that lost binding to VSTB but retained binding to VS147 were isolated from the library using fluorescence-activated cell sorting (FACS). Equilibrium sort rounds were performed in which yeast were incubated at 4 °C for 12 hr in phosphate-buffered saline (PBS) containing 1 mg/mL BSA with the following concentrations of antibody: Sort 1, 10 nM VSTB; Sort 2, 75 nM VSTB; Sort 3, 50 nM VS147; Sort 4, 200 nM VSTB; Sort 5, 5 nM VS147. After incubation with antibody, yeast were pelleted, washed, and resuspended in PBS+BSA with a 1:5000 dilution of chicken anti-c-Myc (Invitrogen, A21281) for 30 mins at 4 °C. Yeast were then washed and pelleted, and labeled on ice with 1:250 dilution of secondary antibodies for binding (anti-mouse 488, ThermoFisher A11059 or anti-human 647, ThermoFisher A21445) and expression (anti-chicken 647, abcam ab150171 or anti-chicken 488, ThermoFisher A11039). Labeled yeast were sorted by FACS using a BD Aria sorter (Stanford FACS Core Facility). Negative sort gates for sorts 1, 2, 4 and positive sort gates for sorts 3, 5 were drawn to isolate populations with desired binding characteristics. Following FACS sort 5, plasmid DNA was recovered from yeast using a Zymoprep kit (Zymo Research Corp), transformed into DH10b electrocompetent E. coli, and isolated using a GeneJET plasmid miniprep kit (ThermoFisher, K0503). Sequencing was performed by MCLAB (Molecular Cloning Laboratories).

[00445] Yeast displaying hVISTA mutants that lost binding to VSTB but retained binding to VS147 were isolated from the library using fluorescence-activated cell sorting (FACS). An alternating positive and negative sort strategy, as opposed to three-color simultaneous sorting, was used because binding of VS147 decreased maximal binding of VSTB (Figure 13B), and therefore simultaneous incubation with both antibodies would not provide an accurate depiction of VSTB binding levels. Equilibrium sort rounds were performed in which yeast were incubated at 4 °C for 12 hr in phosphate-buffered saline (PBS) containing 0.1% mg/mL BSA with the

following concentrations of antibody: Sort 1, 10 nM VSTB; Sort 2, 75 nM VSTB; Sort 3, 50 nM VS147; Sort 4, 200 nM VSTB; Sort 5, 5 nM VS147. After incubation with antibody, yeast were pelleted, washed, and resuspended in PBS+BSA with a 1:5000 dilution of chicken anti-c-Myc (Invitrogen, A21281) for 30 mins at 4 °C. Yeast were then washed and pelleted, and labeled on ice with 1:250 dilution of secondary antibodies for binding (anti-mouse 488, ThermoFisher A11059 or anti-human 647, ThermoFisher A21445) and expression (anti-chicken 647, abcam ab150171 or anti-chicken 488, ThermoFisher A11039). Labeled yeast were sorted by FACS using a BD Aria sorter (Stanford FACS Core Facility). Negative sort gates for sorts 1, 2, 4 and positive sort gates for sorts 3, 5 were drawn to isolate populations with desired binding characteristics. Following FACS sort 5, plasmid DNA was recovered from yeast using a Zymoprep kit (Zymo Research Corp), transformed into DH10b electrocompetent E. coli, and isolated using a GeneJET plasmid miniprep kit (ThermoFisher, K0503). Sequencing was performed by MCLAB (Molecular Cloning Laboratories).

Binding assays

[00446] Single alanine mutants of human VISTA (R54A, R58A, F62A, Q63A, or S124A) were generated using site-directed mutagenesis according to a standard two-stage QuikChange PCR protocol²⁷. PCR fragments were cloned into the pCT yeast surface display vector and individually transformed into EBY100 yeast. The genes for WT human VISTA (33-194, Uniprot) and mouse VISTA (33-191, Uniprot) were also cloned into pCT and transformed into yeast as described above. Binding assays were performed by mixing surface-displayed VISTA on yeast (~50,000 molecules/cell)²⁸ with a titration of target antibody concentrations (VSTB or VS147) in individual eppendorf tubes. Binding reactions were incubated at 4 °C for 12 hr to allow interactions to reach equilibrium. Yeast were labeled with the same reagents using protocols as described for library sorts and analyzed by flow cytometry on a BD Accuri. Binding populations were gated using FlowJo software and geometric means of fluorescence were plotted against concentration and fit to a one-site specific binding curve on GraphPad Prism. Error bars represent standard deviation of the mean for duplicate measurements. Yeast-based affinity measurements are marked as ‘apparent K_d’ due to the limitations of the assay including the avidity effects of displaying numerous copies of the target protein and the deviation from true equilibrium due to multiple washing and labeling steps

[00447] For enzyme-linked immunosorbent assays (ELISAs), recombinant proteins were immobilized on a 96-well flat bottom plate (Corning, CLS3595) by incubation at 4 °C for 12-16

hr. VSIG3 was coated at 15 µg/mL and VSTB and VS147 antibodies were coated at 2 µg/mL in PBS. Wells were washed with PBS + 1% Tween-20 and then blocked with PBS + 2.5% milk powder + 2.5% BSA at room temperature for 2 hr. Soluble His-tagged VISTA protein (WT or Ala triple variant) was added at varying concentrations in PBS + 0.1% BSA + 0.1% Tween-20 and the plate was incubated at room temperature for 2 hr. For VSTB pre-complexed experiments, 1 µM of VISTA was incubated with 1 nM – 5000 nM of VSTB in individual eppendorf tubes overnight and then added to VSIG3-coated ELISA plates. Binding of VISTA was detected indirectly by first adding 1:750 rabbit anti-6-HIS (Bethyl, A190-114F) and then adding 1:7500 anti-rabbit-HRP (Novus Biologicals, NB7160). Substrate solution (1-Step Ultra TMB, ThermoFisher, 34028) was added, reaction was stopped after 15 min with 2M sulfuric acid, and absorbance at 450 nM was read on a microplate reader (Synergy H4, BioTek). Absorbance values of control wells with no coated protein were subtracted from sample wells and corrected values were plotted against VISTA concentration and fit to a one-site specific binding curve on GraphPad Prism. Error bars represent standard deviation of the mean for triplicate measurements. ELISA-based affinity measurements are marked as ‘apparent K_d’ due to limitations of the assay including the deviation from true equilibrium due to multiple washing and detection steps.

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[00448] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing

description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

[00449] The examples set forth above are provided to give those of ordinary skill in the art a complete disclosure and description of how to make and use the embodiments of the compositions, systems and methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Modifications of the above-described modes for carrying out the invention that are obvious to persons of skill in the art are intended to be within the scope of the following claims. All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

[00450] All headings and section designations are used for clarity and reference purposes only and are not to be considered limiting in any way. For example, those of skill in the art will appreciate the usefulness of combining various aspects from different headings and sections as appropriate according to the spirit and scope of the invention described herein.

[00451] All references cited herein are hereby incorporated by reference herein in their entireties and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[00452] Many modifications and variations of this application can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments and examples described herein are offered by way of example only, and the application is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which the claims are entitled.

WHAT IS CLAIMED IS:

1. A method of modulating an immune response in a subject, the method comprising administering to the subject an effective amount of an anti-VISTA antibody comprising:
 - a) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:5;
 - b) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:9 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:13; or
 - c) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:17 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:21.
2. A method of modulating an immune response in a subject, the method comprising administering to the subject an effective amount of an anti-VISTA antibody comprising:
 - a) a vhCDR1 comprising SEQ ID NO:2, a vhCDR2 comprising SEQ ID NO:3, a vhCDR3 comprising SEQ ID NO:4, a vlCDR1 comprising SEQ ID NO:6, a vlCDR2 comprising SEQ ID NO:7, and a vlCDR3 comprising SEQ ID NO:8;
 - b) a vhCDR1 comprising SEQ ID NO:10, a vhCDR2 comprising SEQ ID NO:11, a vhCDR3 comprising SEQ ID NO:12, a vlCDR1 comprising SEQ ID NO:14, a vlCDR2 comprising SEQ ID NO:15, and a vlCDR3 comprising SEQ ID NO:16; or
 - c) a vhCDR1 comprising SEQ ID NO:18, a vhCDR2 comprising SEQ ID NO:19, a vhCDR3 comprising SEQ ID NO:20, a vlCDR1 comprising SEQ ID NO:22, a vlCDR2 comprising SEQ ID NO:23, and a vlCDR3 comprising SEQ ID NO:24.
3. A method of modulating an immune response in a subject, the method comprising administering to the subject an effective amount of an anti-VISTA antibody comprising a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.
4. A method of modulating an immune response in a subject, the method comprising administering to the subject an effective amount of an anti-VISTA antibody comprising a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

5. The method according to any one of claims 1-4, wherein the antibody comprises a constant region with an amino acid sequence at least 90% identical to a human IgG.
6. The method according to claim 5, wherein the human IgG is selected from the group consisting of IgG1, IgG2, IgG3 and IgG4.
7. The method according to claim 6, wherein the IgG is an IgG4.
8. The method according to any one of claims 1-4, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:5; and/or a vhCDR1 comprising SEQ ID NO:2, a vhCDR2 comprising SEQ ID NO:3, a vhCDR3 comprising SEQ ID NO:4, a vlCDR1 comprising SEQ ID NO:6, a vlCDR2 comprising SEQ ID NO:7, and a vlCDR3 comprising SEQ ID NO:8.
9. The method according to any one of claims 1-4, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:9 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:13; and/or a vhCDR1 comprising SEQ ID NO:10, a vhCDR2 comprising SEQ ID NO:11, a vhCDR3 comprising SEQ ID NO:12, a vlCDR1 comprising SEQ ID NO:14, a vlCDR2 comprising SEQ ID NO:15, and a vlCDR3 comprising SEQ ID NO:16.
10. The method according to any one of claims 1-4, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:17 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:21; and/or a vhCDR1 comprising SEQ ID NO:18, a vhCDR2 comprising SEQ ID NO:19, a vhCDR3 comprising SEQ ID NO:20, a vlCDR1 comprising SEQ ID NO:22, a vlCDR2 comprising SEQ ID NO:23, and a vlCDR3 comprising SEQ ID NO:24.
11. A method of treating cancer in a subject, the method comprising administering to the subject an effective amount of an anti-VISTA antibody comprising:
 - a) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:5;

b) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:9 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:13; or

c) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:17 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:21.

12. A method of treating cancer in a subject, the method comprising administering to the subject an effective amount of an anti-VISTA antibody comprising:

a) a vhCDR1 comprising SEQ ID NO:2, a vhCDR2 comprising SEQ ID NO:3, a vhCDR3 comprising SEQ ID NO:4, a vlCDR1 comprising SEQ ID NO:6, a vlCDR2 comprising SEQ ID NO:7, and a vlCDR3 comprising SEQ ID NO:8;

b) a vhCDR1 comprising SEQ ID NO:10, a vhCDR2 comprising SEQ ID NO:11, a vhCDR3 comprising SEQ ID NO:12, a vlCDR1 comprising SEQ ID NO:14, a vlCDR2 comprising SEQ ID NO:15, and a vlCDR3 comprising SEQ ID NO:16; or

c) a vhCDR1 comprising SEQ ID NO:18, a vhCDR2 comprising SEQ ID NO:19, a vhCDR3 comprising SEQ ID NO:20, a vlCDR1 comprising SEQ ID NO:22, a vlCDR2 comprising SEQ ID NO:23, and a vlCDR3 comprising SEQ ID NO:24.

13. The method according to any of claims 11 or 12, wherein the antibody comprises a constant region with an amino acid sequence at least 90% identical to a human IgG.

14. The method according to claim 13, wherein the human IgG is selected from the group consisting of IgG1, IgG2, IgG3 and IgG4.

15. The method according to claim 14, wherein the IgG is an IgG4.

16. The method according to any of claims 11 or 12, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:1 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:5; and/or a vhCDR1 comprising SEQ ID NO:2, a vhCDR2 comprising SEQ ID NO:3, a vhCDR3 comprising SEQ ID NO:4, a vlCDR1 comprising SEQ ID NO:6, a vlCDR2 comprising SEQ ID NO:7, and a vlCDR3 comprising SEQ ID NO:8.

17. The method according to any of claims 11 or 12, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:9 and a light chain

variable region comprising an amino acid sequence of SEQ ID NO:13; and/or a vhCDR1 comprising SEQ ID NO:10, a vhCDR2 comprising SEQ ID NO:11, a vhCDR3 comprising SEQ ID NO:12, a vlCDR1 comprising SEQ ID NO:14, a vlCDR2 comprising SEQ ID NO:15, and a vlCDR3 comprising SEQ ID NO:16.

18. The method according to any of claims 11 or 12, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:17 and a light chain variable region comprising an amino acid sequence of SEQ ID NO:21; and/or a vhCDR1 comprising SEQ ID NO:18, a vhCDR2 comprising SEQ ID NO:19, a vhCDR3 comprising SEQ ID NO:20, a vlCDR1 comprising SEQ ID NO:22, a vlCDR2 comprising SEQ ID NO:23, and a vlCDR3 comprising SEQ ID NO:24.

19. The method according to any one of claims 11 to 18, wherein the cancer expresses VISTA.

20. The method according to any one of claims 11 to 19, wherein the cancer is selected from the group consisting of colorectal cancer, breast cancer, rectal cancer, lung (including non-small cell lung cancer), non-Hodgkin's lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, Kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, melanoma, ovarian cancer, endometrial cancer, cervical cancer, colorectal cancer, mesothelioma, and multiple myeloma.

21. The method according to any one of claims 11 to 20, wherein the antibody is combined with one or more additional therapeutic agents to treat cancer.

22. The method according to claim 21, wherein the additional therapeutic agents are other immune checkpoint inhibitors.

23. The method of claim 22, wherein the other immune checkpoint inhibitors are selected from the group consisting of PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a TIM-3 inhibitor, and a LAG-3 inhibitor.

24. The method according to claim 21, wherein the additional therapeutic agents are tumor targeting antibodies.

25. The method according to claim 24, wherein the tumor targeting antibodies are selected from the group consisting of anti-CD20, anti-EGFR, and anti-Her2.

26. The method according to claim 24 or 25, wherein the tumor targeting antibodies are selected from the group consisting of trastuzumab, rituximab, and cetuximab.

27. The method according to claim 24, wherein the additional therapeutic agent is an integrin-binding polypeptide-Fc fusion.

28. The method according to claim 27, wherein the integrin-binding polypeptide-Fc fusion is NOD-201.

29. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 2; a heavy chain variable region CDR2 comprising SEQ ID NO: 3; a heavy chain variable region CDR3 comprising SEQ ID NO: 4; a light chain variable region CDR1 comprising SEQ ID NO: 6; a light chain variable region CDR2 comprising SEQ ID NO: 7; and a light chain variable region CDR3 comprising SEQ ID NO: 8.

30. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 10; a heavy chain variable region CDR2 comprising SEQ ID NO: 11; a heavy chain variable region CDR3 comprising SEQ ID NO: 12; a light chain variable region CDR1 comprising SEQ ID NO: 14; a light chain variable region CDR2 comprising SEQ ID NO: 15; and a light chain variable region CDR3 comprising SEQ ID NO: 16.

31. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in

an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 18; a heavy chain variable region CDR2 comprising SEQ ID NO: 19; a heavy chain variable region CDR3 comprising SEQ ID NO: 20; a light chain variable region CDR1 comprising SEQ ID NO: 22; a light chain variable region CDR2 comprising SEQ ID NO: 23; and a light chain variable region CDR3 comprising SEQ ID NO: 24.

32. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of response against an antigen, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 2; a heavy chain variable region CDR2 comprising SEQ ID NO: 3; a heavy chain variable region CDR3 comprising SEQ ID NO: 4; a light chain variable region CDR1 comprising SEQ ID NO: 6; a light chain variable region CDR2 comprising SEQ ID NO: 7; and a light chain variable region CDR3 comprising SEQ ID NO: 8.

33. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 10; a heavy chain variable region CDR2 comprising SEQ ID NO: 11; a heavy chain variable region CDR3 comprising SEQ ID NO: 12; a light chain variable region CDR1 comprising SEQ ID NO: 14; a light chain variable region CDR2 comprising SEQ ID NO: 15; and a light chain variable region CDR3 comprising SEQ ID NO: 16.

34. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 18; a heavy chain variable region CDR2 comprising SEQ ID NO: 19; a heavy chain variable region CDR3 comprising SEQ ID NO: 20; a light chain variable region CDR1 comprising SEQ ID NO: 22; a light chain variable region CDR2 comprising SEQ ID NO: 23; and a light chain variable region CDR3 comprising SEQ ID NO: 24.

35. A method of inhibiting growth of VISTA expressing cells comprising contacting the cells with a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit growth of VISTA expressing cells, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 2; a heavy chain variable region CDR2 comprising SEQ ID NO: 3; a heavy chain variable region CDR3 comprising SEQ ID NO: 4; a light chain variable region CDR1 comprising SEQ ID NO: 6; a light chain variable region CDR2 comprising SEQ ID NO: 7; and a light chain variable region CDR3 comprising SEQ ID NO: 8.

36. A method of inhibiting growth of VISTA expressing cells comprising contacting the cells with a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit growth of VISTA expressing cells, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 10; a heavy chain variable region CDR2 comprising SEQ ID NO: 11; a heavy chain variable region CDR3 comprising SEQ ID NO: 12; a light chain variable region CDR1 comprising SEQ ID NO: 14; a light chain variable region CDR2 comprising SEQ ID NO: 15; and a light chain variable region CDR3 comprising SEQ ID NO: 16.

37. A method of inhibiting growth of VISTA expressing cells comprising contacting the cells with a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit growth of VISTA expressing cells, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 18; a heavy chain variable region CDR2 comprising SEQ ID NO: 19; a heavy chain variable region CDR3 comprising SEQ ID NO: 20; a light chain variable region CDR1 comprising SEQ ID NO: 22; a light chain variable region CDR2 comprising SEQ ID NO: 23; and a light chain variable region CDR3 comprising SEQ ID NO: 24.

38. A method of inhibiting the binding of VISTA to VSIG3 on cells in a subject having a disorder by administering to the subject a monoclonal antibody which binds to human VISTA, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 2; a heavy chain variable region CDR2 comprising SEQ ID NO: 3; a heavy chain variable region CDR3 comprising SEQ ID NO: 4; a light chain variable region CDR1 comprising SEQ ID NO:

6; a light chain variable region CDR2 comprising SEQ ID NO: 7; and a light chain variable region CDR3 comprising SEQ ID NO: 8.

39. A method of inhibiting the binding of VISTA to VSIG3 on cells in a subject having a disorder by administering to the subject a monoclonal antibody which binds to human VISTA, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 10; a heavy chain variable region CDR2 comprising SEQ ID NO: 11; a heavy chain variable region CDR3 comprising SEQ ID NO: 12; a light chain variable region CDR1 comprising SEQ ID NO: 14; a light chain variable region CDR2 comprising SEQ ID NO: 15; and a light chain variable region CDR3 comprising SEQ ID NO: 16.

40. A method of inhibiting the binding of VISTA to VSIG3 on cells in a subject having a disorder by administering to the subject a monoclonal antibody which binds to human VISTA, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 18; a heavy chain variable region CDR2 comprising SEQ ID NO: 19; a heavy chain variable region CDR3 comprising SEQ ID NO: 20; a light chain variable region CDR1 comprising SEQ ID NO: 22; a light chain variable region CDR2 comprising SEQ ID NO: 23; and a light chain variable region CDR3 comprising SEQ ID NO: 24.

41. The method of any one of claims 29, 32, 35, or 38, wherein the antibody comprises a heavy chain variable region comprising SEQ ID NO:1.

42. The method of any one of claims 29, 32, 35, or 38, wherein the antibody comprises a light chain variable region comprising SEQ ID NO:5.

43. The method of any one of claims 30, 33, 36, or 39, wherein the antibody comprises a heavy chain variable region comprising SEQ ID NO:9.

44. The method of any one of claims 30, 33, 36, or 39, wherein the antibody comprises a light chain variable region comprising SEQ ID NO:13.

45. The method of any one of claims 31, 34, 37, or 40, wherein the antibody comprises a heavy chain variable region comprising SEQ ID NO:17.

46. The method of any one of claims 31, 34, 37, or 40, wherein the antibody comprises a light chain variable region comprising SEQ ID NO:21.

47. The method of any one of claims 29, 32, 35, or 38, wherein the antibody comprises a heavy chain variable region comprising SEQ ID NO:1 and a light chain variable region comprising SEQ ID NO:5.

48. The method of any one of claims 30, 33, 36, or 39, wherein the antibody comprises a heavy chain variable region comprising SEQ ID NO:9 and a light chain variable region comprising SEQ ID NO:13.

49. The method of any one of claims 31, 34, 37, or 40, wherein the antibody comprises a heavy chain variable region comprising SEQ ID NO:17 and a light chain variable region comprising SEQ ID NO:21.

50. The method of any one of claims 29-49 wherein the immune response is an antigen-specific T cell response.

51. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 1 and 5, respectively.

52. (A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 9 and 13, respectively.

53. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in

an amount effective to induce or enhance an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 17 and 21, respectively.

54. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 1 and 5, respectively.

55. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 9 and 13, respectively.

56. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 17 and 21, respectively.

57. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 1 and 5, respectively.

58. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 9 and 13, respectively.

59. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 17 and 21, respectively.

60. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 1 and 5, respectively.

61. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 9 and 13, respectively.

62. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising heavy and light chain variable region sequences as set forth in SEQ ID NO: 17 and 21, respectively.

63. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises heavy and light chain variable region sequences having at least 95% identity to SEQ ID NO: 1 and 5, respectively.

64. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises heavy and light chain variable region sequences having at least 95% identity to SEQ ID NO: 9 and 13, respectively.

65. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises heavy and light chain variable region sequences having at least 95% identity to SEQ ID NO: 17 and 21, respectively.

66. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody comprises heavy and light chain variable region sequences having at least 95% identity to SEQ ID NO: 1 and 5, respectively.

67. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody comprises heavy and light chain variable region sequences having at least 95% identity to SEQ ID NO: 9 and 13, respectively.

68. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody comprises heavy and light chain variable region sequences having at least 95% identity to SEQ ID NO: 17 and 21, respectively.

69. A method for inducing or enhancing an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount

effective to induce or enhance an immune response, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 2; a heavy chain variable region CDR2 comprising SEQ ID NO: 3; a heavy chain variable region CDR3 comprising SEQ ID NO: 4; a light chain variable region CDR1 comprising SEQ ID NO: 6; a light chain variable region CDR2 comprising SEQ ID NO: 7; and a light chain variable region CDR3 comprising SEQ ID NO: 8.

70. A method for inducing or enhancing an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 10; a heavy chain variable region CDR2 comprising SEQ ID NO: 11; a heavy chain variable region CDR3 comprising SEQ ID NO: 12; a light chain variable region CDR1 comprising SEQ ID NO: 14; a light chain variable region CDR2 comprising SEQ ID NO: 15; and a light chain variable region CDR3 comprising SEQ ID NO: 16.

71. A method for inducing or enhancing an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 18; a heavy chain variable region CDR2 comprising SEQ ID NO: 19; a heavy chain variable region CDR3 comprising SEQ ID NO: 20; a light chain variable region CDR1 comprising SEQ ID NO: 22; a light chain variable region CDR2 comprising SEQ ID NO: 23; and a light chain variable region CDR3 comprising SEQ ID NO: 24.

72. A method for inhibiting the suppression of an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 2; a heavy chain variable region CDR2 comprising SEQ ID NO: 3; a heavy chain variable region CDR3 comprising SEQ ID NO: 4; a light chain variable region CDR1 comprising SEQ ID NO: 6; a light chain variable region CDR2 comprising SEQ ID NO: 7; and a light chain variable region CDR3 comprising SEQ ID NO: 8.

73. A method for inhibiting the suppression of an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 10; a heavy chain variable region CDR2 comprising SEQ ID NO: 11; a heavy chain variable region CDR3 comprising SEQ ID NO: 12; a light chain variable region CDR1 comprising SEQ ID NO: 14; a light chain variable region CDR2 comprising SEQ ID NO: 15; and a light chain variable region CDR3 comprising SEQ ID NO: 16.

74. A method for inhibiting the suppression of an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response, wherein the antibody comprises a heavy chain variable region CDR1 comprising SEQ ID NO: 18; a heavy chain variable region CDR2 comprising SEQ ID NO: 19; a heavy chain variable region CDR3 comprising SEQ ID NO: 20; a light chain variable region CDR1 comprising SEQ ID NO: 22; a light chain variable region CDR2 comprising SEQ ID NO: 23; and a light chain variable region CDR3 comprising SEQ ID NO: 24.

75. The method according to any of the preceding claims, wherein the immune response is antigen-specific T cell response.

76. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

77. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

78. A method of inhibiting growth of VISTA expressing cells comprising contacting the cells with a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit growth of VISTA expressing cells, wherein the antibody comprises a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

79. A method of inhibiting growth of VISTA expressing cells comprising contacting the cells with a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit growth of VISTA expressing cells, wherein the antibody comprises a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

80. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

81. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody binds to the same epitope as an antibody comprising a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

82. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

83. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody competes for binding to human VISTA with an antibody comprising a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

84. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

85. A method for inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response against an antigen, wherein the antibody comprises a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

86. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody comprises a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

87. A method for inhibiting the suppression of an immune response against an antigen in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response against an antigen, wherein the antibody comprises a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

88. A method for inducing or enhancing an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response, wherein the antibody comprises a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

89. A method for inducing or enhancing an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to induce or enhance an immune response, wherein the antibody comprises a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

90. A method for inhibiting the suppression of an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response, wherein the antibody comprises a heavy chain variable region and a light chain variable region as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

91. A method for inhibiting the suppression of an immune response in a subject comprising administering to the subject a monoclonal antibody which binds to human VISTA, in an amount effective to inhibit the suppression of an immune response, wherein the antibody comprises a vhCDR1, a vhCDR2, a vhCDR3, a vlCDR1, a vlCDR2, and a vlCDR3 as provided in Figure 1 and/or Figure 28 and/or Figure 46 and/or Figure 47.

92. The method according to any of the preceding claims, wherein the immune response is antigen-specific T cell response.

Figure 1A
VS7 (anti-VISTA antibody)

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFESSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTAYMELSSLR SEDTAVYYCARPVRSGPDYFQHGWGGTLTVSS	1
vhCDR1	<u>GGTFESSY</u>	2
vhCDR2	<u>GGIPIFGT</u>	3
vhCDR3	<u>PVRSGPDYFQH</u>	4
Variable light (vl) domain	DIQMTQSPSTLSASVGDRTVITCRASQSISSWLAWYQQKPKGKAPKLLIYDASSLESGVPSRFGSGSGTEFTLTISLQPDDFATYYCQQ YNSYSLTFGGGTKVEIK	5
vlCDR1	<u>RASQSISSWLA</u>	6
vlCDR2	<u>DASSLES</u>	7
vlCDR3	<u>QQYNSYSLTF</u>	8

Figure 1B
VS147 (anti-VISTA antibody)

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSCKASGGIFSSYAISWVRQAPGQGLEWMGGIIPFGTANYAQKFQGRVTITADESTSTAYMELSSLRS EDTAVYYCARPVRSGPDYLQHWGQGTLVTVSS	9
vhCDR1	<u>GGIFSSY</u>	10
vhCDR2	<u>GGIIPFGT</u>	11
vhCDR3	<u>PVRSGPDYLQH</u>	12
Variable light (vl) domain	DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPKGKAPKLLIYDASSLESGVPSRFGSGSGTEFTLTISLQPDDFATYYCQQ <u>YNSYSLTFGGGTKVEIK</u>	13
vlCDR1	<u>RASQSISSWLA</u>	14
vlCDR2	<u>DASSLES</u>	15
vlCDR3	<u>QQYNSYSLTF</u>	16

Figure 1C
VS143 (anti-VISTA antibody)

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSCKAPGGIFSSYAISWVROAPGQGLEWMGGIIPFGTANYAQKFQGRVTITADESTSTAYMELSSLRS EDTAVVYCARPVRSGPDYFQHWGQGTLVTVSS	17
vhCDR1	<u>GGIFSSY</u>	18
vhCDR2	<u>GGIIPFGT</u>	19
vhCDR3	<u>PVRSGPDYFQH</u>	20
Variable light (vl) domain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLESGVPSRFGSGSGTEFTLTISSLQPDDFATYYCQQ YNSYSITEGGQTRLEIK	21
vlCDR1	<u>RASQSISSWLA</u>	22
vlCDR2	<u>DASSLES</u>	23
vlCDR3	<u>QQYNSYSITE</u>	24

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REPLACEMENT SHEET

Figure 2A

CH1																	
EU Index	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134
IgG1	A	S	T	K	G	P	S	V	F	P	L	A	P	S	S	K	S
IgG2	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S
IgG3	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S
IgG4	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S

EU Index	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155
IgG1	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T
IgG2	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T
IgG3	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T
IgG4	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T

EU Index	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176
IgG1	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S
IgG2	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S
IgG3	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S
IgG4	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S

EU Index	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197
IgG1	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	T
IgG2	S	L	S	S	V	V	T	V	P	S	S	N	F	G	T	Q	T
IgG3	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	T
IgG4	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	K	T

EU Index	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218
IgG1	V	N	H	K	P	S	N	T	K	V	D	K	K	V	E	P	K
IgG2	V	D	H	K	P	S	N	T	K	V	D	K	T	V	E	R	K
IgG3	V	N	H	K	P	S	N	T	K	V	D	K	F	V	E	L	K
IgG4	V	D	H	K	P	S	N	T	K	V	D	K	F	V	E	S	K

Hinge								Fc >							
EU Index	221				222	223	224	225	226	227	228				
IgG1	D				K	T	H	T	C	P	P				
IgG2					V			E	C	P	P				
IgG3	L	G	D	T	T	H	T	C	P	R	C	P	E	P	K
IgG4							P	P	C	P	S				

EU Index																	
IgG1																	
IgG2																	
IgG3	F	C	F	R	C	P	E	P	K	S	C	D	T	P	P	C	P
IgG4																	

								Fc >							
EU Index								229	230	231	232	233	234	235	236
IgG1								C	P	A	P	E	L	L	G
IgG2								C	P	A	P	R	Y	A	
IgG3	E	P	K	S	C	D	T	P	P	P	C	P	L	L	G
IgG4								C	P	A	P	E	F	L	G

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REPLACEMENT SHEET

Figure 2B

C382																														
EU Index	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257									
kgG1	G	P	S	V	F	L	F	F	P	K	P	K	D	T	L	M	I	S	R	T	P									
kgG2	G	P	S	V	F	L	F	F	P	K	P	K	D	T	L	M	I	S	R	T	P									
kgG3	G	P	S	V	F	L	F	F	P	K	P	K	D	T	L	M	I	S	R	T	P									
kgG4	G	P	S	V	F	L	F	F	P	K	P	K	D	T	L	M	I	S	R	T	P									
EU Index	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278									
kgG1	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	P	N	W	Y									
kgG2	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	P	N	W	Y									
kgG3	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	P	N	W	Y									
kgG4	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	P	N	W	Y									
EU Index	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299									
kgG1	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T									
kgG2	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T									
kgG3	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T									
kgG4	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T									
EU Index	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320									
kgG1	Y	R	V	V	S	V	L	I	V	L	H	G	D	W	L	N	G	K	E	Y	K									
kgG2	Y	R	V	V	S	V	L	I	V	L	H	G	D	W	L	N	G	K	E	Y	K									
kgG3	Y	R	V	V	S	V	L	I	V	L	H	G	D	W	L	N	G	K	E	Y	K									
kgG4	Y	R	V	V	S	V	L	I	V	L	H	G	D	W	L	N	G	K	E	Y	K									
EU Index	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340										
kgG1	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K										
kgG2	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K										
kgG3	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K										
kgG4	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K										
C383																														
EU Index	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361									
kgG1	G	D	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N									
kgG2	G	D	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N									
kgG3	G	D	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N									
kgG4	G	D	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N									
EU Index	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382									
kgG1	G	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E									
kgG2	G	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E									
kgG3	G	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E									
kgG4	G	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E									
EU Index	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403									
kgG1	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S									
kgG2	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S									
kgG3	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S									
kgG4	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S									
EU Index	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424									
kgG1	F	F	L	V	S	K	L	I	V	D	K	S	R	W	Q	G	N	V	F	S										
kgG2	F	F	L	V	S	K	L	I	V	D	K	S	R	W	Q	G	N	V	F	S										
kgG3	F	F	L	V	S	K	L	I	V	D	K	S	R	W	Q	G	N	V	F	S										
kgG4	F	F	L	V	S	K	L	I	V	D	K	S	R	W	Q	G	N	V	F	S										
EU Index	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445									
kgG1	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P									
kgG2	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P									
kgG3	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P									
kgG4	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P									
EU Index	446	447																												
kgG1	G	K																												
kgG2	G	K																												
kgG3	G	K																												
kgG4	G	K																												

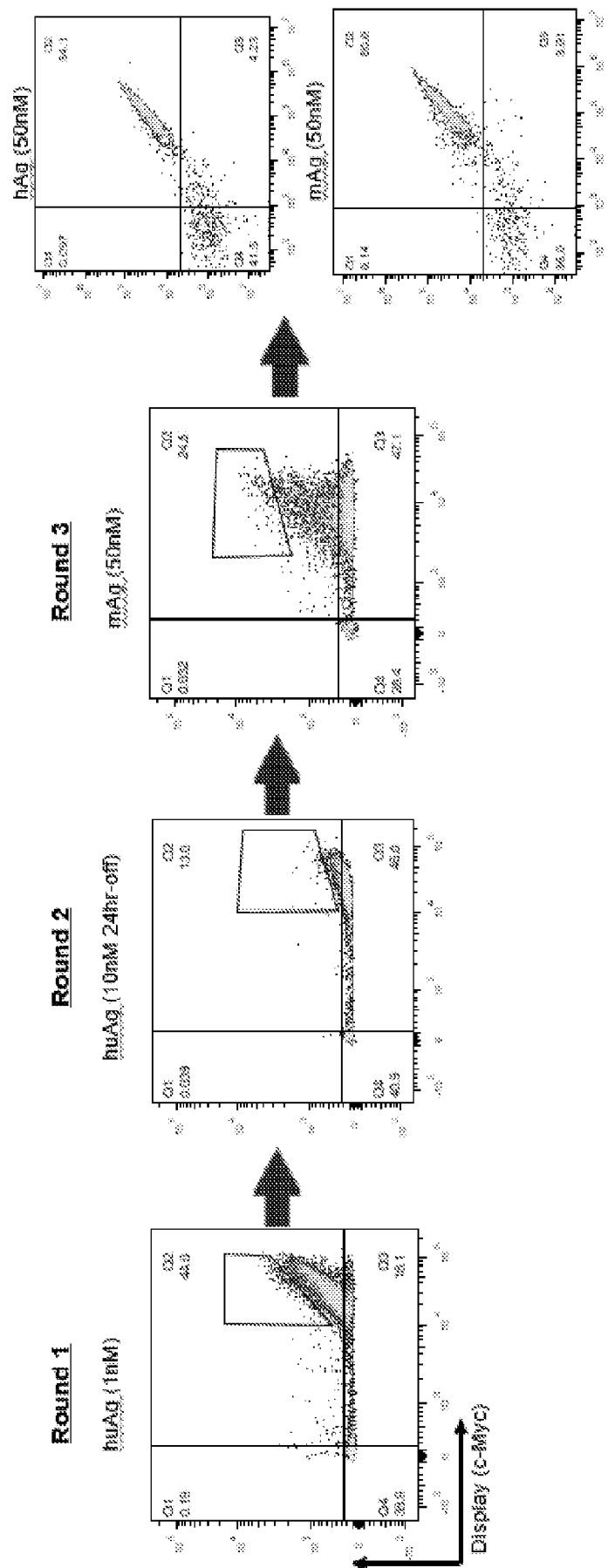
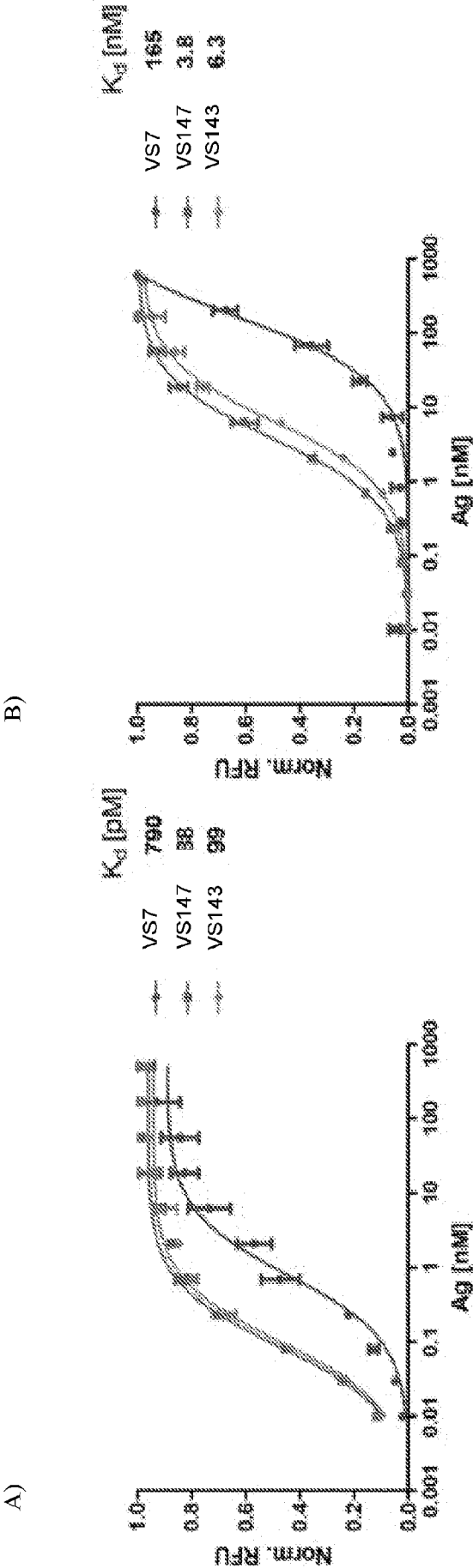


Figure 3

Figure 4A & B



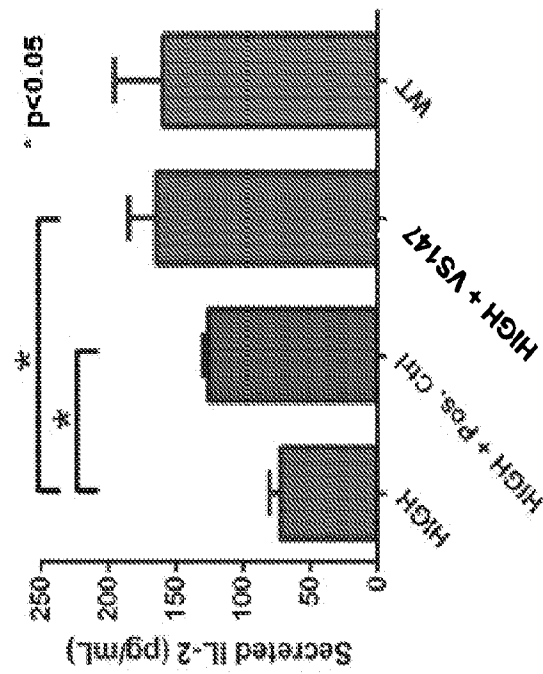


Figure 5

Figure 6

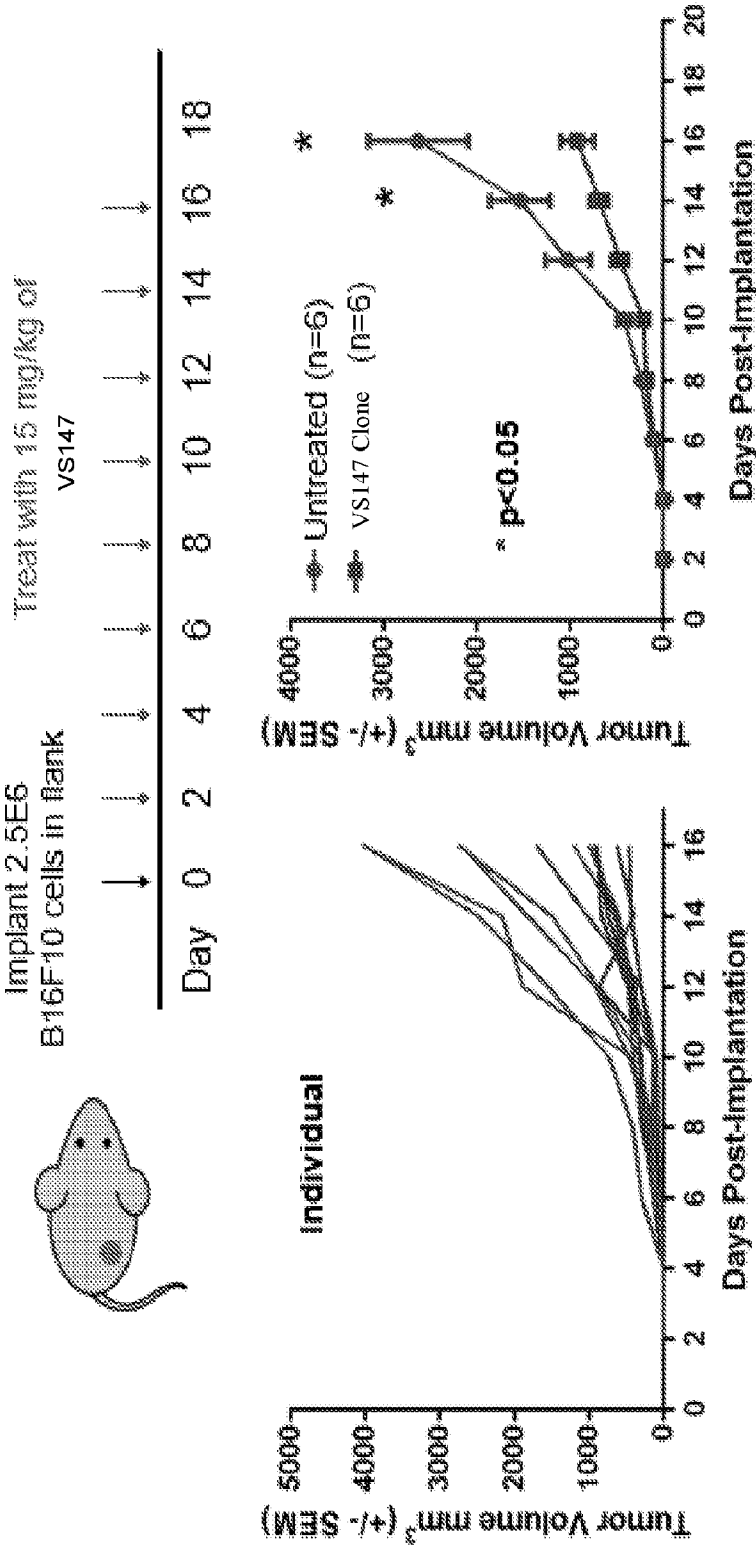


Figure 7

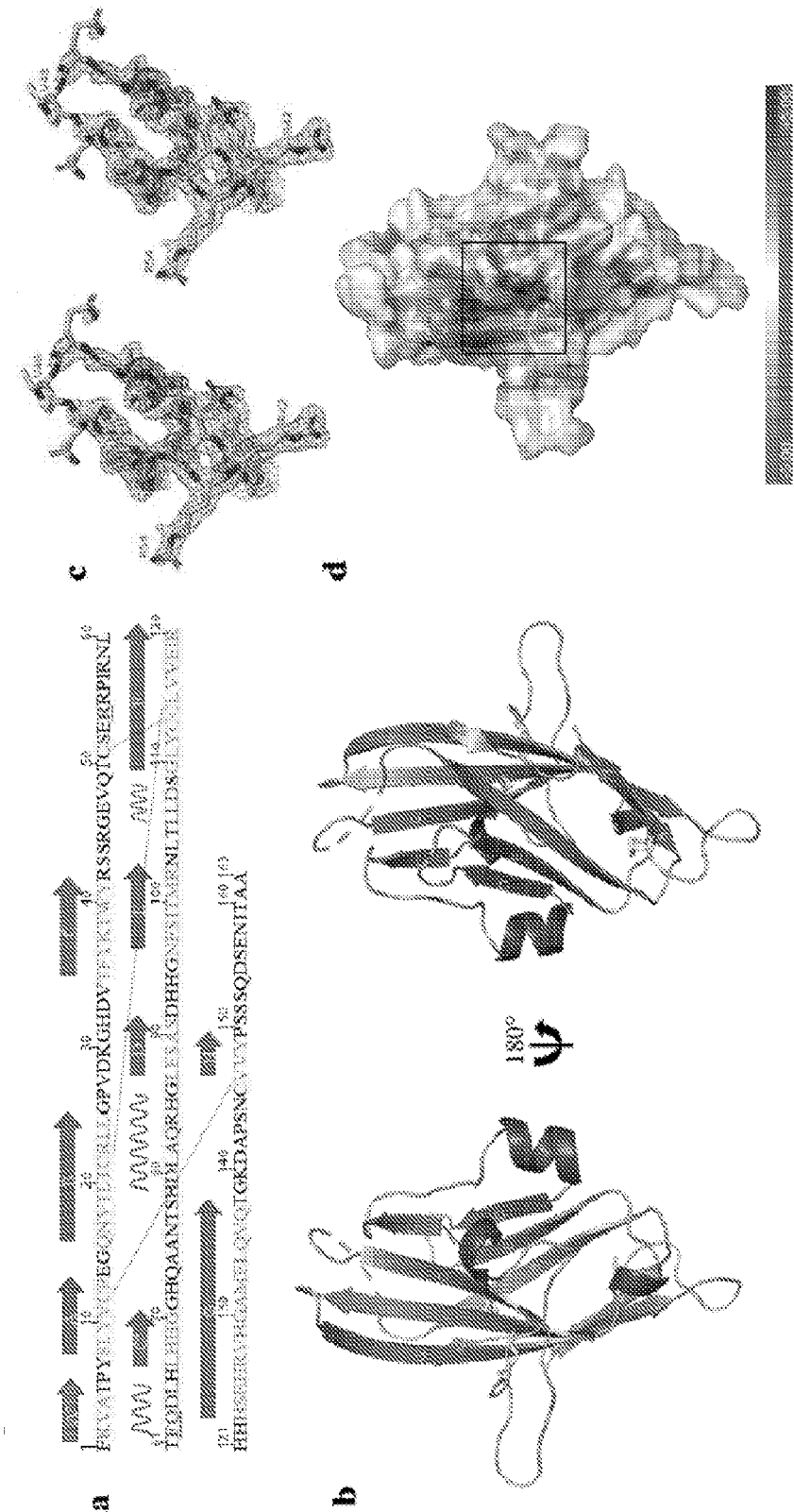


Figure 8

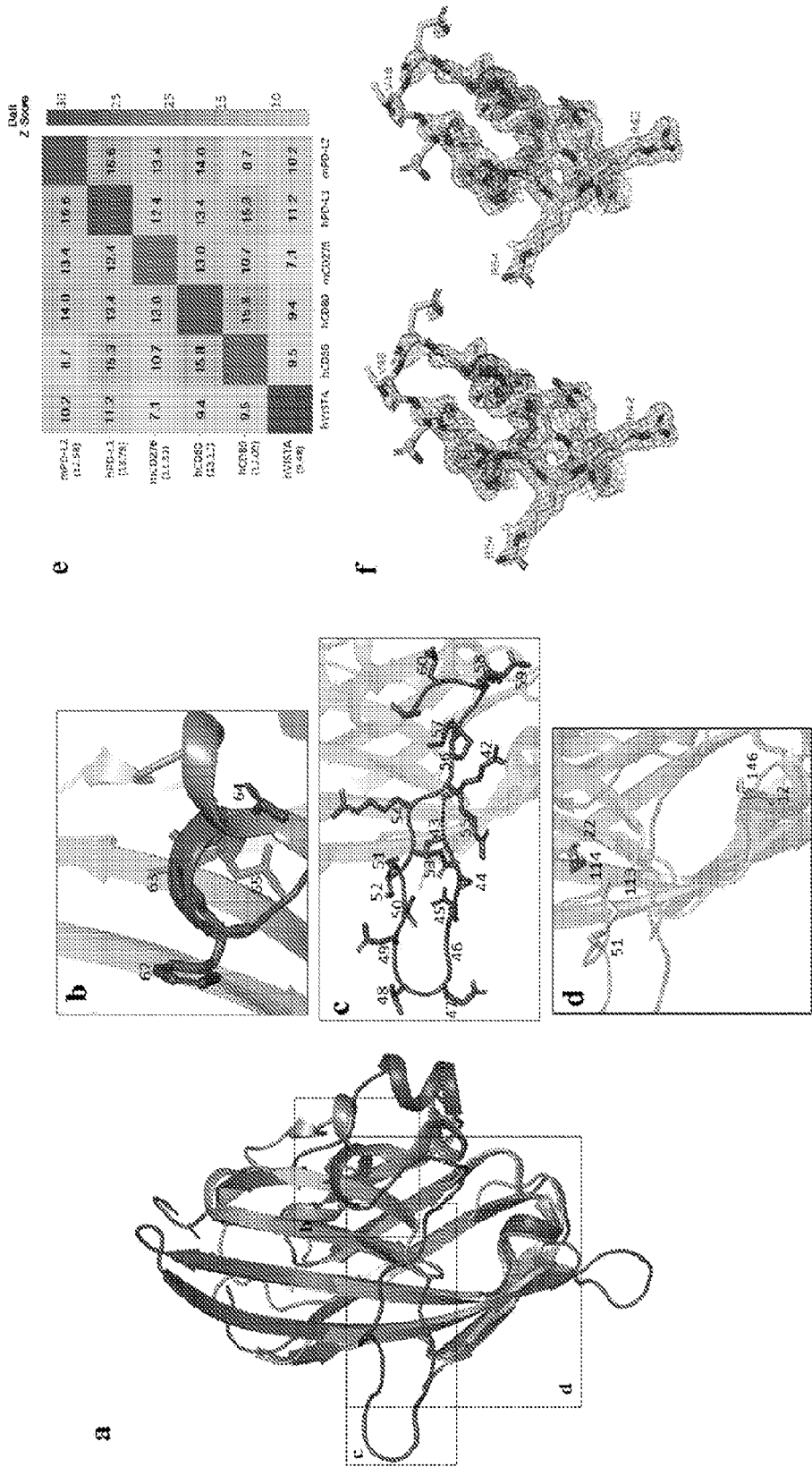


Figure 9

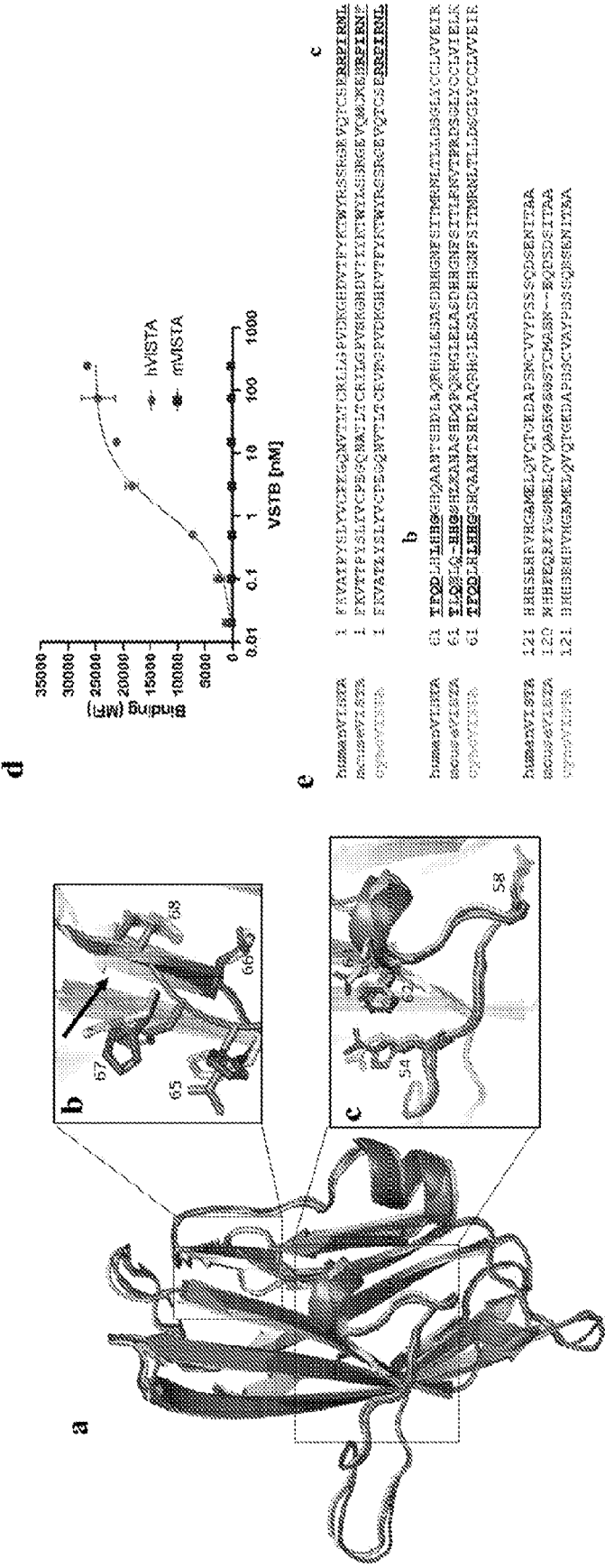


Figure 10

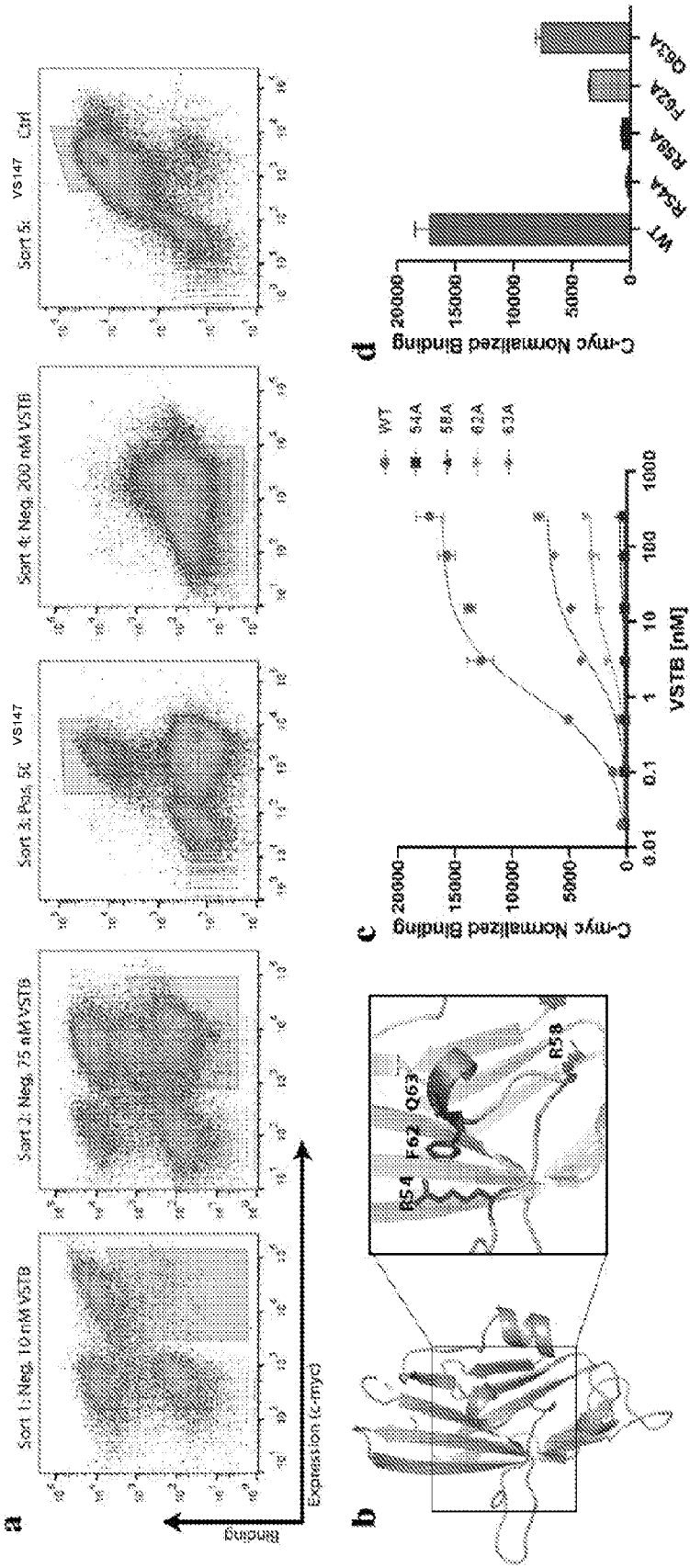
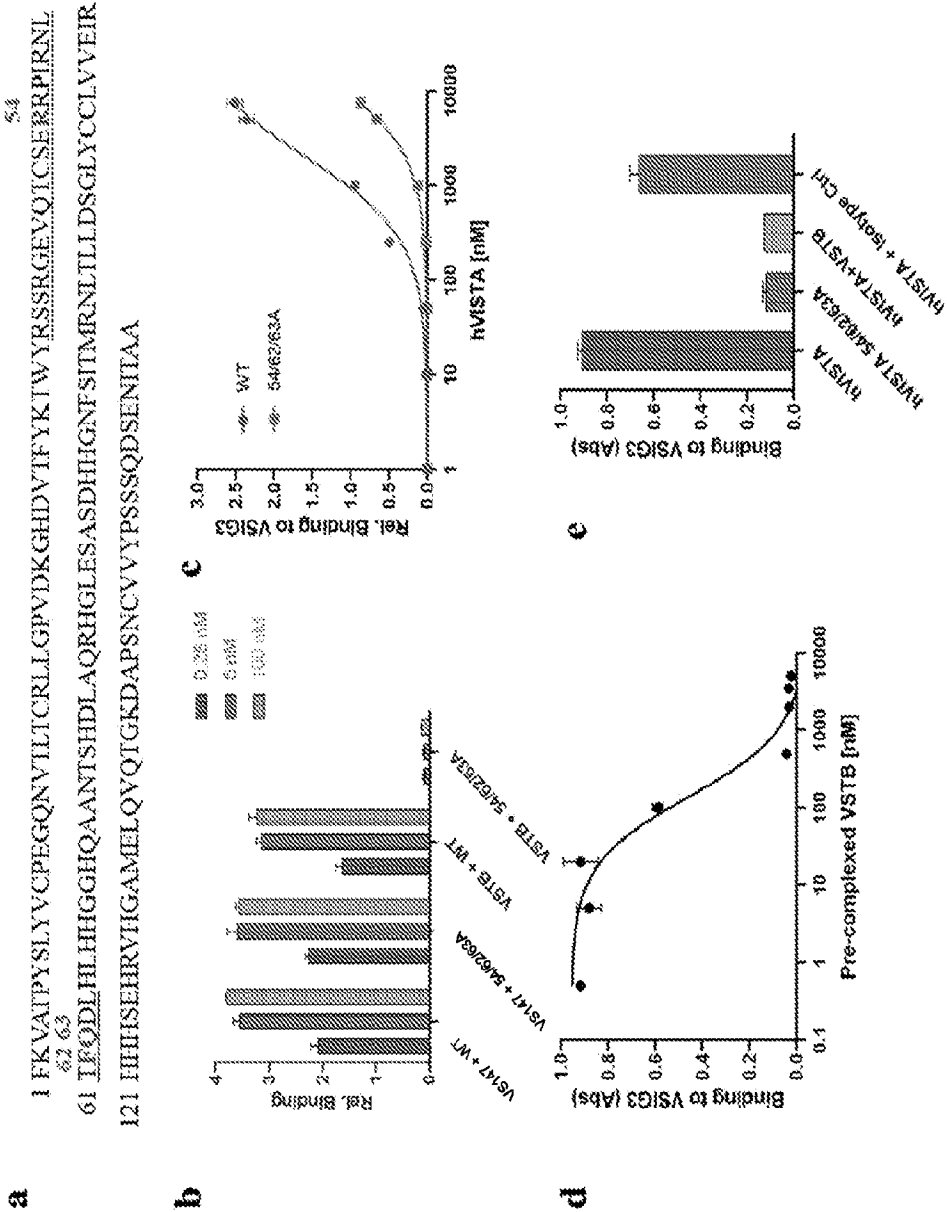


Figure 11



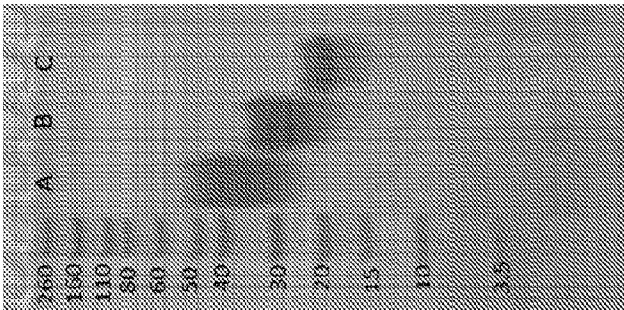
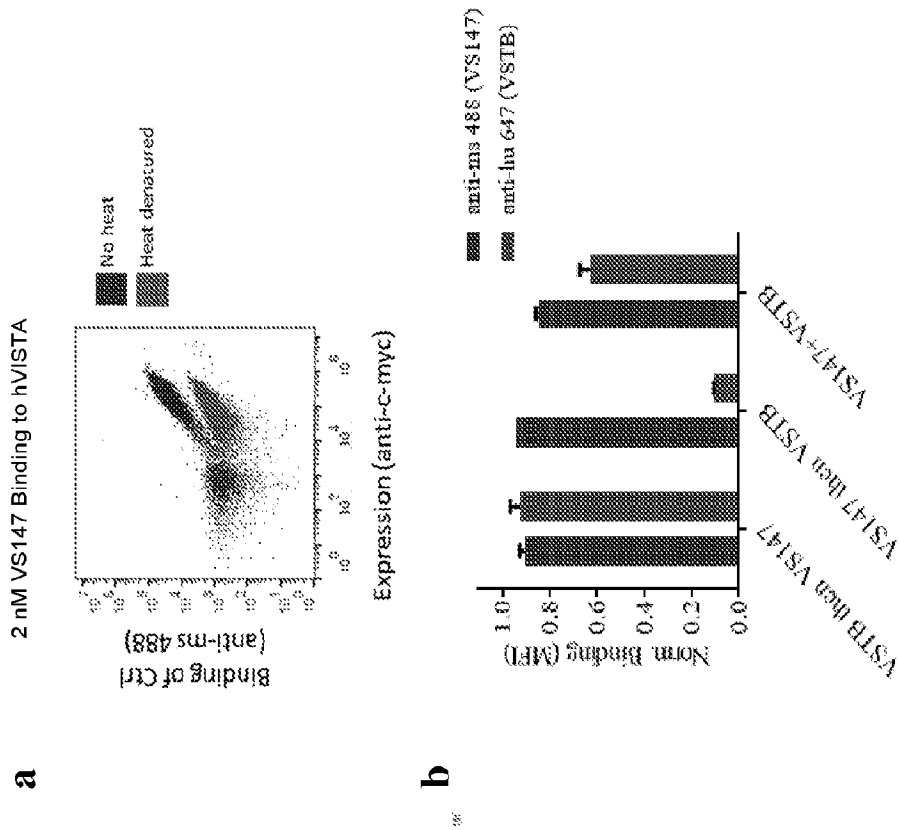


Figure 12

Figure 13



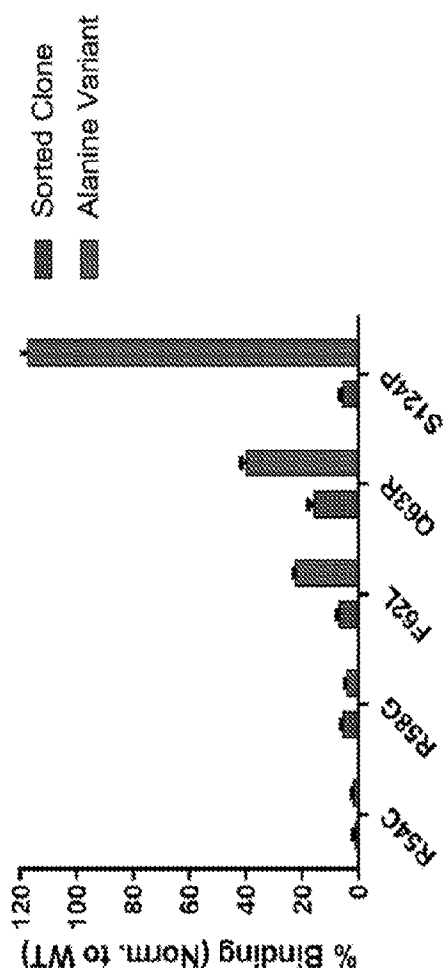
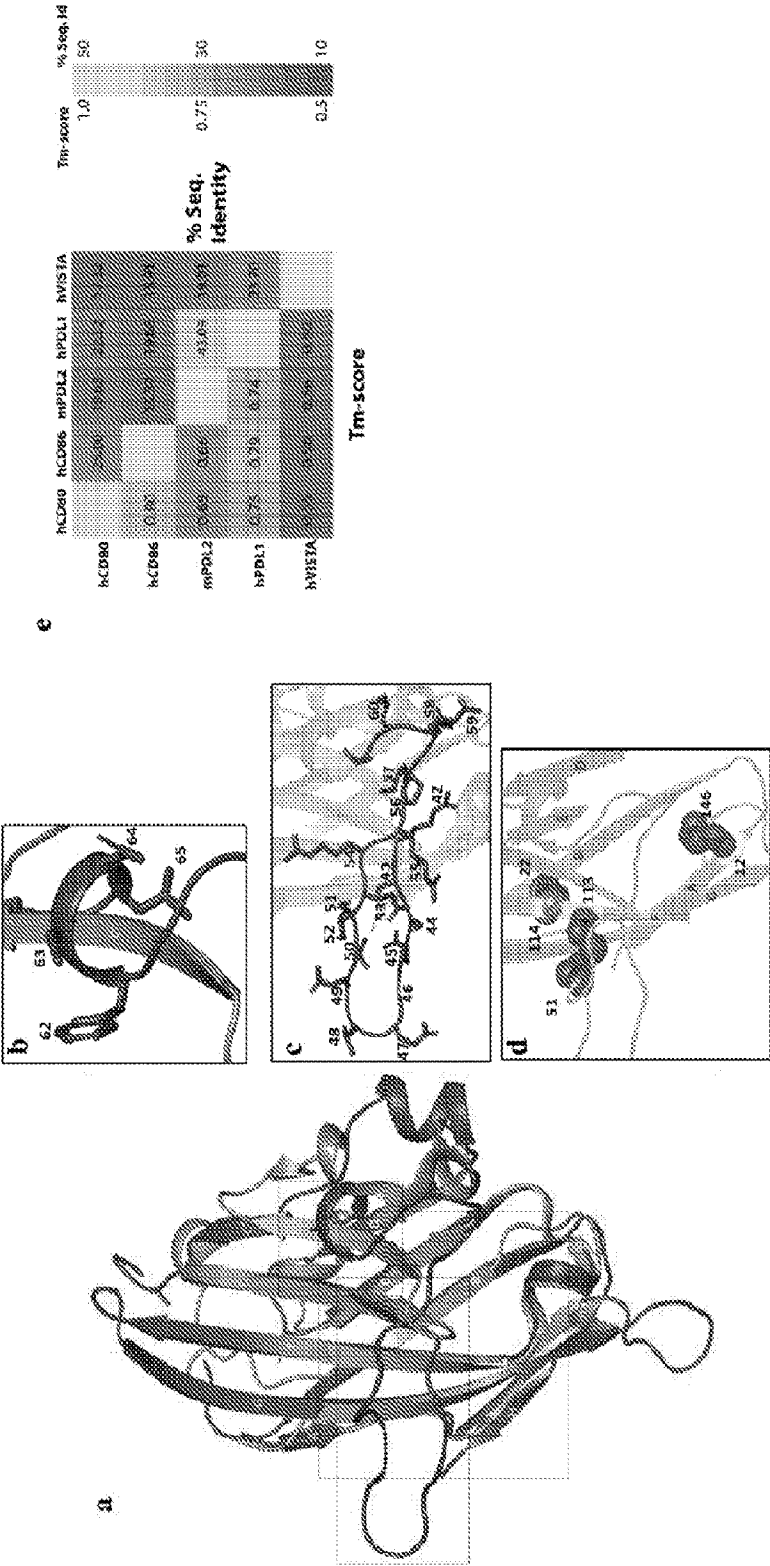


Figure 14

Figure 15

B7-H5 (VISTA)	1	-----FKVATPYSLYVCPEQNVTLTCRLGPPVDKGHDVTFYKTYRSSRGEVQT
B7-1 (CD80)	1	-----VIHVTKEVKEVATLSCGHNV-SV-EELAQTRIYNQKEKKMVLTM
B7-2 (CD86)	1	-----APLKIQAYFNETADLPCQFANSQN-QSISELVVFQDQENLVINE
B7-DC (PD-L2)	1	-----LFTVTPKELYIIIEGNSVTIECNFDTGSN-VNIGAITASLQ-----
B7-H1 (PD-L1)	1	-----FTVTPKDLVVEYGSNMTECKFPVEKQ-LDLAALIVYWMEDKNI IQF
B7-H2 (ICOSL)	1	-----DTQEKEVRAMVGSDELSCACPEGSR-FDINDVYVYQTSSEKTVVT
B7-H3 (CD276)	1	-----AVEVQVPEDPVVAIVGDTATLRCSFSEPPG-FSLAQNLINQLTDTKQ--L
B7-H4 (VTCN1)	1	LIIGFISGRHSITVTTVASAGNIGEDGILSCTFEP--D-IKLSDIVIQWLKEGVIGLVH
B7-H5 (VISTA)	51	CSERRPIRNLTFQDLHLHHGHHQAANTSHDLAQKHGLESAS--DHHGNFSITMRNLTLDD
B7-1 (CD80)	43	MS-----GDMNIWPEYKNRTIFD-----ITNLSIVILALRPSD
B7-2 (CD86)	45	VY-----LQKEKFDVSHSKYMGKTSFDS-----SWTLRLHNLIQKD
B7-DC (PD-L2)	42	-----KVENTSPHREKATILLEEQ--LPLGKASFHPIQVQVVRD
B7-H1 (PD-L1)	50	VH-----GEEDLKVQHSSYRQARLLKQD--LSLGNAAALQITDVKLQD
B7-H2 (ICOSL)	47	YH-----IPQNSSLENVDSRYNRALMSPAG--MLRGDFSRLFNVTPOD
B7-H3 (CD276)	49	VH-----SFTTEGRDQGSAYANRTALFPDL--LAQGNASRLQRVRVAD
B7-H4 (VTCN1)	58	EF-----KEGKDELSEQDEMFRGRTAVFADQ--VIVGNASRLKKNVQLTD
B7-H5 (VISTA)	109	SGLYCCLVVEIRHHHSEHRVHGAMELQVQTGKDAPSNCVVYP--SSSQDSENI TAA
B7-1 (CD80)	77	EGTYECVVLKYEKDAFKREHLAEVTLVSKADFTPTSI SDF--EIPTSNIRRIICS
B7-2 (CD86)	82	KGLYQCI IHHKKPTCMIRI HQMNSLSVLANFSQPEIVPI--SNITENVYINLTCS
B7-DC (PD-L2)	78	EQYQCCI IYGVAWDYK-----YLTGKVKASYRKINTHILKV--PETDEVELTCQ
B7-H1 (PD-L1)	91	AGVYRCMISYGA-DYK-----RITVKVNAPYNKINQRILVVD--PVTSEHELTCQ
B7-H2 (ICOSL)	90	EQKFHCLVLSQSL-CFQEVLSVEVTHVAANFSVPV--SAPHSPSQDELFTTCT
B7-H3 (CD276)	90	EGSFTCFVSIRDG-GSA-----AVSLQVAAPYSKPSMTLEPNKDLRPGDVTITCS
B7-H4 (VTCN1)	101	AGTYKCYI IITSKGKNA-----NLEYK-TGAFSMFEVNV D-----YNASSETLRCE

Figure 16



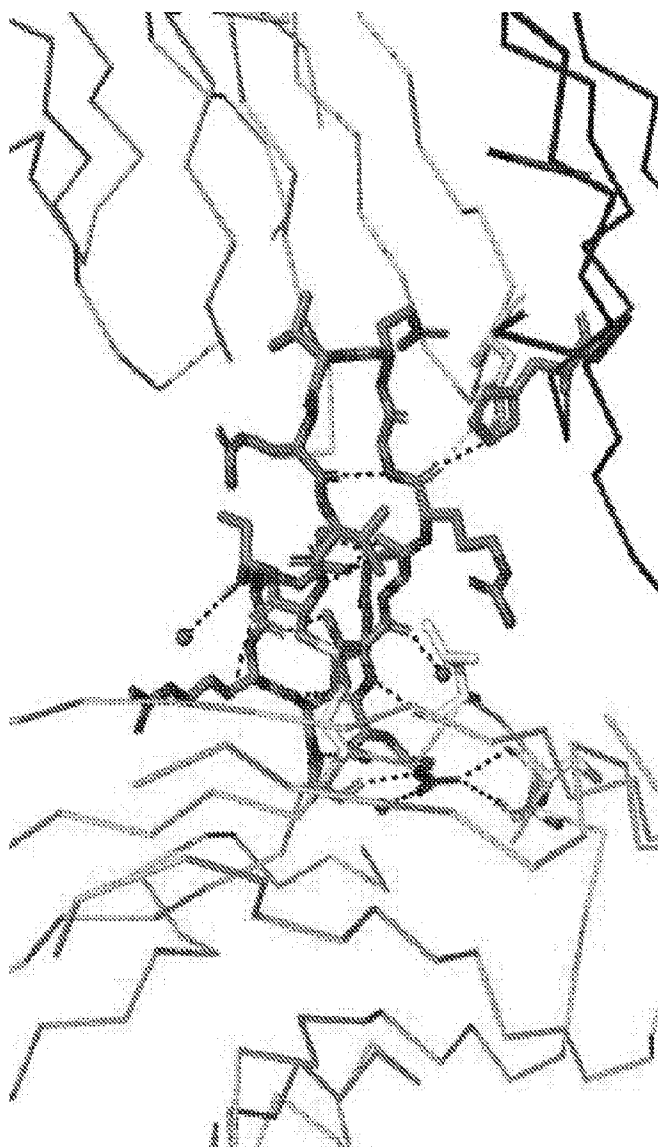


Figure 17

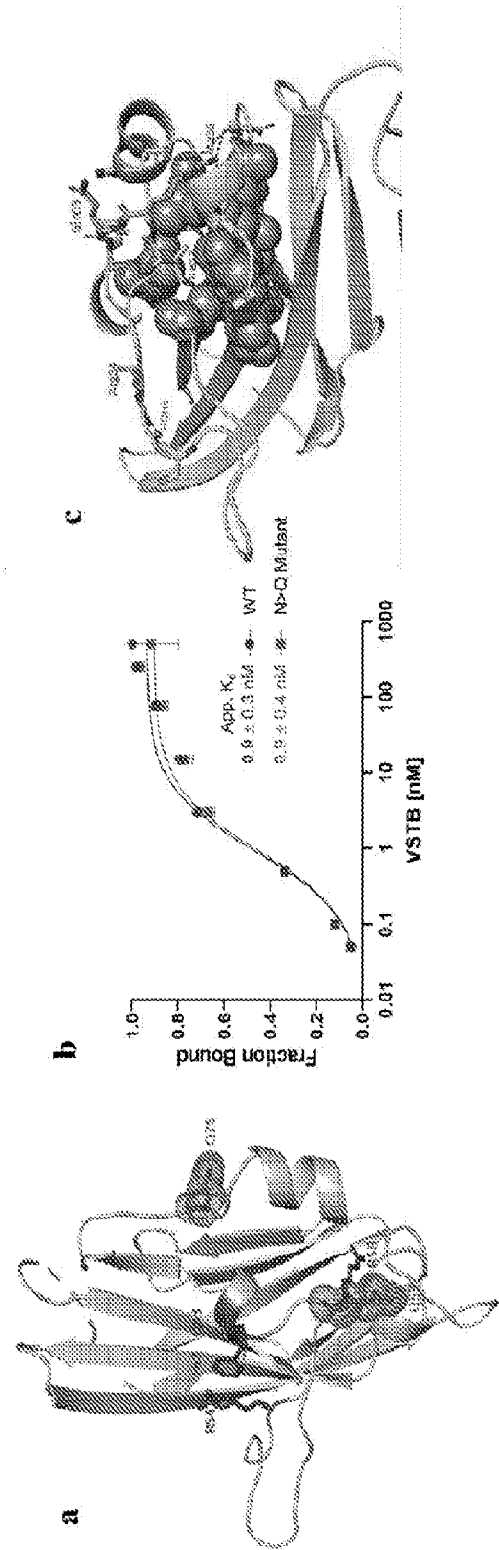


Figure 18

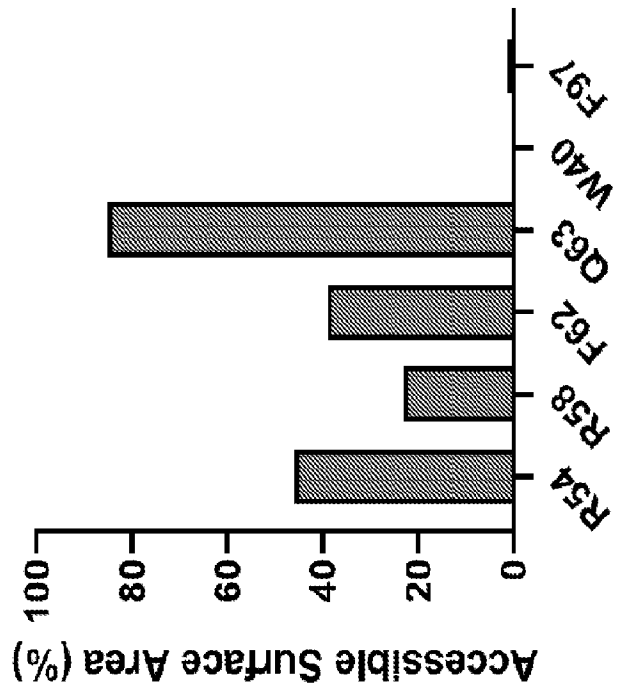


Figure 19

Figure 20

42 Arg	N --- O {55 Arg} = 2.96 O --- N {55 Arg} = 2.76 NE --- O {56 Pro} = 2.99 NH1 --- O {107 Leu} = 2.87 NH1 --- O {109 Ser} = 2.97 NH2 --- O {1 HOH} = 3.12 O {1 HOH} --- N {59 Glu} = 2.96	54 Arg	N --- O {148 Val} = 2.97 O --- N {148 Val} = 3.18 O --- O {7 HOH} = 2.84 O {7 HOH} --- O {5 HOH} = 2.69 O {5 HOH} --- N {62 Phe} = 2.92 O {5 HOH} --- O {55 Arg} = 3.01 NE --- O {6 HOH} = 2.50 O {6 HOH} --- O {52 Ser} = 2.67
43 Ser	N --- O {111 Leu} = 2.76 O --- O {4 HOH} = 2.78 O {4 HOH} --- N {111 Leu} = 2.93 Og --- O {53 Glu} = 2.67	55 Arg	N --- O {42 Arg} = 2.76 O --- N {42 Arg} = 2.96 NH2 --- OE2 {53 Glu} = 2.70 NH2 --- OD1 {33' Asp} = 3.06 NH2 --- OD2 {33' Asp} = 3.09
44 Ser	O --- O {2 HOH} = 2.80 O {2 HOH} --- OE1 {53 Glu} = 2.88 O {2 HOH} --- N {52 Ser} = 3.05 O --- N {51 Cys} = 3.36 Og --- OE2 {53 Glu} = 2.47 OE2 {53 Glu} --- NH2 {55 Arg} = 2.70	56 Pro	O --- NE {42 Arg} = 2.59
45 Arg	O --- NE2 {122' His} = 2.95 O --- NE2 {94' His} = 2.92	57 Ile	N --- O {60 Leu} = 2.81 O --- O {60 Leu} = 2.64
46 Glu	N --- O {49 Gln} = 2.69	58 Arg	NE --- OD1 {108 Asp} = 3.08 NH2 --- OD2 {108 Asp} = 2.80 NH2 --- O {102 Arg} = 2.75 NH1 --- O {102 Arg} = 2.84 NH1 --- O {85 His} = 2.92
51 Cys	S --- S {113 Cys} Disulfide bond O --- OH {41 Tyr} = 2.81		
52 Ser	O --- O {6 HOH} = 2.67 O {6 HOH} --- NE {54 Arg} = 2.50		
53 Glu	OE2 --- NH2 {55 Arg} = 2.70 NH2 {55 Arg} --- OD1 {33 Asp} = 3.06		

Figure 21

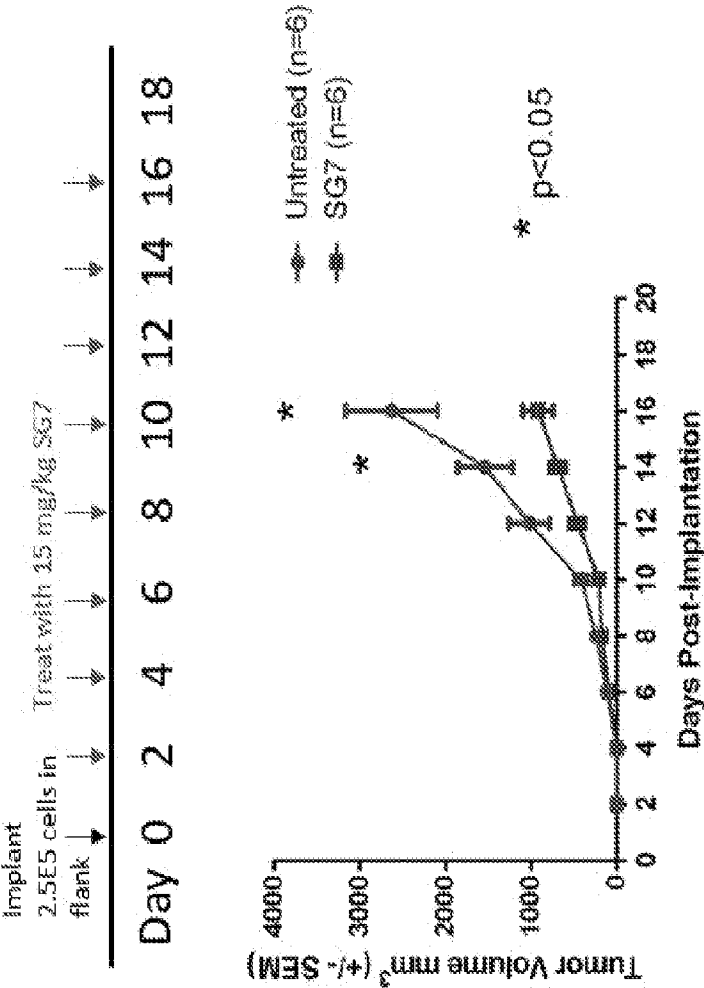


Figure 22

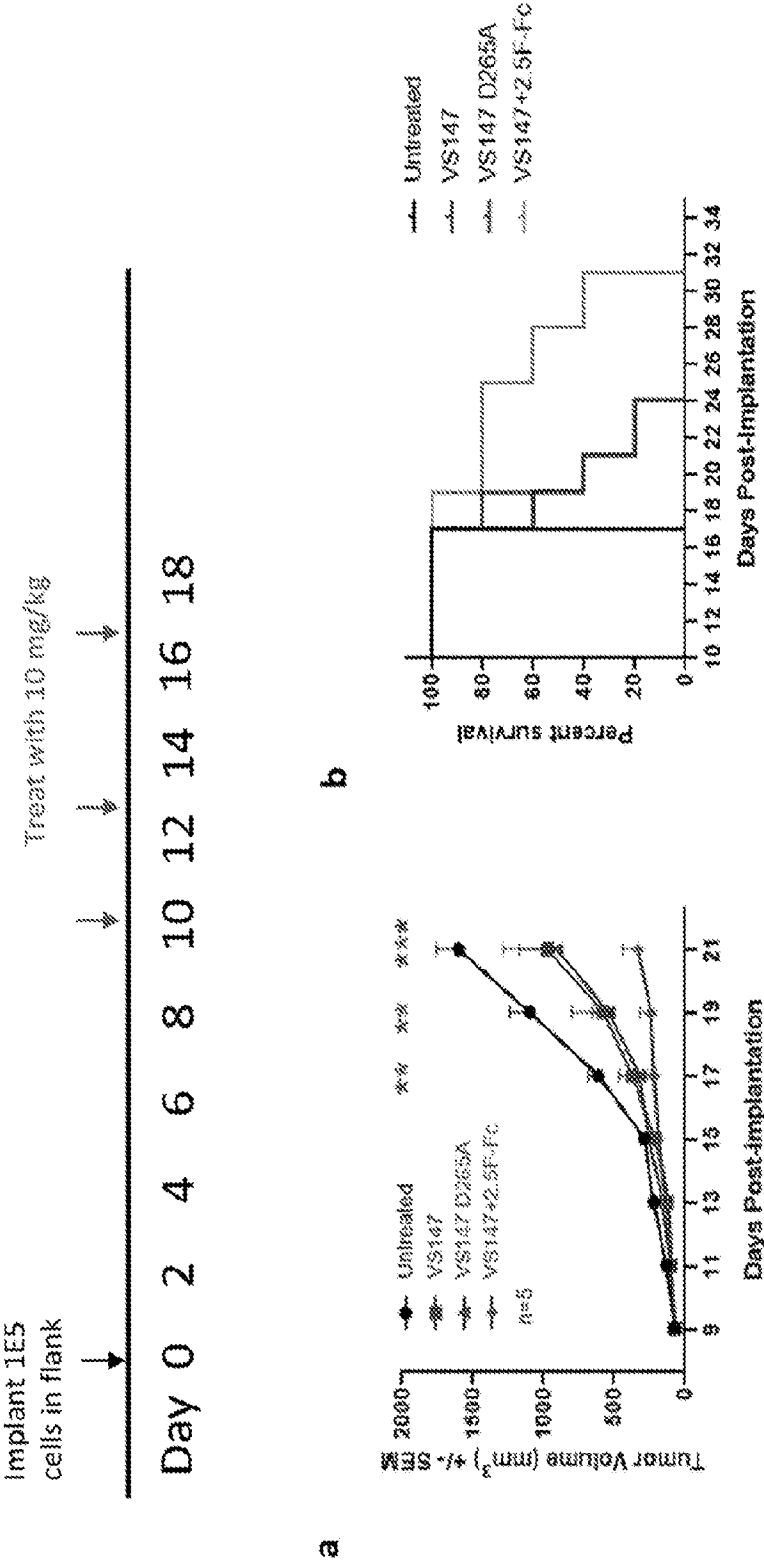


Figure 23

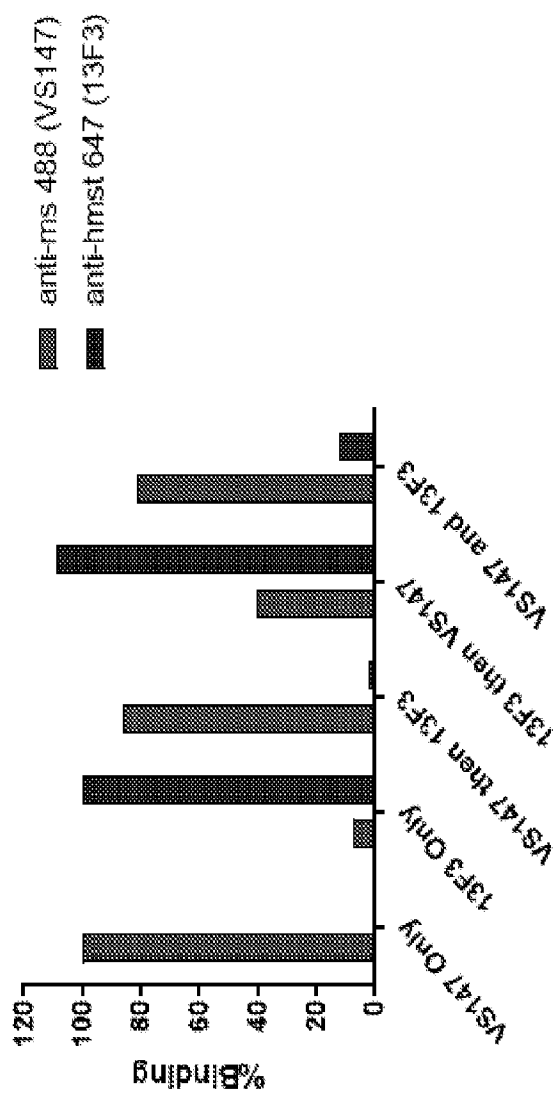
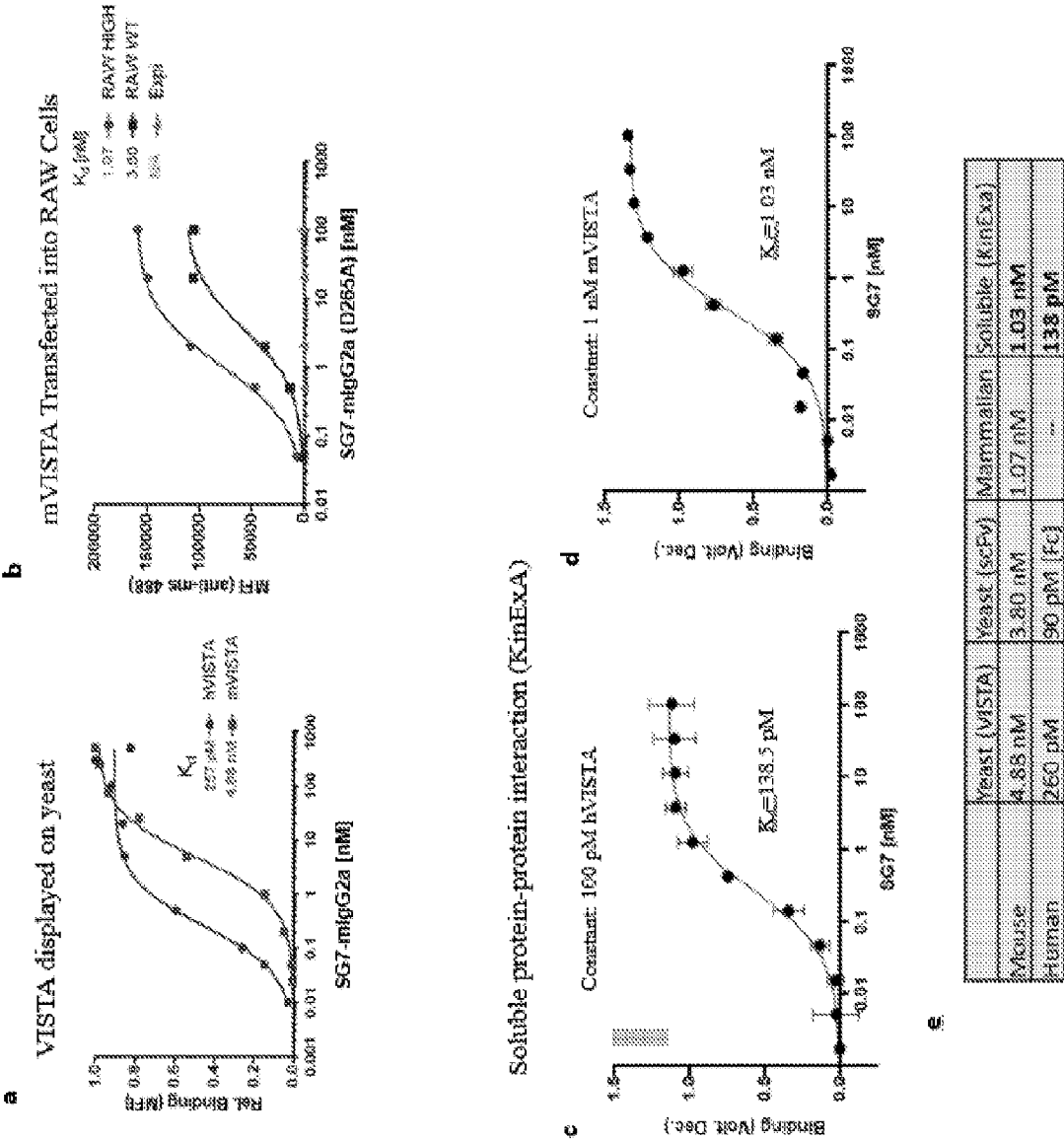


Figure 24



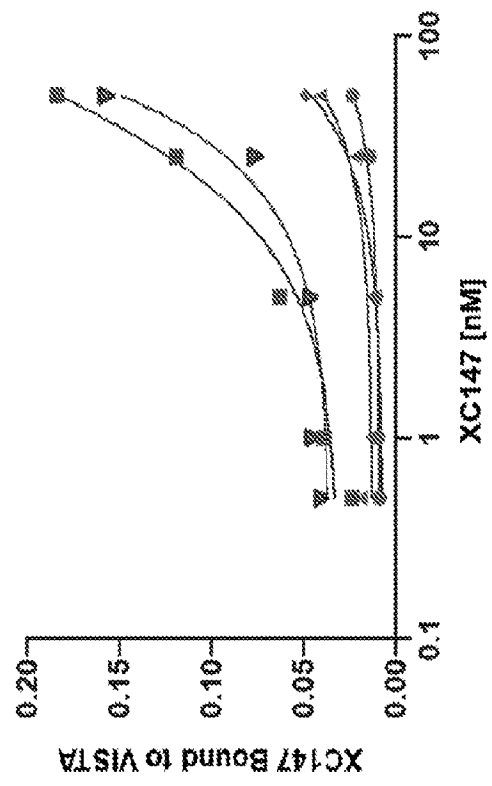


Figure 25

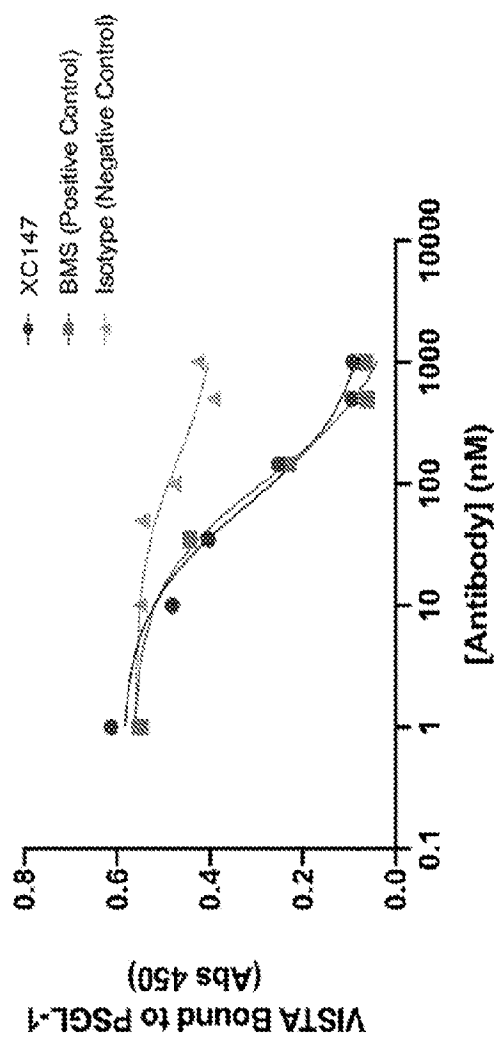


Figure 26

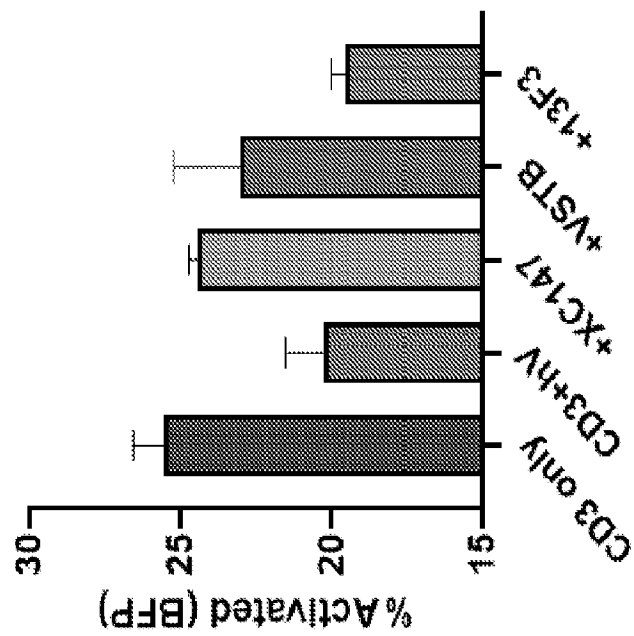


Figure 27

Figure 28A

Sequences:

XC147 NC:

MGWSLLFLFLVAVATGTHSQVLVQSGAEVKKPGSSVKVSCKASGGIESSY AISWVRQAPQGQLEWMGGIPIFGTANYAQKFQGRVTTITADESTSTAYM
ELSSLRSEDTAVYYCARPVRS^{GP}DPYLQ^{HW}GQGTLTVTSSAKTTAPSVYPLAPVCGGTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPA
QSGLYTLSSSVTVTSNTWPSQTITCNVAHPASSTKVDKKIEPRVPIITQNPCLKECPCAAPDLLGGPSVFIFPPKIKDVLMSISPMVTCVVVDVSEDDP
DVQISWFVNNVEVHTAQQTQTHREDYNSTLRVVSALPIQHQQDWMGKKEFKCKVNNRALPSPIEKTISKPRGPPRAPQVYVLP^{PP}AEEMTKKEFSLTCMIT
GFLPAEIAVDWTSNGRTEQNYKNTATVLDSDSGSYFMYSKLRVQKSTWERGSLFACSVVHEGLHNHLTTKTTISRSLGK

XC147 LC:

MDMRVPAQLLGLLLWLPGARCDIQMTQSPSTLSASVGD^{RV}TTITCRASQSISSWLA WYQQKPGKAPKLLIYDASSLES^{GV}PSRFS^{GS}SGTEFTLTITSSLQP
DDFATYYCQQYN^{SY}SLTFGGGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSE^{RQ}NGVLNSWTDQDSK^DSTYSMSSTL
TLTKDEYERHNSYTCEATHK^TSTSPIVKSFNREK

V9.7 (softv version of XC147)

DIQMTQSPSTLSASVGD^{RV}TTITCRASQSISSWLA WYQQKPGKAPKLLIYDASSLES^{GV}PSRFS^{GS}SGTEFTLTITSSLQ^{PD}DFATYYCQQYN^{SY}SLTFGGG
TKVEIKGTTAA^{SG}SSGGSSGAQVQLVQSGAEVKKPGSSVKVSCKASGGIESSY AISWVRQAPQGQLEWMGGIPIFGTANYAQKFQGRVTTITADESTST
AYMELSSLRSEDTAVYYCARPVRS^{GP}DPYLQ^{HW}GQGTLTVTSS

From Round 1 of Sorting

V1:

DIQMTQSPSTLSASVGD^{RV}TTITCRASQSISSWLA WYQQKPGKAPKLLIYDASSLES^{GV}PSRFS^{GS}SGTEFTLTITSSLQ^{PD}DFATYYCQQYN^{SY}SLTFGGG
TRLEIKGTTAA^{SG}SSGGSSGAQVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVA^{VISY}DGSN^{KYY}ADSVKGRFTISRDN
NTLYLQMN^{SL}RSDDTAVYYCARGW^{SG}PSDE^{YV}WGQGTLTVTSS

V2:

QSVVTQPPSVSGAPGQRTVISTCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGN^{SN}RPS^{GV}PD^{RF}SGSKSGTASL^{AIT}GLQAEDEADYYCQSYDSSLSGS
WYFEGGGTKLTVLGTTAA^{SG}SSGGSSGAQLQLQESGPGLVKPSETLSL^{CT}TVSGSIS^{SSSY}YWGWRQHPGKGLEWIGSIYSGSTYYNPSLKSRVTIS
VDTSKNQFSLKLS^{SV}TAADTAVYYCARRSTI^{WG}DGA^{EY}FQHWGQGTLTVTSS

V3:

EIVLTQSPGTLSASVGD^{RV}TTITCRASQSISSWLA WYQQKPGKAPKLLIYDASSLES^{GV}PSRFS^{GS}SGTEFTLTITSSLQ^{PD}DFATYYCQQYN^{SY}SLTFGPGT
KVDIKGTTAA^{SG}SSGGSSGAQVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWMGGIPIFGTANYAQKFQGRVTTITADESTST
AYMELSSLRSEDTAVYYCARSVRS^{GP}DPYLQ^{HW}GQGTLTVTSS

Figure 28B

V4: QSVVTQPPSVSGAPVQRVTISCTIGSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGV~~PDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSGV~~
~~FGTGTKVTVLGTAAAGSSGGSSGAQLQESGPGLVKPSSETLSLCTVSGGSSSSSYWGWIRQPPGKGLEWIGENHSGSTNYPNPSLKSRVTISVDIT~~
~~SKNQFSLKLSSTAAADTAVYYCARVDNDYSYVGSYGMIDVWGGQGLVTVSS~~

V5: QSVVTQPPSVSGAPGQRVTISCTIGSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGV~~PDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSGV~~
~~VFGGGTKLTVLGTAAAGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKKASGGTFESSY~~AISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTIT~~~~
~~ADESTTAYMELSSLRSEDTAVYYCAR~~YDFYGGYTDYWGGQGLVTVSS~~~~

V6: DIQMTQSPSTLSASVGDRTVTITCRASQSISSWLA~~WYQKPGKAPKLLIYDASSLES~~GVPSRFS~~SGSGGTEFTLTISLQPD~~DFATYYCQQYN~~SYSLTFGGG~~
~~TKVEIKGTTAASGSSGGSSGAQVQLVQSGGGLVQPGGSLRLSCAASGTFESSY~~AMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDN~~SK~~
~~NTLYLQMNSLRAEDTAVYYCAR~~LEDWLGGYILEYFDLWGGQGLVTVSS~~~~

V7: DIQMTQSPSTLSASVGDRTVTITCRASQSISSWLA~~WYQKPGKAPKLLIYDASSLES~~GVPSRFS~~SGSGGTEFTLTISLQPD~~DFATYYCQQYN~~SYSLTFGGG~~
~~TKLEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKKASGGTFESSY~~AISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTS~~~~
~~TAYMELSSLRSEDTAVYYCAR~~GVRSGPDYFQHWGGQGLVTVSS~~~~

V8: QSVVTQPPSVSGAPGQRVTISCTIGSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGV~~PDRFSGSKSDTSASLAITGLQAEDEADYYCQSYDSSLSG~~
~~WVEGGGKLTIVLGTAAAGSSGGSSGAQVQLVQSGGGLVQPGGSLRLSCAASGYTFISYYMHWVVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTI~~
~~TADESTTAYMELSSLRSEDTAVYYCAR~~AGVGYWDPLSDAFDVWGGQGLVTVSS~~~~

V9 (parent of XC147):
 DIQMTQSPSTLSASVGDRTVTITCRASQSISSWLA~~WYQKPGKAPKLLIYDASSLES~~GVPSRFS~~SGSGGTEFTLTISLQPD~~DFATYYCQQYN~~SYSLTFGGG~~
~~TKVEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKKASGGTFESSY~~AISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTS~~~~
~~TAYMELSSLRSEDTAVYYCAR~~PVRSGPDYFQHWGGQGLVTVSS~~~~

V10: DIQMTQSPSTLSASVGDRTVTITCRASQSISSWLA~~WYQKPGKAPKLLIYAASSLQSGVPSRFS~~SGSG~~TDFTLTISLQPED~~FATYYCQOOSY~~STPYTFGQG~~
~~TKLEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKKASGGTFESSY~~AISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTS~~~~
~~TAYMELSSLRSEDTAVYYCAR~~GW~~SARYGMDVWGGQGLVTVSS~~~~~~

Figure 28C

V11:
 QSVVTQPPSVSGAPGQRTVITISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVSNRFSGSKSGTASLAIITGLQAEDEADYYCQSYDSSL~~SGV~~
 VFGGGTKLTVLGTAAAGSSGGSSGAQVQLVESGGGLVQPGGSLRLSCAASGFTESNAWMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTIS
 RDNSKNTLYLQMNSLRAEDTAVYYCARLDWPLSYGSGGYYYGMDVWGQGITLVTVSS

V12:
 DIQMTQSPSTLSASVGDRTVITICRASQSISSWLAWYQOKPGKAPKLLIYDASSLES~~GVPSRFS~~SGSGTTEFTLTISLQPD~~DFATYYCQQYNSYSL~~TEGGG
 TKVEIKGTTAASGSSGGSSGAQVQLVESGGGLVQPGGSLRLSCAASGFTESSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTIS
 TSTAYMELRSLRSDDTAVYYCARVWYSGFSD~~FDS~~WGQGITLVTVSS

V13:
 DIQMTQSPSTLSASVGDRTVITICRASQSISSWLAWYQOKPGKAPKLLIYDASSLES~~GVPSRFS~~SGSGTTEFTLTISLQPD~~DFATYYCQQYNSYSL~~TEGGG
 TRLEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKASGCTESSY~~AI~~SWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTS
 TAYMELSSLRSEDTAVYYCARSVRS~~GP~~DPDYFQHWGQGITLVTVSS

V14:
 QSVVTQPPSVSAPGQRTVITISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGV~~PDRFS~~GSKSGTASLAIITGLQAEDEADYYCQSYDSSL~~SGV~~
 VFGGGTKLTVLGTAAAGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKASGCTESSYAMSWVRQAPGQGLEWVSAISGSGGSTYYADSVKGRFTIS
 RDNSKNTLYLQMNSLRAEDTAVYYCARGWVSVTD~~DFDS~~WGQGITLVTVSS

V15:
 DIQMTQSPSSLSASVGDRTVITICRASQSISSWLAWYQOKPGKAPKLLIYDASSLES~~GVPSRFS~~SGSGTTEFTLTISLQPD~~DFATYYCQQYNSYSL~~TEGGG
 TKVEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGASVKVSKASGYT~~FSSY~~AI~~SWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTS~~
 TAYMELSSLRSEDTAVYYCARSVRS~~GP~~DPDYFQHWGQGITLVTVSS

V16:
 QSVVTQPPSVSGAPGQRTVITISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGV~~PDRFS~~GSKSGTATLGITGLQTGDEADYYC~~GTW~~DSSL~~SG~~
 YVEGTGKVTVLGTAAAGSSGGSSGAQVQLVQSGAEVKKPGASVKVSKASGYT~~FSSY~~AI~~SWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGR~~
 VIMTTDTSTAYMELRSLRSDDTAVYYCARD~~F~~GYSSAYFDYWGQGITLVTVSS

V17:
 SYELTQPPSVSVSPGQTASITCSGDKLGDKYACWYQOKPGQSPVLVIYQDSKRPSGIPERFSGNSNGNTATLTISGTQAMDEADYYCQAWDSSTAVEGT
 GTKVTVLGTAAAGSSGGSSGAQVQLVESGGGVVQPGRSLRLSCAASGFTESSYAMHWVRQAPGKGLEWVA~~VISYDGS~~KNKYADSVKGRFTISRDN
 SKNTLYLQMNSLRAEDTAVYYCARYGRRYTPYYWYFDLWGQGITLVTVSS

V18:
 QSVVTQPPSVSGAPGQRTVITISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGV~~PDRFS~~GSKSGTASLAIITGLQAEDEADYYCQSYDSSL~~SGV~~
 VFGGTGKVTVLGTAAAGSSGGSSGAQVQLVESGGGVVQPGRSLRLSCAASGFTESSYAMHWVRQAPGKGLEWVA~~VISYDGS~~KNKYADSVKGRFTI
 SRDNSKNTLYLQMNSLRAEDTAVYYCARGWSTVGD~~DEL~~WGQGITLVTVSS

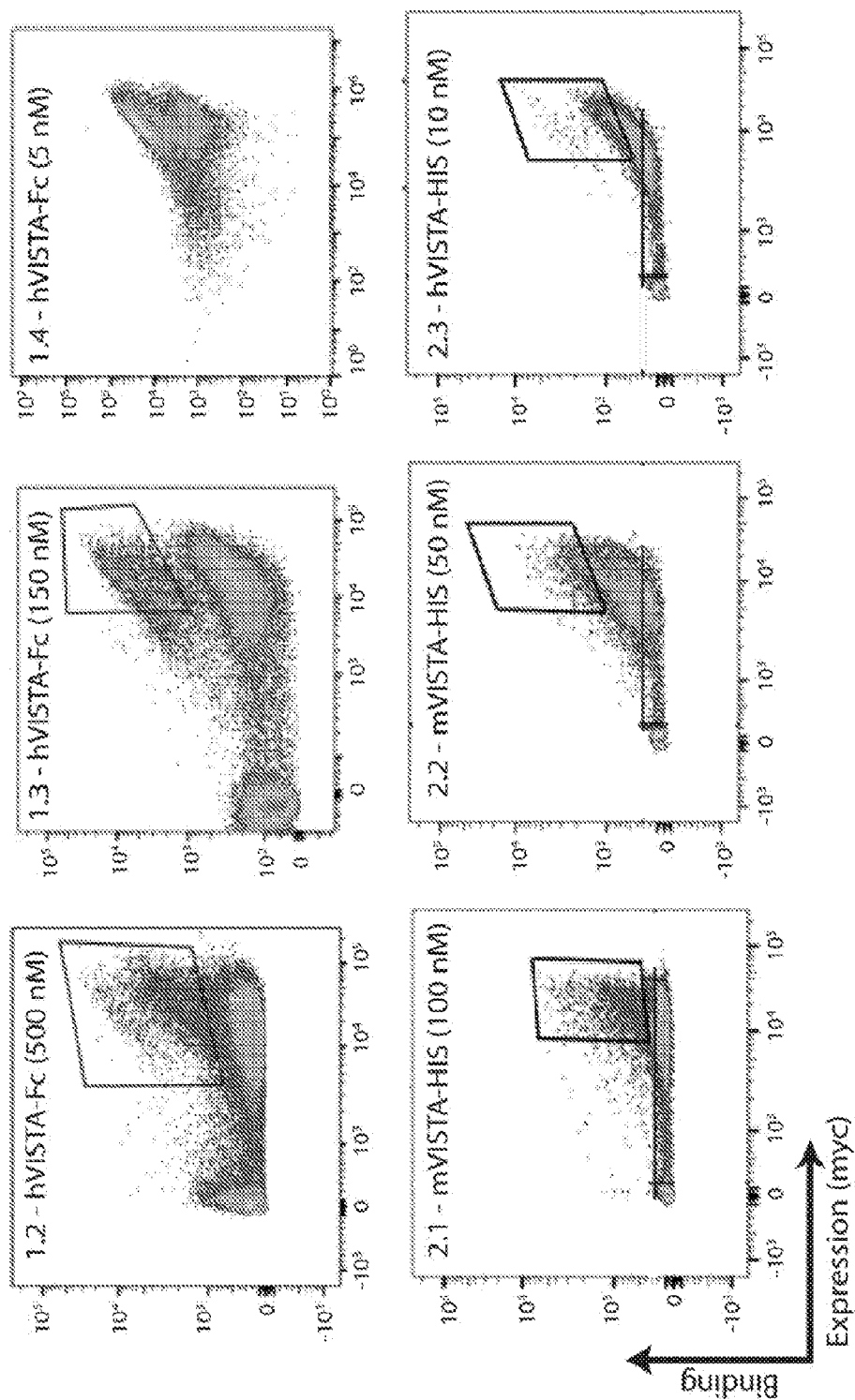


Figure 29

Figure 30

Single clone analysis after sort round 1

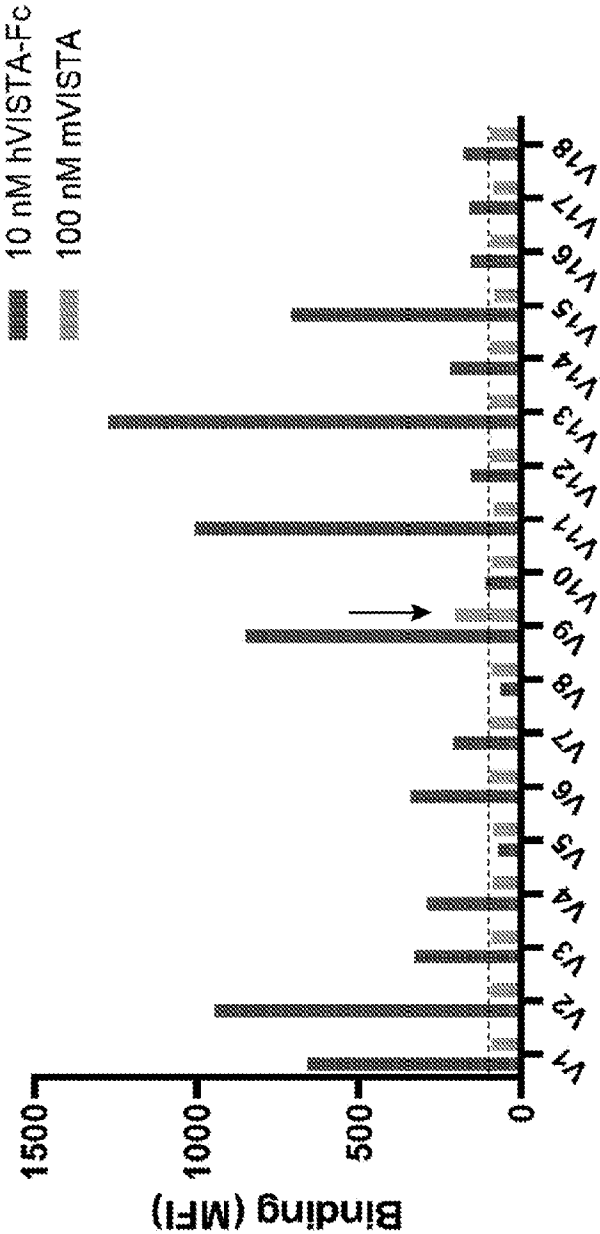


Figure 31

Binding Affinity to mVISTA and hVISTA by KinExA

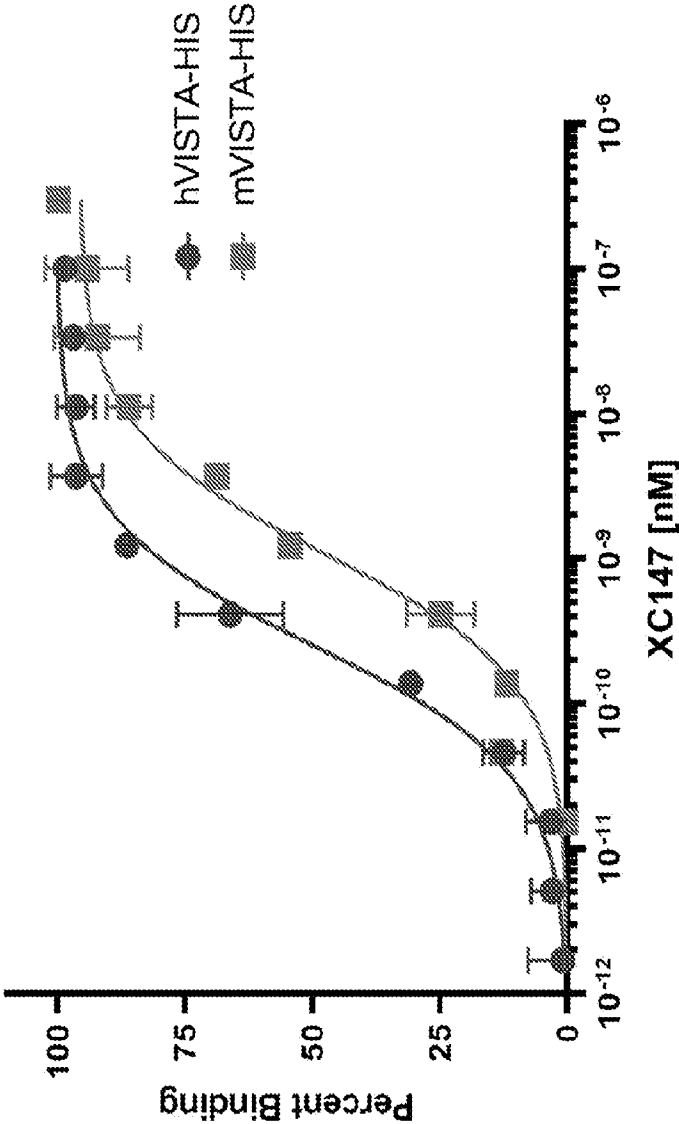


Figure 32

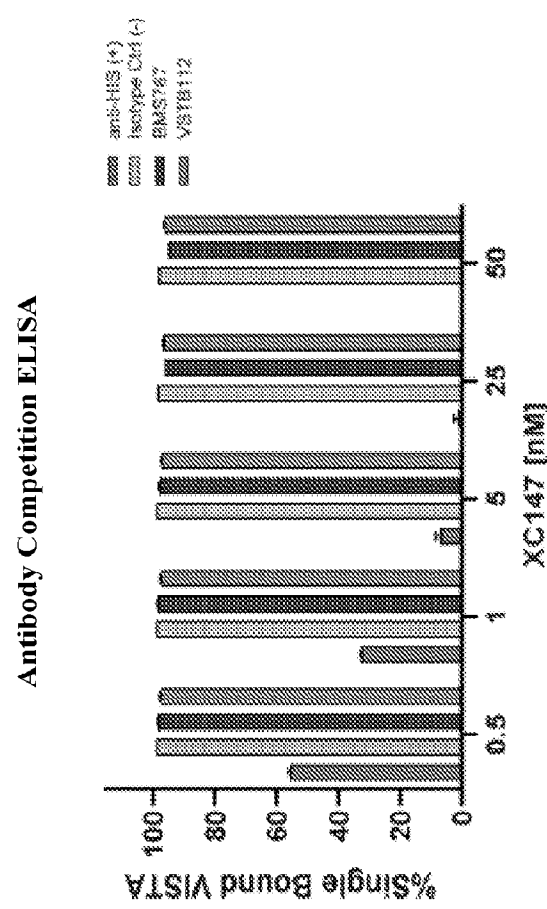


Figure 33

Epitope Binning of XC147 with Competitor Antibodies

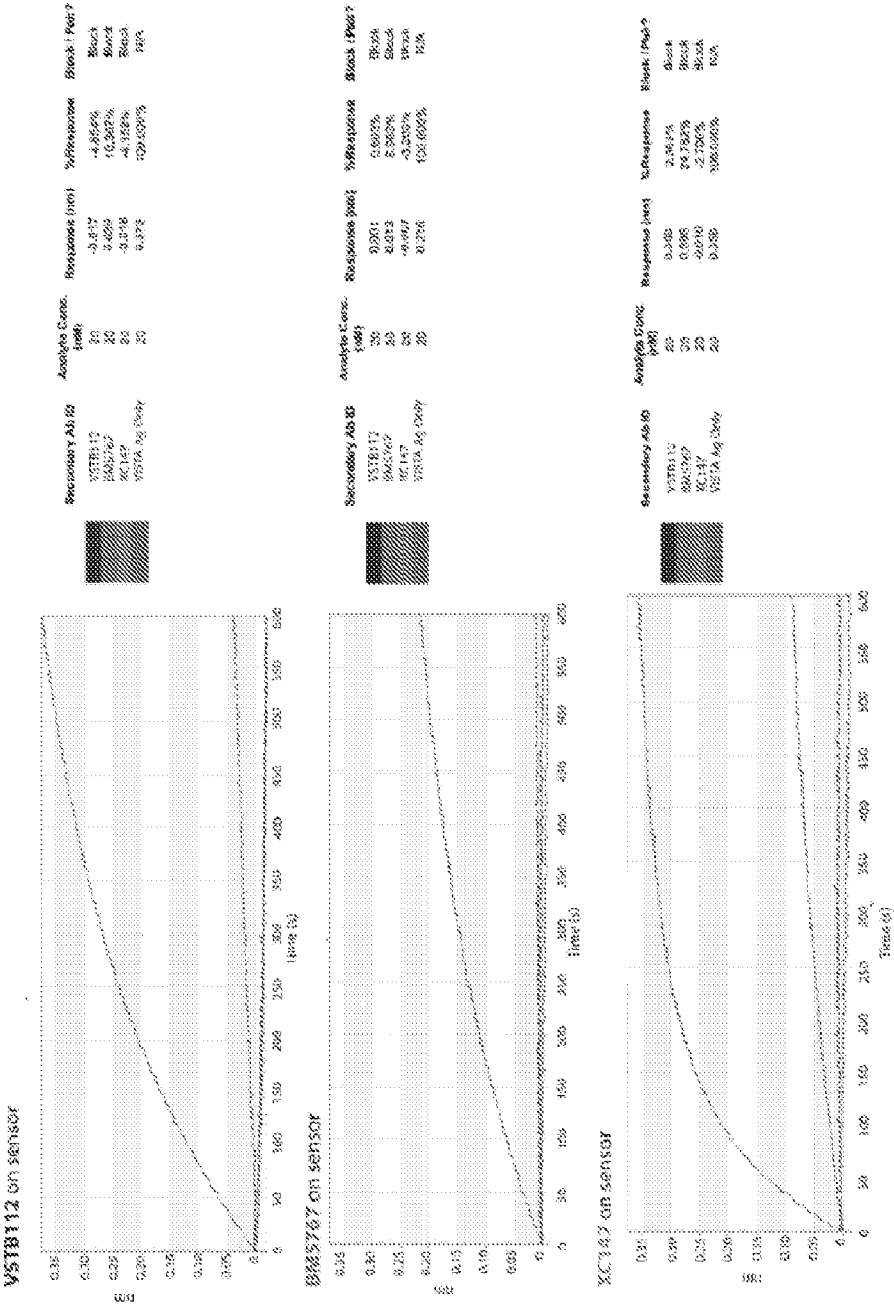


Figure 34

Epitope Binning of XC147 with Competitor Antibodies

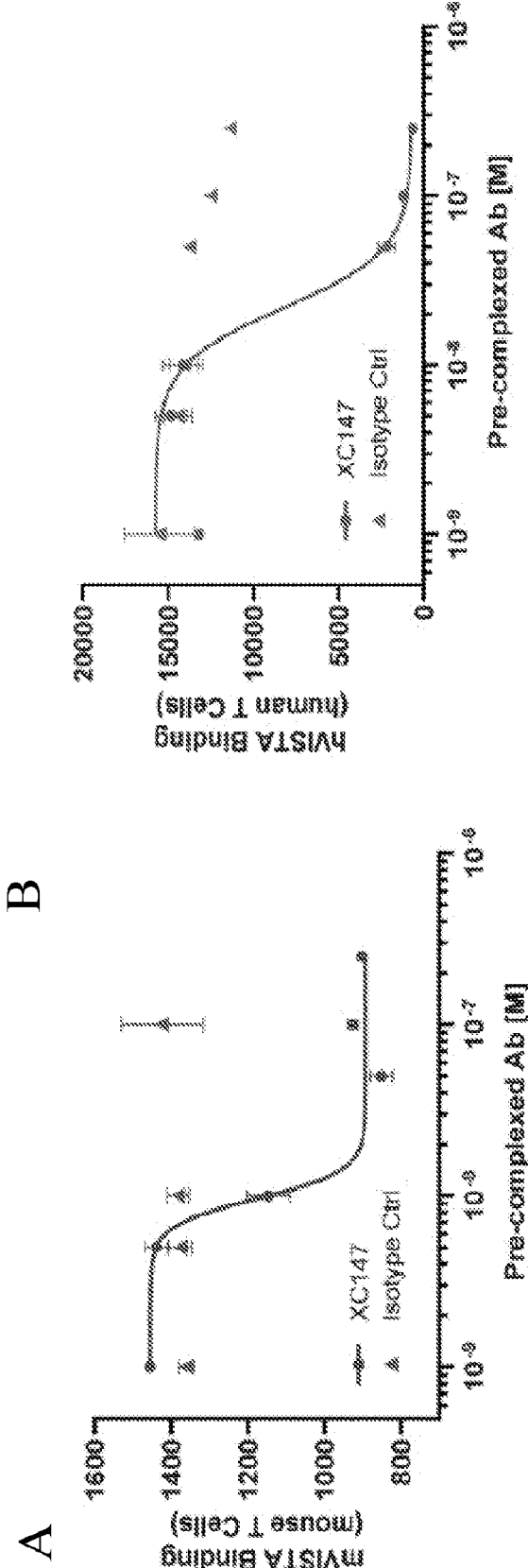
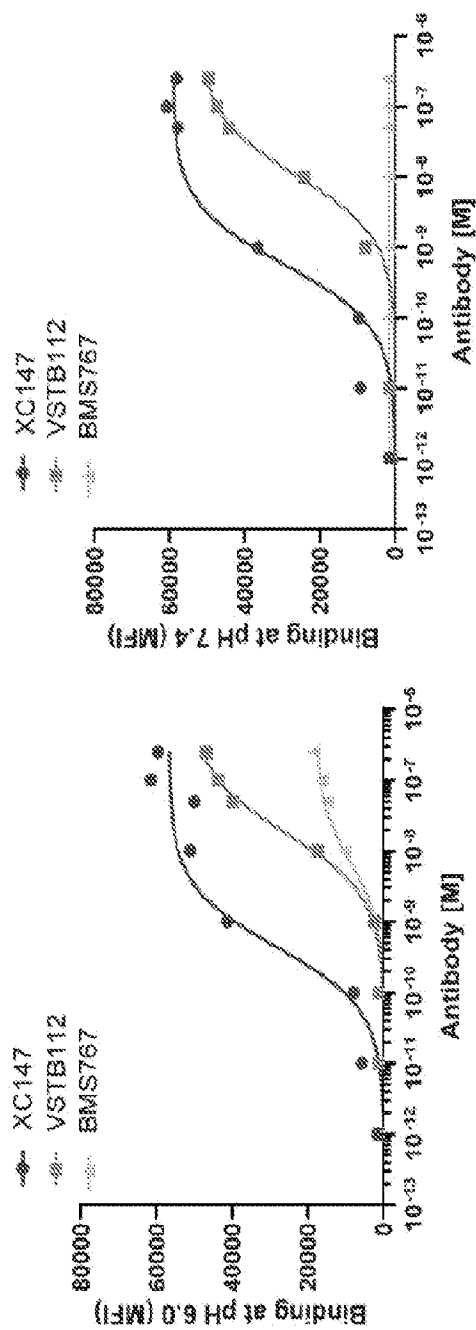


Figure 35

pH Dependence of Antibody Binding to hVISTA



	pH 6.0	pH 7.4
XC147	0.44 +/- 0.3 nM	0.57 +/- 0.4 nM
VSTB112	17 +/- 5 nM	10 +/- 5 nM
BMS767	7 +/- 5 nM	No Binding

Figure 36

Jurkat T Cell Activation Assay

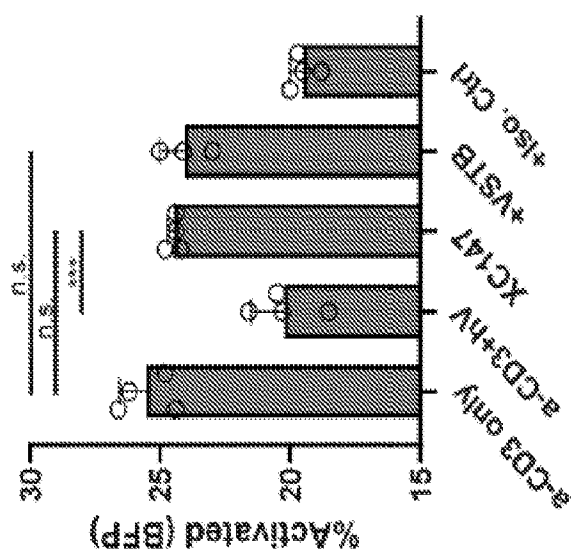


Figure 37

XC147 Epitope Mapping Sorts

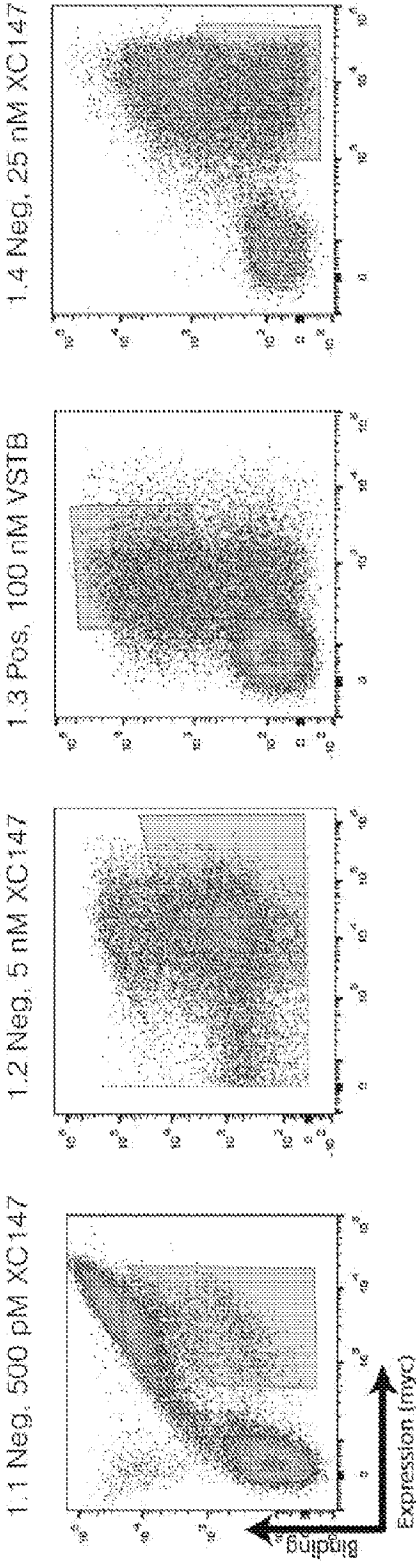


Figure 38

VISTA Enrichment during XC147 Epitope Map Sorts

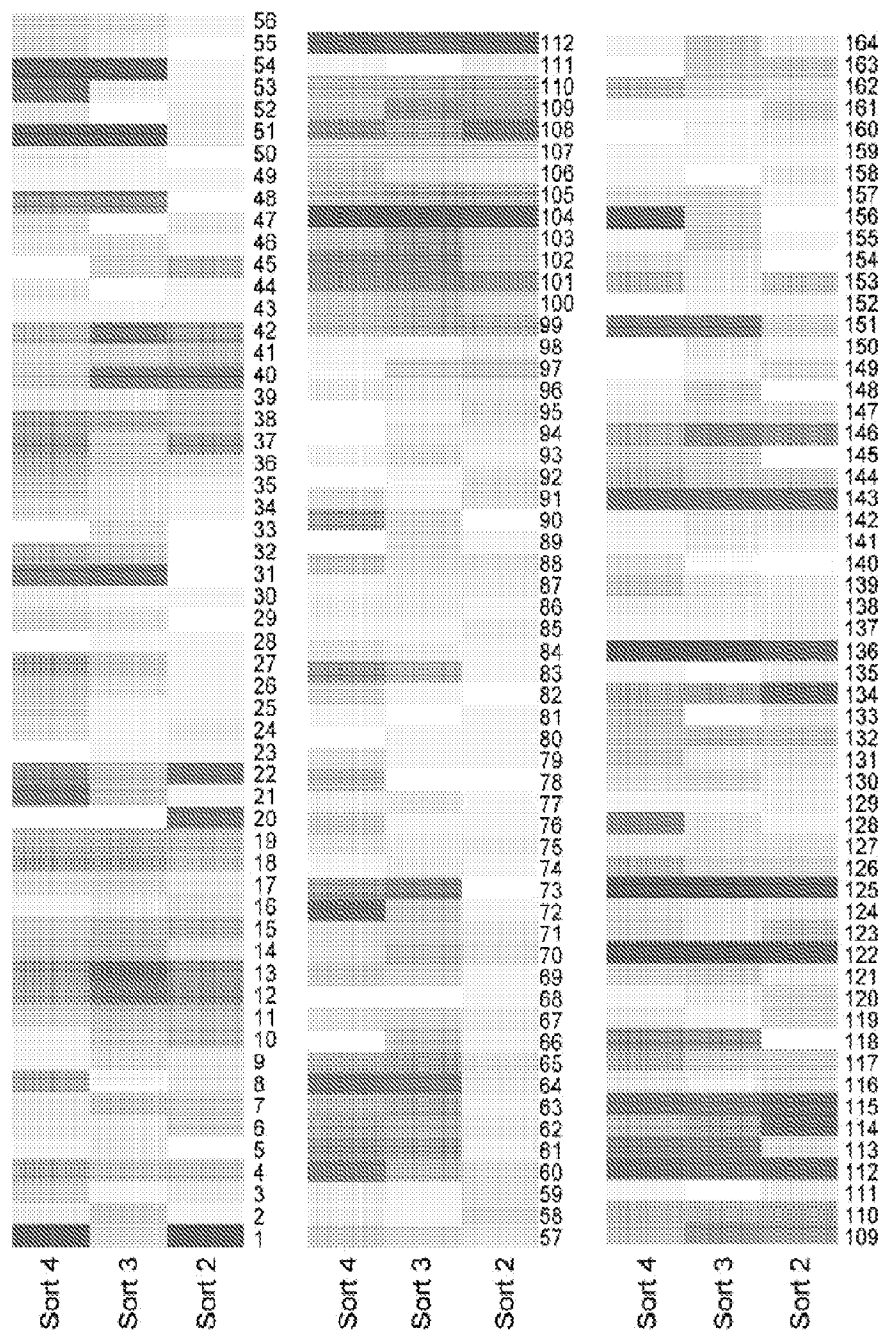


Figure 39

Single clone epitope analysis for XC147, BMS767, and VSTB112

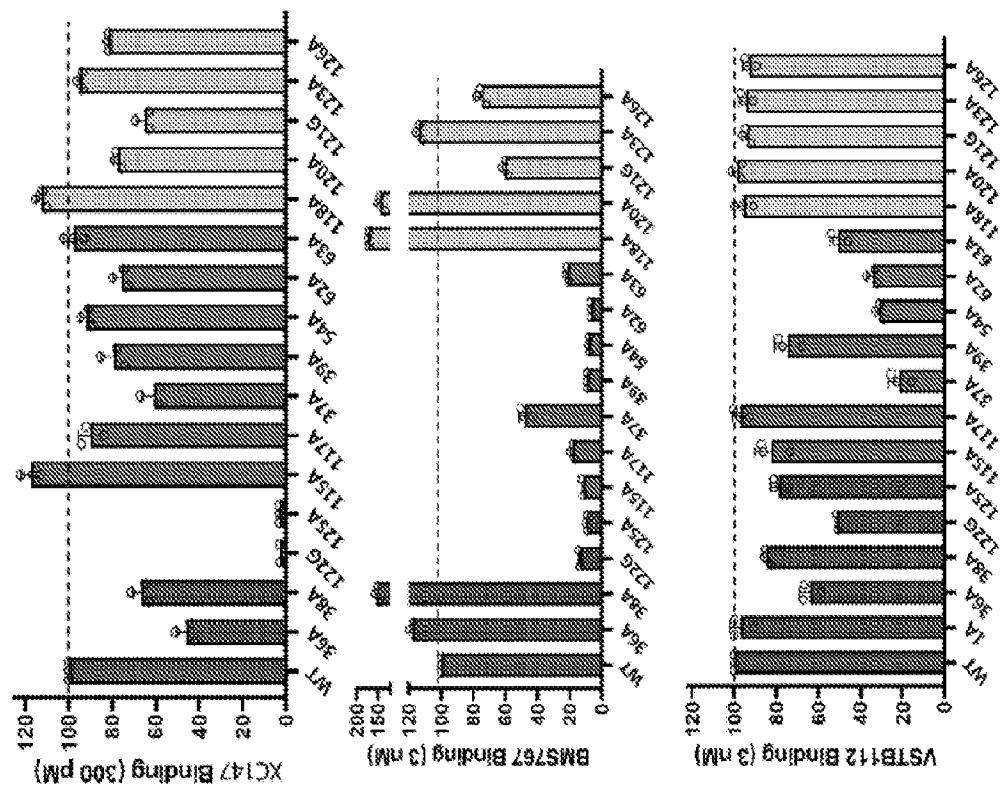


Figure 41

Single clone analysis of mVISTA mutants binding to XC147

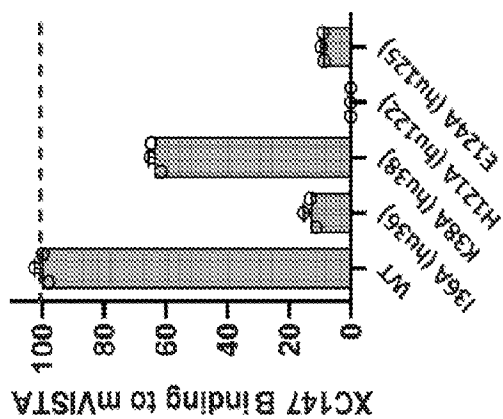


Figure 42

XC147 Competes with binding to PSGL1 and VSIG3

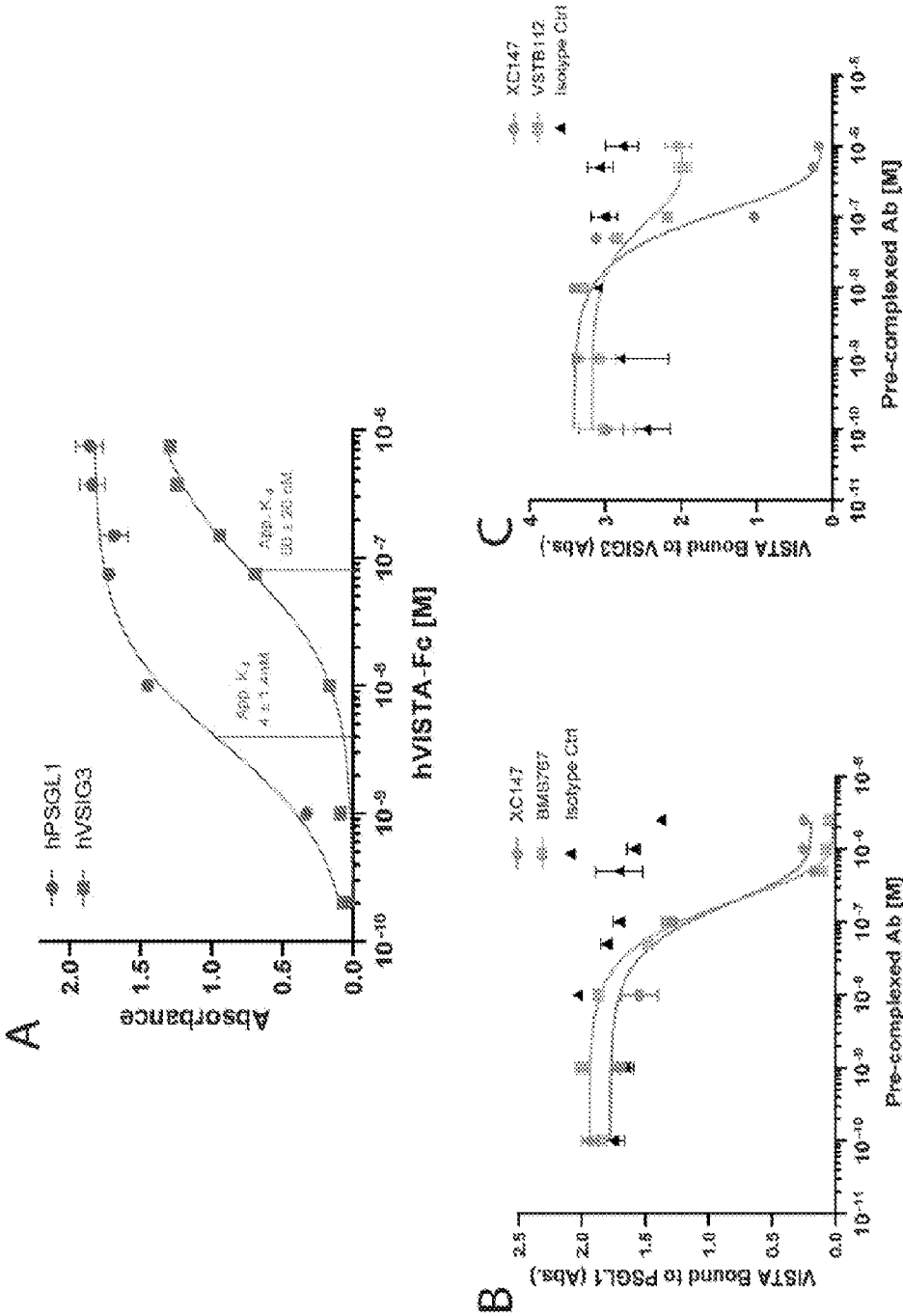


Figure 43

In vivo efficacy of XC147 in B16F10

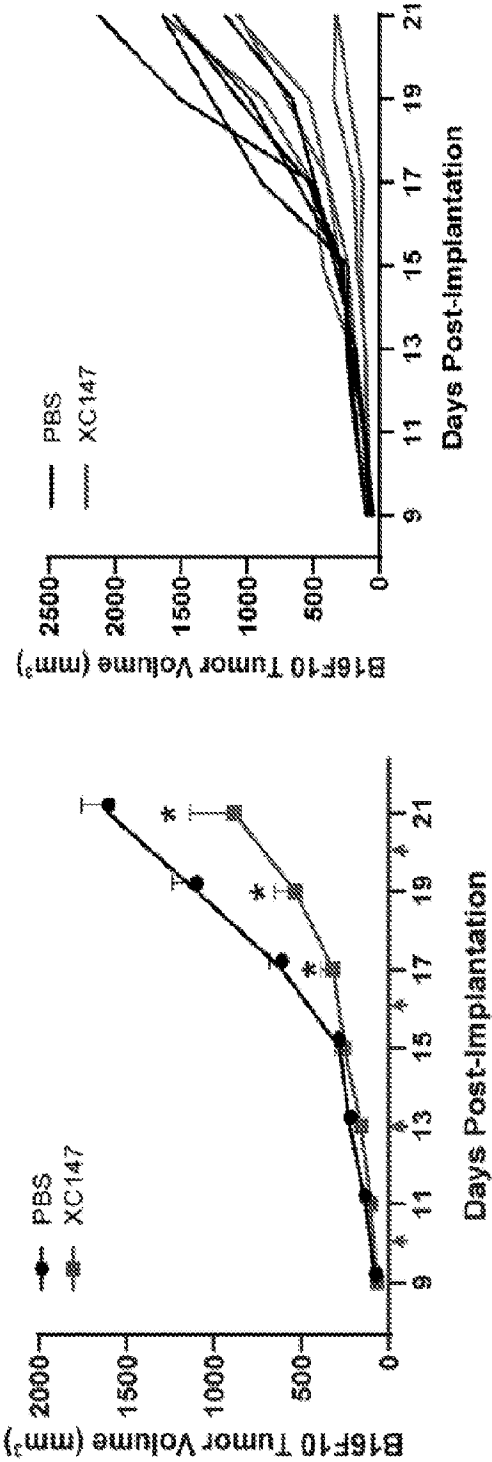


Figure 44

In vivo efficacy of XC147 in combination with PD-1 in MC38

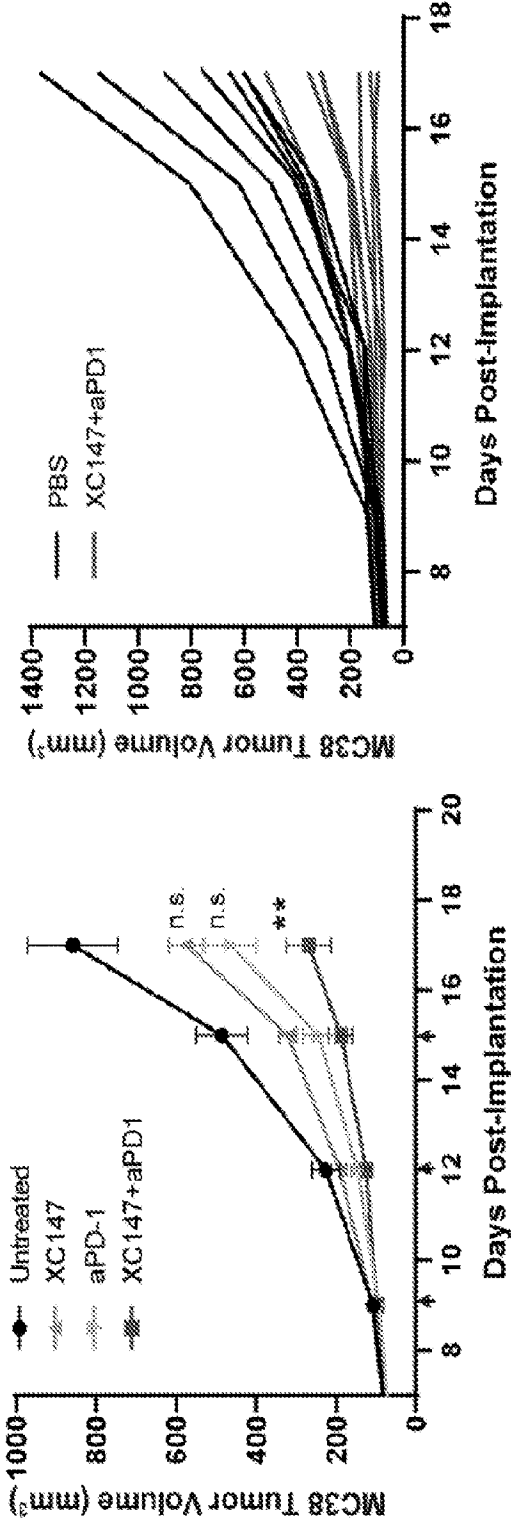
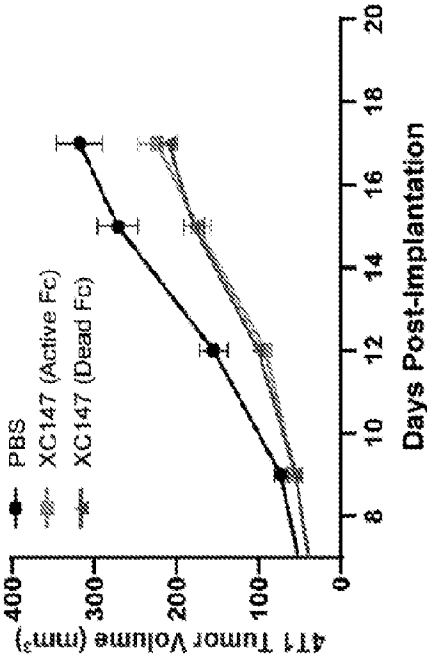


Figure 45

In vivo efficacy of XC147 in 4T1

A



B

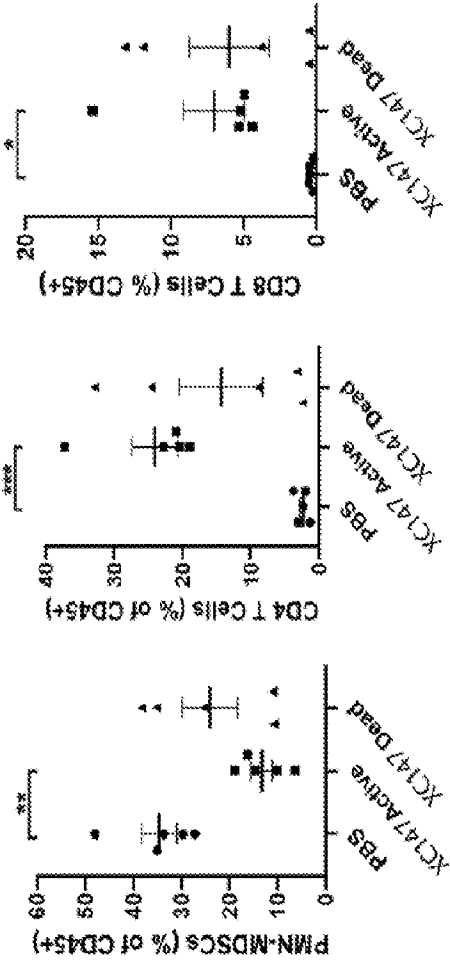


Figure 46A

What	sequence	SEQ ID NO:
Variable heavy (vh) domain Xc147 HC	MGWSLILLFLVAVATGVHSQVQLVQSGAEVKKPGSSVKVSKASGGIFSSYAISWVVRAPGQGGLWMMGGIPIEGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYCARPVRSGPDYLQHWGQGLTVTVSSAKTTAPSVPLAPVCGGTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPALLQSGLYTLSSVTVTSNTWPSQTITCNVAHPASSTKVDDKIEPRVPITQNPCCPLKECPPCAAPDLLGGPSVFIPPKIKDVLMSLSPMVTCVVDVSEDDPDVQISWVFNNEVHTAQTTQTHREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVN NRALPSPIEKTISKPRGPPRAPQVYVLPPEAEEMTKKEFSLTCMITGFLPAEIAVDWTSNGRTEQNYKNTATVLDSDSGSYFMYSKLRV QKSTWERSLFACSVVHEGLHNHLTKTISRSLGK	136
vhCDR1	GGIFSSY	137
vhCDR2	GGIPIFGT	138
vhCDR3	PVRSGPDYLQH	139
Variable light (vl) domain Xc147 LC	MDMRVPAQLLGLLLWLPGARCDIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPKGAPKLLIYDASSLES ~~GVPSR~~ FGSGSGTEFTLTISSLQPD ~~FATYYC~~ QQYN ~~SYSLTF~~ EGGGTKVEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSE QNGVLNSWTDQDSKDYMSSTLTLT ~~TKDEYERHNSY~~ TCEAT ~~HK~~ TSTSPIVKSFNRNEC	140
viCDR1	RASQSISSWLA	141
viCDR2	DASSLES	142
viCDR3	QQYN ~~SYSLTF~~	143
V9.7 (sequence version of Xc147):	DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPKGAPKLLIYDASSLES ~~GVPSR~~ FGSGSGTEFTLTISSLQPD ~~FATYYC~~ QQYN ~~SYSLTF~~ EGGGTKVEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKASGGIFSSYAISWVVRAPGQGGLWMMGGIPIEGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYCARPVRSGPDYLQHWGQGLTVTVSS	144

Figure 46B

What	sequence	SEQ ID NO:
V1	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSLQPD ~~DFATYYCQQ~~ <u>YNSYSITFGQGRLEIKGTTAASGSSGGSSGAEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVA</u> <u>VISYD</u> <u>GSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRSDDTAVYYCARGW</u> <u>SGPSDFDVWGQG</u> TLTVSS	145
vhCDR1	<u>GFTFSSY</u>	146
vhCDR2	<u>AVISYDGSN</u>	147
vhCDR3	<u>GWSGPSDFDV</u>	148
vICDR1	<u>RASQSISSWLA</u>	149
vICDR2	DASSLES	150
vICDR3	<u>QQYNSYSITE</u>	151

Figure 46C

What	sequence	SEQ ID NO:
V2	QSWTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVDPDRFSGSKSGTSASLAITGLQAEDEADY YQSYDSSLGSGSWVEGGGKLTVLGTTAASGSSGSSGGAQLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQHPGKGLE WIGSIYSGSTYYNPSLKSRTISVDTSKNQFSLKLSVTAADTAVYYCARRSTIWGDGAIEYFQHWGQGLTVTVSS	152
vhCDR1	<u>GSISSSY</u>	153
vhCDR2	<u>GSIYSGS</u>	154
vhCDR3	<u>RSTIWGDGAIEYFQH</u>	155
viCDR1	<u>TGSSNIGAGYDVH</u>	156
viCDR2	<u>GNSNRP</u>	157
viCDR3	<u>QSYDSSLGSGWVE</u>	158

Figure 46D

What	sequence	SEQ ID NO:
V3	EIVLTQSPGTLASVGDRTVITCRASQSISSWLAWYQQKPKAPKLLIYDASSLESGVPSRFGSGSGTEFTLTISLQPD ~~DFATYYCQQY~~ <u>NSYSFTEGPGTKVDIKGTTAASGSSGGSSSGAEVQLVESGGGLVQPGGSLRLSCAASGFTESSYAMS~~WVRQAPGKGLEWMGGIPIE~~</u> <u>GTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSVRSGPDYFQH</u> WGQGT ~~LVTVSS~~	159
vhCDR1	GFTESSY	160
vhCDR2	GGIPIFGT	161
vhCDR3	SVRSGPDYFQH	162
vICDR1	RASQSISSWLA	163
vICDR2	DASSLES	164
vICDR3	QQYNSYSFTF	165

Figure 46E

What	sequence	SEQ ID NO:
V4	QSWVTQPPSVSGAPVQRVTISCTGSSSNIGAGYDVHWHYQQLPGTAPKLLIYGNSNRPSGVPD ¹⁶⁶ RFSGSKGTSASLAITGLQAEADY YQ ¹⁶⁶ QSYDSSLGVEGTGKVTVLGTTAASGSSGGSSGGAQLQEQESGPGLVKPS ¹⁶⁶ ETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIG EINHSGSTNYPNPSLKSRVTISVDTSKNQFSLKSSVTAADTAVYYCARVDNDY ¹⁶⁶ SVGSGMDVWGGQGLTVTVSS	166
vhCDR1	<u>GSISSSSY</u>	167
vhCDR2	<u>GEINHS¹⁶⁸GS</u>	168
vhCDR3	<u>VDNDYSYVGSYGMDV</u>	169
vICDR1	<u>TGSSSNIGAGYDVH</u>	170
vICDR2	<u>GNSNRP</u>	171
vICDR3	<u>QSYDSSLGVE</u>	172

Figure 46F

What	sequence	SEQ ID NO:
V5	QSWVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGV ¹⁷³ PD ¹⁷³ RFSGSKSGTSASLAITGLQAEDEADY Y ¹⁷³ CQSYDSSLSGV ¹⁷³ VEGGGKLTVLGTTAASGSSGGSSGGAQVQLVQSGAEVKKPGSSVKV ¹⁷³ CKASGGTFSSYAISWVRQAPGQGLEW MGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARYDFVGGYTDYWGQGT ¹⁷³ LVTVSS	173
vhCDR1	GGTFSSY	174
vhCDR2	GGIPIFGT	175
vhCDR3	YDFVGGYTDY	176
vICDR1	TGSSSNIGAGYDVH	177
vICDR2	GNSNRP	178
vICDR3	QSYDSSLSGVVF	179

Figure 46G

What	sequence	SEQ ID NO:
V6	DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSLQPD ~~DFATYYCQQ~~ <u>YNSYSLTEGGG</u> TKVEIKGTTAASGSSGSSGA ~~EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGS~~ <u>GGSTYYADSVKGRFTISRDN</u> SKNTLYLQMNSLRAEDTAVYYCAR ~~LEDWLGGYILEYFDL~~ WGQGTLLTVSS	180
vhCDR1	GFTFSY	181
vhCDR2	SAISGSGGS	182
vhCDR3	LEDWLGGYILEYFDL	183
viCDR1	RASQSISSWLA	184
viCDR2	DASSLES	185
viCDR3	<u>QQYNSYSLTE</u>	186

Figure 46H

What	sequence	SEQ ID NO:
V7	DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSRFSGSGSGTEFTLT~~ ISSLPD ~~DFATYYCQQYN~~ SYSLT ~~FGGGTKLEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKV~~ SCKASGGTFESSYAISWVRQAPGGGLEWMGGIIPF ~~GTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGVRS~~ GGPDYFQHWGGQGLTVTVSS	187
vhCDR1	GGTFSSY	188
vhCDR2	GGIPIFGT	189
vhCDR3	GVRSGPDYFQH	190
vICDR1	RASQSISSWLA	191
vICDR2	DASSLES	192
vICDR3	QQYNSYSLTF	193

Figure 461

What	sequence	SEQ ID NO:
V8	QSWVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVDPDRFSGSKSDTSASLAITGLQAEDEADY YQSYDSSLSGWVEGGGTKLTVLGTTAASGSSGGSSGA ~~EVQLVESGGGLVQPGGSLRLSCAASGYTFTSY~~ MYMHWVRQAPGQGGLF WMGIINPSSGGSTSYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARAGVGYWDPLSDAFDVWGGQGLTVTVSS	194
vhCDR1	GYTFTSY	195
vhCDR2	GIINPSSGS	196
vhCDR3	AGVGYWDPLSDAFDV	197
viCDR1	TGSSNIGAGYDVH	198
viCDR2	GNSNRP	199
viCDR3	QSYDSSLSGWVF	200

Figure 46J

What	sequence	SEQ ID NO:
V9 (parent of XC147)	DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSLQPD ~~DFATYYCQQ~~ <u>YNSYSLTEGGG</u> TKVEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSCKASGGTFESSYAISWVRQAPGQGLEWMG <u>GIIPIE</u> <u>GTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARPVRSGPDYFQH</u> HWGQGTLTVSS	201
vhCDR1	GGTFSSY	202
vhCDR2	GGIPIFGT	203
vhCDR3	PVRSGPDYFQH	204
viCDR1	RASQSISSWLA	205
viCDR2	DASSLES	206
viCDR3	QQYNSYSLTF	207

Figure 46K

What	sequence	SEQ ID NO:
V10	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGGTDFTLTISSLQPEDFATYYCQQ SYSTPYTEGGQGTKLEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVVRQAPGQGLEWMGGIPIF GTANYAQKFQGRVTITADESTSTAYMIELSSLRSEDTAVYYCARGW/SARYGMDVW/GQGTLTVTVSS	208
vhCDR1	GGTFSSY	209
vhCDR2	GGIPIFGT	210
vhCDR3	GWSARYGMDVW	211
vICDR1	RASQSISSYLN	212
vICDR2	AASSLOS	213
vICDR3	QQSYSTPYTF	214

Figure 46L

What	sequence	SEQ ID NO:
V11	QSVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWHYQQLPGTAPKLLIYGNSNRPSGVSNRFSGSKSGTSASLAITGLQAEDEADY YQSYDSSLSGVVEGGGKLTVLGTTAASGSSGGSSGAEVQLVESGGGLVQPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLE WVSAISGSGGGSTYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARLDWPLSYGGSGYYYGMDVWGQGTLLTVSS	215
vhCDR1	GFTFSNA	216
vhCDR2	SAISGSGGS	217
vhCDR3	LDWPLSYGGSGYYYGMDV	218
vICDR1	TGSSSNIGAGYDVH	219
vICDR2	GNSNRP	220
vICDR3	QSYDSSLSGVVF	221

Figure 46M

What	sequence	SEQ ID NO:
V12	DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSLQPD ~~DFATYYCQQ~~ <u>YNSYSLTEGGG</u> TKVEIKGTTAASGSSGGSSGA ~~EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGISWVRQA~~ PGQG ~~LEWMGWISAY~~ <u>NGNTNYAQKLQGRVTMTTDTSTAYMELRSLRSDDTAVYCARVWYSGFSD</u> FDSWGQGTLVTVSS	222
vhCDR1	GFTFSY	223
vhCDR2	GWISAYNGN	224
vhCDR3	VWYSGFSDFDS	225
vICDR1	RASQSISSWLA	226
vICDR2	DASSLES	227
vICDR3	QQYN ~~SYSLTF~~	228

Figure 46N

What	sequence	SEQ ID NO:
V13	DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSLQPD ~~DFATYYCQQ~~ <u>YNSYSITFGQGT</u> RL ~~EIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSCKASGGTFESSYAISWVRQA~~ PGQGLEW/MGGIIPF <u>GTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSVRSGPDYFQH</u> WGQGTLTVSS	229
vhCDR1	GGTFSSY	230
vhCDR2	GGIPIFGT	231
vhCDR3	SVRSGPDYFQH	232
viCDR1	RASQSISSWLA	233
viCDR2	DASSLES	234
viCDR3	QQYNSYSITF	235

Figure 460

What	sequence	SEQ ID NO:
V14	QSWVTQPPSVSAAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVDPDRFSGSKSGTSASLAITGLQAEADY YQSYDSSLSGVVEGGGKLTVLGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSCCKASGFTFSSYAMSWVRQAPGGQGLEW VSAISGGGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGWVSVTDDFDSWGQGTLVTVSS	236
vhCDR1	GFTFSSY	237
vhCDR2	SAISGGGS	238
vhCDR3	GWVSVTDDFDS	239
vICDR1	TGSSSNIGAGYDVH	240
vICDR2	GNSNRP	241
vICDR3	QSYDSSLSGVVF	242

Figure 46P

What	sequence	SEQ ID NO:
V15	DIQMTQSPSSLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FGSGSGTEFTLTISSLQPD ~~DFATYYCQQ~~ YNSYSLTEGGGKVEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGASVKVSCKASGYTFSSYAISWVRQAPGQGLEWMGGIIPF GTANYAQKFQGRVTITADESTSTAYMIELSSLRSEDTAVYYCARSVRSGPDYFQH ~~WGQGTL~~ TVSS	243
vhCDR1	GYTFSSY	244
vhCDR2	GGIPIFGT	245
vhCDR3	SVRSGPDYFQH	246
vICDR1	RASQSISSWLA	247
vICDR2	DASSLES	248
vICDR3	QQYNSYSLTF	249

Figure 46Q

What	sequence	SEQ ID NO:
V16	QSWVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVDPDRFSGSKSGTSATLGITGLQTGDEADY YCGTWDSSLSGYVEGTGKVTVLGTTAASGSSGGSSGAQVQLVQSGAEVKKPGASVKVSCKASGYTFTISYGISWVRQAPGGQGLEW MGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDEFGYYSSAYFDYWGQGTLVTVSS	250
vhCDR1	GYTFTSY	251
vhCDR2	GWISAYNGN	252
vhCDR3	DFGYSSAYFDY	253
vICDR1	TGSSSNIGAGYDVH	254
vICDR2	GNSNRP	255
vICDR3	GTWDSSLSGYVF	256

Figure 46R

What	sequence	SEQ ID NO:
V17	SYELTQPPSVSPGQTASITCSGD ~~KL~~ GDKYACWYQQKPGQSPVLVIYQDSKRPSGIPERFSGSNSGNTATLTISGTQAMDEADYYCQ <u>AWDSSTAV</u> EGTGKVTVLGTTAASGSSGGSSGAQVQLVESGGGVQPGRSLRLSCAASGFTFESSYAMHWVRQAPGKGLEWVAVI <u>SYDGSN</u> KYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARYGRRYTPYYWYFDLWGQGTLVTVSS	257
vhCDR1	GFTFSSY	258
vhCDR2	AVISYDGSN	259
vhCDR3	YGRRYTPYYWYFDL	260
viCDR1	SGDKLGD ~~K~~ YAC	261
viCDR2	QDSKRP	262
viCDR3	QAWDSSTAVF	263

Figure 46S

What	sequence	SEQ ID NO:
V18	QSWVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVDPDRFSGSKSGTSASLAITGLQAEADY YQSYDSSLSGWFGTGTKVTVLGTTAASGSSGGSSGAQVQLVESGGGVVQPGRSRLSCAASGFTFSSYAMHHWV/RQAPGKGLEW VAVISYDGSNKYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARGWSTVGDDFDLWGQGTLLTVSS	264
vhCDR1	GFTFSY	265
vhCDR2	AVISYDGSN	266
vhCDR3	GWSTVGDDFDL	267
vICDR1	TGSSSNIGAGYDVH	268
vICDR2	GNSNRP	269
vICDR3	QSYDSSLSGYVF	270

Figure 46T
VISTA 0.5.9

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARPVRSGPDYFQHWGQGTLVTVSSGSEQKLISEEDL	271
vhCDR1	<u>GGTFSSYA</u>	272
vhCDR2	<u>IPIFGTA</u>	273
vhCDR3	<u>CARPVRSGPDYFQHW</u>	274
Variable light (vl) domain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FSGSGSGTEFTLTISLQPDDFAITYYCCQQYN ~~SYSLTF~~ GGGKVEIK	275
vlCDR1	<u>RASQSISSWLA</u>	276
vlCDR2	<u>DASSLES</u>	277
vlCDR3	<u>QQYN~~SYSLTF~~</u>	278
Full Protein Sequence	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FSGSGSGTEFTLTISLQPDDFAITYYCCQQYN ~~SYSLTF~~ GGGKVEIKGTTASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARPVRSGPDYFQHWGQGTLVTVSSGSEQKLISEEDL	279

Figure 46U
VISTA1.4.1

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSCKAPGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARPVRSGPDYLOHWGQGTLVTVSS	280
vhCDR1	<u>GGTFSSYA</u>	281
vhCDR2	<u>IPIFGTA</u>	282
vhCDR3	<u>CARPVRSGPDYLOHW</u>	283
Variable light (vl) domain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPPRF~~ SGSGSGAEFTLTISSLQPEDDFA ~~FATY~~ YCQQYN ~~SYSLTF~~ GGG ~~TKVEIK~~	284
vlCDR1	<u>RASQSISSWLA</u>	285
vlCDR2	<u>DASSLES</u>	286
vlCDR3	<u>QQYN~~SYSLTF~~</u>	287
Full Protein Sequence	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPPRF~~ SGSGSGAEFTLTISSLQPEDDFA ~~FATY~~ YCQQYN ~~SYSLTF~~ GGG ~~TKVEIK~~ TAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSCKAPGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARPVRSGPDYLOHWGQGTLVTVSS	288

Figure 46V
VISTA1.4.2

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSKAPGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYCARPVRSGPDYLOHWGQGTLVTVSS	289
vhCDR1	<u>GGTFSSYA</u>	290
vhCDR2	<u>IPIFGTA</u>	291
vhCDR3	<u>CARPVRSGPDYLOHW</u>	292
Variable light (vl) domain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FSGSGSGTEFTLTISLQPDDFAITYCQQYNSYSLT ~~TFGGG~~ TKVEIK	293
vlCDR1	<u>RASQSISSWLA</u>	294
vlCDR2	<u>DASSLES</u>	295
vlCDR3	<u>QQYNSYSLT~~TF~~</u>	296
Full Protein Sequence	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FSGSGSGTEFTLTISLQPDDFAITYCQQYNSYSLT ~~TFGGG~~ TKVEIKGTTASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKAPGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYCARPVRSGPDYLOHWGQGTLVTVSS	297

Figure 46W
VISTA1.4.3

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSKAPGGIFSSYAISWVRQAPGQGLEWMGGMGGIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYCARPVRS ~~GGPDYFQHW~~ GQGTLVTVSS	298
vhCDR1	<u>GGIFSSYA</u>	299
vhCDR2	<u>IPIFGTA</u>	300
vhCDR3	<u>CARPVRS~~GGPDYFQHW~~</u>	301
Variable light (vl) domain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLA ~~WYQQKPGKAPKLLIYDASSLES~~ GVPSRFSGSGSGTEFTLTISLQPDDEFATYYCQQYNYSY ~~SITFGQGTRLEIK~~	302
vlCDR1	<u>RASQSISSWLA</u>	303
vlCDR2	<u>DASSLES</u>	304
vlCDR3	<u>QQYNYSY~~SITF~~</u>	305
Full Protein Sequence (scFv)	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLA ~~WYQQKPGKAPKLLIYDASSLES~~ GVPSRFSGSGSGTEFTLTISLQPDDEFATYYCQQYNYSY <del>SITFGQGTRLEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKAPGGIFSSYAISWVRQAPGQGLEWMGGMGGIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYCARPVRS~~GGPDYFQHW~~GQGTLVTVSS</del>	306

Figure 46X
VISTA1.4.4

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSKASGGIFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFRGRVTITA DESTSTAYMELSSLRSEDTAVYYC <u>CARPVRSGPDYFQHWGQGT</u> LVTVSS	307
vhCDR1	<u>GGIFSSYA</u>	308
vhCDR2	<u>IIPIFGTA</u>	309
vhCDR3	<u>CARPVRSGPDYFQHW</u>	310
Variable light (vl) domain	DIQMTQSPSTLSASVGGGRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES <u>GVPSRFS</u> SGSGTEFTLT ISLQPDDEFATYYCQQYNYSY <u>SITFGQG</u> TRLEIK	311
vlCDR1	<u>RASQSISSWLA</u>	312
vlCDR2	<u>DASSLES</u>	313
vlCDR3	<u>QQYNYSY</u> <u>SITF</u>	314
Full Protein Sequence	DIQMTQSPSTLSASVGGGRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES <u>GVPSRFS</u> SGSGTEFTLT ISLQPDDEFATYYCQQYNYSY <u>SITFGQG</u> TRLEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSK ASGGIFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFRGRVTITADESTSTAYMELSSLRSEDTAVYYC <u>ARPVRSGPDYFQHWGQGT</u> LVTVSS	315

Figure 46Y
VISTA1.4.5

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSKASGGIFSSYAISWVRQAPGQGLEWMGGLIPIFGTANYAQKFFQGRVTITADESTAYMELSSLRSEDTAVYYCARPVRSGPDYFQHWGQGTLVTVSS	316
vhCDR1	<u>GGIFSSYA</u>	317
vhCDR2	<u>IPIFGTA</u>	318
vhCDR3	<u>CARPVRSGPDYFQHW</u>	319
Variable light (vl) domain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FSGSGSGTEFTLTISLQPDDFAITYYCCQQYN ~~SYSLTF~~ GGGKVEIK	320
vlCDR1	<u>RASQSISSWLA</u>	321
vlCDR2	<u>DASSLES</u>	322
vlCDR3	<u>QQYN~~SYSLTF~~</u>	323
Full Protein Sequence	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FSGSGSGTEFTLTISLQPDDFAITYYCCQQYN ~~SYSLTF~~ GGGKVEIKGTTASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKASGGIFSSYAISWVRQAPGQGLEWMGGLIPIFGTANYAQKFFQGRVTITADESTAYMELSSLRSEDTAVYYCARPVRSGPDYFQHWGQGTLVTVSS	324

Figure 46Z
VISTA1.4.6

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSC EASGGIFESSYAISWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYC ARAVRSGPDYFQHWGQGT LVTVSS	325
vhCDR1	<u>GGIFESSYA</u>	326
vhCDR2	<u>IIP</u> IFGT A	327
vhCDR3	<u>CARAVRSGPDYFQHW</u>	328
Variable light (vl) domain	DIQMTQSPSSLSASVGD RVTTITCRASQSISSWLA WYQQKPGKAPKLLIYD ASSLES GVPSRFSGSGSGTEFTLTIS SSLQ PDDEFATYYC QQYN SYSLT TFGGG TKVEIK	329
vlCDR1	<u>RASQSISSWLA</u>	330
vlCDR2	<u>DASSLES</u>	331
vlCDR3	<u>QQYNSYSLTF</u>	332
Full Protein Sequence	DIQMTQSPSSLSASVGD RVTTITCRASQSISSWLA WYQQKPGKAPKLLIYD ASSLES GVPSRFSGSGSGTEFTLTIS SSLQ PDDEFATYYC QQYN SYSLT TFGGG TKVEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSC EASGGIFESSYAISWVRQAPGQGLEWMGGIIP IFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYC ARAVRSGPDYFQHWGQGT LVTVSS	333

Figure 46AA
VISTA1.4.7 (VS147)

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSKASGGIFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYCARPVRSGPDYLOHWGQGTLVTVSS	334
vhCDR1	<u>GGIFSSYA</u>	335
vhCDR2	<u>IIPIFGTA</u>	336
vhCDR3	<u>CARPVRSGPDYLOHW</u>	337
Variable light (vl) domain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FSGSGSGTEFTLTISLQPDDFAITYYCCQQYN ~~SYSLTF~~ GGGKVEIK	338
vlCDR1	<u>RASQSISSWLA</u>	339
vlCDR2	<u>DASSLES</u>	340
vlCDR3	<u>QQYN~~SYSLTF~~</u>	341
Full Protein Sequence	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FSGSGSGTEFTLTISLQPDDFAITYYCCQQYN ~~SYSLTF~~ GGGKVEIKGTTASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKASGGIFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYCARPVRSGPDYLOHWGQGTLVTVSS	342

Figure 46BB
VISTA1.4.8

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVRVSKVSGGIFSSYAISWVVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYCARPVRSRSGPDYFQHWGQGTLVTVSS	343
vhCDR1	<u>GGIFSSYA</u>	344
vhCDR2	<u>IIPIFGTA</u>	345
vhCDR3	<u>CARPVRSRSGPDYFQHW</u>	346
Variable light (vl) domain	DIQMTQSPSTLSASVGDRVTVTTCRASQSSSWLAWYQQKPGKAPKLLIYDASSLESQVPSRFSGSGSGTEFTLTISSLQPEDDFATYYCQQYNSSYSLTFGGGTKVEIK	347
vlCDR1	<u>RASQSSSWLA</u>	348
vlCDR2	<u>DASSLES</u>	349
vlCDR3	<u>QQYNSSYSLTF</u>	350
Full Protein Sequence	DIQMTQSPSTLSASVGDRVTVTTCRASQSSSWLAWYQQKPGKAPKLLIYDASSLESQVPSRFSGSGSGTEFTLTISSLQPEDDFATYYCQQYNSSYSLTFGGGTKVEIKGTTASGSSGGSSGAQVQLVQSGAEVKKPGSSVRVSKVSGGIFSSYAISWVVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYCARPVRSRSGPDYFQHWGQGTLVTVSS	351

Figure 46CC
V1

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRSDDTAVYYCARGWSGPSDFDVWGQGLVTVSS	352
vhCDR1	<u>GFTFSYA</u>	353
vhCDR2	<u>ISYDGSNK</u>	354
vhCDR3	<u>CARGWSGPSDFDVWG</u>	355
Variable light (vl) domain	DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLESVPSRFSGSGTEFTLTISLQPDDEFATYYCQQYNYSYITFGQGRLEIK	356
vlCDR1	<u>RASQSISSWLA</u>	357
vlCDR2	<u>DASSLES</u>	358
vlCDR3	<u>QQYNYSITE</u>	359
Full Protein Sequence	DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLESVPSRFSGSGTEFTLTISLQPDDEFATYYCQQYNYSYITFGQGRLEIKGTTAASGSSGSSGAEVQLVESGGGLVQPGGSLRLSCAASGFTFSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRSDDTAVYYCARGWSGPSDFDVWGQGLVTVSS	360

Figure 46DD
V2

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWRQHPGKGLEWIGSIYSGSTYYNP ³⁶¹ PSLKSRVTISVD TSKNQFSLKLSSTVAADTAVYYCARRSTIWGDGA ³⁶¹ EYFQHWGQGTLVTVSS	361
vhCDR1	<u>GGSISSSSYY</u>	362
vhCDR2	<u>IYYSGST</u>	363
vhCDR3	<u>CARRSTIWGDGA³⁶⁴EYF</u>	364
Variable light (vl) domain	QSVVTQPPSVSGAPGQRTITISCTGSSSNIGAGYDVH ³⁶⁵ WYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSA SLAITGLQAEDEADYYCQSYDSSLSGSWVFGGGTKLTVL	365
vlCDR1	<u>TGSSSNIGAGYDVH</u>	366
vlCDR2	<u>GNSNRPS</u>	367
vlCDR3	<u>QSYDSSLSGSWVF</u>	368
Full Protein Sequence	QSVVTQPPSVSGAPGQRTITISCTGSSSNIGAGYDVH ³⁶⁹ WYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSA SLAITGLQAEDEADYYCQSYDSSLSGSWVFGGGTKLTVLGT ³⁶⁹ TAAAGSSGGSSGAQLQLQESGPGLVKPSET LSLTCTVSGGSISSSSYYWGWRQHPGKGLEWIGSIYSGSTYYNP ³⁶⁹ PSLKSRVTISVDTSKNQFSLKLSSTVTA DTAVYYCARRSTIWGDGA ³⁶⁹ EYFQHWGQGTLVTVSS	369

Figure 46EE
V3

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGFTFESSYAMSWVRQAPGKGLEWMGGIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYCARSVRSGPDYFQHWGQGTLVTVSS	370
vhCDR1	<u>GFTFESSYA</u>	371
vhCDR2	<u>IPIPIFGTA</u>	372
vhCDR3	<u>CARSVRSGPDYFQHW</u>	373
Variable light (vl) domain	EIVLTQSPGTLSASVGDVRVITITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLESVPSRFSGSGSGTEFTLTISLQPDDEFATYYCQQYNYSYSEFTFGPGTKVDIK	374
vlCDR1	<u>RASQSISSWLA</u>	375
vlCDR2	<u>DASSLES</u>	376
vlCDR3	<u>QQYNYSYSEFTF</u>	377
Full Protein Sequence	EIVLTQSPGTLSASVGDVRVITITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLESVPSRFSGSGSGTEFTLTISLQPDDEFATYYCQQYNYSYSEFTFGPGTKVDIKGTTAASGSSGSSSGAEVQLVESGGGLVQPGGSLRLSCAASGFTFESSYAMSWVRQAPGKGLEWMGGIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYCARSVRSGPDYFQHWGQGTLVTVSS	378

Figure 46FF
V4

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIROPKGLEWIGENHSGSTNYPNPSLKSRVTISVD TSKNQFSLKLSSTVTAADTAVYYCARVDNDYSYVGSYGMDVWGQGTLVTVSS	379
vhCDR1	<u>GGSISSSSYY</u>	380
vhCDR2	<u>INHSGST</u>	381
vhCDR3	<u>CARVDNDYSYVGSYG</u>	382
Variable light (vl) domain	QSVVTQPPSVSGAPVQRTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSA SLAITGLQAEDEADYYCQSYDSSLGSGVFGTGTKVTVL	383
vlCDR1	<u>TGSSSNIGAGYDVH</u>	384
vlCDR2	<u>GNSNRPS</u>	385
vlCDR3	<u>QSYDSSLGSGVF</u>	386
Full Protein Sequence	QSVVTQPPSVSGAPVQRTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSA SLAITGLQAEDEADYYCQSYDSSLGSGVFGTGTKVTVLGTTAASGSSGGSSGAQLQLQESGPGLVKPSETLS LTCTVSGGSISSSSYYWGWIROPKGLEWIGENHSGSTNYPNPSLKSRVTISVDTSKNQFSLKLSSTVTAADT AVYYCARVDNDYSYVGSYGMDVWGQGTLVTVSS	387

Figure 46GG
V5

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARYDFVGGYTDYWGGQTLVTVSS	388
vhCDR1	<u>GGTFSSYA</u>	389
vhCDR2	<u>IPIFGTA</u>	390
vhCDR3	<u>CARYDFVGGYTDYWG</u>	391
Variable light (vl) domain	QSVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSA SLAITGLQAEDEADYYCQSYDSSLSGVVFSGGTKLTVL	392
vlCDR1	<u>TGSSSNIGAGYDVH</u>	393
vlCDR2	<u>GNSNRPS</u>	394
vlCDR3	<u>QSYDSSLSGVVF</u>	395
Full Protein Sequence	QSVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSA SLAITGLQAEDEADYYCQSYDSSLSGVVFSGGTKLTVLGTAAAGSSGGSSGAQVQLVQSGAEVKKPGSS VKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSE DTAVYYCARYDFVGGYTDYWGGQTLVTVSS	396

Figure 46HH
V6

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGFTFTSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLEDWLGGYILEYFDLWGQGTLVTVSS	397
vhCDR1	<u>GFTFTSSYA</u>	398
vhCDR2	<u>ISGSGGST</u>	399
vhCDR3	<u>CARLEDWLGGYILEY</u>	400
Variable light (vl) domain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FSGSGSGTEFTLTISLQPD ~~DF~~ ATYYCQQYN ~~SYSLTF~~ GGGKVEIK	401
vlCDR1	<u>RASQSISSWLA</u>	402
vlCDR2	<u>DASSLES</u>	403
vlCDR3	<u>QQYN~~SYSLTF~~</u>	404
Full Protein Sequence	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FSGSGSGTEFTLTISLQPD ~~DF~~ ATYYCQQYN ~~SYSLTF~~ GGGKVEIKGTTAASGSSGGSSGAEVQLVESGGGLVQPGGSLRLSCAASGFTFTSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLEDWLGGYILEYFDLWGQGTLVTVSS	405

Figure 46II
V7

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGVRS ~~GP~~ DYFQHWGQGTLVTVSS	406
vhCDR1	<u>GGTFSSYA</u>	407
vhCDR2	<u>IIPIFGTA</u>	408
vhCDR3	<u>CARGVRS~~GP~~DYFQHW</u>	409
Variable light (vl) domain	DIQMTQSPSTLSASVGD ~~R~~ VTTITCRASQSISSWLA ~~W~~ YYQQKPGKAPKLLIYDASSLES ~~GV~~ PSRFSGSGSGTEFTLTITSLQPDDFA ~~TY~~ YCQQYN ~~SY~~ SLTFGGG ~~TK~~ LEIK	410
vlCDR1	<u>RASQSISSWLA</u>	411
vlCDR2	<u>DASSLES</u>	412
vlCDR3	<u>QQYN~~SY~~SLTF</u>	413
Full Protein Sequence	DIQMTQSPSTLSASVGD ~~R~~ VTTITCRASQSISSWLA ~~W~~ YYQQKPGKAPKLLIYDASSLES ~~GV~~ PSRFSGSGSGTEFTLTITSLQPDDFA ~~TY~~ YCQQYN ~~SY~~ SLTFGGG ~~TK~~ LEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGVRS ~~GP~~ DYFQHWGQGTLVTVSS	414

Figure 46JJ
V8

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSY ⁴¹⁵ YMHWVRQAPGQGLEWMGII ⁴¹⁵ NPSSGGSTSYAQKFQGRVTIT ADESTTAYMELSSLRSEDTAVYYCARAGVGYWDPLSDAFDVWGQGTLVTVSS	415
vhCDR1	GYTFTSY ⁴¹⁶	416
vhCDR2	INPSGGST ⁴¹⁷	417
vhCDR3	CARAGVGYWDPLSDA ⁴¹⁸	418
Variable light (vl) domain	QSVVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVH ⁴¹⁹ WYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSDTSA SLAITGLQAEDEADYYCQSYDSSL ⁴¹⁹ SGWVFE ⁴¹⁹ GGTKLTVL	419
vlCDR1	TGSSSNIGAGYDVH ⁴²⁰	420
vlCDR2	GNSNRPS ⁴²¹	421
vlCDR3	QSYDSSLSGWVF ⁴²²	422
Full Protein Sequence	QSVVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVH ⁴²³ WYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSDTSA SLAITGLQAEDEADYYCQSYDSSL ⁴²³ SGWVFE ⁴²³ GGTKLTVLGTAAAGSSGGSSGAEVQLVESGGGLVQPGGS LRLSCAASGYTFTSY ⁴²³ YMHWVRQAPGQGLEWMGII ⁴²³ NPSSGGSTSYAQKFQGRVTIT ADESTTAYMELSSLRSEDTAVYYCARAGVGYWDPLSDAFDVWGQGTLVTVSS	423

Figure 46KK
V8

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTIT ADESTSTAYMELSSLRSEDTAVYYCARPVRSGPDYFQHWGQGTLVTVSS	424
vhCDR1	GGTFSSYA	425
vhCDR2	IIPIFGTA	426
vhCDR3	CARPVRSGPDYFQHW	427
Variable light (vl) domain	DIQMTQSPSTLSASVGDRVTITCRASQISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FGSGSGTEFTLT ISSLQPD ~~DF~~ ATYYCQQYN ~~SYSLTF~~ GGGKVEIK	428
vlCDR1	RASQISSWLA	429
vlCDR2	DASSLES	430
vlCDR3	QQYN ~~SYSLTF~~	431
Full Protein Sequence	DIQMTQSPSTLSASVGDRVTITCRASQISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FGSGSGTEFTLT ISSLQPD ~~DF~~ ATYYCQQYN ~~SYSLTF~~ GGGKVEIKGTTASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKK ASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYY CARPVRSGPDYFQHWGQGTLVTVSS	432

Figure 46LL
V9

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYCARGWSARYGMDVWGQGTLVTVSS	433
vhCDR1	GGTFSSYA	434
vhCDR2	IIPFGTA	435
vhCDR3	CARGWSARYGMDVWG	436
Variable light (vl) domain	DIQMTQSPSSLASVGDVRVTITCRASQSISSYLNWYQQKPKGKAPKLLIYAASSLQSGVPSRFSGSGTDFTLTISLQPEDFATYYCCQQSYSTPYTFGGQGTKLEIK	437
vlCDR1	RASQSISSYLN	438
vlCDR2	AASSLQS	439
vlCDR3	QQSYSTPYTF	440
Full Protein Sequence	DIQMTQSPSSLASVGDVRVTITCRASQSISSYLNWYQQKPKGKAPKLLIYAASSLQSGVPSRFSGSGTDFTLTISLQPEDFATYYCCQQSYSTPYTFGGQGTKLEIKGTAAAGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYCARGWSARYGMDVWGQGTLVTVSS	441

Figure 46MM
V10

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGWSARYGMDVWGQGTLVTVSS	442
vhCDR1	GGTFSSYA	443
vhCDR2	IPIFGTA	444
vhCDR3	CARGWSARYGMDVWG	445
Variable light (vl) domain	DIQMTQSPSSLASVVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGTDFTLTISLQPEDFATYYCQQSYSTPYTFGGQGTKLEIK	446
vlCDR1	RASQSISSYLN	447
vlCDR2	AASSLQS	448
vlCDR3	QQSYSTPYTF	449
Full Protein Sequence	DIQMTQSPSSLASVVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGTDFTLTISLQPEDFATYYCQQSYSTPYTFGGQGTKLEIKGTAAAGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGWSARYGMDVWGQGTLVTVSS	450

Figure 46NN
V11

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEWVSAISGGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC <u>ARLDWPLSYGGSGY</u> YYGMDVWGQGTLLVTVSS	451
vhCDR1	GFTFSNAW	452
vhCDR2	ISGGGGST	453
vhCDR3	CARLDWPLSYGGSGY	454
Variable light (vl) domain	QSVVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVSNRFSGSKSGTSA SLAITGLQAEDEADYYCQSYDSSLSGVVFEGGTKLTVL	455
vlCDR1	TGSSSNIGAGYDVH	456
vlCDR2	GNSNRPS	457
vlCDR3	QSYDSSLSGVVF	458
Full Protein Sequence	QSVVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVSNRFSGSKSGTSA SLAITGLQAEDEADYYCQSYDSSLSGVVFEGGTKLTVLGTAAAGSSGGSSGAEVQLVESGGGLVQPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEWVSAISGGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC <u>ARLDWPLSYGGSGY</u> YYGMDVWGQGTLLVTVSS	459

Figure 4600
V12

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	EVQLVESGGGLVQPGGSLRLSCAASGFTSSYGISWVRQAPGQGLEWMGWISAYNGNTN ^Y AQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARVWYSGFSD ^{FD} SW/GQGTLVTVSS	460
vhCDR1	GFTFSSYG	461
vhCDR2	ISAYNGNT	462
vhCDR3	CARVWYSGFSD ^{FD} SW	463
Variable light (vl) domain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLA ^W YYQQKPGKAPKLLIYDASSLES ^{GV} PSRFSGSGSGTEFTLT ^{ISS} LQPD ^{DF} ATYYCQQYN ^{SY} SLTFGGG ^{TK} VEIK	464
vlCDR1	RASQSISSWLA	465
vlCDR2	DASSLES	466
vlCDR3	QQYN ^{SY} SLTF	467
Full Protein Sequence	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLA ^W YYQQKPGKAPKLLIYDASSLES ^{GV} PSRFSGSGSGTEFTLT ^{ISS} LQPD ^{DF} ATYYCQQYN ^{SY} SLTFGGG ^{TK} VEIKGTTAASGSSGGSSGA ^{EV} QLVESGGGLVQPGGSLRLSCAASGFTFSSYGISWVRQAPGQGLEWMGWISAYNGNTN ^Y AQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARVWYSGFSD ^{FD} SW/GQGTLVTVSS	468

Figure 46PP
V13

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSVRSGPDYFQHWGQGTLVTVSS	469
vhCDR1	GGTFSSYA	470
vhCDR2	IIPIFGTA	471
vhCDR3	CARSVRSGPDYFQHW	472
Variable light (vl) domain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FSGSGSGTEFTLTITSSLPDDFAFYCYCQQYNYSITFGQGTRLEIK	473
vlCDR1	RASQSISSWLA	474
vlCDR2	DASSLES	475
vlCDR3	QQYNYSITF	476
Full Protein Sequence	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLES ~~GVPSR~~ FSGSGSGTEFTLTITSSLPDDFAFYCYCQQYNYSITFGQGTRLEIKGTTAASGSSGGSSGAQVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSVRSGPDYFQHWGQGTLVTVSS	477

Figure 46QQ
V14

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVSCKASGFTFESSYAMSWVRQAPGQGLEWVSAISGSGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCARGWVSVTDDEDSWGQGTLVTVSS	478
vhCDR1	GFTFESSYA	479
vhCDR2	ISGSGGST	480
vhCDR3	CARGWVSVTDDEDSW	481
Variable light (vl) domain	QSVVVTQPPSVSAAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVDPDRFSGSKSGTSA SLAITGLQAEDEADYYCQSYDSSLSGVVFEGGTKLTVL	482
vlCDR1	TGSSSNIGAGYDVH	483
vlCDR2	GNSNRPS	484
vlCDR3	QSYDSSLSGVVF	485
Full Protein Sequence	QSVVVTQPPSVSAAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVDPDRFSGSKSGTSA SLAITGLQAEDEADYYCQSYDSSLSGVVFEGGTKLTVLGTAAAGSSGGSSGAQVQLVQSGAEVKKPGSS VKVSCKASGFTFESSYAMSWVRQAPGQGLEWVSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRA EDTAVYYCARGWVSVTDDEDSWGQGTLVTVSS	486

Figure 46RR
V15

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGASVKVCKASGYTFSSYAISWVVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYCARSVRSGPDYFQHWGQGTLVTVSS	487
vhCDR1	GYTFSSYA	488
vhCDR2	IIPIFGTA	489
vhCDR3	CARSVRSGPDYFQHW	490
Variable light (vl) domain	DIQMTQSPSSLASVGDRAVITTCRASQSISSWLAWYQQKPGKAPKLLIYDASSLESQVPSRFSGSGTEFTLTISLQPDDEFATYYCQQYNISYSLTFGGGTVKVEIK	491
vlCDR1	RASQSISSWLA	492
vlCDR2	DASSLES	493
vlCDR3	QQYNISYSLTF	494
Full Protein Sequence	DIQMTQSPSSLASVGDRAVITTCRASQSISSWLAWYQQKPGKAPKLLIYDASSLESQVPSRFSGSGTEFTLTISLQPDDEFATYYCQQYNISYSLTFGGGTVKVEIKGTTASGSSGGSSGAQVQLVQSGAEVKKPGASVKVCKASGYTFSSYAISWVVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTAYMELSSLRSEDTAVYYCARSVRSGPDYFQHWGQGTLVTVSS	495

Figure 46SS
V16

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVQSGAEVKKPGASVKVCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTINYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARD <u>FGYYSSAYFDYWGQGT</u> LVTVSS	496
vhCDR1	GYTFTSYG	497
vhCDR2	ISAYNGNT	498
vhCDR3	CARD <u>FGYYSSAYFDY</u>	499
Variable light (vl) domain	QSVVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSA TLGITGLQTGDEADYYCGTWDSSLSGYVFGTGTKVTVL	500
vlCDR1	TGSSSNIGAGYDVH	501
vlCDR2	GNSNRPS	502
vlCDR3	GTWDSSLSGYVF	503
Full Protein Sequence	QSVVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSA TLGITGLQTGDEADYYCGTWDSSLSGYVFGTGTKVTVLGTTAASGSSGGSSGAQVQLVQSGAEVKKPGAS VKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTINYAQKLQGRVTMTTDTSTSTAYMELRSL RSDDTAVYYCARD <u>FGYYSSAYFDYWGQGT</u> LVTVSS	504

Figure 46TT
V17

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYGRRYTPYYWYEDLWGQGTLVTVSS	505
vhCDR1	GFTFSSYA	506
vhCDR2	ISYDGSNK	507
vhCDR3	CARYGRRYTPYYWYF	508
Variable light (vl) domain	SYELTQPPSVSVSPGQTASITCSGDKLGDKYACWYQQKPGQSPVLVIYQDSKRPSGIPERFSGSNSGNTATLT ISGTQAMDEADYYCYCAWDSSSTAVFGTGTKVTVL	509
vlCDR1	SGDKLGDKYAC	510
vlCDR2	QDSKRPS	511
vlCDR3	QAWDSSTAVF	512
Full Protein Sequence	SYELTQPPSVSVSPGQTASITCSGDKLGDKYACWYQQKPGQSPVLVIYQDSKRPSGIPERFSGSNSGNTATLT ISGTQAMDEADYYCYCAWDSSSTAVFGTGTKVTVLGTTAASGSSGGSSGAQVQLVESGGGVVQPGRSRLS CAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDT AVYYCARYGRRYTPYYWYEDLWGQGTLVTVSS	513

Figure 46UU
V18

What	sequence	SEQ ID NO.
Variable heavy (vh) domain	QVQLVESGGGVVQPGRLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGWSTVGDDFDLWGQGTLVTVSS	514
vhCDR1	GFTFSSYA	515
vhCDR2	ISYDGSNK	516
vhCDR3	CARGWSTVGDDFDLW	517
Variable light (vl) domain	QSVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSA SLAITGLQAEDEADYYCQSYDSSLGYYVFGTGTGVTVL	518
vlCDR1	TGSSSNIGAGYDVH	519
vlCDR2	GNSNRPS	520
vlCDR3	QSYDSSLGYYVF	521
Full Protein Sequence	QSVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPDRFSGSKSGTSA SLAITGLQAEDEADYYCQSYDSSLGYYVFGTGTGVTVLGTAAAGSSGGSSGAQVQLVESGGGVVQPGRLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGWSTVGDDFDLWGQGTLVTVSS	522

Figure 47A

Clone Name	VL	VH	vhCDR1	vhCDR2	vhCDR3	vCDR1	vCDR2	vCDR3	Full Protein Sequence
VISTA 0.5.9:	DIQMTQSP STLSASV GDRVTITCR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPSRFSG SGSGTEFTL TISSLPDD FATYYCQQ YNSYSLTF GGGKVEIK	QVQLVQSGAE VKKPGSSVKVS CKASGGTFSSY AISWVRQAPGQ GLEWMGGIPIF GTANYAQKFQ GRVTITADESTS TAYMELSSLS EDTAVYYCARP VRSGPDYFQH WGQGTLVTVS SGSEKLISEED L	GGTFSS YA	IIPIFGT A	CARPVRSGP DYFQHW	RASQSISS WLA	DASSLE S	QQYN SYSLT F	DIQMTQSPSTLSASVGDRTVTI TCRASQSISSWLAAYQQKPG KAPKLLIYDASSLESVPSRF SGSGTEFTLTISLQPDFA TYCCQQYNSYSLTFGGGKTV EIKGTTAASGSSGSSSGAQV QLVQSGAEVKKPGSSVKVSC KASGGTFSSYAIWVRQAPG QGLEWMGGIPIFGTANYAQ KFQGRVTITADESTSTAYME LSSLRSEDTAVYYCARPVR GPDYFQHWGGQTLVTVSSGS EQKLISEEDL
VISTA 1.4.1:	DIQMTQSP STLSASV GDRVTITCR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPPRFSG SGSGAEFT LTISSLPDD DEATYYCQ QYNSYSLT FEGGKVEIK	QVQLVQSGAE VKKPGSSVKVS CKAPGGTFSSY AISWVRQAPGQ GLEWMGGIPIF GTANYAQKFQ GRVTITADESTS TAYMELSSLS EDTAVYYCARP VRSGPDYFQH WGQGTLVTVS S	GGTFSS YA	IIPIFGT A	CARPVRSGP DYFQHW	RASQSISS WLA	DASSLE S	QQYN SYSLT F	DIQMTQSPSTLSASVGDRTVTI TCRASQSISSWLAAYQQKPG KAPKLLIYDASSLESVPSRF SGSGAEFTLTISLQPDFA ATYYCQQYNSYSLTFGGGKTV VEIKGTTAASGSSGSSSGAQ VQLVQSGAEVKKPGSSVKVS CKAPGGTFSSYAIWVRQAP QGLEWMGGIPIFGTANYA QKFQGRVTITADESTSTAYM ELSSLRSEDTAVYYCARPVR SGPDYFQHWGGQTLVTVSS

Figure 47AB

Clone Name	VL	VH	vhCDR1	vhCDR2	vhCDR3	vCDR1	vCDR2	vCDR3	Full Protein Sequence
VISTA 1.4.2:	DIQMTQSP STLSASV/G DRVITICR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPSRFSG SGSGTEFTL TISSLQPD FATYYCQQ YNSYSLTF GGGKVEI K	QVQLVQSGAE VKKPGSSVKVS CKAPGGTFSS AISWVRQAPGQ GLEWMGGIPIF GTANYAQKFQ GRVTITADEST TAYMELSSLRS EDTAVYYCARP VRSGPDYLQH WGQGTLVTVS S	GGTFSS YA	IIPIFGT A	CARPVRSGP DYLQHW	RASQSISS WLA	DASSLE S	QQYN SYSLT F	DIQMTQSPSTLSASVGDRTVTI TCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESQVPSRF SGSGSGTEFTLTISLQPDFA TYQCQQYNSYSLTFGGGKTV EIKGTTAASGSSGSSGAQV QLVQSGAEVKKPGSSVKVSC KAPGGTFSSYAIWVRQAPG QGLEWMGGIPIFGTANYAQ KFQGRVTITADESTSTAYME LSSLRSEDTAVYYCARPVS GPDYQLQHWGGQGLVTVSS
VISTA 1.4.3:	DIQMTQSP STLSASV/G DRVITICR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPSRFSG SGSGTEFTL TISSLQPD FATYYCQQ YNSYSLTF GGGKVEI K	QVQLVQSGAE VKKPGSSVKVS CKAPGGTFSS AISWVRQAPGQ GLEWMGGIPIF GTANYAQKFQ GRVTITADEST TAYMELSSLRS EDTAVYYCARP VRSGPDYFQH WGQGTLVTVS S	GGTFSS YA	IIPIFGT A	CARPVRSGP DYFQHW	RASQSISS WLA	DASSLE S	QQYN SYSLT F	DIQMTQSPSTLSASVGDRTVTI TCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESQVPSRF SGSGSGTEFTLTISLQPDFA TYQCQQYNSYSLTFGGGKTV EIKGTTAASGSSGSSGAQV QLVQSGAEVKKPGSSVKVSC KAPGGTFSSYAIWVRQAPG QGLEWMGGIPIFGTANYAQ KFQGRVTITADESTSTAYME LSSLRSEDTAVYYCARPVS GPDYQLQHWGGQGLVTVSS
VISTA 1.4.4:	DIQMTQSP STLSASV/G DRVITICR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPSRFSG SGSGTEFTL TISSLQPD FATYYCQQ YNSYSLTF GGGKVEI K	QVQLVQSGAE VKKPGSSVKVS CKAPGGTFSS AISWVRQAPGQ GLEWMGGIPIF GTANYAQKFQ GRVTITADEST TAYMELSSLRS EDTAVYYCARP VRSGPDYFQH WGQGTLVTVS S	GGTFSS YA	IIPIFGT A	CARPVRSGP DYFQHW	RASQSISS WLA	DASSLE S	QQYN SYSLT F	DIQMTQSPSTLSASVGDRTVTI TCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESQVPSRF SGSGSGTEFTLTISLQPDFA TYQCQQYNSYSLTFGGGKTV EIKGTTAASGSSGSSGAQV QLVQSGAEVKKPGSSVKVSC KAPGGTFSSYAIWVRQAPG QGLEWMGGIPIFGTANYAQ KFQGRVTITADESTSTAYME LSSLRSEDTAVYYCARPVS GPDYQLQHWGGQGLVTVSS

Figure 47C

Clone Name	VL	VH	vHCDR1	vHCDR2	vHCDR3	vCDR1	vCDR2	vCDR3	Full Protein Sequence
VISTA 1.4.5:	AWYQQKP GKAPKLLI YDASSLES GVPSRFSG SGSGTEFTL TISSLQPD FATYYCQQ YNSYSITFG QGTRLEIK	GLEWMGGIPIF GTANYAQKFR GRVTITADESTS TAYMELSSLRS EDTAVYYCARP VRSGPDYFQH WGQGTILVTVS S							TYYCQQYNSYSITFGQGTRL EIKGTTAASGSSGSSSSGAQV QLVQSGAEVKKPGSSSVK VSC KASGGIFSSY AISWVRQAPG QGLEWMGGIPIFGTANYAQ KFRGRVTITADESTSTAYME LSSLRSEDTAVYYCARPVR GPDYFQHWGGQGTILVTVSS
	DIQMTQSP STLSASVG DRVITICR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPSRFSG SGSGTEFTL TISSLQPD FATYYCQQ YNSYSITFG GGGKVEI K	QVQLVQSGAE VKKPGSSSVKVS CKASGGIFSSY AISWVRQAPGQ GLEWMGGIPIF GTANYAQKFO GRVTITADESTS TAYMELSSLRS EDTAVYYCARP VRSGPDYFQH WGQGTILVTVS S	GGIFSSY A	IIPIFGT A	CARPVRSGP DYFQHW	RASQSISS WLA	DASSLE S	QQYN SYSLT F	DIQMTQSPSTLSASVGDRTI TCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESVPSRF SGSGGTEFTLTISLQPDFA TYYCQQYNSYSITFGGGTKV EIKGTTAASGSSGSSSSGAQV QLVQSGAEVKKPGSSSVK VSC KASGGIFSSY AISWVRQAPG QGLEWMGGIPIFGTANYAQ KFQGRVTITADESTSTAYME LSSLRSEDTAVYYCARPVR GPDYFQHWGGQGTILVTVSS
VISTA 1.4.6:	DIQMTQSP SSLASAVG DRVITICR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPSRFSG	QVQLVQSGAE VKKPGSSSVKVS CEASGGIFSSYA ISWVRQAPGQ LEWMGGIPIFG TANYAQKFOG RVITITADESTST AYMELSSLRSE	GGIFSSY A	IIPIFGT A	CARAVRSGP DYFQHW	RASQSISS WLA	DASSLE S	QQYN SYSLT F	DIQMTQSPSSLSASVGDRTI TCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESVPSRF SGSGGTEFTLTISLQPDFA TYYCQQYNSYSITFGGGTKV EIKGTTAASGSSGSSSSGAQV QLVQSGAEVKKPGSSSVK VSC EASGGIFSSY AISWVRQAPGQ

Figure 47D

Clone Name	VL	VH	vhCDR1	vhCDR2	vhCDR3	vCDR1	vCDR2	vCDR3	Full Protein Sequence
	SGSGTEFTL TISSLQPD FATYYCQQ YNSYSLTF GGGKVEI K	DTAVYYCARA VRSGPDYFQH WGQGTILVTVS S							GLEWMGGIPIFGTANYAQK FQGRVTITADESTSTAYMELS SLRSEDNAVYYCARAVRSGP DYFQHWGQGTILVTVSS
VISTA 1.4.7 (VS147):	DIQMTQSP STLSASV DRVTITCR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPSRFSG SGSGTEFTL TISSLQPD FATYYCQQ YNSYSLTF GGGKVEI K	QVQLVQSGAE VKKPGSSVKVS CKASGGIFSSY AISWVRQAPGQ GLEWMGGIPIF GTANYAQKFQ GRVTITADEST TAYMELSSLRS EDTAVYYCARP VRSGPDYFQH WGQGTILVTVS S	GGIFSSY A	IIPIFGT A	CARPVRSGP DYLQHW	RASQSISS WLA	DASSLE S	QQYN SYSLT F	DIQMTQSPSTLSASVGDRTV TCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESVPSRF SGSGSGTEFTLTISLQPDFA TYYCQQYNYSYSLTFGGGK EIKGTTAASGSSGSSSGAQV QLVQSGAEVKKPGSSVKVSC KASGGIFSSY AISWVRQAPG QGLEWMGGIPIFGTANYAQ KFQGRVTITADESTSTAYME LSSLRSEDNAVYYCARPVR S GPDYQLQHWGQGTILVTVSS
VISTA 1.4.8:	DIQMTQSP STLSASV DRVTITCR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPSRFSG SGSGTEFTL TISSLQPD FATYYCQQ YNSYSLTF GGGKVEI K	QVQLVQSGAE VKKPGSSVRVS CKVSGGIFSSY AISWVRQAPGQ GLEWMGGIPIF GTANYAQKFQ GRVTITADEST TAYMELSSLRS EDTAVYYCARP VRSGPDYFQH	GGIFSSY A	IIPIFGT A	CARPVRSGP DYFQHW	RASQSISS WLA	DASSLE S	QQYN SYSLT F	DIQMTQSPSTLSASVGDRTV VTCRASQSISSWLAWYQQK GKAPKLLIYDASSLESVPSR FSGSGSGTEFTLTISLQPD ATYYCQQYNYSYSLTFGGGK VEIKGTTAASGSSGSSSGAQ VQLVQSGAEVKKPGSSVRVS CKVSGGIFSSY AISWVRQAPG QGLEWMGGIPIFGTANYAQ KFQGRVTITADESTSTAYME

Figure 47E

Clone Name	VL	VH	vhCDR1	vhCDR2	vhCDR3	vCDR1	vCDR2	vCDR3	Full Protein Sequence
	YNSYSLTF GGGTKVEI K	WGQGTSLVTVSS							LSSLRSEDTAVYYCARPVRS GPDYFQHWGQGTLVTVSS
V1	DIQMTQSP STLSASV DRVTITCR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPSRFSG SGSGTEFTL TISSLQPD FATYYCQQ YNSYSITFG QGTRLEIK	EVQLVESGGGL VQPGGSLRLSC AASGTFSSYA MHWVRQAPGK GLEWVAISYD GSNKYYADSV KGRFTISRDN KNTLYQMNS LRSDDTAVYYC ARGWSGSPD VWGQGTSLVTVSS	G-- FTFSSY A	ISYDGS NK	CARGWSGSPS DFDVWG	RASQS--- ISSWLA	DASSLE S	QQYN SYS--- ITF	DIQMTQSPSTLSASVGDRTIT TCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESQVPSRF SGSGSGTEFTLTITSLQPDDFA TYYCQQYNYSYITFGQGTSL EIKGTTAASGSSGSSSGAEV QLVESGGGLVQPGGSLRLSC AASGTFSSYAMHWVRQAP GKLEWVAISYDGSNKYY ADSVKGRFTISRDNKNTLY LQMNSLRSDDTAVYYCARG WSGSPDFTDVWGQGTSLVTVSS
V2	QSVVTQPP SVSGAPGQ RVTISCTGS SSNIGAGY DVHWYQQ LPGTAPKL LIYGNNSNRP SGVPDRFS GSKSGTSA SLAITGLQA EDEADYYC QSYDSSL GSWVFGG GTKLTIVL	QLQLQESGPG VKPSETLSLTCT VSGGSISSSY WGWIRQHPGK GLEWIGSIYS GSTYYNPSLKS RVTISVDTSKN QFSLKLSVTA ADTAVYYCAR RSTIWGDGA FQHWGQGT TVSS	GGSISS SY	IYYS- GST	CARRSTIWG DGAIEYF	TGSSSNIG AGYDVH	GNSNR PS	QSYDS SLSGS WVF	QSVVTQPPSVSGAPGQRVTIS CTGSSSNIGAGYDVHWYQQ PGTAPKLLIYGNNSNRP RVSGSGSGTSAITGLQAE DEADYYCQSYDSSSLSGSWVF GGGTKLTVLGTAAASGSSG SSSGAQLQLQESGPGLVKPS TLSTCTVSGGSISSSYW WIRQHPGKLEWIGSIYS TYYNPSLKSRVTISVDTSKN FSLKLSVTAADTAVYYCAR RSTIWGDGAIEYFQHWGQGT LTVSS

Figure 47F

Clone Name	VL	VH	vhCDR1	vhCDR2	vhCDR3	vCDR1	vCDR2	vCDR3	Full Protein Sequence
V3	EIVLTQSPG TLSASVGD RVITICRAS QSISSWLA WYQKPG KAPKLLIY DASSLESG VPSRFSGS GSGTEFTLT ISSLPDDF ATYYCQY NSYSFTGPG GTKVDIK	EVQLVESGGGL VQPGGSLRLSC AASGFTSSYA MSWVRQAPGK GLEWMGGIPIF GTANYAQKFQ GRVTITADEST TAYMELSSLRS EDTAVYYCARS VRSGPDYFQH WGQGTLVTVS S	G-- FTFSSY A	IIPIFGT A	CARSVRSGP DYFQHW	RASQS--- ISSWLA	DASSLE S	QQYN SYS--- FTF	EIVLTQSPGTLASVGD TLCRASQSISSWLA WYQKPG KAPKLLIYDASSLESG VPSRFSGSGSGTEFTLT TISSLPDDFA TYYCQYNSYSFTFG PGTKV DIKGTAAAGSSGSSG AEV QLVESGGGLVQPGGSL RLSC AASGFTSSYAMSWVR QAPG KGLEWMGGIPIFGTAN YAAQ KFQGRVTITADESTST AYME LSSLRSEDTAVYYCAR SVRS GPDYFQHWGGQGLV TVSS
V4	QSVVTQPP SVSGAPVQ RVTISCTGS SSNIGAGY DVHWYQQ LPGTAPKL LIYGNSNRP SGVPDRFS GSKSGTSA SLAITGLQA EDEADYYC QSYDSSL GVFGTGTK VTVL	QLQLQESGPG VKPSETLSLTCT VSGGSISSSY WGWIRQPPGK GLEWIGEINHS GSTNYPNPSLKS RVTISVDTSKN QFSLKLSVTA ADTAVYYCAR VDNDYSYVGS YGMMDVWGQG TLVTVSS	GGSISS SY	INHS- GST	CARVDNDY SYVGSYG	TGSSSNIG AGYDVH	GNSNR PS	QSYDS SLSG-- VF	QSVVTQPPSVSGAPV QRVVTIS CTGSSSNIGAGYDVH WYQQL PGTAPKLLIYGNSNRP SGVDP RFSGSKSGTASLAITGL QAE DEADYYCQSYDSSLSG VFGT GTKVTVLGTTAASGSSG SS SGAQLQLQESGPGGLV KPSET LSLTCTVSGGSISSSY YWG WRQPPGKGLEWIGEIN HSGS TNYNPSLKSRVTISVD TSKNQ FSLKLSVTAADTAVYYC AR VDNDYSYVGSYGMDV WGQ GTLVTVSS
V5	QSVVTQPP SVSGAPGQ RVTISCTGS SSNIGAGY	QVQLVQSGAE VKKPGSSVKVS CKASGGTFSSY AISWVRQAPGQ	GG-- TFSSYA	IIPIFGT A	CARYDFVG GYTDYWG	TGSSSNIG AGYDVH	GNSNR PS	QSYDS SLSG- VVF	QSVVTQPPSVSGAPG QRVVTIS CTGSSSNIGAGYDVH WYQQL PGTAPKLLIYGNSNRP SGVDP RFSGSKSGTASLAITGL QAE

Figure 47G

Clone Name	VL	VH	vhCDR1	vhCDR2	vhCDR3	vCDR1	vCDR2	vCDR3	Full Protein Sequence
	DVHWYQQ LPGTAPKL LIYGNENRP SGVPDRFS GSKSGTSA SLAITGLQA EDEADYYC QSYDSSL GVVFGGGT KLTVL	GLEWMGGIPIF GTANYAQKFQ GRVTITADESTS TAYMELSSLRS EDTAVYYCAR YDFVGGYTDY WGQGTLVTVS S							DEADYYCQSYDSSLSGVVFG GGTKLTVLGTAAASGSSGS SSGAQVQLVQSGAEVKKPGS SVKVSCKASGGTFSSYAISW VRQAPGQGLEWMGGIPIFGT ANYAQKFQGRVTITADESTS TAYMELSSLRSSEDTAVYYCA RYDFVGGYTDYWGQGTLVTV VSS
V6	DIQMTQSP STLSASV DRVTITCR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPSRFSG SGSGTEFTL TISSLQPD FATYYCQ YNSYSLTF GGGKVEI K	EVQLVESGGGL VQPGGSLRLSC AASGTFSSYA MSWVRQAPGK GLEWVSAISGS GGSTYYADSV KGRFTISRDN KNTLYLQMNS LRAEDTAVYY CARLEDWLGG YILEYFDLWGQ GTLVTVSS	G-- FTFSSY A	ISGSGG ST	CARLEDWL GGYILEY	RASQS--- ISSWLA	DASSLE S	QQYN SYS--- LTF	DIQMTQSPSTLSASVGDRTV TCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESVPSRF SGSGGTEFTLTISLQPDFA TYQCQQYNYSYSLTFGGGK EIKGTTAASGSSGSSSGAEV QLVESGGGLVQPGGSLRLSC AASGTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYAD SVKGRFTISRDNKNTLYLQ MNSLRAEDTAVYYCARLED WLGGYILEYFDLWGQGTLV TVSS
V7	DIQMTQSP STLSASV DRVTITCR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES	QVQLVQSGAE VKKPGSSVKVS CKASGGTFSSY AISWVRQAPGQ GLEWMGGIPIF GTANYAQKFQ GRVTITADESTS	GG-- TFSSYA	IIPIFGT A	CARGVRS DYPQHW	RASQS--- ISSWLA	DASSLE S	QQYN SYS--- LTF	DIQMTQSPSTLSASVGDRTV TCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESVPSRF SGSGGTEFTLTISLQPDFA TYQCQQYNYSYSLTFGGGK EIKGTTAASGSSGSSSGAEV QLVQSGAEVKKPGSSVKVSC

Figure 47H

Clone Name	VL	VH	vhCDR1	vhCDR2	vhCDR3	vCDR1	vCDR2	vCDR3	Full Protein Sequence
	GVPSRFSG SGSGTEFTL TISSLQPD FATYYCQQ YNSYSLTF GGGKLEI K	TAYMELSSLRS EDTAVYYCAR GVRSGPDYFQH WGQGTLLVTVS S							KASGGTFSSY AISWVRQAPG QGLEWMGGIPIFGTANYAQ KFQGRVTITADESTSTAYME LSSLRSEDTAVYYCARGVRS GPDYFQHWGQGTLLVTVSS
V8	QSVVTQPP SVSGAPGQ RVTISCTGS SSNIGAGY DVHWYQQ LPGTAPKL LIYGNNSRNP SGVPDRFS GSKSDTSA SLAITGLQA EDEADYYC QSYDSSL GWVFGGG TKLTVL	EVQLVESGGGL VQPGGSLRLSC AASGYTFTSY Y MHWRQAPGQ GLEWMGIINPS GGSTSYAQKFQ GRVTITADESTS TAYMELSSLRS EDTAVYYCAR AGVGYWDPLS DAFDVWGQGT LVTVSS	G-- YTFTSY Y	INPSGG ST	CARAGVGY WDPLSDA	TGSSSNIG AGYDVH	GNSNR PS	QSYDS SLSG- WVF	QSVVTQPPSVSGAPGQRTIS CTGSSNIGAGYDVHWYQQ PGTAPKLLIYGNNSRPSGVPD RFSGSKSDTASLAITGLQAE DEADYYCQSYDSSLSGWVF GGGTKLTVLGTAAAGSSGG SSSGAEVQLVESGGGLVQPG GSLRLSCAASGYTFTSYMH WVRQAPGQGLEWMGIINPSG GSTSYAQKFQGRVTITADEST STAYMELSSLRSEDTAVYYC ARAGVGYWDPLSDAFDVG QGTLVTVSS
V9	DIQMTQSP STLSASV/G DRVITTCR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPSRFSG SGSGTEFTL TISSLQPD	QVQLVQSGAE VKKPGSSVKVS CKASGGTFSSY AISWVRQAPGQ GLEWMGGIPIF GTANYAQKFQ GRVTITADESTS TAYMELSSLRS EDTAVYYCARP VRSQPDYFQH	GG-- TFSSYA	IIPIFGT A	CARPVRS DYPQHW	RASQS--- ISSWLA	DASSLE S	QQYN SYS--- LTF	DIQMTQSPSTLSASVGDRTI TCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESQVPSRF SGSGSGTEFTLTISLQPDFA TYQCQQYNISYSLTFGGGKTV EIKGTAAAGSSGGSSSGAQV QLVQSGAEVKKPGSSVKVSC KASGGTFSSY AISWVRQAPG QGLEWMGGIPIFGTANYAQ KFQGRVTITADESTSTAYME

Figure 471

Clone Name	VL	VH	vhCDR1	vhCDR2	vhCDR3	vCDR1	vCDR2	vCDR3	Full Protein Sequence
V10	FATYYCQQ YNSYSLTF GGGKVEI K	WGQGTLVTVS S							LSSLRSEDTAVYYCARPVRSGPDYFQHWGGQGLVTVSS
	DIQMTQSP SSLSASV DRVTITCR ASQSISSYL NWYQQKP GKAPKLLI YAASSLQS GVPSRFSG SGSGTDFT LTISSLQPE DEATYYCQ QSYSTPYT FGQGTKLEI K	QVQLVQSGAE VKKPGSSVKVS CKASGGTFSSY AISWVRQAPGQ GLEWMGGIPIF GTANYAQKFQ GRVTITADESTS TAYMELSSLRS EDTAVYYCAR GWSARYGMDV WGQGTLVTVS S	GG-- TFSSYA	IIPIFGT A	CARGWSAR YGMDEVWG	RASQS--- ISSYLN	AASSL QS	QQSYS TP--- YTF	DIQMTQSPSSLSASVGDRTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFAITYYCQSYSTPYTFGGQGTKLEIKGTAAAGSSGSSGAQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMEISSLRSEDTAVYYCARGWSARYGMDVWGQGLVTVSS
V11	QSVVTQPP SVSGAPGQ RVTISCTGS SSNIGAGY DVHWYQQ LPGTAPKL LIYGNNSNRP SGVSNRFS GSKSGTSA SLAITGLQA EDEADYYC QSYDSSL S	EVQLVESGGGL VQPGGSLRLSC AASGFTFSNAW MSWVRQAPGK GLEWVSAISGS GGSTYYADSV KGRFTISRDN KNTLYLQMNS LRAEDTAVYY CARLDWPLSY GGSGYYYG DVWGQGLVTV VSS	G-- FTFSNA W	ISGSGG ST	CARLDWPLS YGGSGY	TGSSSNIG AGYDVH	GNSNR PS	QSYDS SLSG- VVF	QSVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNNSNRPSGVSNRFSGSKSGTASLAIITGLQAEDEADYYCQSYDSSLSGVVFVGGTKLTVLGTAAAGSSGSSSGAEVQLVESGGGLVQPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEWVSAISGSGSTYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARLDWPLSYGGSGYYYGMDVWGQGLVTVSS

Figure 47J

Clone Name	VL	VH	vhCDR1	vhCDR2	vhCDR3	vCDR1	vCDR2	vCDR3	Full Protein Sequence
V12	GVVFGGGT KLTVL DIQMTQSP STLSASVG DRVTITCR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPSRFSG SGSGTEFTL TISSLQPD FATYYCQQ YNSYSITF GGGTKVEI K	EVQLVESGGGL VQPGGSLRLSC AASGFTFSSYGI SWVRQAPGQG LEWMGWISAY NGNTNYAQKL QGRVTMTDTS TSTAYMELRSL RSDDTAVYYC ARVWYSGFSD DSWGQGTLVT VSS	G-- FTFSSY G	ISAYNG NT	CARVWYSG FSDFDWSW	RASQS--- ISSWLA	DASSLE S	QQYN SYS--- LTF	DIQMTQSPSTLSASVGDRTVTI TCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESVPSRF SGSGTEFTLTISLQPDFA TYVCCQQYNSYSITFGGGTKV EIKGTTAASGSSGSSSGAEV QLVESGGGLVQPGGSLRLSC AASGFTFSSYGISWVRQAPG QGLEWMGWISAYNGNTNYA QKLQGRVTMTDSTSTAY MELRSLRSDDTAVYYCARV WYSGFSDFDWSWGQGTLLVTV SS
V13	DIQMTQSP STLSASVG DRVTITCR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPSRFSG SGSGTEFTL TISSLQPD FATYYCQQ YNSYSITF QGTRLEIK	QVQLVQSGAE VKKPGSSVKVS CKASGFTFSSY AISWVRQAPGQ GLEWMGGIPIF GTANYAQKFQ GRVTITADEST TAYMELSSLRS EDTAVYYCARS VRSGPDYFQH WGQGTLVTVS S	GG-- TFSSYA	IIPIFGT A	CARSVRSGP DYFQHW	RASQS--- ISSWLA	DASSLE S	QQYN SYS--- ITF	DIQMTQSPSTLSASVGDRTVTI TCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESVPSRF SGSGTEFTLTISLQPDFA TYVCCQQYNSYSITFGGTRL EIKGTTAASGSSGSSSGAQV QLVQSGAEVKKPGSSVKVSC KASGFTFSSYAIWVRQAPG QGLEWMGGIPIFGTANYAQ KFQGRVTITADESTSTAYME LSSLRSEDTAVYYCARSVRS GPDYFQHWGGQGTLLVTVSS

Figure 47K

Clone Name	VL	VH	vhCDR1	vhCDR2	vhCDR3	vCDR1	vCDR2	vCDR3	Full Protein Sequence
V14	QSVVTQPP SVSAAPGQ RVTISCTGS SSNIGAGY DVHWYQQ LPGTAPKL LIYGNSNR SGVPDRFS GSKSGTSA SLAITGLQA EDEADYYC QSYDSSL GVVFGGGT KLTVL	QVQLVQSGAE VKKPGSSVKVS CKASGFTFSY AMSWVRQAPG QGLEWVSAISG SGGSTYYADSV KGRFTISRDN KNTLYLQMS LRAEDTAVYY CARGWVSVTD DFDSWGQGTL VTVSS	G-- FTFSY A	ISGSGG ST	CARGWVSV TDDFDSW	TGSSSNIG AGYDVH	GNSNR PS	QSYDS SLSG- VVF	QSVVTQPPSVSAAPGQQRVTIS CTGSSSNIGAGYDVHWYQQQL PGTAPKLLIYGNSNRPSGVDP RFGSKSGTASLAITGLQAE DEADYYCQSYDSSLSGVVFG GGTKLTVLGTAAAGSSGGS SSGAQVQLVQSGAEVKKPGS SVKVSCKASGFTFSYAMSW VRQAPGQGLEWVSAISGSG STYYADSVKGRFTISRDN NTLYLQMSLRAEDTAVYY CARGWVSVTDDFDSWGQGT LTVSS
V15	DIQMTQSP SSLSASV DRVTITCR ASQSISSWL AWYQQKP GKAPKLLI YDASSLES GVPSRFSG SGSGTEFTL TISSLQPD FATYYCQ YNSYSLTF GGGKVEI K	QVQLVQSGAE VKKPGASVKVS CKASGYTFSY AISWVRQAPGQ GLEWMGGIPIF GTANYAQKFQ GRVTITADESTS TAYMELSSLRS EDTAVYYCARS VRSGPDYFQH WGQGTLVTVS S	G-- YTFSSY A	IIPIGT A	CARSVRSGP DYFQHW	RASQS--- ISSWLA	DASSLE S	QOYN SYS--- LTF	DIQMTQSPSSLSASVGDRTVTI TCRASQSISSWLAWYQQKPG KAPKLLIYDASSLESVPSRF SGSGSGTEFTLTISLQPDFA TYYCQQYNYSYSLTFGGGKTV EIKGTTAASGSSGSSSGAQV QLVQSGAEVKKPGASVKVSC KASGYTFSYAISWVRQAPG QGLEWMGGIPIFGTANYAQ KFQGRVTITADESTAYME LSSLRSEDTAVYYCARSVRS GPDYFQHWGQGTLVTVSS
V16	QSVVTQPP SVSGAPGQ RVTISCTGS	QVQLVQSGAE VKKPGASVKVS CKASGYTFSY	G-- YTFTSY G	ISAYNG NT	CARDFGYYS SAYFDY	TGSSSNIG AGYDVH	GNSNR PS	GTWD SSLG- YVF	QSVVTQPPSVSGAPGQQRVTIS CTGSSSNIGAGYDVHWYQQQL PGTAPKLLIYGNSNRPSGVDP

Figure 47L

Clone Name	VL	VH	vhCDR1	vhCDR2	vhCDR3	vCDR1	vCDR2	vCDR3	Full Protein Sequence
V17	SSNIGAGY DVHWYQQ LPGTAPKL LIYGNSNR SGVPDRFS GSKSGTSA TLGITGLQT GDEADYYC GTWDSLS GYVFGTGT KVTVL	GISWVRQAPGQ GLEWMGWISA YNGNTNYAQK LQGRVTMTD TSTSTAYMELR SLRSDDTAVYY CARDFGYYSSA YFDYWGGQTL VTVSS	G-- FTFSSY A	ISYDGS NK	CARYGRRY TPYYWYF	SGDKL--- GDKYAC	QDSKR PS	QAWD SST--- AVF	RFSGSKSGTSATLGITGLQTG DEADYYCGTWDSSL SGYVF GTGKVTVLGTTAASGSSGG SSSGAQVQLVQSGAEVKKPG ASVKVSCASGYTFTSYGIS WVRQAPGQGLEWMGWISA YNGNTNYAQKQGRVTMTT DTSTSTAYMELRSLRSDDTA VYYCARDFGYYSSAYFDYW GQGTLVTVSS
V18	QSVVTQPP SVSGAPGQ RVTISCTGS SSNIGAGY DVHWYQQ LPGTAPKL	QVQLVESGGG VVPGRSLRLS CAASGFTFSSY AMHWVRQAPG KGLEWVAVISY DGSNKYYADS VKGRFTISRDN SKNTLYLQMNS LRAEDTAVYY CARYGRRYTPY YWYFDLWGQG TLVTVSS	G-- FTFSSY A	ISYDGS NK	CARGWSTV GDDFDLW	TGSSSNIG AGYDVH	GNSNR PS	QSYDS SLSG- YVF	QSVVTQPPSVSGAPGQRVTIS CTGSSSNIGAGYDVHWYQQ PGTAPKLLIYGNSNRPSGVPD RFSGSKSGTSASLAITGLQAE DEADYYCQSYDSSL SGYVFG TGKVTVLGTTAASGSSGG

Figure 47M

Clone Name	VL	VH	vhCDR1	vhCDR2	vhCDR3	vCDR1	vCDR2	vCDR3	Full Protein Sequence
	LIYGNSNRP SGVPDRFS GSKSGTSA SLAITGLQA EDEADYYC QSYDSSL GYVFGTGT KVTVL	VKGRFTISRDN SKNTLYLQMNS LRAEDTAVYY CARGWSTVGD DFDLWGQGT LTVSS							SSGAQVQLVESGGGVVQPGR SLRLSCAASGFTFSSYAMHW VRQAPGKGLEWVAVISYDGS NKYYADSVKGRFTISRDNK NTLYLQMNSLRAEDTAVYY CARGWSTVGDDEFDLWGQGT LTVSS