



(12) **DEMANDE DE BREVET CANADIEN**
CANADIAN PATENT APPLICATION

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2020/05/29
(87) Date publication PCT/PCT Publication Date: 2020/12/03
(85) Entrée phase nationale/National Entry: 2021/11/29
(86) N° demande PCT/PCT Application No.: US 2020/035399
(87) N° publication PCT/PCT Publication No.: 2020/243623
(30) Priorités/Priorities: 2019/05/31 (US62/855,590);
2019/09/13 (US62/900,105); 2020/01/22 (US62/964,487)

(51) Cl.Int./Int.Cl. *C07K 16/18* (2006.01),
A61K 39/00 (2006.01), *A61P 35/00* (2006.01)
(71) Demandeur/Applicant:
THE COUNCIL OF THE QUEENSLAND INSTITUTE OF
MEDICAL RESEARCH, AU
(72) Inventeurs/Inventors:
PULUKKUNAT, DILEEP K., US;
WYKES, MICHELLE, AU
(74) Agent: GOWLING WLG (CANADA) LLP

(54) Titre : ACTIVATION DE MOLECULES DE LIAISON A L'ANTICORPS ANTI-GAL9
(54) Title: ACTIVATING ANTI-GAL9 BINDING MOLECULES

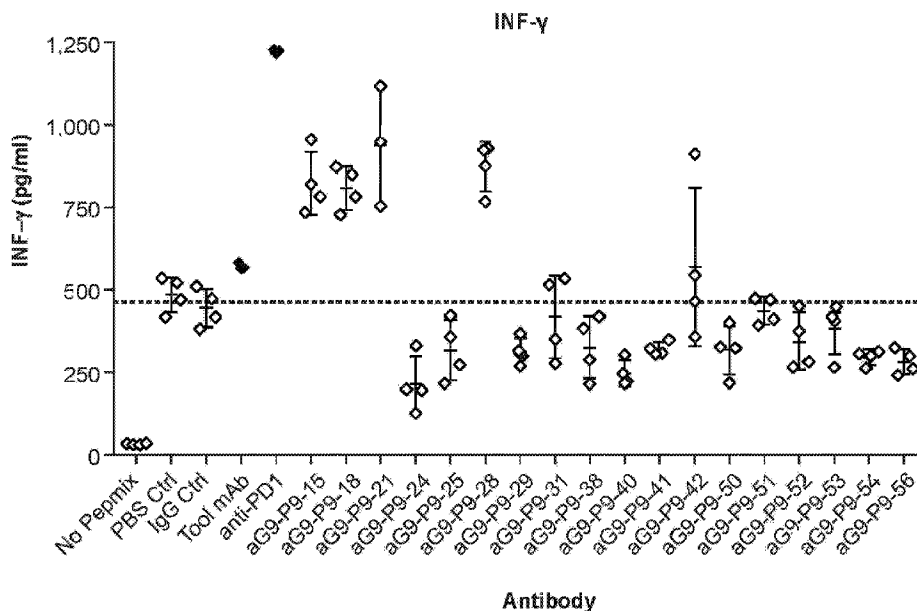


FIG. 3A

(57) Abrégé/Abstract:

Anti-GAL9 antibody constructs, pharmaceutical compositions comprising the constructs, and methods of use thereof are presented.



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

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

03 December 2020 (03.12.2020)



(10) International Publication Number

WO 2020/243623 A1

(51) International Patent Classification:

A61P 35/00 (2006.01) A61P 43/00 (2006.01)

A61P 37/06 (2006.01) A61P 29/00 (2006.01)

A61P 37/02 (2006.01) G01N 33/68 (2006.01)

(21) International Application Number:

PCT/US2020/035399

(22) International Filing Date:

29 May 2020 (29.05.2020)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/855,590 31 May 2019 (31.05.2019) US

62/900,105 13 September 2019 (13.09.2019) US

62/964,487 22 January 2020 (22.01.2020) US

(71) Applicant: THE COUNCIL OF THE QUEENSLAND

INSTITUTE OF MEDICAL RESEARCH [AU/AU];

300 Herston Road, Herston, Brisbane, QLD, 4006 (AU).

(72) Inventor; and

(71) Applicant: PULUKKUNAT, Dileep, K. [US/US]; 9414

Cobalt Street, Middleton, WI 53562 (US).

(72) Inventor: WYKES, Michelle; c/o The Council of The Queensland Institute of Medical Research, 300 Herston Road, Herston, Brisbane, QLD, 4006 (AU).

(74) Agent: HARRADINE, Kelly, A. et al.; Fenwick & West LLP, 801 California Street, Mountain View, CA 94041 (US).

(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,

(54) Title: ACTIVATING ANTI-GAL9 BINDING MOLECULES

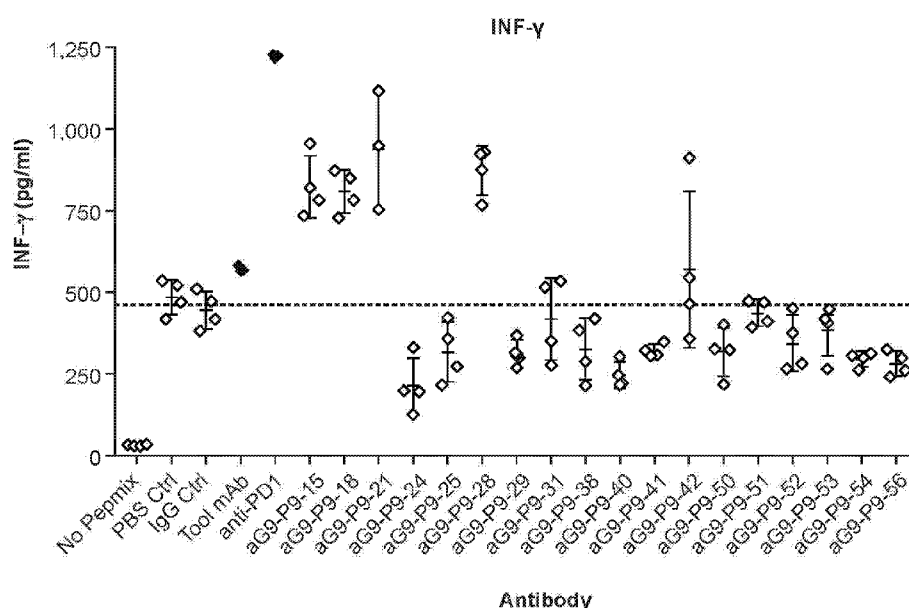


FIG. 3A

(57) Abstract: Anti-GAL9 antibody constructs, pharmaceutical compositions comprising the constructs, and methods of use thereof are presented.

[Continued on next page]



WO 2020/243623 A1

WO 2020/243623 A1



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:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

ACTIVATING ANTI-GAL9 BINDING MOLECULES

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of prior co-pending U.S. Provisional Patent Application No. 62/964,487, filed on January 22, 2020, U.S. Provisional Patent Application No. 62/900,105, filed on September 13, 2019, and U.S. Provisional Patent Application No. 62/855,590, filed on May 31, 2019.

2. SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated herein by reference in its entirety. Said ASCII copy, created on April 9, 2020, is named 42700WO_CRF_sequencelisting.txt, and is 389,339 bytes in size.

3. BACKGROUND

[0003] Immune therapy has great potential for the treatment of cancer. However, tumors can become resistant to immune therapy, for example by recruiting immunosuppressive cells or signaling molecules to the tumor microenvironment or by co-opting immune checkpoint signaling pathways.

[0004] Galectin-9 (GAL9) is an S-type lectin beta-galactoside-binding protein with N- and C- terminal carbohydrate-binding domains connected by a linker peptide. GAL9 has been implicated in modulating cell-cell and cell-matrix interactions. GAL9 has been shown to bind soluble PD-L2, and at least some of the immunological effects of PD-L2 have been suggested to be mediated through binding of multimeric PD-L2 to GAL9, rather than through PD-1 (WO 2016/008005, which is incorporated herein by reference in its entirety). However, mechanisms by which GAL9 and PD-L2 impact immune effector function are not yet fully characterized.

[0005] There remains a need for therapeutic agents that can enhance immune effector function and reduce immunosuppressive or T cell exhaustion pathways. Such therapeutic agents may be useful for improving cancer immune therapy.

4. SUMMARY

[0006] In a first aspect the disclosure provides a Galectin-9 (GAL9) antigen binding molecule comprising a first antigen binding site specific (ABS) for a first epitope of a first GAL9 antigen, wherein the first antigen binding site comprises all three VH CDRs from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

[0007] In a second aspect, the disclosure provides a Galectin-9 (GAL9) antigen binding molecule, comprising a first antigen binding site specific for a first epitope of a first GAL9 antigen, wherein the first antigen binding site comprises all three VL CDRs from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

[0008] In a third aspect, the disclosure provides a Galectin-9 (GAL9) antigen binding molecule, comprising a first antigen binding site specific for a first epitope of a first GAL9 antigen, wherein the first antigen binding site comprises all three VH CDRs and all three VL CDRs from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

[0009] In a fourth aspect, the disclosure provides a Galectin-9 (GAL9) antigen binding molecule, comprising a first antigen binding site specific for a first epitope of a first GAL9 antigen, comprising the VL sequence and the VH sequence from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

[0010] In some embodiments, the GAL9 antigen binding molecule comprises a full immunoglobulin heavy chain “IgG1” sequence comprising the VH sequence and a full immunoglobulin light chain sequence comprising the VL sequence, wherein the VH sequence and the VL sequence are from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

[0011] In some embodiments, the GAL9 antigen binding molecule comprises a full immunoglobulin heavy chain “IgG4” sequence comprising the VH sequence and a full immunoglobulin light chain sequence comprising the VL sequence, wherein the VH sequence and the VL sequence are from any one of the ABS clones selected from P9-02B, P9-04, P9-

05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

[0012] In some embodiments, the GAL9 antigen binding molecule comprises a full immunoglobulin heavy chain “IgG3” sequence comprising the VH sequence and a full immunoglobulin light chain sequence comprising the VL sequence, wherein the VH sequence and the VL sequence are from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

[0013] In some embodiments, the GAL9 antigen binding molecule can comprise a GAL9 antigen that is a human GAL9 antigen.

[0014] In some embodiments, the GAL9 antigen binding molecule can further comprises a second antigen binding site.

[0015] In certain embodiments, the second antigen binding site is specific for the GAL9 antigen. In other embodiments, the second antigen binding site is identical to the first antigen binding site.

[0016] In other embodiments, the second antigen binding site is specific for a second epitope of the first GAL9 antigen.

[0017] In some embodiments, the second antigen binding site comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from another ABS clone selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

[0018] In some embodiments, the second antigen binding site comprises the VL sequence and the VH sequence from the other ABS clone.

[0019] In some embodiments, the second antigen binding site comprises a full immunoglobulin heavy chain sequence comprising the VH sequence and a full immunoglobulin light chain sequence comprising the VL sequence from the other ABS clone.

[0020] In some embodiments, the second antigen binding site is specific for an antigen other than the first GAL9 antigen.

[0021] In some embodiments, the first antigen binding site comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from any one of the ABS clones selected from: P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

[0022] In some embodiments, the first antigen binding site comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from any one of the ABS clones selected from: P9-18, P9-15, P9-21, and P9-28.

[0023] In some embodiments the first antigen binding site comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from ABS clone P9-15.

[0024] In some embodiments, the first antigen binding site comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from ABS clone P9-18.

[0025] In some embodiments, the first antigen binding site comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from ABS clone P9-21.

[0026] In some embodiments, the first antigen binding site comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from ABS clone P9-22.

[0027] In some embodiments, the first antigen binding site comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from ABS clone P9-28.

[0028] In some embodiments, the GAL9 antigen binding molecule comprises an antibody format selected from the group consisting of: full-length antibodies, Fab fragments, F(ab)'₂ fragments, Fvs, scFvs, tandcFvs, diabodies, scDiabodies, DARTs, single chain VHH camelid antibodies, tandAbs, minibodies, and B-bodies. B-bodies are described in US pre-grant publication number US 2018/0118811, which is incorporated herein by reference in its entirety.

[0029] In some embodiments, the GAL9 antigen binding molecule increases TNF- α secretion by activated immune cells, wherein the increase is greater than an 20, 30, 40, 50, 60, 70, or 80-fold increase relative to activated immune cells treated with a control agent.

[0030] In some embodiments, the GAL9 antigen binding molecule increases IFN- γ secretion by activated immune cells, wherein the increase is greater than an 1.2-fold increase relative to activated immune cells treated with a control agent.

[0031] In some embodiments, the GAL9 antigen binding molecule increases CD40L surface expression of activated CD8⁺ T-cells, wherein the increase is greater than a 2-fold increase relative to activated CD8⁺ T-cells treated with a control agent.

[0032] In some embodiments, the GAL9 antigen binding molecule increases OX40 surface expression of Activated CD8⁺ T-cells, wherein the increase is greater than a 2-fold increase relative to activated CD8⁺ T-cells treated with a control agent.

[0033] In some embodiments, the GAL9 antigen binding molecule increases IL-12 production of activated dendritic cells (DCs), wherein the increase is greater than an 20-fold increase relative to activated DCs treated with a control agent.

[0034] In some embodiments, GAL9 antigen binding molecule increases PD-L2 surface expression on activated dendritic cells (DCs), wherein the increase is greater than an 4-fold increase relative to activated DCs treated with a control agent.

[0035] In some embodiments, the control agent is a negative control agent or positive control agent.

[0036] In some embodiments, the control agent is a control antibody.

[0037] In some embodiments, the control antibody is selected from the group consisting of: an ECA42 clone anti-GAL9 antibody, an RG9.1 clone anti-GAL9 antibody, an RG9.35 clone anti-GAL9 antibody, an anti-PD1 antibody, and a non-GAL9 binding isotype control antibody.

[0038] In some embodiments, the activated immune cells, activated CD8⁺ T-cells, or activated DCs were activated by peptide stimulation, e.g., by a peptide or plurality of peptides known to induce an immune response.

[0039] In a fifth aspect, the disclosure provides a GAL9 antigen binding molecule increases TNF- α secretion by activated immune cells, wherein the increase is greater than an 80-fold increase relative to activated immune cells treated with a control agent.

[0040] In a sixth aspect, the disclosure provides a GAL9 antigen binding molecule increases IFN- γ secretion by activated immune cells, wherein the increase is greater than an 1.2-fold increase relative to activated immune cells treated with a control agent.

[0041] In a seventh aspect, the disclosure provides a GAL9 antigen binding molecule increases CD40L surface expression of Activated CD8⁺ T-cells, wherein the increase is greater than a 2-fold increase relative to activated CD8⁺ T-cells treated with a control agent.

[0042] In an eighth aspect, the disclosure provides a GAL9 antigen binding molecule increases OX40 surface expression of Activated CD8⁺ T-cells, wherein the increase is greater than a 2-fold increase relative to activated CD8⁺ T-cells treated with a control agent.

[0043] In a ninth aspect, the disclosure provides a GAL9 antigen binding molecule increases IL-12 production of activated dendritic cells (DCs), wherein the increase is greater than an 20-fold increase relative to activated DCs treated with a control agent.

[0044] In a tenth aspect, the disclosure provides a GAL9 antigen binding molecule increases PD-L2 surface expression on activated dendritic cells (DCs), wherein the increase is greater than an 4-fold increase relative to activated DCs treated with a control agent.

[0045] In an eleventh aspect, the disclosure provides a GAL9 antigen binding molecule demonstrates one or more of the following properties: A) increases TNF- α secretion by activated immune cells, wherein the increase is greater than an 80-fold increase relative to

activated immune cells treated with a control agent; B) increases IFN- γ secretion by activated immune cells, wherein the increase is greater than an 1.2-fold increase relative to activated immune cells treated with a control agent; C) increases CD40L surface expression of activated CD8⁺ T-cells, wherein the increase is greater than a 2-fold increase relative to activated CD8⁺ T-cells treated with a control agent; D) increases OX40 surface expression of activated CD8⁺ T-cells, wherein the increase is greater than a 2-fold increase relative to activated CD8⁺ T-cells treated with a control agent; E) increases IL-12 production of activated dendritic cells (DCs), wherein the increase is greater than an 20-fold increase relative to activated DCs treated with a control agent; F) increases PD-L2 surface expression on activated dendritic cells (DCs), wherein the increase is greater than an 4-fold increase relative to activated DCs treated with a control agent.

[0046] In some embodiments, the control agent is a negative control agent or positive control agent.

[0047] In some embodiments, the control agent is a control antibody.

[0048] In some embodiments, the control antibody is selected from the group consisting of: an ECA42 clone anti-GAL9 antibody, an RG9.1 clone anti-GAL9 antibody, an RG9.35 clone anti-GAL9 antibody, an anti-PD1 antibody, and a non-GAL9 binding isotype control antibody.

[0049] In some embodiments, the activated immune cells, activated CD8⁺ T-cells, or activated DCs were activated by peptide stimulation, e.g., by a peptide or plurality of peptides known to induce an immune response.

[0050] In some embodiments, the GAL9 antigen binding molecule of the fifth-eleventh aspects provided herein comprise a first antigen binding site specific for a first epitope of a first GAL9 antigen, wherein the first antigen binding site comprises all three VH CDRs and all three VL CDRs from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

[0051] In some embodiments, the VL sequence and the VH sequence from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

[0052] In some certain embodiments, the GAL9 antigen binding molecule comprises a full immunoglobulin heavy chain sequence comprising the VH sequence and a full immunoglobulin light chain sequence comprising the VL sequence, wherein the VH sequence

and the VL sequence are from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

[0053] In some embodiments, the GAL9 antigen is a human GAL9 antigen.

[0054] In some embodiments, the GAL9 antigen binding molecule further comprises a second antigen binding site.

[0055] In some embodiments, the second antigen binding site is specific for the GAL9 antigen.

[0056] The GAL9 antigen binding molecule of claim 48, wherein the second antigen binding site is identical to the first antigen binding site.

[0057] In some embodiments, the second antigen binding site is specific for a second epitope of the first GAL9 antigen.

[0058] In some embodiments, the second antigen binding site comprises all three VH CDRs and all three VL CDRs from another ABS clone selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

[0059] In some embodiments, the second antigen binding site comprises the VL sequence and the VH sequence from the other ABS clone.

[0060] In some embodiments, the second antigen binding site comprises a full immunoglobulin heavy chain sequence comprising the VH sequence and a full immunoglobulin light chain sequence comprising the VL sequence from the other ABS clone.

[0061] In some embodiments, the second antigen binding site is specific for an antigen other than the first GAL9 antigen.

[0062] In some embodiments, the first antigen binding site comprises all three VH CDRs and all three VL CDRs from any one of the ABS clones selected from: P9-10, P9-15, P9-18, P9-21, P9-22, and P9-28.

[0063] In some embodiments, the first antigen binding site comprises all three VH CDRs and all three VL CDRs from any one of the ABS clones selected from: P9-10, P9-15, P9-18, P9-21, P9-22, and P9-28.

[0064] In some embodiments, the first antigen binding site comprises all three VH CDRs and all three VL CDRs from ABS clone P9-15.

[0065] In some embodiments, the first antigen binding site comprises all three VH CDRs and all three VL CDRs from ABS clone P9-18.

[0066] In some embodiments, the first antigen binding site comprises all three VH CDRs and all three VL CDRs from ABS clone P9-21.

[0067] In some embodiments, the first antigen binding site comprises all three VH CDRs and all three VL CDRs from ABS clone P9-22.

[0068] In some embodiments, the first antigen binding site comprises all three VH CDRs and all three VL CDRs from ABS clone P9-28.

[0069] In some embodiments, the GAL9 antigen binding molecule comprises an antibody format selected from the group consisting of: full-length antibodies, Fab fragments, Fvs, scFvs, tandem scFvs, Diabodies, scDiabodies, DARTs, tandAbs, minibodies, and B-bodies.

[0070] In a twelfth aspect, the disclosure provides a GAL9 antigen binding molecule which binds to the same epitope as a GAL9 antigen binding molecule of any one of the preceding claims.

[0071] In a thirteenth aspect, the disclosure provides a GAL9 antigen binding molecule which competes for binding with a GAL9 antigen binding molecule of any one of the preceding claims.

[0072] In some embodiments, the GAL9 antigen binding molecule is purified.

[0073] In a fourteenth aspect, the disclosure provides a pharmaceutical composition comprising the GAL9 antigen binding molecule of any one of the preceding claims and a pharmaceutically acceptable diluent.

[0074] In a fifteenth aspect, the disclosure provides a method for treating a subject with cancer, the method comprising administering a therapeutically effective amount of the pharmaceutical composition as provided herein to the subject.

[0075] In some embodiments, the cancer is selected from the group consisting of: pancreatic cancer, ovarian cancer, breast cancer, lung cancer, gastric cancer, melanoma, Ewing sarcoma, chronic lymphocytic leukemia, mantle cell lymphoma, B-ALL, hematological cancer, head and neck squamous cell carcinoma, prostate cancer, colon cancer, renal cancer, and uterine cancer.

[0076] In some embodiments, cancer is selected from the group consisting of: the breast cancer, colon cancer, lung cancer and prostate cancer, cancers of the blood and lymphatic systems (including Hodgkin's disease, leukemias, lymphomas, multiple myeloma, and Waldenstrom's disease), skin cancers (including malignant melanoma), cancers of the digestive tract (including head and neck cancers, esophageal cancer, stomach cancer, cancer of the pancreas, liver cancer, colon and rectal cancer, anal cancer), cancers of the genital and urinary systems (including kidney cancer, bladder cancer, testis cancer, prostate cancer),

cancers in women (including breast cancer, ovarian cancer, gynecological cancers and choriocarcinoma) as well as in brain, bone carcinoid, nasopharyngeal, retroperitoneal, thyroid and soft tissue tumors.

[0077] In some embodiments, the cancer is a viral induced tumor caused by a cancer virus. In some embodiments, the cancer virus is a Epstein-Barr virus (EBV), Hepatitis B virus, Hepatitis C virus, Human papilloma virus, Human T-lymphotropic virus 1 (HTLV-1), Kaposi sarcoma associated-herpesvirus (KHSV), Merkel cell polyomavirus, or Cytomegalovirus.

5. BRIEF DESCRIPTION OF THE DRAWINGS

[0078] **FIG. 1** shows results of administering immune-activating anti-GAL9 (α -GAL9) antibodies in a colon cancer tumor model. BALB/c mice were implanted subcutaneously with CT26 tumor line cells and treated with control rat IgG, or anti-GAL9 antibodies P9-18 or P9-21. All treatments were intraperitoneal (I.P.), 200 μ g, on days 7, 11, 15, and 19. n=10/group. Tumor growth was assessed by measuring tumor volume. Mice treated with P9-18 and P9-21 demonstrated reduced growth of the implanted CT26 tumors as compared to treatment with control IgG.

[0079] **FIG. 2** shows results of administering immune-activating anti-GAL9 antibodies in a melanoma tumor model. C57BL/6 mice were implanted subcutaneously with B16.F0 tumor line cells and treated with control IgG or α -GAL9 antibodies P9-18 or P9-21. All treatments were intraperitoneal (I.P.), 200 μ g, on days 7, 11, 15, and 19. n=10/group. Tumor growth was assessed by measuring tumor volume. Mice treated with P9-18 and P9-21 demonstrated reduced growth of the implanted B16.F0 tumors as compared to treatment with control IgG.

[0080] **FIGs. 3A and 3B** show $\text{INF-}\gamma$ (**3A**) and $\text{TNF-}\alpha$ (**3B**) secretion from activated PBMCs stimulated *in vitro* with various GAL9 antibody candidates, a known comparator Tool antibody (Tool mAb), an anti-PD-1 antibody, a control antibody (IgG Ctrl), and a vehicle control (PBS Ctrl). Black diamond shapes show secretion from activated PBMCs stimulated by comparator Tool mAb and anti-PD-1 antibody, positive controls.

[0081] **FIG. 4** shows levels of immune stimulatory markers CD27, CD40L, ICOS, 4-1BB, and OX40 on the surface of activated CD8^+ T cells stimulated *in vitro* with various GAL9 antibody candidates or an IgG control antibody.

[0082] **FIG. 5** shows representative flow cytometry plots quantifying IL-12 production by DCs stimulated *in vitro* with control IgG or α -GAL9 candidate P9-18, along with a staining control.

[0083] **FIGs. 6A and 6B** show representative flow cytometry plots of TNF- α secretion by CD56⁺ NK cells following 72 hours' stimulation with control antibody P9-55 (Clone 55), anti-GAL9 candidate antibody P9-15 (Clone 15), or α -GAL9 candidate antibody P9-18 (Clone 18) at dosages 5 μ g (**FIG. 6A**) or 20 μ g (**FIG. 6B**).

[0084] **FIGs. 7A-7E** show illustrative examples of Martin numbering scheme with various CDR definitions – Chothia, AbM, Kabat, Contact, IMGT – as applied to the P9-28 anti-GAL9 candidate antibody provided herein. **FIGs. 7A-7E** each disclose SEQ ID NOS 187 and 188, respectively, in order of appearance.

[0085] **FIGs. 8A-8C** show representative confocal microscopy images demonstrating co-localization and clustering of GAL9 and PD-L2 on DCs after treatment with IgG control (**FIG. 8A**), P9-18 (**FIG. 8B**), and P9-21 (**FIG. 8C**). The blue staining shows DNA (DAPI), red staining shows PD-L2, green staining shows CD11c, and yellow staining shows GAL9. Non-labeled microscopy images are bright field; rendered in gray scale in the attached figures.

[0086] **FIGs. 9A and 9B** show representative confocal images demonstrating retention of PD-L2 and PD-L1 on the surface of CT26 tumor cells after treatment with anti-GAL9 P9-18 (**FIG. 9B**) compared to IgG control (**FIG. 9A**). The speckles in the images highlight increased expression of PD-L2 and PD-L1 ligands. The blue staining shows DNA (DAPI), the red staining shows PD-L2, and the green staining shows PD-L1; rendered in gray scale in the attached figures.

[0087] **FIGs. 10A-E** show representative data from an EBV-infected humanized mouse model treated with anti-GAL9 P9-15. **FIG. 10A** shows a schematic of the protocol with treatment timeline. **FIG. 10B** shows images of spleens from mice treated with IgG control and P9-15. Arrows point to uncontrolled tumor growth in IgG control mice. **FIG. 10C** shows bar graphs of the weights of spleens. **FIG. 10D** shows bar graphs of the number of cells per spleen. **FIG. 10E** shows bar graphs of spleen viral load.

[0088] **FIG. 11** shows representative data from an EBV-infected humanized mouse model treated with anti-GAL9 P9-28. **FIG. 10A** shows a schematic of the protocol with treatment timeline. **FIG. 11** shows images of spleens from IgG control and anti-GAL9 P9-28 treated mice. Arrows point to uncontrolled tumor growth in IgG control treated mice.

[0089] **FIG. 12A** shows *in vivo* evaluation of tumor growth in a CT26 tumor model with P9-18-IgG1 (diamond $\diamond$), sFc-P9-18-IgG2a (upside-down triangle ∇), P9-18-IgG2a (circle $\circ$), and IgG (IgG2a) control #1 (black squares $\blacksquare$).

[0090] FIG. 12B shows an *in vivo* evaluation of immune memory in previously treated CT26 tumors with sFc-P9-18 IgG2a (upside-down triangle ▽), P9-18 IgG2a (circle ○), and IgG (IgG2a) control #2 (black diamond ◆).

[0091] FIG. 13 shows a bar graph of the mean percentage of PD-L1⁺ or PD-L2⁺ tumor-associated dendritic cells (CD11c⁺) and the mean cell surface expression (GMI) of PD-L1 or PD-L2 on tumor-associated dendritic cells (CD11c⁺) after treatment with anti-GAL9 P9-18 or control.

[0092] FIG. 14 shows bar graphs of the mean percentage of PD-L1⁺ or PD-L2⁺ tumor cells and the mean cell surface expression level of PD-L1 or PD-L2 (GMI) on tumor cells after treatment with anti-GAL9 P9-18 or IgG control.

6. DETAILED DESCRIPTION

6.1. Definitions

[0093] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them below.

[0094] By “**antigen binding site**” or “**ABS**” is meant a region of a GAL9 binding molecule that specifically recognizes or binds to a given antigen or epitope.

[0095] As used herein, the terms “**treat**” or “**treatment**” are used in their broadest accepted clinical sense. The terms include, without limitation, lessening a sign or symptom of disease; improving a sign or symptom of disease; alleviation of symptoms; diminishment of extent of disease; stabilized (i.e., not worsening) state of disease; delay or slowing of disease progression; amelioration or palliation of the disease state; remission (whether partial or total), whether detectable or undetectable; cure; prolonging survival as compared to expected survival if not receiving treatment. Unless explicitly stated otherwise, “treat” or “treatment” do not intend prophylaxis or prevention of disease.

[0096] By “**subject**” or “**individual**” or “**animal**” or “**patient**” or “**mammal**,” is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, and so on. Unless otherwise stated, “patient” intends a human “subject.”

[0097] The term “**sufficient amount**” means an amount sufficient to produce a desired effect, e.g., an amount sufficient to modulate protein aggregation in a cell.

[0098] The term “**therapeutically effective amount**” is an amount that is effective to ameliorate a symptom of a disease.

[0099] The term “**prophylactically effective amount**” is an amount that is effective to prevent a symptom of a disease.

6.2. Other interpretational conventions

[0100] Unless otherwise specified, all references to sequences herein are to amino acid sequences.

[0101] Unless otherwise specified, antibody constant region residue numbering is according to the Eu index as described at

www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html#refs

(accessed Aug. 22, 2017), which is hereby incorporated by reference in its entirety, and residue numbers identify the residue according to its location in an endogenous constant region sequence regardless of the residue’s physical location within a chain of the GAL9 binding molecules described herein.

[0102] Unless otherwise specified as “Kabat CDR”, “Chothia CDR”, “Contact CDR”, or “IMGT CDR”, all references to “**CDRs**” are to CDRs defined using the Martin (AbM) definition.

[0103] By “**endogenous sequence**” or “native sequence” is meant any sequence, including both nucleic acid and amino acid sequences, which originates from an organism, tissue, or cell and has not been artificially modified or mutated.

[0104] Polypeptide chain numbers (*e.g.*, a “first” polypeptide chains, a “second” polypeptide chain. *etc.* or polypeptide “chain 1,” “chain 2,” *etc.*) are used herein as a unique identifier for specific polypeptide chains that form a binding molecule and is not intended to connote order or quantity of the different polypeptide chains within the binding molecule.

[0105] In this disclosure, “**comprises,**” “**comprising,**” “**containing,**” “**having,**” “**includes,**” “**including,**” and linguistic variants thereof have the meaning ascribed to them in U.S. Patent law, permitting the presence of additional components beyond those explicitly recited.

[0106] As used herein, the singular forms “a,” “an,” and “the” include the plural referents unless the context clearly indicates otherwise. The terms “include,” “such as,” and the like are intended to convey inclusion without limitation, unless otherwise specifically indicated.

[0107] **Ranges** provided herein are understood to be shorthand for all of the values within the range, inclusive of the recited endpoints. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50.

[0108] Unless specifically stated or otherwise apparent from context, as used herein the term “**about**” is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value.

6.3. General Overview

[0109] The present disclosure provides Galectin-9 (GAL9) antigen binding molecules, such as anti-GAL9 antibodies and antigen-binding fragments thereof; compositions comprising the GAL9-binding molecules; and pharmaceutical compositions comprising the GAL9-binding molecules. The disclosure particularly provides various GAL9 antigen binding molecules that are stimulatory, acting as activators of the immune system, increasing secretion and production of various cytokines in various immune cells and increasing surface expression of stimulatory molecules.

[0110] Also provided by the disclosure are methods of treating a disease or condition in a subject by administering an immune-stimulatory Galectin-9 antibody binding molecule. The methods provided by the disclosure are particularly useful for the treatment of a proliferative disease or cancer. In some embodiments, the cancer is a viral-induced cancer, for example, a cancer caused by an infection by an oncovirus or tumor virus. In some embodiments, the compositions and methods provided by the disclosure can be used for the treatment of a disease or condition that is immunosuppressive, such as malaria, HIV or AIDs, or the like.

6.4. GAL9 antigen binding molecules

[0111] In a first aspect, antigen binding molecules are provided. In every embodiment, the antigen binding molecule includes at least a first antigen binding site specific for a GAL9

antigen; the binding molecules are therefore termed GAL9 antigen binding molecules or GAL9 binding molecules.

[0112] The GAL9 antigen binding molecules described herein bind specifically to GAL9 antigens.

[0113] As used herein, “GAL9 antigens” refer to Galectin-9 family members and homologs. GAL9 is also referred to as LGALS9, HUAT, LGALS9A, tumor antigen HOM-HD-21, and ecalectin. In particular embodiments, the GAL9 binding molecule has antigen binding sites that specifically bind to at least a portion of more than one GAL9 domain, such as the junction between a first and a second GAL9 domain.

[0114] In specific embodiments, the GAL9 antigen is human. GenBank Accession # NP_033665.1 describes a canonical human GAL9 protein, including its sequences and domain features, and is hereby incorporated by reference in its entirety. SEQ ID NO:6 provides the full-length GAL9 protein sequence.

```
MAFSGSQAPYLSPAVPFSGTIQGGLQDGLQITVNGTVLSSSGTRFAVNFQTGFSGND
IAFHFNPRFEDGGYVVCNTRQNGSWGPEERKTHMPFQKGMPFDLCFLVQSSDFKVMV
NGILFVQYFHRVPFHRVDTISVNGSVQLSYISFQNPRTVPVQPAFSTVPFSPVCFP
PRPRGRRQKPPGVWPANPAPITQTVIHTVQSAPGQMFSTPAIPPMYPHPAYPMPFI
TTILGGLYPSKSILLSGTVLPSAQRFHINLCSGNHIAFHLNPRFDENAVVRNTQIDN
SWGSEERSLPRKMPFVRGQSFSVWILCEAHCLKVAVDGQHLFEYYHRLRNLPINRL
EVGGDIQLTHVQT [SEQ ID NO:6]
```

[0115] In various embodiments, the GAL9 binding molecule additionally binds specifically to at least one antigen additional to a GAL9 antigen.

6.4.1. Functional characteristics of the GAL9 antigen binding molecules

[0116] In some embodiments, upon contact therewith, the GAL9 antigen binding molecule increases cytokine secretion by activated immune cells, e.g., activated human immune cells. In some embodiments, the immune cells are peripheral blood mononuclear cells (PBMCs). In some embodiments, the immune cells are T cells. In some embodiments, the T cells are effector T cells. In some embodiments, the T cells are CD8⁺ T cells. In some embodiments,

the T cells are CD4⁺ T cells. In some embodiments, the immune cells are natural killer (NK) cells. In some embodiments, the immune cells are dendritic cells (DC).

[0117] The impact of the GAL9 antigen binding molecule on immune cell cytokine secretion may be determined by any suitable means. For instance, the impact of the GAL9 antigen binding molecule on immune cell cytokine secretion may be determined in vivo, ex vivo, or in vitro. In some embodiments, cytokine secretion is determined in activated immune cells contacted with a GAL9 antigen binding molecule, as compared to activated immune cells contacted with a control agent, e.g., a control antigen binding molecule or vehicle control. The immune cells may be activated by peptide stimulation. For example, the immune cells may be activated by a peptide or plurality of peptides known to induce an immune response. The control agent can be a negative control or a positive control. In some embodiments, the GAL9 antigen binding molecule increases cytokine secretion in immune cells, relative to a negative control agent or negative control antigen binding molecule. In some embodiments, the negative control antigen binding molecule is an isotype control binding molecule that does not bind GAL9. In some embodiments, the positive control antibody is an anti-PD1 antibody, such as nivolumab. In some embodiments, the positive control antibody is a GAL9 control antibody. The GAL9 control antibody can be Gal9 antibody clone RG9.1 (Cat. No. BE0218, InVivoMab Antibodies) or RG9.35. RG9.1 and RG9.35 are both described in Fukushima A, Sumi T, Fukuda K, Kumagai N, Nishida T, *et al.* (2008), “Roles of galectin-9 in the development of experimental allergic conjunctivitis in mice,” *Int Arch Allergy Immunol* 146: 36–43, which is hereby incorporated by reference in its entirety. The GAL9 control antibody can be Gal9 antibody clone ECA42 (Cat. No. LS-C179449, LifeSpan BioScience). In some embodiments, the GAL9 antigen binding molecule increases cytokine secretion in immune cells, relative to the positive control antibody.

[0118] Cytokine secretion by the immune cells can be assessed by any suitable means. By way of example only, cytokine secretion by in vitro or ex vivo immune cell culture models may be assessed by analyzing cytokine content of the cultured cell supernatants, e.g., by cytokine bead array.

[0119] In some embodiments, the cytokine is IFN- γ . In some embodiments, the GAL9 antigen binding molecule increases IFN- γ secretion in activated immune cells by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 105%, 110%, 115%, or 120%. In some embodiments, the GAL9 antigen binding molecule increases IFN- γ secretion in activated immune cells by at least 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35%-40%, 40%-45%, 45%-50%, 50%-55%, 55%-60%,

60%-65%, 70% -75%, 75%-80%, 80%-85%, 85%-90%, 90%-95%, 95%-100%, 100%-105%, 105%-110%, 110%-115%, or 115%-120%.

[0120] In some embodiments, the cytokine is TNF- α . In some embodiments, the GAL9 antigen binding molecule increases TNF- α secretion in activated immune cells by at least 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500%, 550%, 600%, 650%, 700%, 750%, 800%, 850%, 900%, 950%, 10,00%, 10,500%, 11,000%, 11,500%, 12,000%, 12,500%, 13,000%, 13,500%, 14,000%, 14,500%, 15,000%, 15,500%, 16,000%, 16,500%, 17,000%, 17,500%, 18,000%, 18,500%, 19,000%, 19,500%, 20,000%, 20,500%, 30,000%, 30,500%, 40,000%, 40,500%, 50,000%, 50,500%, 60,000%, 60,500%, 70,000%, 70,500%, 80,000%, 80,500%, 90,000%, or 90,500% as compared to a negative control agent described herein. In some embodiments, the GAL9 antigen binding molecule increases TNF- α secretion in activated immune cells by at least 100% -150%, 150% -200%, 200% -250%, 250%-300%, 300%-350%, 350%-400%, 400%-450%, 500%-550%, 550%-600%, 600%-650%, 650%-700%, 700%-750%, 750%- 800%, 800%-850%, 850%-900%, 900%- 950%, 950%-10,000%, 10,000%-10,500%, 10,500%-11,000%, 11,000-11,500%, 11,500-12,000%, 12,000% -12,500%, 13,000%-13,500%, 13,500%-14,000%, 14,000%-14,500%, 14,500%-15,000%, 15,000- 15,500%, 15,550%-16,000%, 16,000%-16,500%, 17,000%-17,500%, 17,500%-18,000%, 17,500%-18,500%, 18,500%-19,000%, 19,000%-19,500%, 19,500%-20,000%, 20,000%-20,500%, 20,500%-30,000%, 30,000%-30,500%, 30,500%-40,000%, 40,000%- 40,500%, 45,500%-50,000%, 50,000%-50,500%, 55,500%-60,000%, 60,000%-60,500%, 70,000%-70,500%, 70,500%-80,000%, 80,000%-80,500%, 85,000%-90,000%, or 90,000%-90,500% as compared to a negative control agent described herein.

[0121] In various embodiments, the activated immune cells are T-cells, CD8⁺ T cells, NK cells, CD4⁺ T cells, or Dendritic Cells (DC).

[0122] In some embodiments, the GAL9 antigen binding molecule increases surface expression of one or more costimulatory molecules on immune cells, e.g., human immune cells. In certain embodiments, the GAL9 antigen binding molecule increases surface expression of the one or more costimulatory molecules in activated immune cells. In particular embodiments, the immune cells are T cells. In specific embodiments, the activated immune cells are CD8⁺ T cells. In certain embodiments, the activated immune cell is an NK cell. In certain embodiments, the activated immune cell is a dendritic cell.

[0123] In some embodiments, the one or more costimulatory molecules is selected from 4-1BB, CD27, CD40L, ICOS, and OX40. In some embodiments, the one or more

costimulatory molecules is selected from 4-1BB, CD27, CD40L, and OX40. In some embodiments, the one or more costimulatory molecules is selected from 4-1BB, CD40L, and OX40.

[0124] The impact of the GAL9 antigen binding molecule on surface expression of the one or more costimulatory molecules may be determined by any suitable means. For instance, the impact of the GAL9 antigen binding molecule on surface expression of the one or more costimulatory molecules may be determined in vivo, ex vivo, or in vitro.

[0125] In some embodiments, the GAL9 antigen binding molecule increases surface expression of the one or more costimulatory molecules in activated immune cells as compared to activated immune cells treated with a control agent. Exemplary control agents are described herein. In particular embodiments, the control agent is an isotype control binding molecule that does not bind GAL9.

[0126] In some embodiments, the GAL9 antigen binding molecule increases CD40L surface expression of activated CD8⁺ T-cells, relative to activated CD8⁺ T-cells treated with the control agent. In some embodiments, activated CD8⁺ T-cells treated with the GAL9 antigen binding molecule exhibits at least about a 0.1X increase, 0.2X increase, 0.3X increase, 0.4X increase, 0.5X increase, 0.6X increase, 0.7X increase, 0.8X increase, 0.9X increase, 1X increase, 2X increase, 3X increase, 4X increase, 5X increase, 6X increase, 7X increase, 8X increase, 9X increase, 10X increase, or greater than 10X increase in CD40L surface expression relative to activated CD8⁺ T-cells treated with the control agent. In some embodiments, activated CD8⁺ T-cells treated with the GAL9 antigen binding molecule exhibits about a 0.1X-10X increase, a 0.5X-5X increase, a 1X-4X increase, or about a 1.5X-2.5X increase in CD40L surface expression, relative to activated CD8⁺ T-cells treated with the control agent.

[0127] In some embodiments, the GAL9 antigen binding molecule increases OX40 surface expression of activated CD8⁺ T-cells, relative to activated CD8⁺ T-cells treated with the control agent. In some embodiments, activated CD8⁺ T-cells treated with the GAL9 antigen binding molecule exhibits about at least a 0.1X increase, 0.2X increase, 0.3X increase, 0.4X increase, 0.5X increase, 0.6X increase, 0.7X increase, 0.8X increase, 0.9X increase, 1X increase, 2X increase, 3X increase, 4X increase, 5X increase, 6X increase, 7X increase, 8X increase, 9X increase, 10X increase, or greater than 10X increase in OX40 surface expression relative to activated CD8⁺ T-cells treated with the control agent. In some embodiments, activated CD8⁺ T-cells treated with the GAL9 antigen binding molecule exhibits about a

0.1X-10X increase, a 0.5X-5X increase, or about a 1.0X-2.0X increase in OX40 surface expression, relative to activated CD8⁺ T-cells treated with the control agent.

[0128] In some embodiments, the GAL9 antigen binding molecule increases 4-1BB surface expression of activated CD8⁺ T-cells, relative to activated CD8⁺ T-cells treated with the control agent. In some embodiments, activated CD8⁺ T-cells treated with the GAL9 antigen binding molecule exhibits about at least a 0.1X increase, 0.2X increase, 0.3X increase, 0.4X increase, 0.5X increase, 0.6X increase, 0.7X increase, 0.8X increase, 0.9X increase, 1X increase, 2X increase, 3X increase, 4X increase, 5X increase, 6X increase, 7X increase, 8X increase, 9X increase, 10X increase, or greater than 10X increase in 4-1BB surface expression relative to activated CD8⁺ T-cells treated with the control agent. In some embodiments, activated CD8⁺ T-cells treated with the GAL9 antigen binding molecule exhibits about a 0.1X-10X increase, a 0.2X-2X increase, or about a 0.5X-1X increase in 4-1BB surface expression, relative to activated CD8⁺ T-cells treated with the control agent.

[0129] In some embodiments, the GAL9 antigen binding molecule increases CD27 surface expression of activated CD8⁺ T-cells, relative to activated CD8⁺ T-cells treated with the control agent. In some embodiments, activated CD8⁺ T-cells treated with the GAL9 antigen binding molecule exhibits about at least a 1% increase, 2% increase, 3% increase, 4% increase, 5% increase, 6% increase, 7% increase, 8% increase, 9% increase, 10% increase, 11% increase, 12% increase, 13% increase, 14% increase, 15% increase, 16% increase, 17% increase, 18% increase, 19% increase, 20% increase, 21% increase, 22% increase, 23% increase, 24% increase, 25% increase, 26% increase, 27% increase, 28% increase, 29% increase, 30% increase, 31% increase, 32% increase, 33% increase, 34% increase, 35% increase, 36% increase, 37% increase, 38% increase, 39% increase, 40% increase, 41% increase, 42% increase, 43% increase, 44% increase, 45% increase, 46% increase, 47% increase, 48% increase, 49% increase, 50% increase, 51% increase, 52% increase, 53% increase, 54% increase, 55% increase, 56% increase, 57% increase, 58% increase, 59% increase, 60% increase, 61% increase, 62% increase, 63% increase, 64% increase, 65% increase, 66% increase, 67% increase, 68% increase, 69% increase, 70% increase, 71% increase, 72% increase, 73% increase, 74% increase, 75% increase, 76% increase, 77% increase, 78% increase, 79% increase, 80% increase, 81% increase, 82% increase, 83% increase, 84% increase, 85% increase, 86% increase, 87% increase, 88% increase, 89% increase, 90% increase, 91% increase, 92% increase, 93% increase, 94% increase, 95% increase, 96% increase, 97% increase, 98% increase, 99% increase, or 100% increase in CD27 surface expression, relative to activated CD8⁺ T-cells treated with the control agent.

In some embodiments, activated CD8⁺ T-cells treated with the GAL9 antigen binding molecule exhibits about at least a 1%-100% increase, a 5%-50% increase, a 10%-40% increase, or about a 20%-30% increase in CD27 surface expression, relative to activated CD8⁺ T-cells treated with the control agent.

[0130] In some embodiments, the GAL9 antigen binding molecule increases ICOS surface expression of activated CD8⁺ T-cells, relative to activated CD8⁺ T-cells treated with the control agent. In some embodiments, activated CD8⁺ T-cells treated with the GAL9 antigen binding molecule exhibits about at least a 1% increase, 2% increase, 3% increase, 4% increase, 5% increase, 6% increase, 7% increase, 8% increase, 9% increase, 10% increase, 11% increase, 12% increase, 13% increase, 14% increase, 15% increase, 16% increase, 17% increase, 18% increase, 19% increase, 20% increase, 21% increase, 22% increase, 23% increase, 24% increase, 25% increase, 26% increase, 27% increase, 28% increase, 29% increase, 30% increase, 31% increase, 32% increase, 33% increase, 34% increase, 35% increase, 36% increase, 37% increase, 38% increase, 39% increase, 40% increase, 41% increase, 42% increase, 43% increase, 44% increase, 45% increase, 46% increase, 47% increase, 48% increase, 49% increase, 50% increase, 51% increase, 52% increase, 53% increase, 54% increase, 55% increase, 56% increase, 57% increase, 58% increase, 59% increase, 60% increase, 61% increase, 62% increase, 63% increase, 64% increase, 65% increase, 66% increase, 67% increase, 68% increase, 69% increase, 70% increase, 71% increase, 72% increase, 73% increase, 74% increase, 75% increase, 76% increase, 77% increase, 78% increase, 79% increase, 80% increase, 81% increase, 82% increase, 83% increase, 84% increase, 85% increase, 86% increase, 87% increase, 88% increase, 89% increase, 90% increase, 91% increase, 92% increase, 93% increase, 94% increase, 95% increase, 96% increase, 97% increase, 98% increase, 99% increase, or 100% increase in ICOS surface expression, relative to activated CD8⁺ T-cells treated with the control agent. In some embodiments, activated CD8⁺ T-cells treated with the GAL9 antigen binding molecule exhibits about at least a 1%-100% increase, a 5%-50% increase, a 10%-40% increase, or about a 20%-30% increase in ICOS surface expression, relative to activated CD8⁺ T-cells treated with the control agent.

[0131] In some embodiments, the GAL9 antigen binding molecule increases retention of PD-L1, PD-L2, or both PD-L1 and PD-L2 on the surface of tumor cells. In some embodiments, the increased retention of PD-L1, PD-L2, or both PD-L1 and PD-L2 on the surface of tumor cells is demonstrated by microscopy techniques, e.g., confocal microscopy.

[0132] In some embodiments, the GAL9 antigen binding molecule increases PD-L2 expression on the surface of dendritic cells (DCs). In some embodiments, the GAL9 antigen binding molecule decreases PD-L1 expression on the surface of dendritic cells (DCs). In some embodiments, the DCs are activated DCs. Activation of immune cells, including DCs is described herein. Surface expression of proteins, including PD-L1 and PD-L2 on DCs can be assessed by any suitable means. For example, the percentage of DCs that exhibit detectable surface PD-L1 and/or PD-L2 may be measured by, e.g., flow cytometry. In some embodiments, a population of dendritic cells treated with the GAL9 antigen binding molecule exhibits a greater percentage of cells positive for surface PD-L2 as compared to a control population of dendritic cells treated with a control agent. Exemplary control agents are described herein. In some embodiments, the control agent is an isotype antigen binding molecule that does not bind GAL9. In some embodiments, the population of dendritic cells treated with the GAL9 antigen binding molecule exhibits about a 0.1X-100X, a 0.5X-20X, a 1X-10X, or about a 5X-6X increase in the percentage of DCs exhibiting detectable surface PD-L2 expression, relative to a control population of dendritic cells treated with the control agent, e.g., the isotype control antigen binding molecule. In some embodiments, the population of dendritic cells treated with the GAL9 antigen binding molecule exhibits about a 1%-50% decrease, a 5%-30% decrease, or about a 10%-20% decrease in the percentage of DCs exhibiting detectable surface PD-L1 expression, relative to a control population of dendritic cells treated with the control agent, e.g., the isotype control antigen binding molecule.

[0133] In some embodiments, the GAL9 antigen binding molecule increases cell surface aggregation of PD-L2 in dendritic cells (DCs). In some embodiments, the DCs are activated DCs. Activation of immune cells, including DCs is described herein. In some embodiments, the increase in cell surface aggregation of PD-L2 is relative to DCs treated with a control agent. Control agents are described herein. In some embodiments, the control agent is an isotype antigen binding molecule that does not bind GAL9. Cell surface aggregation of PD-L2 in DCs may be assessed by any suitable means, e.g., confocal microscopy.

[0134] In some embodiments, the GAL9 antigen binding molecule increases IL-12 production by DCs. The DCs may be activated DCs. In some embodiments, the GAL9 antigen binding molecule increases IL-12 production in DCs, relative to DCs treated with a control agent. Exemplary control agents are described herein. In some embodiments, the control agent is an isotype antigen binding molecule that does not bind GAL9. In some embodiments, a population of DCs treated with the GAL9 antigen binding molecule exhibits

about a 0.1X-100X increase, a 10X-75X increase, a 20X-40X increase, a 25X-35X increase, or about a 28X increase in the percentage of DCs that are IL-12 positive, as compared to a population of DCs treated with the control agent.

[0135] In some embodiments, the GAL9 antigen binding molecule induces clustering of GAL9 and PD-L2 on the surface of the immune cell. In some embodiments, the immune cells can be DCs. In some embodiments, the immune cells can be NK cells.

[0136] In some embodiments, the GAL9 antigen binding molecule reduces tumor burden in a subject. The subject can be a mammal. The mammal can be a mouse. In some embodiments, the mammal is a human. In some embodiments, the GAL9 antigen binding molecule prevents growth of a tumor in the subject. The tumor can be, e.g., a colon tumor. In some embodiments, the GAL9 antigen binding molecule reduces tumor growth. In some embodiments, the GAL9 antigen binding molecule reduces tumor growth by about 25%, 50%, or more than 50%. In some embodiments, the tumor is a melanoma tumor. In some embodiments, the reduction in tumor growth is relative to a subject treated with a control agent. Exemplary control agents are described herein. In some embodiments, the control agent is an isotype antigen binding molecule that does not bind GAL9.

6.4.2. Variable Regions

[0137] The GAL9 binding molecules described herein have variable region domain amino acid sequences of an antibody, including VH and VL antibody domain sequences. VH and VL sequences are described in greater detail below in **Sections 6.4.2.1 and 6.4.2.2**, respectively.

6.4.2.1. VH Regions

[0138] The VH amino acid sequences in the GAL9 binding molecules described herein are antibody heavy chain variable domain sequences. In a typical antibody arrangement in both nature and in the GAL9 binding molecules described herein, a specific VH amino acid sequence associates with a specific VL amino acid sequence to form an antigen-binding site. In various embodiments, VH amino acid sequences are mammalian sequences, including human sequences, synthesized sequences, or combinations of non-human mammalian, mammalian, and/or synthesized sequences, as described in further detail above in **Sections 6.4.2.3 and 6.4.2.4**. In various embodiments, VH amino acid sequences are mutated sequences of naturally occurring sequences.

6.4.2.2. VL Regions

[0139] The VL amino acid sequences useful in the GAL9 binding molecules described herein are antibody light chain variable domain sequences. In a typical arrangement in both natural antibodies and the antibody constructs described herein, a specific VL amino acid sequence associates with a specific VH amino acid sequence to form an antigen-binding site. In various embodiments, the VL amino acid sequences are mammalian sequences, including human sequences, synthesized sequences, or combinations of human, non-human mammalian, mammalian, and/or synthesized sequences, as described in further detail below in **Sections 6.4.2.3 and 6.4.2.4.**

[0140] In various embodiments, VL amino acid sequences are mutated sequences of naturally occurring sequences. In certain embodiments, the VL amino acid sequences are lambda (λ) light chain variable domain sequences. In certain embodiments, the VL amino acid sequences are kappa (κ) light chain variable domain sequences. In a preferred embodiment, the VL amino acid sequences are kappa (κ) light chain variable domain sequences.

6.4.2.3. Complementarity Determining Regions

[0141] The VH and VL amino acid sequences comprise highly variable sequences termed “**complementarity determining regions**” (CDRs), typically three CDRs (CDR1, CDR2, and CDR3). In a variety of embodiments, the CDRs are mammalian sequences, including, but not limited to, mouse, rat, hamster, rabbit, camel, donkey, goat, and human sequences. In a preferred embodiment, the CDRs are human sequences. In various embodiments, the CDRs are naturally occurring sequences. In various embodiments, the CDRs are naturally occurring sequences that have been mutated to alter the binding affinity of the antigen-binding site for a particular antigen or epitope. In certain embodiments, the naturally occurring CDRs have been mutated in an *in vivo* host through affinity maturation and somatic hypermutation. In certain embodiments, the CDRs have been mutated *in vitro* through methods including, but not limited to, PCR-mutagenesis and chemical mutagenesis. In various embodiments, the CDRs are synthesized sequences including, but not limited to, CDRs obtained from random sequence CDR libraries and rationally designed CDR libraries. Martin numbering scheme was used to determine the CDR boundaries. See **FIGs. 7A-7E.**

[0142] In various embodiments, CDRs identified as binding an antigen of interest are further mutated (*i.e.*, “affinity matured”) to achieve a desired binding characteristic, such as an increased affinity for the antigen of interest relative to the original CDR. For example,

targeted introduction of diversity into the CDRs, including those CDRs identified to bind an antigen of interest, can be introduced using degenerate oligonucleotides. Various randomization schemes can be employed. For example, “soft-randomization” can be used that provides a high bias towards the identity of wild-type sequence at a given amino acid position, such as allowing a given position in CDRs to vary among all twenty amino acids while biasing towards the wild-type sequence by doping the four bases at each codon position at non-equivalent level. As an illustrative example of soft-randomization, if achieving approximately 50% of the wild-type sequence is desired, each base of each codon is kept 70% wild-type and 10% each of other nucleotides and the degenerate oligonucleotides are used to make a focused phage library around the selected CDRs with the resulting phage particles used for phage panning under various stringent selection conditions depending on the need.

6.4.2.4. Framework Regions and CDR Grafting

[0143] The VH and VL amino acid sequences comprise “framework region” (FR) sequences. FRs are generally conserved sequence regions that act as a scaffold for interspersed CDRs (see **Section 6.4.2.3**), typically in a FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 arrangement (from N-terminus to C-terminus). In a variety of embodiments, the FRs are mammalian sequences, including, but not limited to mouse, rat, hamster, rabbit, camel, donkey, goat, and human sequences. In a preferred embodiment, the FRs are human sequences. In various embodiments, the FRs are naturally occurring sequences. In various embodiments, the FRs are synthesized sequences including, but not limited, rationally designed sequences.

[0144] In a variety of embodiments, the FRs and the CDRs are both from the same naturally occurring variable domain sequence. In a variety of embodiments, the FRs and the CDRs are from different variable domain sequences, wherein the CDRs are grafted onto the FR scaffold with the CDRs providing specificity for a particular antigen. In certain embodiments, the grafted CDRs are all derived from the same naturally occurring variable domain sequence. In certain embodiments, the grafted CDRs are derived from different variable domain sequences. In certain embodiments, the grafted CDRs are synthesized sequences including, but not limited to, CDRs obtained from random sequence CDR libraries and rationally designed CDR libraries. In certain embodiments, the grafted CDRs and the FRs are from the same species. In certain embodiments, the grafted CDRs and the FRs are from different species. In a preferred grafted CDR embodiment, an antibody is “humanized”, wherein the grafted CDRs are non-human mammalian sequences including, but not limited to, mouse, rat,

hamster, rabbit, camel, donkey, and goat sequences, and the FRs are human sequences. Humanized antibodies are discussed in more detail in U.S. Pat. No. 6,407,213, the entirety of which is hereby incorporated by reference for all it teaches. In various embodiments, portions or specific sequences of FRs from one species are used to replace portions or specific sequences of another species' FRs.

6.4.3. Exemplary amino acid sequences of the GAL9 binding molecules

[0145] In various embodiment, the GAL9 binding molecule comprises a particular VH CDR3 (CDR-H3) sequence and a particular VL CDR3 (CDR-L3) sequence.

[0146] In some embodiments, the GAL9 binding molecule comprises the CDR-H3 and the CDR-L3 from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58. VH CDR amino acid sequences of the ABS clones are disclosed in **Table 3**. VL CDR amino acid sequences of the ABS clones are disclosed in **Table 4**. For clarity, each GAL9 ABS clone is assigned a unique ABS clone number which is used throughout this disclosure.

[0147] In one currently preferred embodiment, the GAL9 binding molecule comprises the CDR-H3 and CDR-L3 of ABS clone P9-28.

[0148] In some embodiments, the GAL9 binding molecule comprises all three VH CDRs from one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58. In one currently preferred embodiment, the GAL9 binding molecule comprises all three VH CDRs from ABS clone P9-28.

[0149] In some embodiments, the GAL9 binding molecule comprises all three VL CDRs from one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58. In one currently preferred embodiment, the GAL9 binding molecule comprises all three VL CDRs from ABS clone P9-28.

[0150] In some embodiments, the GAL9 binding molecule comprises all six CDRs from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58. In one currently preferred embodiment, the GAL9 binding molecule comprises all six CDRs from ABS clone P9-28.

[0151] In some embodiments, the GAL9 binding molecule comprises a VH amino acid sequence, a VL amino acid sequence, or a VH and VL amino acid sequence from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58. Full immunoglobulin heavy chain and immunoglobulin light chain sequences, as well as VH and VL amino acid sequences, are provided in **Table 6**. In certain currently preferred embodiments, the GAL9 binding molecule comprises a VH amino acid sequence, a VL amino acid sequence, or a VH and VL amino acid sequence from ABS clone P9-28.

[0152] In some embodiments, the GAL9 binding molecule comprises the full IgG heavy chain sequence and the full IgG light chain sequence from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58. In one currently preferred embodiment, the GAL9 binding molecule comprises the full IgG heavy chain sequence and the full IgG light chain sequence from ABS clone P9-28.

6.4.4. Constant Regions

[0153] In the GAL9 binding molecules, the GAL9 binding molecule can have a constant region domain sequence. Constant region domain amino acid sequences, as described herein, are sequences of a constant region domain of an antibody. Constant regions can refer to CH1, CH2, CH3, CH4, or CL constant domain.

[0154] In a variety of embodiments, the constant region sequences are mammalian sequences, including, but not limited to, mouse, rat, hamster, rabbit, camel, donkey, goat, and human sequences. In a preferred embodiment, the constant region sequences are human sequences. In certain embodiments, the constant region sequences are from an antibody light chain. In particular embodiments, the constant region sequences are from a lambda or kappa light chain. In certain embodiments, the constant region sequences are from an antibody heavy chain. In particular embodiments, the constant region sequences are an antibody heavy chain sequence that is an IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, or IgM isotype. In a specific embodiment, the constant region sequences are from an IgG isotype. In a preferred embodiment, the constant region sequences are from an IgG1 isotype.

[0155] Exemplary constant regions and modifications thereof are described in WO2018075692, which is hereby incorporated by reference in its entirety.

6.4.4.1. CH1 and CL Regions

[0156] CH1 amino acid sequences, as described herein, are sequences of the second domain of an antibody heavy chain, with reference from the N-terminus to C-terminus of a native antibody heavy chain architecture. In certain embodiments, the CH1 sequences are endogenous sequences. In a variety of embodiments, the CH1 sequences are mammalian sequences, including, but not limited to mouse, rat, hamster, rabbit, camel, donkey, goat, and human sequences. In a preferred embodiment, the CH1 sequences are human sequences. In certain embodiments, the CH1 sequences are from an IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, or IgM isotype. In a preferred embodiment, the CH1 sequences are from an IgG1 isotype. In preferred embodiments, the CH1 sequence is UniProt accession number P01857 amino acids 1-98.

[0157] The CL amino acid sequences useful in the GAL9 binding molecules described herein are antibody light chain constant domain sequences, with reference to a native antibody light chain architecture. In certain embodiments, the CL sequences are endogenous sequences. In a variety of embodiments, the CL sequences are mammalian sequences, including, but not limited to mouse, rat, hamster, rabbit, camel, donkey, goat, and human sequences. In a preferred embodiment, CL sequences are human sequences.

[0158] In certain embodiments, the CL amino acid sequences are lambda (λ) light chain constant domain sequences. In particular embodiments, the CL amino acid sequences are human lambda light chain constant domain sequences. In preferred embodiments, the lambda (λ) light chain sequence is UniProt accession number P0CG04.

[0159] In certain embodiments, the CL amino acid sequences are kappa (κ) light chain constant domain sequences. In a preferred embodiment, the CL amino acid sequences are human kappa (κ) light chain constant domain sequences. In a preferred embodiment, the kappa light chain sequence is UniProt accession number P01834.

[0160] In certain embodiments, the CH1 sequence and the CL sequences are both endogenous sequences. In certain embodiments, the CH1 sequence and the CL sequences separately comprise respectively orthogonal modifications in endogenous CH1 and CL sequences, as discussed below in greater detail in **Section 6.4.4.1**. CH1 and CL sequences can also be portions thereof, either of an endogenous or modified sequence, such that a domain having the CH1 sequence, or portion thereof, can associate with a domain having the CL sequence, or portion thereof.

6.4.4.2. CH1 and CL Orthogonal Modifications

[0161] In certain embodiments, the CH1 sequence and the CL sequences separately comprise respectively orthogonal modifications in endogenous CH1 and CL sequences. Orthogonal mutations, in general, are described in more detail below in **Sections 6.4.6.1-6.4.6.3**.

[0162] In particular embodiments, the orthogonal modifications in endogenous CH1 and CL sequences are an engineered disulfide bridge selected from engineered cysteines at position 138 of the CH1 sequence and position 116 of the CL sequence, at position 128 of the CH1 sequence and position 119 of the CL sequence, or at position 129 of the CH1 sequence and position 210 of the CL sequence, as numbered and discussed in more detail in U.S. Pat. No. 8,053,562 and U.S. Pat. No. 9,527,927, each incorporated herein by reference in its entirety. In a preferred embodiment, the engineered cysteines are at position 128 of the CH1 sequence and position 118 of the CL Kappa sequence, as numbered by the Eu index.

[0163] In a series of preferred embodiments, the mutations that provide non-endogenous cysteine amino acids are a F118C mutation in the CL sequence with a corresponding A141C in the CH1 sequence, or a F118C mutation in the CL sequence with a corresponding L128C in the CH1 sequence, or a S162C mutations in the CL sequence with a corresponding P171C mutation in the CH1 sequence, as numbered by the Eu index.

[0164] In a variety of embodiments, the orthogonal mutations in the CL sequence and the CH1 sequence are charge-pair mutations. In specific embodiments the charge-pair mutations are a F118S, F118A or F118V mutation in the CL sequence with a corresponding A141L in the CH1 sequence, or a T129R mutation in the CL sequence with a corresponding K147D in the CH1 sequence, as numbered by the Eu index and described in greater detail in Bonisch *et al.* (*Protein Engineering, Design & Selection*, 2017, pp. 1–12), herein incorporated by reference for all that it teaches. In a series of preferred embodiments, the charge-pair mutations are a N138K mutation in the CL sequence with a corresponding G166D in the CH1 sequence, or a N138D mutation in the CL sequence with a corresponding G166K in the CH1 sequence, as numbered by the Eu index.

6.4.4.3. CH2 Regions

[0165] In the GAL9 binding molecules described herein, the GAL9 binding molecules can have a CH2 amino acid sequence. CH2 amino acid sequences, as described herein, are CH2 amino acid sequences of the third domain of an antibody heavy chain, with reference from the N-terminus to C-terminus of a native antibody heavy chain architecture. In a variety of embodiments, the CH2 sequences are mammalian sequences, including but not limited to

mouse, rat, hamster, rabbit, camel, donkey, goat, and human sequences. In a preferred embodiment, the CH2 sequences are human sequences. In certain embodiments, the CH2 sequences are from an IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, or IgM isotype. In a preferred embodiment, the CH2 sequences are from an IgG1 isotype.

[0166] In certain embodiments, the CH2 sequences are endogenous sequences. In particular embodiments, the sequence is UniProt accession number P01857 amino acids 111-223.

[0167] In a series of embodiments, a GAL9 binding molecule has more than one paired set of CH2 domains that have CH2 sequences, wherein a first set has CH2 amino acid sequences from a first isotype and one or more orthologous sets of CH2 amino acid sequences from another isotype. The orthologous CH2 amino acid sequences, as described herein, are able to interact with CH2 amino acid sequences from a shared isotype, but not significantly interact with the CH2 amino acid sequences from another isotype present in the GAL9 binding molecule. In particular embodiments, all sets of CH2 amino acid sequences are from the same species. In preferred embodiments, all sets of CH2 amino acid sequences are human CH2 amino acid sequences. In other embodiments, the sets of CH2 amino acid sequences are from different species. In particular embodiments, the first set of CH2 amino acid sequences is from the same isotype as the other non-CH2 domains in the GAL9 binding molecule. In a specific embodiment, the first set has CH2 amino acid sequences from an IgG isotype and the one or more orthologous sets have CH2 amino acid sequences from an IgM or IgE isotype. In certain embodiments, one or more of the sets of CH2 amino acid sequences are endogenous CH2 sequences. In other embodiments, one or more of the sets of CH2 amino acid sequences are endogenous CH2 sequences that have one or more mutations. In particular embodiments, the one or more mutations are orthogonal knob-hole mutations, orthogonal charge-pair mutations, or orthogonal hydrophobic mutations. Orthologous CH2 amino acid sequences useful for the GAL9 binding molecules are described in more detail in international PCT applications WO2017/011342 and WO2017/106462, herein incorporated by reference in their entirety.

6.4.4.4. CH3 Regions

[0168] CH3 amino acid sequences, as described herein, are sequences of the C-terminal domain of an antibody heavy chain, with reference from the N-terminus to C-terminus of a native antibody heavy chain architecture.

[0169] In a variety of embodiments, the CH3 sequences are mammalian sequences, including, but not limited to, mouse, rat, hamster, rabbit, camel, donkey, goat, and human

sequences. In a preferred embodiment, the CH3 sequences are human sequences. In certain embodiments, the CH3 sequences are from an IgA1, IgA2, IgD, IgE, IgM, IgG1, IgG2, IgG3, IgG4 isotype or CH4 sequences from an IgE or IgM isotype. In a specific embodiment, the CH3 sequences are from an IgG isotype. In a preferred embodiment, the CH3 sequences are from an IgG1 isotype.

[0170] In certain embodiments, the CH3 sequences are endogenous sequences. In particular embodiments, the CH3 sequence is UniProt accession number P01857 amino acids 224-330. In various embodiments, a CH3 sequence is a segment of an endogenous CH3 sequence. In particular embodiments, a CH3 sequence has an endogenous CH3 sequence that lacks the N-terminal amino acids G224 and Q225. In particular embodiments, a CH3 sequence has an endogenous CH3 sequence that lacks the C-terminal amino acids P328, G329, and K330. In particular embodiments, a CH3 sequence has an endogenous CH3 sequence that lacks both the N-terminal amino acids G224 and Q225 and the C-terminal amino acids P328, G329, and K330. In preferred embodiments, a GAL9 binding molecule has multiple domains that have CH3 sequences, wherein a CH3 sequence can refer to both a full endogenous CH3 sequence as well as a CH3 sequence that lacks N-terminal amino acids, C-terminal amino acids, or both.

[0171] In certain embodiments, the CH3 sequences are endogenous sequences that have one or more mutations. In particular embodiments, the mutations are one or more orthogonal mutations that are introduced into an endogenous CH3 sequence to guide specific pairing of specific CH3 sequences, as described in more detail below in **Sections 6.4.6.1-6.4.6.3**.

[0172] In certain embodiments, the CH3 sequences are engineered to reduce immunogenicity of the antibody by replacing specific amino acids of one allotype with those of another allotype and referred to herein as isoallotype mutations, as described in more detail in Stickler *et al.* (*Genes Immun.* 2011 Apr; 12(3): 213–221), which is herein incorporated by reference for all that it teaches. In particular embodiments, specific amino acids of the G1m1 allotype are replaced. In a preferred embodiment, isoallotype mutations D356E and L358M are made in the CH3 sequence.

[0173] In some embodiments, an IgG1 CH3 amino acid sequence comprises the following mutational changes: P343V; Y349C; and a tripeptide insertion, 445P, 446G, 447K. In other preferred embodiments, domain B has a human IgG1 CH3 sequence with the following mutational changes: T366K; and a tripeptide insertion, 445K, 446S, 447C. In still other preferred embodiments, domain B has a human IgG1 CH3 sequence with the following mutational changes: Y349C and a tripeptide insertion, 445P, 446G, 447K.

[0174] In some embodiments, an IgG1 CH3 amino acid sequence comprises a 447C mutation incorporated into an otherwise endogenous CH3 sequence.

6.4.5. Antigen Binding Sites

[0175] In some embodiments, a VL or VH amino acid sequence and a cognate VL or VH amino acid sequence are associated and form a first antigen binding site (ABS). The antigen binding site (ABS) is capable of specifically binding an epitope of an antigen. Antigen binding by an ABS is described in greater detail below in **Section 6.4.5.1**.

[0176] In alternative embodiments, e.g., wherein the GAL9 binding molecule is a single domain antibody, a VH or VL amino acid sequence forms the first ABS.

[0177] In some embodiments, the GAL9 antigen binding molecule comprises a second ABS. In some embodiments, the second ABS is specific for the same GAL9 antigen as the first ABS. In some embodiments, the second ABS specifically binds the same epitope of the same GAL9 antigen as the first ABS. In some embodiments, the second ABS is identical to the first ABS.

[0178] In some embodiments, the second ABS is specific for a different epitope of the first GAL9 antigen. For example if the first ABS comprises CDRs or variable domains from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58, the second ABS may comprise CDRs or variable domains from another ABS clone selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

[0179] In some embodiments, the GAL9 antigen binding molecule is multispecific, e.g., the second ABS of the GAL9 antigen binding molecule specifically binds an antigen that is different than the GAL9 antigen specifically bound by the first ABS.

6.4.5.1. Binding of Antigen by ABS

[0180] An ABS, and the GAL9 binding molecule comprising such ABS, is said to “**recognize**” the epitope (or more generally, the antigen) to which the ABS specifically binds, and the epitope (or more generally, the antigen) is said to be the “**recognition specificity**” or “**binding specificity**” of the ABS.

[0181] The ABS is said to bind to its specific antigen or epitope with a particular affinity. As described herein, “**affinity**” refers to the strength of interaction of non-covalent intermolecular forces between one molecule and another. The affinity, i.e. the strength of the

interaction, can be expressed as a dissociation equilibrium constant (K_D), wherein a lower K_D value refers to a stronger interaction between molecules. K_D values of antibody constructs are measured by methods well known in the art including, but not limited to, bio-layer interferometry (*e.g.*, Octet/FORTEBIO[®]), surface plasmon resonance (SPR) technology (*e.g.*, Biacore[®]), and cell binding assays. For purposes herein, affinities are dissociation equilibrium constants measured by bio-layer interferometry using Octet/FORTEBIO[®].

[0182] “**Specific binding**,” as used herein, refers to an affinity between an ABS and its cognate antigen or epitope in which the K_D value is below $10^{-6}M$, $10^{-7}M$, $10^{-8}M$, $10^{-9}M$, or $10^{-10}M$.

[0183] The number of ABSs in a GAL9 binding molecule as described herein defines the “**valency**” of the GAL9 binding molecule. A GAL9 binding molecule having a single ABS is “**monovalent**”. A GAL9 binding molecule having a plurality of ABSs is said to be “**multivalent**”. A multivalent GAL9 binding molecule having two ABSs is “**bivalent**.” A multivalent GAL9 binding molecule having three ABSs is “**trivalent**.” A multivalent GAL9 binding molecule having four ABSs is “**tetravalent**.”

[0184] In various multivalent embodiments, all of the plurality of ABSs have the same recognition specificity. Such a GAL9 binding molecule is a “**monospecific**” “multivalent” binding construct. In other multivalent embodiments, at least two of the plurality of ABSs have different recognition specificities. Such GAL9 binding molecules are multivalent and “**multispecific**”. In multivalent embodiments in which the ABSs collectively have two recognition specificities, the GAL9 binding molecule is “**bispecific**.” In multivalent embodiments in which the ABSs collectively have three recognition specificities, the GAL9 binding molecule is “**trispecific**.”

[0185] In multivalent embodiments in which the ABSs collectively have a plurality of recognition specificities for different epitopes present on the same antigen, the GAL9 binding molecule is “**multiparatopic**.” Multivalent embodiments in which the ABSs collectively recognize two epitopes on the same antigen are “**biparatopic**.”

[0186] In various multivalent embodiments, multivalency of the GAL9 binding molecule improves the avidity of the GAL9 binding molecule for a specific target. As described herein, “**avidity**” refers to the overall strength of interaction between two or more molecules, *e.g.* a multivalent GAL9 binding molecule for a specific target, wherein the avidity is the cumulative strength of interaction provided by the affinities of multiple ABSs. Avidity can be measured by the same methods as those used to determine affinity, as described above. In certain embodiments, the avidity of a GAL9 binding molecule for a specific target is such

that the interaction is a specific binding interaction, wherein the avidity between two molecules has a K_D value below $10^{-6}M$, $10^{-7}M$, $10^{-8}M$, $10^{-9}M$, or $10^{-10}M$. In certain embodiments, the avidity of a GAL9 binding molecule for a specific target has a K_D value such that the interaction is a specific binding interaction, wherein the one or more affinities of individual ABSs do not have has a K_D value that qualifies as specifically binding their respective antigens or epitopes on their own. In certain embodiments, the avidity is the cumulative strength of interaction provided by the affinities of multiple ABSs for separate antigens on a shared specific target or complex, such as separate antigens found on an individual cell. In certain embodiments, the avidity is the cumulative strength of interaction provided by the affinities of multiple ABSs for separate epitopes on a shared individual antigen.

6.4.6. Orthogonal Modifications

[0187] In the GAL9 binding molecules described herein, a GAL9 binding molecule can have constant region domains comprising orthogonal modifications. Constant region domain amino acid sequences are described in greater detail above in **Section 6.4.4**.

[0188] “Orthogonal modifications” or synonymously “orthogonal mutations” as described herein are one or more engineered mutations in an amino acid sequence of an antibody domain that increase the affinity of binding of a first domain having orthogonal modification for a second domain having a complementary orthogonal modification. In certain embodiments, the orthogonal modifications decrease the affinity of a domain having the orthogonal modifications for a domain lacking the complementary orthogonal modifications. In certain embodiments, orthogonal modifications are mutations in an endogenous antibody domain sequence. In a variety of embodiments, orthogonal modifications are modifications of the N-terminus or C-terminus of an endogenous antibody domain sequence including, but not limited to, amino acid additions or deletions. In particular embodiments, orthogonal modifications include, but are not limited to, engineered disulfide bridges, knob-in-hole mutations, and charge-pair mutations, as described in greater detail below in **Sections 6.4.6.1-6.4.6.3**. In particular embodiments, orthogonal modifications include a combination of orthogonal modifications selected from, but not limited to, engineered disulfide bridges, knob-in-hole mutations, and charge-pair mutations. In particular embodiments, the orthogonal modifications can be combined with amino acid substitutions that reduce immunogenicity, such as isoallotype mutations, as described in greater detail above in **Section 6.4.4.4**.

6.4.6.1. Orthogonal Engineered Disulfide Bridges

[0189] In a variety of embodiments, the orthogonal modifications comprise mutations that generate engineered disulfide bridges between a first and a second domain. As described herein, “**engineered disulfide bridges**” are mutations that provide non-endogenous cysteine amino acids in two or more domains such that a non-native disulfide bond forms when the two or more domains associate. Engineered disulfide bridges are described in greater detail in Merchant *et al.* (*Nature Biotech* (1998) 16:677-681), the entirety of which is hereby incorporated by reference for all it teaches. In certain embodiments, engineered disulfide bridges improve orthogonal association between specific domains. In a particular embodiment, the mutations that generate engineered disulfide bridges are a K392C mutation in one of a first or second CH3 domains, and a D399C in the other CH3 domain. In a preferred embodiment, the mutations that generate engineered disulfide bridges are a S354C mutation in one of a first or second CH3 domains, and a Y349C in the other CH3 domain. In another preferred embodiment, the mutations that generate engineered disulfide bridges are a 447C mutation in both the first and second CH3 domains that are provided by extension of the C-terminus of a CH3 domain incorporating a KSC tripeptide sequence.

6.4.6.2. Orthogonal Knob-Hole Mutations

[0190] In a variety of embodiments, orthogonal modifications comprise knob-hole (synonymously, knob-in-hole) mutations. As described herein, knob-hole mutations are mutations that change the steric features of a first domain's surface such that the first domain will preferentially associate with a second domain having complementary steric mutations relative to association with domains without the complementary steric mutations. Knob-hole mutations are described in greater detail in U.S. Pat. No. 5,821,333 and U.S. Pat. No. 8,216,805, each of which is incorporated herein in its entirety. In various embodiments, knob-hole mutations are combined with engineered disulfide bridges, as described in greater detail in Merchant *et al.* (*Nature Biotech* (1998) 16:677-681), incorporated herein by reference in its entirety. In various embodiments, knob-hole mutations, isoallotype mutations, and engineered disulfide mutations are combined.

[0191] In certain embodiments, the knob-in-hole mutations are a T366Y mutation in a first domain, and a Y407T mutation in a second domain. In certain embodiments, the knob-in-hole mutations are a F405A in a first domain, and a T394W in a second domain. In certain embodiments, the knob-in-hole mutations are a T366Y mutation and a F405A in a first

domain, and a T394W and a Y407T in a second domain. In certain embodiments, the knob-in-hole mutations are a T366W mutation in a first domain, and a Y407A in a second domain. In certain embodiments, the combined knob-in-hole mutations and engineered disulfide mutations are a S354C and T366W mutations in a first domain, and a Y349C, T366S, L368A, and aY407V mutation in a second domain. In a preferred embodiment, the combined knob-in-hole mutations, isoallotype mutations, and engineered disulfide mutations are a S354C and T366W mutations in a first domain, and a Y349C, D356E, L358M, T366S, L368A, and aY407V mutation in a second domain.

6.4.6.3. Orthogonal Charge-pair Mutations

[0192] In a variety of embodiments, orthogonal modifications are charge-pair mutations. As used herein, charge-pair mutations are mutations that affect the charge of an amino acid in a domain's surface such that the domain will preferentially associate with a second domain having complementary charge-pair mutations relative to association with domains without the complementary charge-pair mutations. In certain embodiments, charge-pair mutations improve orthogonal association between specific domains. Charge-pair mutations are described in greater detail in U.S. Pat. No. 8,592,562, U.S. Pat. No. 9,248,182, and U.S. Pat. No. 9,358,286, each of which is incorporated by reference herein for all they teach. In certain embodiments, charge-pair mutations improve stability between specific domains. In a preferred embodiment, the charge-pair mutations are a T366K mutation in a first domain, and a L351D mutation in the other domain.

[0193] In specific embodiments, the orthogonal mutations are charge-pair mutations at the VH/VL interface. In preferred embodiments, the charge-pair mutations at the VH/VL interface are a Q39E in VH with a corresponding Q38K in VL, or a Q39K in VH with a corresponding Q38E in VL, as described in greater detail in Igawa *et al.* (*Protein Eng. Des. Sel.*, 2010, vol. 23, 667–677), herein incorporated by reference for all it teaches.

6.4.7. Trivalent and Tetravalent GAL9 binding molecules

[0194] In another series of embodiments, the GAL9 binding molecules have three antigen binding sites and are therefore termed “**trivalent**.” In a variety of embodiments, the GAL9 binding molecules have 4 antigen binding sites and are therefore termed “**tetravalent**.”

6.5. GAL9 binding molecule architecture

[0195] The antigen binding sites described herein, including specific CDR subsets, can be formatted into any binding molecule architecture including, but not limited to, full-length antibodies, Fab fragments, Fvs, scFvs, tandem scFvs, Diabodies, scDiabodies, DARTs, tandAbs, minibodies, camelid VHH, and other antibody fragments or formats known to those skilled in the art. Exemplary antibody and antibody fragment formats are described in detail in Brinkmann *et al.* (*MABS*, 2017, Vol. 9, No. 2, 182–212), herein incorporated by reference for all that it teaches. The antigen binding sites described herein, including specific CDR subsets, can also be formatted into a “B-body” format, as described in more detail in US pre-grant publication no. US 2018/0118811 and International Application Pub. No. WO 2018/075692, each of which is herein incorporated by reference in their entireties.

6.6. Further modification

[0196] In a further series of embodiments, the GAL9 binding molecule has additional modifications.

6.6.1. Antibody-Drug Conjugates

[0197] In various embodiments, the GAL9 binding molecule is conjugated to a therapeutic agent (i.e. drug) to form a GAL9 binding molecule-drug conjugate. Therapeutic agents include, but are not limited to, chemotherapeutic agents, imaging agents (e.g. radioisotopes), immune modulators (e.g. cytokines, chemokines, or checkpoint inhibitors), and toxins (e.g. cytotoxic agents). In certain embodiments, the therapeutic agents are attached to the GAL9 binding molecule through a linker peptide, as discussed in more detail below in **Section 6.6.3.**

[0198] Methods of preparing antibody-drug conjugates (ADCs) that can be adapted to conjugate drugs to the GAL9 binding molecules disclosed herein are described, e.g., in US patent no. 8,624,003 (pot method), US patent no. 8,163,888 (one-step), US patent no. 5,208,020 (two-step method), US patent No. 8,337,856, US patent no. 5,773,001, US patent no. 7,829,531, US patent no. 5,208,020, US patent no. 7,745,394, WO 2017/136623, WO 2017/015502, WO 2017/015496, WO 2017/015495, WO 2004/010957, WO 2005/077090, WO 2005/082023, WO 2006/065533, WO 2007/030642, WO 2007/103288, WO 2013/173337, WO 2015/057699, WO 2015/095755, WO 2015/123679, WO 2015/157286, WO 2017/165851, WO 2009/073445, WO 2010/068759, WO 2010/138719, WO 2012/171020, WO 2014/008375, WO 2014/093394, WO 2014/093640, WO 2014/160360,

WO 2015/054659, WO 2015/195925, WO 2017/160754, Storz (*MAbs*, 2015 Nov-Dec; 7(6): 989–1009), Lambert *et al.* (*Adv Ther*, 2017 34: 1015), Diamantis *et al.* (*British Journal of Cancer*, 2016, 114, 362–367), Carrico *et al.* (*Nat Chem Biol*, 2007. 3: 321-2), We *et al.* (*Proc Natl Acad Sci USA*, 2009. 106: 3000-5), Rabuka *et al.* (*Curr Opin Chem Biol.*, 2011 14: 790-6), Hudak *et al.* (*Angew Chem Int Ed Engl.*, 2012: 4161-5), Rabuka *et al.* (*Nat Protoc.*, 2012 7:1052-67), Agarwal *et al.* (*Proc Natl Acad Sci USA.*, 2013, 110: 46-51), Agarwal *et al.* (*Bioconjugate Chem.*, 2013, 24: 846–851), Barfield *et al.* (*Drug Dev. and D.*, 2014, 14:34-41), Drake *et al.* (*Bioconjugate Chem.*, 2014, 25:1331-41), Liang *et al.* (*J Am Chem Soc.*, 2014, 136:10850-3), Drake *et al.* (*Curr Opin Chem Biol.*, 2015, 28:174-80), and York *et al.* (*BMC Biotechnology*, 2016, 16(1):23), each of which is hereby incorporated by reference in its entirety for all that it teaches.

6.6.2. Additional Binding Moieties

[0199] In various embodiments, the GAL9 binding molecule has modifications that comprise one or more additional binding moieties. In certain embodiments the binding moieties are antibody fragments or antibody formats including, but not limited to, full-length antibodies, Fab fragments, Fvs, scFvs, tandem scFvs, Diabodies, scDiabodies, DARTs, tandAbs, minibodies, camelid VHH, and other antibody fragments or formats known to those skilled in the art. Exemplary antibody and antibody fragment formats are described in detail in Brinkmann *et al.* (*MABS*, 2017, Vol. 9, No. 2, 182–212), herein incorporated by reference for all that it teaches.

[0200] In particular embodiments, the one or more additional binding moieties are attached to the C-terminus of the first or third polypeptide chain. In particular embodiments, the one or more additional binding moieties are attached to the C-terminus of both the first and third polypeptide chain. In particular embodiments, the one or more additional binding moieties are attached to the C-terminus of both the first and third polypeptide chains. In certain embodiments, individual portions of the one or more additional binding moieties are separately attached to the C-terminus of the first and third polypeptide chains such that the portions form the functional binding moiety.

[0201] In particular embodiments, the one or more additional binding moieties are attached to the N-terminus of any of the polypeptide chains (e.g. the first, second, third, fourth, fifth, or sixth polypeptide chains). In certain embodiments, individual portions of the additional binding moieties are separately attached to the N-terminus of different polypeptide chains such that the portions form the functional binding moiety.

[0202] In certain embodiments, the one or more additional binding moieties are specific for a different antigen or epitope of the ABSs within the GAL9 binding molecule. In certain embodiments, the one or more additional binding moieties are specific for the same antigen or epitope of the ABSs within the GAL9 binding molecule. In certain embodiments, wherein the modification is two or more additional binding moieties, the additional binding moieties are specific for the same antigen or epitope. In certain embodiments, wherein the modification is two or more additional binding moieties, the additional binding moieties are specific for different antigens or epitopes.

[0203] In certain embodiments, the one or more additional binding moieties are attached to the GAL9 binding molecule using *in vitro* methods including, but not limited to, reactive chemistry and affinity tagging systems, as discussed in more detail below in **Section 6.6.3**. In certain embodiments, the one or more additional binding moieties are attached to the GAL9 binding molecule through Fc-mediated binding (e.g. Protein A/G). In certain embodiments, the one or more additional binding moieties are attached to the GAL9 binding molecule using recombinant DNA techniques, such as encoding the nucleotide sequence of the fusion product between the GAL9 binding molecule and the additional binding moieties on the same expression vector (e.g., plasmid).

6.6.3. Functional/Reactive Groups

[0204] In various embodiments, the GAL9 binding molecule has modifications that comprise functional groups or chemically reactive groups that can be used in downstream processes, such as linking to additional moieties (e.g., drug conjugates and additional binding moieties, as discussed in more detail above in **Sections 6.6.1. and 6.6.2.**) and downstream purification processes.

[0205] In certain embodiments, the modifications are chemically reactive groups including, but not limited to, reactive thiols (e.g. maleimide based reactive groups), reactive amines (e.g., *N*-hydroxysuccinimide based reactive groups), “click chemistry” groups (e.g. reactive alkyne groups), and aldehydes bearing formylglycine (FGly). In certain embodiments, the modifications are functional groups including, but not limited to, affinity peptide sequences (e.g., HA, HIS, FLAG, GST, MBP, and Strep systems etc.). In certain embodiments, the functional groups or chemically reactive groups have a cleavable peptide sequence. In particular embodiments, the cleavable peptide is cleaved by means including, but not limited to, photocleavage, chemical cleavage, protease cleavage, reducing conditions, and pH conditions. In particular embodiments, protease cleavage is carried out by intracellular

proteases. In particular embodiments, protease cleavage is carried out by extracellular or membrane associated proteases. ADC therapies adopting protease cleavage are described in more detail in Choi *et al.* (*Theranostics*, 2012; 2(2): 156–178.), the entirety of which is hereby incorporated by reference for all it teaches.

6.6.4. Reduced Effector Function

[0206] In certain embodiments, the GAL9 binding molecule has one or more engineered mutations in an amino acid sequence of an antibody domain that reduce the effector functions naturally associated with antibody binding. Effector functions include, but are not limited to, cellular functions that result from an Fc receptor binding to an Fc portion of an antibody, such as antibody-dependent cellular cytotoxicity (ADCC, also referred to as antibody-dependent cell-mediated cytotoxicity), complement fixation (*e.g.* C1q binding), antibody dependent cellular-mediated phagocytosis (ADCP), and opsonization. Exemplary engineered mutations that reduce the effector functions are described in more detail in U.S. Pub. No. 2017/0137530, Armour, *et al.* (*Eur. J. Immunol.* 29(8) (1999) 2613-2624), Shields, *et al.* (*J. Biol. Chem.* 276(9) (2001) 6591-6604), and Oganessian, *et al.* (*Acta Crystallographica D* 64 (2008) 700-704), each of which is herein incorporated by reference in its entirety.

6.7. Methods of Purification

[0207] A method of purifying a GAL9 binding molecule is provided herein. Purification steps include, but are not limited to, purifying the GAL9 binding molecules based on protein characteristics, such as size (*e.g.*, size exclusion chromatography), charge (*e.g.*, ion exchange chromatography), or hydrophobicity (*e.g.*, hydrophobicity interaction chromatography). In one embodiment, cation exchange chromatography is performed. Other purification methods known to those skilled in the art can be performed including, but not limited to, use of Protein A, Protein G, or Protein A/G reagents. Multiple iterations of a single purification method can be performed. A combination of purification methods can be performed.

6.7.1. Assembly and Purity of Complexes

[0208] In the embodiments of the present invention, at least four distinct polypeptide chains associate together to form a complete complex, *i.e.*, the GAL9 binding molecule. However, incomplete complexes can also form that do not contain the at least four distinct polypeptide chains. For example, incomplete complexes may form that only have one, two, or three of the polypeptide chains. In other examples, an incomplete complex may contain more than three

polypeptide chains, but does not contain the at least four distinct polypeptide chains, *e.g.*, the incomplete complex inappropriately associates with more than one copy of a distinct polypeptide chain. The method of the invention purifies the complex, *i.e.*, the completely assembled GAL9 binding molecule, from incomplete complexes.

[0209] Methods to assess the efficacy and efficiency of the purification steps are well known to those skilled in the art and include, but are not limited to, SDS-PAGE analysis, ion exchange chromatography, size exclusion chromatography, and mass spectrometry. Purity can also be assessed according to a variety of criteria. Examples of criterion include, but are not limited to: 1) assessing the percentage of the total protein in an eluate that is provided by the completely assembled GAL9 binding molecule, 2) assessing the fold enrichment or percent increase of the method for purifying the desired products, *e.g.*, comparing the total protein provided by the completely assembled GAL9 binding molecule in the eluate to that in a starting sample, 3) assessing the percentage of the total protein or the percent decrease of undesired products, *e.g.*, the incomplete complexes described above, including determining the percent or the percent decrease of specific undesired products (*e.g.*, unassociated single polypeptide chains, dimers of any combination of the polypeptide chains, or trimers of any combination of the polypeptide chains). Purity can be assessed after any combination of methods described herein.

6.8. Methods of Manufacturing

[0210] The GAL9 binding molecules described herein can readily be manufactured by expression using standard cell free translation, transient transfection, and stable transfection approaches currently used for antibody manufacture. In specific embodiments, Expi293 cells (ThermoFisher) can be used for production of the GAL9 binding molecules using protocols and reagents from ThermoFisher, such as ExpiFectamine, or other reagents known to those skilled in the art, such as polyethylenimine as described in detail in Fang *et al.* (*Biological Procedures Online*, 2017, 19:11), herein incorporated by reference for all it teaches.

[0211] The expressed proteins can be readily separated from undesired proteins and protein complexes using various purification strategies including, but not limited to, use of Protein A, Protein G, or Protein A/G reagents. Further purification can be affected using ion exchange chromatography as is routinely used in the art.

6.9. Pharmaceutical Compositions

[0212] In another aspect, pharmaceutical compositions are provided that comprise a GAL9 binding molecule as described herein and a pharmaceutically acceptable carrier or diluent. In typical embodiments, the pharmaceutical composition is sterile.

[0213] In various embodiments, the pharmaceutical composition comprises the GAL9 binding molecule at a concentration of 0.1 mg/ml – 100 mg/ml. In specific embodiments, the pharmaceutical composition comprises the GAL9 binding molecule at a concentration of 0.5 mg/ml, 1 mg/ml, 1.5 mg/ml, 2 mg/ml, 2.5 mg/ml, 5 mg/ml, 7.5 mg/ml, or 10 mg/ml. In some embodiments, the pharmaceutical composition comprises the GAL9 binding molecule at a concentration of more than 10 mg/ml. In certain embodiments, the GAL9 binding molecule is present at a concentration of 20 mg/ml, 25 mg/ml, 30 mg/ml, 35 mg/ml, 40 mg/ml, 45 mg/ml, or even 50 mg/ml or higher. In particular embodiments, the GAL9 binding molecule is present at a concentration of more than 50 mg/ml.

[0214] In various embodiments, the pharmaceutical compositions are described in more detail in U.S. Pat No. 8,961,964, U.S. Pat No. 8,945,865, U.S. Pat No. 8,420,081, U.S. Pat No. 6,685,940, U.S. Pat No. 6,171,586, U.S. Pat No. 8,821,865, U.S. Pat No. 9,216,219, US application 10/813,483, WO 2014/066468, WO 2011/104381, and WO 2016/180941, each of which is incorporated herein in its entirety.

6.10. Methods of Treatment

[0215] In another aspect, methods of treatment are provided, the methods comprising administering a GAL9 binding molecule as described herein to a patient with a disease or condition in an amount effective to treat the patient.

6.10.1. Subjects

[0216] In some embodiments, the subject can be a mammal. In some embodiments, the mammal is a mouse. In a preferred embodiment, the mammal is a human.

6.10.2. Combination therapy

[0217] The GAL9 binding molecule can be used alone or in combination with other therapeutic agents or procedures to treat or prevent a disease or condition. The GAL9 binding molecule can be administered either simultaneously or sequentially with a second therapeutic agent, dependent upon the disease to be treated.

[0218] In some embodiments, the anti-GAL9 binding molecules is used in combination with an agent or procedure that is used in the clinic or is within the current standard of care to treat or prevent a disease or condition, such as proliferative disease or cancer. In some embodiments, the GAL9 binding molecule is administered in combination with an immune checkpoint inhibitor, such as an anti-PD-L1 antibody, anti-PD-1 antibody, anti-CTLA4 antibody, anti-LAB3 antibody, anti-TIM1 antibody, anti-TIGIT antibody, anti-PVRIG antibody.

6.10.3. Proliferative Diseases

[0219] In some embodiments, the treatment comprises administration one or more GAL9 binding molecule as described herein to a subject with a proliferative disease in an amount effective to treat the subject.

[0220] In some embodiments, the treatment comprises administration of an effective amount of one or more GAL9 binding molecules as described herein for the treatment of cancer and/or precancer. In some embodiments, the treatment comprises administration of an effective amount of one or more GAL9 binding molecules as described herein, in combination with another cancer therapeutic and/or treatment regimen (radiation, surgery, or the like, etc.).

[0221] In various embodiments, the cancer is a cancer of the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestinal, gum, head, kidney, liver, lung, nasopharynx, neck, head and neck, ovary, prostate, pancreas, skin, stomach, testis, tongue, or uterus.

[0222] In some embodiments, the cancerous or pre-cancerous tumor is a neoplasm, malignant tumor, carcinoma, undifferentiated tumor, giant and spindle cell carcinoma, small cell carcinoma, papillary carcinoma, squamous cell carcinoma, head and neck squamous cell carcinoma, lymphoepithelial carcinoma, basal cell carcinoma, pilomatrix carcinoma, transitional cell carcinoma, papillary transitional cell carcinoma, adenocarcinoma, gastrinoma, malignant, cholangiocarcinoma, hepatocellular carcinoma, combined hepatocellular carcinoma and cholangiocarcinoma, trabecular adenocarcinoma, adenoid cystic carcinoma, adenocarcinoma in adenomatous polyp, adenocarcinoma, familial polyposis coli, solid carcinoma, carcinoid tumor, malignant, bronchiolo-alveolar adenocarcinoma, papillary adenocarcinoma, chromophobe carcinoma, acidophil carcinoma, oxyphilic adenocarcinoma, basophil carcinoma, clear cell adenocarcinoma, granular cell

carcinoma, follicular adenocarcinoma, papillary and follicular adenocarcinoma, nonencapsulating sclerosing carcinoma, adrenal cortical carcinoma, endometroid carcinoma, skin appendage carcinoma, apocrine adenocarcinoma, sebaceous adenocarcinoma, ceruminous adenocarcinoma, mucoepidermoid carcinoma, cystadenocarcinoma, pancreatic adenocarcinoma, pancreatic ductal adenocarcinoma, cystadenocarcinomas, pancreatic neuroendocrine tumors (PanNETs), adenosquamous carcinomas of the pancreas, signet ring cell carcinomas of the pancreas, hepatoid carcinomas of the pancreas, colloid carcinomas of the pancreas, undifferentiated carcinomas of the pancreas, and undifferentiated carcinomas with osteoclast-like giant cells of the pancreas, acinar cell carcinomas of the pancreas, solid pseudopapillary neoplasms of the pancreas, pancreatoblastoma, rare exocrine cancers of the pancreas, pancreatic serous cystadenomas, pancreatic mucinous cystic neoplasms, papillary cystadenocarcinoma, papillary serous cystadenocarcinoma, mucinous cystadenocarcinoma, mucinous adenocarcinoma, signet ring cell carcinoma, infiltrating duct carcinoma, medullary carcinoma, lobular carcinoma, inflammatory carcinoma, Paget's disease, mammary, acinar cell carcinoma, adenosquamous carcinoma, adenocarcinoma w/squamous metaplasia, thymoma, malignant, ovarian stromal tumor, malignant thecoma, malignant granulosa cell tumor, malignant androblastoma, malignant sertoli cell carcinoma, leydig cell tumor, malignant lipid cell tumor, malignant paraganglioma, malignant extra-mammary paraganglioma, malignant pheochromocytoma, glomangiosarcoma, malignant melanoma, amelanotic melanoma, superficial spreading melanoma, melanoma in giant pigmented nevus, epithelioid cell melanoma, blue nevus, malignant sarcoma, fibrosarcoma, fibrous histiocytoma, malignant myxosarcoma, liposarcoma, leiomyosarcoma, rhabdomyosarcoma, embryonal rhabdomyosarcoma, alveolar rhabdomyosarcoma, stromal sarcoma, mixed tumor, malignant mullerian mixed tumor, nephroblastoma, hepatoblastoma, carcinosarcoma, mesenchymoma, malignant brenner tumor, malignant phyllodes tumor, malignant synovial sarcoma, mesothelioma, malignant dysgerminoma, embryonal carcinoma, teratoma, malignant struma ovarii, malignant choriocarcinoma, mesonephroma, malignant hemangiosarcoma, hemangioendothelioma, malignant Kaposi's sarcoma, hemangiopericytoma, malignant, lymphangiosarcoma, osteosarcoma, juxtacortical osteosarcoma, chondrosarcoma, chondroblastoma, malignant mesenchymal chondrosarcoma, giant cell tumor of bone, Ewing's sarcoma, odontogenic tumor, malignant ameloblastic odontosarcoma, ameloblastoma, malignant ameloblastic fibrosarcoma, pinealoma, malignant chordoma, glioma, malignant ependymoma, astrocytoma, protoplasmic astrocytoma, fibrillary astrocytoma, astroblastoma, glioblastoma, oligodendroglioma,

oligodendroblastoma, primitive neuroectodermal, cerebellar sarcoma, ganglioneuroblastoma, neuroblastoma, retinoblastoma, olfactory neurogenic tumor, meningioma, malignant, neurofibrosarcoma, neurilemmoma, malignant granular cell tumor, malignant lymphoma, Hodgkin's disease, Hodgkin's paraganuloma, malignant lymphoma, small lymphocytic, malignant lymphoma, large cell, diffuse, malignant lymphoma, follicular, mycosis fungoides, other specified Non-Hodgkin's lymphomas, malignant histiocytosis, multiple myeloma, mast cell sarcoma, immunoproliferative small intestinal disease, leukemia, lymphoid leukemia, plasma cell leukemia, erythroleukemia, lymphosarcoma cell leukemia, myeloid leukemia, basophilic leukemia, eosinophilic leukemia, monocytic leukemia, mast cell leukemia, megakaryoblastic leukemia, myeloid sarcoma, or hairy cell leukemia.

[0223] In some embodiments, the cancer is a viral-induced cancer, for example, a cancer caused by an infection from an oncovirus or a tumor virus (which are also known as a "cancer virus"). In some embodiments, the cancer virus is a DNA virus. In some embodiments, the cancer virus is an RNA virus.

[0224] In some embodiments, the cancerous or pre-cancerous tumor is associated or caused by a cancer virus. Non-limiting examples of a cancer virus include: a Epstein-Barr virus (EBV), a Hepatitis B virus, a Hepatitis C virus, a Human papilloma virus, a Human T-lymphotropic virus 1 (HTLV-1), a Kaposi sarcoma associated-herpesvirus (KHSV), a Merkel cell polyomavirus, or a Cytomegalovirus.

[0225] In some embodiments, the cancerous or pre-cancerous tumor is associated or caused by a cancer virus that directly induces transformation of the infected host cell, thereby regulating the host cell's growth and survival or alternatively initiating a DNA damage response which in turn increases genetic instability and accelerates the acquisition of the cancer causing mutations in the genome of the host cell.

[0226] In some embodiments, the cancerous or pre-cancerous tumor is associated or caused by a cancer virus that induces chronic inflammation in a host. For example, infections with HBV and HCV can induce chronic liver inflammation associated with oxidative DNA damage followed by cirrhosis resulting in some cases in the development of hepatocellular carcinoma.

[0227] In some embodiments, the cancerous or pre-cancerous tumor is associated or caused by a cancer virus that is not oncogenic but inhibits the host's immune system, disrupting immunosurveillance and thereby allowing for the emergence of mutated malignant cells, for example HIV-infected patients.

[0228] In some embodiments, the treatment comprises administration one or more GAL9 binding molecules as described herein to a subject with an infectious disease(s), such as infection with HIV, HCV, HBV, EBV, or HPV.

[0229] In some embodiments, the treatment comprises administration one or more GAL9 binding molecules as described herein to a subject with HIV or AIDs in an amount effective to treat the subject.

6.10.4. Administration

[0230] The GAL9 binding molecule may be administered to a subject by any route known in the art. For example, the GAL9 binding molecule is administered to a human subject via, e.g., intravenous, subcutaneous, intramuscular, intradermal, intraarterial, intraperitoneal, intranasal, parenteral, pulmonary, topical, oral, sublingual, intratumoral, peritumoral, intralesional, intrasynovial, intrathecal, intra-cerebrospinal, or perilesional administration. The GAL9 binding molecule may be administered to a subject *per se* or as a pharmaceutical composition. Exemplary pharmaceutical compositions are described herein.

6.11. Examples

[0231] The following examples are provided by way of illustration, not limitation. In particular, the methods for the expression and purification of the various antigen-binding proteins and their use in various assays as described in more detail below are non-limiting and illustrative.

6.11.1. Methods

6.11.1.1. Expi293 Expression

[0232] Various antigen-binding proteins tested were expressed using the Expi293 transient transfection system according to manufacturer's instructions (Thermo Fisher Scientific). Briefly, plasmids coding for individual chains were mixed at 1:1 mass ratio, unless otherwise stated, and transfected into Expi 293 cells with ExpiFectamine 293 transfection kit. Cells were cultured at 37°C with 8% CO₂, 100% humidity and shaking at 125 rpm. Transfected cells were fed once after 16-18 hours of transfections. The cells were harvested at day 5 by centrifugation at 2000 g for 10 minutes. The supernatant was collected for affinity chromatography purification.

6.11.1.2. ExpiCHO Expression

[0233] Various GAL9 antigen-binding proteins are tested and expressed using the ExpiCHO transient transfection system according to manufacturer's instructions. Briefly, plasmids coding for individual chains are mixed at, for example, a 1:1 mass ratio, and transfected with ExpiFectamine CHO transfection kit into ExpiCHO.

[0234] Cells are cultured at 37°C with 8% CO₂, 100% humidity and shaking at 125 rpm. Transfected cells are generally be fed once after 16-18 hours of transfections. The cells are harvested at day 5 by centrifugation at 2000 g for 10 minutes. The supernatant is then collected for affinity chromatography purification.

6.11.1.3. Protein A Purification

[0235] Cleared supernatants containing the various antigen-binding proteins were separated using either a Protein A (ProtA) resin or an anti-CH1 resin on a Gravity flow purifier. In examples where a head-to-head comparison was performed, supernatants containing the various antigen-binding proteins were split into two equal samples. For ProtA purification, a 1 mL Protein A column (GE Healthcare) was equilibrated with PBS (5 mM sodium potassium phosphate pH 7.4, 150 mM sodium chloride). The sample was loaded onto the column at 5 ml/min. The sample was eluted using 0.1M sodium acetate pH 3.5. The elution was monitored by absorbance at 280 nm and the elution peaks were pooled for analysis. The elution was monitored by absorbance at 280 nm and the elution peaks were pooled for analysis.

6.11.1.4. SDS-Page Analysis

[0236] Samples containing the various separated antigen-binding proteins were analyzed by reducing and non-reducing SDS-PAGE for the presence of complete product, incomplete product, and overall purity. 2 µg of each sample was added to 15 µL SDS loading buffer. Reducing samples were incubated in the presence of 10 mM reducing agent at 75°C for 10 minutes. Non-reducing samples were incubated at 70°C for 5 minutes without reducing agent. The reducing and non-reducing samples were loaded into a 4-15% gradient TGX gel (BioRad) with running buffer and run for 30 minutes at 220 volts. Upon completion of the run, the gel was washed with deionized (DI) water and stained using GelCode Blue Safe Protein Stain (ThermoFisher). The gels were destained with DI water prior to analysis. Densitometry analysis of scanned images of the destained gels was performed using standard image analysis software to calculate the relative abundance of bands in each sample.

6.11.1.5. IEX Chromatography

[0237] Samples containing the various separated antigen-binding proteins were analyzed by cation exchange chromatography for the ratio of complete product to incomplete product and impurities. Cleared supernatants were analyzed with a 5-ml MonoS (GE Lifesciences) on an AKTA Purifier FPLC. The MonoS column was equilibrated with buffer A (10 mM MES pH 6.0). The samples were loaded onto the column at 2 ml/min. The sample was eluted using a 0-30% gradient with buffer B (10 mM MES pH 6.0, 1 M sodium chloride) over 6 column bed volumes (CV). The elution was monitored by absorbance at 280 nm and the purity of the samples were calculated by peak integration to identify the abundance of the monomer peak and contaminants peaks. The monomer peak and contaminant peaks were separately pooled for analysis by SDS-PAGE as described above.

[0238] Analytical SEC Chromatography of each sample at 1 mg/mL was loaded onto the column at 1 ml/min. The sample was eluted using an isocratic flow of PBS for 1.5 CV. The elution was monitored by absorbance at 280 nm and the elution peaks were analyzed by peak integration.

6.11.1.6. Mass Spectrometry

[0239] Samples containing the various separated antigen-binding proteins were analyzed by mass spectrometry to confirm the correct species by molecular weight. All analysis was performed by a third-party research organization. Briefly, samples were treated with a cocktail of enzymes to remove glycosylation. Samples were tested in the reduced format to specifically identify each chain by molecular weight and under non-reducing conditions to identify the molecular weights of all complexes in the samples. Mass spec analysis was used to identify the number of unique products based on molecular weight.

6.11.1.7. Antibody discovery by phage display

[0240] Phage display of human Fab libraries was carried out using standard protocols. Human GAL9 protein was purchased from Acro Biosystems (Human Gal9 His-tag Cat # LG9-H5244) and biotinylated using EZ-Link NHS-PEG₁₂-Biotin (ThermoScientific Cat# 21312) using standard protocols. Phage clones were screened for the ability to bind the GAL9 protein by phage ELISA using standard protocols.

[0241] Briefly, Fab-formatted phage libraries were constructed using expression vectors capable of replication and expression in phage (also referred to as a phagemid). Both the heavy chain and the light chain were encoded for in the same expression vector, where the

heavy chain was fused to a truncated variant of the phage coat protein pIII. The light chain and heavy chain-pIII fusion were expressed as separate polypeptides and assembled in the bacterial periplasm, where the redox potential enables disulfide bond formation, to form the phage display antibody containing the candidate ABS.

[0242] The library was created using sequences derived from a specific human heavy chain variable domain (VH3-23) and a specific human light chain variable domain (Vκ-1). For the screened library, all three CDRs of the VH domain were diversified to match the positional amino acid frequency by CDR length found in the human antibody repertoire. Light chain variable domains within the screened library were generated with diversity introduced solely into the VL CDR3 (L3); the light chain VL CDR1 (L1) and CDR2 (L2) retained the human germline sequence.

[0243] The heavy chain scaffold (SEQ ID NO:2), light chain scaffold (SEQ ID NO:4), full heavy chain Fab polypeptide (SEQ ID NO:1), and full light chain Fab polypeptide (SEQ ID NO:3) used in the phage display library are shown below, where a lower case “x” represents CDR amino acids that were varied to create the library.

Phage display VH scaffold [SEQ ID NO:2]:

EVQLVESGGGLVQPGGSLRLSCAASGFTFxxxxIHWVRQAPGKGLEWVAXXXXXXXXXXX
xxxYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARXXXXXXXXXXXXXXXXXDY
WGQGTLVTVSSAS

Phage display VL scaffold [SEQ ID NO:4]:

DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSG
VPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQxxxxxxTFGGGTKVEIKRT

Phage display heavy chain Fab polypeptide [SEQ ID NO:1]:

EVQLVESGGGLVQPGGSLRLSCAASGFTFxxxxIHWVRQAPGKGLEWVAXXXXXXXXXXX
xxxYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARXXXXXXXXXXXXXXXXXDY
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTC

Phage display light chain Fab polypeptide [SEQ ID NO:3]:

DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSG
VPSRFGSGRSGTDFTLTITSSLPEDFATYYCQQxxxxxxTFGQGTKVEIKRTVAAPS
VFIFPPSDSQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0244] Diversity was created through Kunkel mutagenesis using primers to introduce diversity into VH CDR1 (H1), VH CDR2 (H2), VH CDR3 (H3) and VL CDR3 (L3) to mimic the diversity found in the natural antibody repertoire, as described in more detail in Kunkel, TA (*PNAS* January 1, 1985. 82 (2) 488-492), incorporated herein by reference in its entirety. Briefly, single-stranded DNA was prepared from isolated phage using standard procedures and Kunkel mutagenesis was carried out. Chemically synthesized DNA was then electroporated into MC1061F- cells. Phagemid obtained from overnight culture was digested with restriction enzymes (Bam HI and Xba I) to remove the wild-type sequence. The digested sample was electroporated into TG1 cells, followed by recovery. Recovered cells were sub-cultured and infected with M13K07 helper phage to produce the phage library.

[0245] Phage panning was performed using standard procedures. Briefly, the first round of phage panning was performed with target immobilized on streptavidin magnetic beads which were subjected to $\sim 5 \times 10^{12}$ phages from the prepared library in a volume of 1 mL in PBST-2% BSA. After a one-hour incubation, the bead-bound phage were separated from the supernatant using a magnetic stand. Beads were washed three times to remove non-specifically bound phage and were then added to ER2738 cells (5 mL) at $OD_{600} \sim 0.6$. After 20 minutes, infected cells were sub-cultured in 25 mL 2xYT + Ampicillin and M13K07 helper phage (final concentration, $\sim 10^{10}$ pfu/ml) and allowed to grow overnight at 37 °C with vigorous shaking. The next day, phage were prepared using standard procedures by PEG precipitation. Pre-clearance of phage specific to SAV-coated beads was performed prior to panning. The second round of panning was performed using the KingFisher magnetic bead handler with 100 nM bead-immobilized antigen using standard procedures. In total, 3-4 rounds of phage panning were performed to enrich in phage displaying Fabs specific for the target antigen. Target-specific enrichment was confirmed using polyclonal and monoclonal phage ELISA. DNA sequencing was used to determine isolated Fab clones containing a candidate ABS.

[0246] The VL and VH domains identified in the phage screen described above were reformatted into a bivalent monospecific native human full-length IgG1 architecture.

Native human full-length IgG1 heavy chain architecture [SEQ ID NO:5]:

EVQLVESGGGLVQPGGSLRLSCAASGFTFxxxxIHWVRQAPGKGLEWVAXXXXXXXXXXX
 xxxYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARXXXXXXXXXXXXXXXXDY
 WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL
 TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVKDKKVEPKSC
 DKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
 YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
 KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
 [SEQ ID NO: 5]

Native human full-length IgG1 light chain architecture:

Equivalent to phage display light chain Fab, *see* [SEQ ID NO:3].

6.11.1.8. Octet Determination of Binding Kinetics

[0247] To measure qualitative binding affinity in the GAL9 binder discovery campaigns, IgG1 reformatted binders were immobilized to a biosensor on an Octet (Pall ForteBio) biolayer interferometer.

[0248] Soluble GAL9 antigen was then added to the system and binding measured. Qualitative binding affinity was assessed by visualizing the slope of the dissociation phase of the octet sensogram from weakest (+) to strongest (+++). A slow off rate represented by a negligible drop in the dissociation phase of the sensogram and indicated a tight binding antibody (+++). To obtain accurate kinetic constants for monovalent affinities, a dilution series involving of at least five concentrations of the GAL9 analyte (ranging from approximately 10 to 20X K_D to 0.1X K_D value, 2-fold dilutions) were measured in the association step. In the dissociation step, the sensor was dipped into buffer solution that did not contain the GAL9 analyte, permitting the bound complex on the surface of the sensor to dissociate. Octet kinetic analysis software was used to calculate the kinetic and equilibrium

binding constants based on the rate of association and dissociation curves. Analysis was performed globally (global fit) where kinetic constants were derived simultaneously from all analyte concentration included in the experiment.

6.11.1.9. Epitope Binning

[0249] Anti-GAL9 candidates formatted into a bivalent monospecific native human full-length IgG1, as described above, were tested for GAL9 binding in a pair-wise manner using an octet-based ‘tandem’ assay. Briefly, biotinylated GAL9 was immobilized on a streptavidin sensor and two anti-GAL9 candidates were bound in tandem. A competitive blocking profile was generated determining whether a given anti-GAL9 candidate blocked binding of a panel of other anti-GAL9 candidates to GAL9. Anti-GAL9 candidates that competed for the same or non-overlapping binding regions were grouped together and referred to as belonging to the same bin.

6.11.1.10. PBMC activation and Galectin 9 antibody treatment

[0250] Individual aliquots of PepMix HCMVA (pp65) (>90%) Protein ID: P06725 (Cat. No. PM-PP65-2, JPT Peptide Technologies) were prepared according to manufacturer’s instructions. PepMix™ HCMVA (pp65) is a complete protein-spanning mixture of overlapping 15mer peptides of the 65 kDa phosphoprotein (pp65) (Swiss-Prot ID: P06725) of human cytomegalovirus (HHV-5). Aliquots of PepMix were used for immunostimulation of PBMCs to assess immune cell responses.

[0251] Frozen human peripheral blood mononuclear cells (PBMCs) were thawed according to standard conditions, then resuspended in growth media (10% FBS in RPMI).

[0252] Resuspended PBMCs were seeded at 5×10^5 cells in 96-well plates. Cells were incubated with 2 µg/mL PepMix™ HCMVA (pp65) plus 40 µg/mL of candidate GAL9 antibodies or control antibodies in growth media for 24 hours at 37°C, 5% CO₂.

6.11.1.11. LEGENDplex Human Th Cytokine Assay

[0253] Cytokine secretion by PBMCs and by specific immune cell subpopulations was assessed by cytokine bead array at 24 hours and 72 hours after PBMC activation by PepMix HCMVA (pp65) and Galectin 9 antibody treatment as follows.

[0254] 200 µl cell culture supernatant was collected and centrifuged to pellet cell debris. The resulting supernatants were analyzed using the LEGENDplex™ Human Th1 Panel (5-plex)

(Cat. No. 740009, Biolegend). The LEGENDplex™ Human Th1 Panel is a bead-based assay that allows for simultaneous quantification of human cytokines IL-2, IL-6, IL-10, IFN- γ and TNF- α using flow cytometry.

[0255] Briefly, cytokine standards and capture bead mixtures were prepared according to manufacturer's instructions. Assay master mixes of 1:1:1 capture bead mixture: biotinylated detection antibodies, and assay buffer were prepared.

[0256] 12.5 μ l of supernatant samples or cytokine standards were incubated with 37.5 μ l assay master mix. Plates were sealed, covered with foil, and shaken at 600 rpm for 2 hours at room temperature. Wells were then incubated, with shaking at 600 rpm, with streptavidin-phycoerythrin (SA-PE) for 30 minutes at room temperature. Beads were then washed twice and resuspended before proceeding to flow cytometry analysis according to manufacturer's instructions.

6.11.1.12. PBMC Staining with Marker Antibodies

[0257] Following PBMC activation and Galectin 9 antibody treatment as described above, PBMC immune cells were stained with marker antibodies according to the following procedures.

[0258] Cells were resuspended at 5×10^6 cells/mL in growth media (10% FBS in RPMI). 200 μ L of resuspended cells were aliquoted to 96 well plates, then incubated with Fixable Viability Dye eFluor® 780 for 30 minutes at 2-8°C to irreversibly label dead cells. Cells were then washed and then incubated with human Fc Block solution (Cat. No. 14-9161-73, eBiosciences) for 10 minutes at room temperature.

[0259] An antibody cocktail working solution was prepared according to the following table.

Table 1 Antibody Staining Working Solutions		
	Antibody	Dilution
T cell surface markers	BV510 anti-human CD3 (Cat. No. 563109, BD Biosciences)	1 in 20
	PerCP/Cy5.5 anti-human CD56 (Cat. No. 362505, BD Biosciences)	1 in 20
Monocyte surface markers	FITC anti-human CD14 (Cat. No. 367115, BD Biosciences)	1 in 20

Table 1 Antibody Staining Working Solutions		
	Antibody	Dilution
	Alexa Fluor® 700 anti-human CD16 (Cat. No. 302025, Biolegend)	1 in 20
Dendritic cell surface makers	Brilliant Violet 421™ anti-human CD11c (Cat. No. 301627, Biolegend)	1 in 20
	Alexa Fluor 647 anti-human CD123 (Cat. No. 306023, Biolegend)	1 in 40
	BV510 anti-human Lineage Cocktail (CD3, CD14, CD16, CD19, CD20, CD56) (Cat. No. 348807, Biolegend)	1 in 10
	FITC anti-human HLA-DR (Cat. No. 307603, Biolegend)	1 in 20
B cell surface markers	PerCP/Cy5.5 anti-human CD19 (Cat. No. 363015, Biolegend)	1 in 20
Galectin-9	PE anti-human galectin 9 (Cat. No. 348905, Biolegend)	1 in 10

[0260] Wells were incubated with 10 µL of diluted antibody cocktail for 30 minutes at 2-8°C. Cells were then washed and resuspended and analyzed by flow cytometry analysis.

[0261] To analyze immune stimulatory markers CD27, CD40L, ICOS, 4-1BB, and OX40 the same protocol provided above was followed but cells were incubated with the alternative antibody cocktail as detailed in **Table 2** below:

Table 2 Antibody Staining Working Solutions	
Antibody	Dilution
FITC anti-human CD134 (OX40) (Cat. No. 350006, BioLegend)	1 in 50
PerCP/Cy5.5 anti-human CD3 (Cat. No. 560835, BD Biosciences)	1 in 100
AF700 anti-human CD4 (Cat. No. 344622, BioLegend)	1 in 100

Table 2 Antibody Staining Working Solutions	
Antibody	Dilution
eFluor™ Fixable Viability Dye (Cat. No. 65-0865-14, eBioscience™)	1 in 2000
BV421 anti-human CD8 (Cat. No. 344748, BioLegend)	1 in 100
BV650 anti-human CD137 (4-1BB) (Cat. No. 309828, BioLegend)	1 in 50
BV711 anti-human ICOS (Cat. No. 563833, BD Biosciences)	1 in 100
PE anti-human CD154 (CD40L) (Cat. No. 310806, BioLegend)	1 in 50
PE/Cy7 anti- mouse/rat/human CD27 (Cat. No. 124216, BioLegend)	1 in 100

6.11.2. Example 1: GAL9 Binding Arm Discovery Campaign

[0262] A chemically synthetic Fab phage library with diversity introduced into the Fab CDRs was screened against GAL9 antigens using a monoclonal phage ELISA format as described above. Phage clones expressing Fabs that recognized GAL9 were sequenced.

[0263] The campaign initially identified 52 GAL9 binding candidates (antigen binding site clones). Functional assays conducted after the variable regions of these clones had been reformatted into a bivalent monospecific human IgG1 format identified 22 antibodies having immune activating properties.

[0264] **Table 3** lists the VH CDR1/2/3 sequences from the 22 activating ABS clones, showing only the residues of the CDRs that had been varied in constructing the library.

Table 4 lists the VL CDR1/2/3 sequences from the identified ABS clones; the light chain CDR1 and CDR2 sequences are invariant, and only the residues of CDR3 that were varied in constructing the library are shown.

Table 3
Candidate hGAL9 VH Antigen Binding Sites

ABS clone	CDR1 (variant residues)	SEQ ID NO	CDR2 (variant residues)	SEQ ID NO	CDR3 (variant residues)	SEQ ID NO
P9-02B	SGYF	9	RISSYGGHTD	61	AQYVPGL	113
P9-04	GSYF	11	YISPTWGYTY	63	AWFGFAF	115
P9-05	TSYY	12	WIWPIGGYTY	64	DAGSGF	116
P9-08	SRYA	15	AIYSPTGYTD	67	EGYIGM	119
P9-09	SSYF	16	WIYSSGGYTY	68	FTPDLGYGL	120
P9-10	DYYV	17	AIDSYWGDTY	69	FYFYGF	121
P9-15	RYYW	21	AIFPSSGITT	73	GWPWGL	125
P9-16	SYYW	22	DIYPSSGYTY	74	GWYAAAYGM	126
P9-18	SWYV	23	AIYPYHSKTY	75	GYWYGM	127
P9-19	GYYY	24	WISPSGSVTA	76	GYGGWGM	128
P9-20	RYYY	25	GIYPYGGYTS	77	GYVEGVL	129
P9-21	SYYY	26	RIHPPSGYTD	78	GYVFGVM	130
P9-22	SSYY	27	AIYPFSGGT	79	GYVYVVM	131
P9-27	WGYG	32	AIYPYGGSTY	84	LSDIYHSFSGM	136
P9-28	GFYY	33	FIDPHGGSTY	85	LSYPGVL	137
P9-31	SQYA	36	RIYPDSGYTY	88	PYHQYAEGM	140
P9-32	SAYW	37	LIGPDGGYTY	89	QASRGL	141
P9-36	GTYT	39	SILSGGGYTV	91	RVYPGF	143
P9-39	SFYG	42	WIYPYGGFTD	94	SGFFAF	146
P9-49	SWYE	50	RIGPYSSYTY	102	TYYPYSGM	154
P9-54	STYF	55	WISPSGSHTG	107	VRYPGVM	159
P9-58	SRYT	58	FISSDSGYTQ	110	TMSYSAL	162

Table 4
Candidate hGAL9 VL Antigen Binding Sites

ABS clone	CDR1 (invariant)	SEQ ID NO	CDR2 (invariant)	SEQ ID NO	CDR3 (variant residues)	SEQ ID NO
P9-02B	RASQSVSSA	165	SASSLYS	217	SYPTLG	269
P9-04	RASQSVSSA	167	SASSLYS	219	VYSSPL	271
P9-05	RASQSVSSA	168	SASSLYS	220	YYYSLL	272
P9-08	RASQSVSSA	171	SASSLYS	223	SYSALY	275
P9-09	RASQSVSSA	172	SASSLYS	224	ADPSLV	276
P9-10	RASQSVSSA	173	SASSLYS	225	YSWSLW	277
P9-15	RASQSVSSA	177	SASSLYS	229	RDRSPY	281
P9-16	RASQSVSSA	178	SASSLYS	230	YALRPL	282
P9-18	RASQSVSSA	179	SASSLYS	231	YTDAPW	283
P9-19	RASQSVSSA	180	SASSLYS	232	YDTSPY	284
P9-20	RASQSVSSA	181	SASSLYS	233	YYGSLA	285
P9-21	RASQSVSSA	182	SASSLYS	234	SHWYPF	286
P9-22	RASQSVSSA	183	SASSLYS	235	YKSSPW	287
P9-27	RASQSVSSA	188	SASSLYS	240	RYSTPV	292
P9-28	RASQSVSSA	189	SASSLYS	241	GYSTLV	293
P9-31	RASQSVSSA	192	SASSLYS	244	YYSPLL	296
P9-32	RASQSVSSA	193	SASSLYS	245	WSSPLH	297
P9-36	RASQSVSSA	195	SASSLYS	247	DSWGLW	299
P9-39	RASQSVSSA	198	SASSLYS	250	VQTSLA	302
P9-49	RASQSVSSA	206	SASSLYS	258	SFSSPV	310
P9-54	RASQSVSSA	211	SASSLYS	263	WYPSLI	315
P9-58	RASQSVSSA	214	SASSLYS	266	GFGFLV	318

[0265] Table 5 presents the full CDR sequences, according to multiple art-accepted definitions, for the 22 candidate anti-GAL9 immune-activating antibodies.

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
P9-02B					
CDR-H1	Chothia	GFTFSGY---	26 - 32	7	319
	AbM	GFTFSGYFIH	26 - 35	10	320
	Kabat	-----GYFIH	31 - 35	5	321
	Contact	----SGYFIH	30 - 35	6	322
	IMGT	GFTFSGYF--	26 - 33	8	323
CDR-H2	Chothia	-----SSYGGH----- ---	52 - 57	6	324
	AbM	---RISSYGGHTD----- ---	50 - 59	10	325
	Kabat	--- RISSYGGHTDYADSVKG	50 - 66	17	326
	Contact	WVARISSYGGHTD----- ---	47 - 59	13	327
	IMGT	----ISSYGGHT----- ---	51 - 58	8	328
CDR-H3	Chothia	--AQYVPGLDY	99 - 107	9	329
	AbM	--AQYVPGLDY	99 - 107	9	330
	Kabat	--AQYVPGLDY	99 - 107	9	331
	Contact	ARAQYVPGLD-	97 - 106	10	332
	IMGT	ARAQYVPGLDY	97 - 107	11	333
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	334
	AbM	RASQSVSSAVA--	24 - 34	11	335
	Kabat	RASQSVSSAVA--	24 - 34	11	336
	Contact	-----SSAVAWY	30 - 36	7	337
	IMGT	---QSVSSA-----	27 - 32	6	338
CDR-L2	Chothia	----SASSLYS	50 - 56	7	339
	AbM	----SASSLYS	50 - 56	7	340
	Kabat	----SASSLYS	50 - 56	7	341
	Contact	LLIYSASSLY-	46 - 55	10	342
	IMGT	----SA-----	50 - 51	2	343
CDR-L3	Chothia	QQSYPTLGT	89 - 97	9	344
	AbM	QQSYPTLGT	89 - 97	9	345
	Kabat	QQSYPTLGT	89 - 97	9	346
	Contact	QQSYPTLG-	89 - 96	8	347
	IMGT	QQSYPTLGT	89 - 97	9	348

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
P9-04					
CDR-H1	Chothia	GFTFGSY---	26 - 32	7	349
	AbM	GFTFGSYFIH	26 - 35	10	350
	Kabat	-----SYFIH	31 - 35	5	351
	Contact	----GSYFIH	30 - 35	6	352
	IMGT	GFTFGSYF--	26 - 33	8	353
CDR-H2	Chothia	-----SPTWGY----- ---	52 - 57	6	354
	AbM	---YISPTWGYTY----- ---	50 - 59	10	355
	Kabat	--- YISPTWGYTYADSVKG	50 - 66	17	356
	Contact	WVAYISPTWGYTY----- ---	47 - 59	13	357
	IMGT	----ISPTWGYT----- ---	51 - 58	8	358
CDR-H3	Chothia	--AWFGFAFDY	99 - 107	9	359
	AbM	--AWFGFAFDY	99 - 107	9	360
	Kabat	--AWFGFAFDY	99 - 107	9	361
	Contact	ARAWFGFAFD-	97 - 106	10	362
	IMGT	ARAWFGFAFDY	97 - 107	11	363
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	364
	AbM	RASQSVSSAVA--	24 - 34	11	365
	Kabat	RASQSVSSAVA--	24 - 34	11	366
	Contact	-----SSAVAWY	30 - 36	7	367
	IMGT	---QSVSSA----	27 - 32	6	368
CDR-L2	Chothia	----SASSLYS	50 - 56	7	369
	AbM	----SASSLYS	50 - 56	7	370
	Kabat	----SASSLYS	50 - 56	7	371
	Contact	LLIYSASSLY-	46 - 55	10	372
	IMGT	----SA-----	50 - 51	2	373
CDR-L3	Chothia	QQVYSSPLT	89 - 97	9	374
	AbM	QQVYSSPLT	89 - 97	9	375
	Kabat	QQVYSSPLT	89 - 97	9	376
	Contact	QQVYSSPL-	89 - 96	8	377
	IMGT	QQVYSSPLT	89 - 97	9	378
P9-05					
CDR-H1	Chothia	GFTFTSY---	26 - 32	7	379
	AbM	GFTFTSYIIH	26 - 35	10	380

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	Kabat	-----SYIIH	31 - 35	5	381
	Contact	-----TSYIIH	30 - 35	6	382
	IMGT	GFTFTSY--	26 - 33	8	383
CDR-H2	Chothia	-----WPIGGY----- ---	52 - 57	6	384
	AbM	---WIWPIGGYTY---- ---	50 - 59	10	385
	Kabat	--- WIWPIGGYTYADSVKG	50 - 66	17	386
	Contact	WVAWIWPIGGYTY---- ---	47 - 59	13	387
	IMGT	-----IWPIGGYT----- ---	51 - 58	8	388
CDR-H3	Chothia	--DAGSGFDY	99 - 106	8	389
	AbM	--DAGSGFDY	99 - 106	8	390
	Kabat	--DAGSGFDY	99 - 106	8	391
	Contact	ARDAGSGFD-	97 - 105	9	392
	IMGT	ARDAGSGFDY	97 - 106	10	393
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	394
	AbM	RASQSVSSAVA--	24 - 34	11	395
	Kabat	RASQSVSSAVA--	24 - 34	11	396
	Contact	-----SSAVAWY	30 - 36	7	397
	IMGT	---QSVSSA----	27 - 32	6	398
CDR-L2	Chothia	----SASSLYS	50 - 56	7	399
	AbM	----SASSLYS	50 - 56	7	400
	Kabat	----SASSLYS	50 - 56	7	401
	Contact	LLIYSASSLY-	46 - 55	10	402
	IMGT	----SA-----	50 - 51	2	403
CDR-L3	Chothia	QQYYYSLLT	89 - 97	9	404
	AbM	QQYYYSLLT	89 - 97	9	405
	Kabat	QQYYYSLLT	89 - 97	9	406
	Contact	QQYYYSLL-	89 - 96	8	407
	IMGT	QQYYYSLLT	89 - 97	9	408
P9-08					
CDR-H1	Chothia	GFTFSRY---	26 - 32	7	409
	AbM	GFTFSRYAIH	26 - 35	10	410
	Kabat	-----RYAIH	31 - 35	5	411
	Contact	----SRYAIH	30 - 35	6	412
	IMGT	GFTFSRYA--	26 - 33	8	413
CDR-H2	Chothia	-----YSPTGY----- ---	52 - 57	6	414

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	AbM	---AIYSPTGYTD--- ---	50 - 59	10	415
	Kabat	--- AIYSPTGYTDYADSVKG	50 - 66	17	416
	Contact	WVAAIYSPTGYTD--- ---	47 - 59	13	417
	IMGT	-----IYSPTGYT----- ---	51 - 58	8	418
CDR-H3	Chothia	--EGYIGMDY	99 - 106	8	419
	AbM	--EGYIGMDY	99 - 106	8	420
	Kabat	--EGYIGMDY	99 - 106	8	421
	Contact	AREGYIGMD-	97 - 105	9	422
	IMGT	AREGYIGMDY	97 - 106	10	423
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	424
	AbM	RASQSVSSAVA--	24 - 34	11	425
	Kabat	RASQSVSSAVA--	24 - 34	11	426
	Contact	-----SSAVAWY	30 - 36	7	427
	IMGT	---QSVSSA----	27 - 32	6	428
CDR-L2	Chothia	----SASSLYS	50 - 56	7	429
	AbM	----SASSLYS	50 - 56	7	430
	Kabat	----SASSLYS	50 - 56	7	431
	Contact	LLIYSASSLY-	46 - 55	10	432
	IMGT	----SA-----	50 - 51	2	433
CDR-L3	Chothia	QQSYSALYT	89 - 97	9	434
	AbM	QQSYSALYT	89 - 97	9	435
	Kabat	QQSYSALYT	89 - 97	9	436
	Contact	QQSYSALY-	89 - 96	8	437
	IMGT	QQSYSALYT	89 - 97	9	438
P9-09					
CDR-H1	Chothia	GFTFSSY---	26 - 32	7	439
	AbM	GFTFSSYFIH	26 - 35	10	440
	Kabat	-----SYFIH	31 - 35	5	441
	Contact	----SSYFIH	30 - 35	6	442
	IMGT	GFTFSSYF--	26 - 33	8	443
CDR-H2	Chothia	-----YSSGGY----- ---	52 - 57	6	444
	AbM	---WIYSSGGYTY---- ---	50 - 59	10	445
	Kabat	--- WIYSSGGYTYADSVKG	50 - 66	17	446

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	Contact	WVAWIYSSGGYTY---- ---	47 - 59	13	447
	IMGT	-----IYSSGGYT----- ---	51 - 58	8	448
CDR-H3	Chothia	--FTPSDLGYGLDY	99 - 110	12	449
	AbM	--FTPSDLGYGLDY	99 - 110	12	450
	Kabat	--FTPSDLGYGLDY	99 - 110	12	451
	Contact	ARFTPSDLGYGLD-	97 - 109	13	452
	IMGT	ARFTPSDLGYGLDY	97 - 110	14	453
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	454
	AbM	RASQSVSSAVA--	24 - 34	11	455
	Kabat	RASQSVSSAVA--	24 - 34	11	456
	Contact	-----SSAVAWY	30 - 36	7	457
	IMGT	---QSVSSA----	27 - 32	6	458
CDR-L2	Chothia	----SASSLYS	50 - 56	7	459
	AbM	----SASSLYS	50 - 56	7	460
	Kabat	----SASSLYS	50 - 56	7	461
	Contact	LLIYSASSLY-	46 - 55	10	462
	IMGT	----SA-----	50 - 51	2	463
CDR-L3	Chothia	QQADPSLVT	89 - 97	9	464
	AbM	QQADPSLVT	89 - 97	9	465
	Kabat	QQADPSLVT	89 - 97	9	466
	Contact	QQADPSLV-	89 - 96	8	467
	IMGT	QQADPSLVT	89 - 97	9	468
P9-10					
CDR-H1	Chothia	GFTFDYY---	26 - 32	7	469
	AbM	GFTFDYYVIH	26 - 35	10	470
	Kabat	-----YYVIH	31 - 35	5	471
	Contact	----DYYVIH	30 - 35	6	472
	IMGT	GFTFDYYV--	26 - 33	8	473
CDR-H2	Chothia	-----DSYWGD----- ---	52 - 57	6	474
	AbM	---AIDSYWGDY---- ---	50 - 59	10	475
	Kabat	--- AIDSYWGDYYADSVKG	50 - 66	17	476
	Contact	WVA/AIDSYWGDY---- ---	47 - 59	13	477
	IMGT	----IDSYWGDT----- ---	51 - 58	8	478
CDR-H3	Chothia	--FYFYGFDY	99 - 106	8	479

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	AbM	--FYFYGFDY	99 - 106	8	480
	Kabat	--FYFYGFDY	99 - 106	8	481
	Contact	ARFYFYGFD-	97 - 105	9	482
	IMGT	ARFYFYGFDY	97 - 106	10	483
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	484
	AbM	RASQSVSSAVA--	24 - 34	11	485
	Kabat	RASQSVSSAVA--	24 - 34	11	486
	Contact	-----SSAVAWY	30 - 36	7	487
	IMGT	---QSVSSA----	27 - 32	6	488
CDR-L2	Chothia	----SASSLYS	50 - 56	7	489
	AbM	----SASSLYS	50 - 56	7	490
	Kabat	----SASSLYS	50 - 56	7	491
	Contact	LLIYSASSLY-	46 - 55	10	492
	IMGT	----SA-----	50 - 51	2	493
CDR-L3	Chothia	QQYSWSLWT	89 - 97	9	494
	AbM	QQYSWSLWT	89 - 97	9	495
	Kabat	QQYSWSLWT	89 - 97	9	496
	Contact	QQYSWSLW-	89 - 96	8	497
	IMGT	QQYSWSLWT	89 - 97	9	498
P9-15					
CDR-H1	Chothia	GFTFRYY---	26 - 32	7	499
	AbM	GFTFRYYWIH	26 - 35	10	500
	Kabat	-----YYWIH	31 - 35	5	501
	Contact	----RYYWIH	30 - 35	6	502
	IMGT	GFTFRYYW--	26 - 33	8	503
CDR-H2	Chothia	-----FPSGGI----- ---	52 - 57	6	504
	AbM	---AIFPSGGITT---- ---	50 - 59	10	505
	Kabat	--- AIFPSGGITTYADSVKG	50 - 66	17	506
	Contact	WVA AIFPSGGITT---- ---	47 - 59	13	507
	IMGT	----IFPSGGIT----- ---	51 - 58	8	508
CDR-H3	Chothia	--GWPWGLDY	99 - 106	8	509
	AbM	--GWPWGLDY	99 - 106	8	510
	Kabat	--GWPWGLDY	99 - 106	8	511
	Contact	ARGWPWGLD-	97 - 105	9	512

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	IMGT	ARGWPWGLDY	97 - 106	10	513
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	514
	AbM	RASQSVSSAVA--	24 - 34	11	515
	Kabat	RASQSVSSAVA--	24 - 34	11	516
	Contact	-----SSAWAY	30 - 36	7	517
	IMGT	---QSVSSA----	27 - 32	6	518
CDR-L2	Chothia	----SASSLYS	50 - 56	7	519
	AbM	----SASSLYS	50 - 56	7	520
	Kabat	----SASSLYS	50 - 56	7	521
	Contact	LLIYSASSLY-	46 - 55	10	522
	IMGT	----SA-----	50 - 51	2	523
CDR-L3	Chothia	QQRDRSPYT	89 - 97	9	524
	AbM	QQRDRSPYT	89 - 97	9	525
	Kabat	QQRDRSPYT	89 - 97	9	526
	Contact	QQRDRSPY-	89 - 96	8	527
	IMGT	QQRDRSPYT	89 - 97	9	528
P9-16					
CDR-H1	Chothia	GFTFSYY---	26 - 32	7	529
	AbM	GFTFSYYWIH	26 - 35	10	530
	Kabat	-----YYWIH	31 - 35	5	531
	Contact	----SYWYIH	30 - 35	6	532
	IMGT	GFTFSYYW--	26 - 33	8	533
CDR-H2	Chothia	-----YPSSGY----- ---	52 - 57	6	534
	AbM	---DIYPSSGYTY---- ---	50 - 59	10	535
	Kabat	--- DIYPSSGYTYADSVKG	50 - 66	17	536
	Contact	WVADIYPSSGYTY---- ---	47 - 59	13	537
	IMGT	----IYPSSGYT----- ---	51 - 58	8	538
CDR-H3	Chothia	--GWYAAYGMDY	99 - 108	10	539
	AbM	--GWYAAYGMDY	99 - 108	10	540
	Kabat	--GWYAAYGMDY	99 - 108	10	541
	Contact	ARGWYAAYGMD-	97 - 107	11	542
	IMGT	ARGWYAAYGMDY	97 - 108	12	543
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	544
	AbM	RASQSVSSAVA--	24 - 34	11	545
	Kabat	RASQSVSSAVA--	24 - 34	11	546
	Contact	-----SSAWAY	30 - 36	7	547

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	IMGT	---QSVSSA----	27 - 32	6	548
CDR-L2	Chothia	----SASSLYS	50 - 56	7	549
	AbM	----SASSLYS	50 - 56	7	550
	Kabat	----SASSLYS	50 - 56	7	551
	Contact	LLIYSASSLY-	46 - 55	10	552
	IMGT	----SA-----	50 - 51	2	553
CDR-L3	Chothia	QQYALRPLT	89 - 97	9	554
	AbM	QQYALRPLT	89 - 97	9	555
	Kabat	QQYALRPLT	89 - 97	9	556
	Contact	QQYALRPL-	89 - 96	8	557
	IMGT	QQYALRPLT	89 - 97	9	558
P9-18					
CDR-H1	Chothia	GFTFSWY---	26 - 32	7	559
	AbM	GFTFSWYVIH	26 - 35	10	560
	Kabat	-----WYVIH	31 - 35	5	561
	Contact	----SWYVIH	30 - 35	6	562
	IMGT	GFTFSWYV--	26 - 33	8	563
CDR-H2	Chothia	-----YPYHSK----- ---	52 - 57	6	564
	AbM	---AIYPYHSKTY----- ---	50 - 59	10	565
	Kabat	--- AIYPYHSKTYADSVKG	50 - 66	17	566
	Contact	WVAAIYPYHSKTY----- ---	47 - 59	13	567
	IMGT	-----IYPYHSKT----- ---	51 - 58	8	568
CDR-H3	Chothia	--GYWYGMDY	99 - 106	8	569
	AbM	--GYWYGMDY	99 - 106	8	570
	Kabat	--GYWYGMDY	99 - 106	8	571
	Contact	ARGYWYGMD-	97 - 105	9	572
	IMGT	ARGYWYGMDY	97 - 106	10	573
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	574
	AbM	RASQSVSSAVA--	24 - 34	11	575
	Kabat	RASQSVSSAVA--	24 - 34	11	576
	Contact	-----SSAWAY	30 - 36	7	577
	IMGT	---QSVSSA----	27 - 32	6	578
CDR-L2	Chothia	----SASSLYS	50 - 56	7	579
	AbM	----SASSLYS	50 - 56	7	580

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	Kabat	----SASSLYS	50 - 56	7	581
	Contact	LLIYSASSLY-	46 - 55	10	582
	IMGT	----SA-----	50 - 51	2	583
CDR-L3	Chothia	QQYTDAPWT	89 - 97	9	584
	AbM	QQYTDAPWT	89 - 97	9	585
	Kabat	QQYTDAPWT	89 - 97	9	586
	Contact	QQYTDAPW-	89 - 96	8	587
	IMGT	QQYTDAPWT	89 - 97	9	588
P9-19					
CDR-H1	Chothia	GFTFGYY---	26 - 32	7	589
	AbM	GFTFGYYYYIH	26 - 35	10	590
	Kabat	-----YYYYIH	31 - 35	5	591
	Contact	-----GYYYYYIH	30 - 35	6	592
	IMGT	GFTFGYYYY--	26 - 33	8	593
CDR-H2	Chothia	-----SPSGSV----- ---	52 - 57	6	594
	AbM	---WISPSGSVTA---- ---	50 - 59	10	595
	Kabat	--- WISPSGSVTAYADSVKG	50 - 66	17	596
	Contact	WVAWISPSGSVTA---- ---	47 - 59	13	597
	IMGT	-----ISPSGSVT----- ---	51 - 58	8	598
CDR-H3	Chothia	--GYYGWGM DY	99 - 108	10	599
	AbM	--GYYGWGM DY	99 - 108	10	600
	Kabat	--GYYGWGM DY	99 - 108	10	601
	Contact	ARGYYGWGM D-	97 - 107	11	602
	IMGT	ARGYYGWGM DY	97 - 108	12	603
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	604
	AbM	RASQSVSSAVA--	24 - 34	11	605
	Kabat	RASQSVSSAVA--	24 - 34	11	606
	Contact	-----SSAVAWY	30 - 36	7	607
	IMGT	---QSVSSA----	27 - 32	6	608
CDR-L2	Chothia	----SASSLYS	50 - 56	7	609
	AbM	----SASSLYS	50 - 56	7	610
	Kabat	----SASSLYS	50 - 56	7	611
	Contact	LLIYSASSLY-	46 - 55	10	612
	IMGT	----SA-----	50 - 51	2	613
CDR-L3	Chothia	QQYDTSPYT	89 - 97	9	614
	AbM	QQYDTSPYT	89 - 97	9	615

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	Kabat	QQYDTSPYT	89 - 97	9	616
	Contact	QQYDTSPY-	89 - 96	8	617
	IMGT	QQYDTSPYT	89 - 97	9	618
P9-20					
CDR-H1	Chothia	GFTFRYY---	26 - 32	7	619
	AbM	GFTFRYYYYIH	26 - 35	10	620
	Kabat	-----YYYYIH	31 - 35	5	621
	Contact	----RYYYYIH	30 - 35	6	622
	IMGT	GFTFRYYY--	26 - 33	8	623
CDR-H2	Chothia	-----YPYGGY----- ---	52 - 57	6	624
	AbM	---GIYPYGGYTS----- ---	50 - 59	10	625
	Kabat	--- GIYPYGGYTSYADSVKG	50 - 66	17	626
	Contact	WVAGIYPYGGYTS----- ---	47 - 59	13	627
	IMGT	-----IYPYGGYT----- ---	51 - 58	8	628
CDR-H3	Chothia	--GYVEGVLDY	99 - 108	10	629
	AbM	--GYVEGVLDY	99 - 108	10	630
	Kabat	--GYVEGVLDY	99 - 108	10	631
	Contact	ARGYYVEGVLD-	97 - 107	11	632
	IMGT	ARGYYVEGVLDY	97 - 108	12	633
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	634
	AbM	RASQSVSSAVA--	24 - 34	11	635
	Kabat	RASQSVSSAVA--	24 - 34	11	636
	Contact	-----SSAVAWY	30 - 36	7	637
	IMGT	---QSVSSA----	27 - 32	6	638
CDR-L2	Chothia	----SASSLYS	50 - 56	7	639
	AbM	----SASSLYS	50 - 56	7	640
	Kabat	----SASSLYS	50 - 56	7	641
	Contact	LLIYSASSLY-	46 - 55	10	642
	IMGT	----SA-----	50 - 51	2	643
CDR-L3	Chothia	QQYYGSLAT	89 - 97	9	644
	AbM	QQYYGSLAT	89 - 97	9	645
	Kabat	QQYYGSLAT	89 - 97	9	646
	Contact	QQYYGSLA-	89 - 96	8	647
	IMGT	QQYYGSLAT	89 - 97	9	648

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
P9-21					
CDR-H1	Chothia	GFTFSYY---	26 - 32	7	649
	AbM	GFTFSYYYYIH	26 - 35	10	650
	Kabat	-----YYYIH	31 - 35	5	651
	Contact	----SYYYYIH	30 - 35	6	652
	IMGT	GFTFSYYY--	26 - 33	8	653
CDR-H2	Chothia	-----HPPSGY----- ---	52 - 57	6	654
	AbM	---RIHPPSGYTD----- ---	50 - 59	10	655
	Kabat	--- RIHPPSGYTDYADSVKG	50 - 66	17	656
	Contact	WVARIHPPSGYTD----- ---	47 - 59	13	657
	IMGT	-----IHPPSGYT----- ---	51 - 58	8	658
CDR-H3	Chothia	--GYVFGVMDY	99 - 108	10	659
	AbM	--GYVFGVMDY	99 - 108	10	660
	Kabat	--GYVFGVMDY	99 - 108	10	661
	Contact	ARGYYVFGVMD-	97 - 107	11	662
	IMGT	ARGYYVFGVMDY	97 - 108	12	663
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	664
	AbM	RASQSVSSAVA--	24 - 34	11	665
	Kabat	RASQSVSSAVA--	24 - 34	11	666
	Contact	-----SSAVAWY	30 - 36	7	667
	IMGT	---QSVSSA----	27 - 32	6	668
CDR-L2	Chothia	----SASSLYS	50 - 56	7	669
	AbM	----SASSLYS	50 - 56	7	670
	Kabat	----SASSLYS	50 - 56	7	671
	Contact	LLIYSASSLY-	46 - 55	10	672
	IMGT	----SA-----	50 - 51	2	673
CDR-L3	Chothia	QQSHWYPFT	89 - 97	9	674
	AbM	QQSHWYPFT	89 - 97	9	675
	Kabat	QQSHWYPFT	89 - 97	9	676
	Contact	QQSHWYPF-	89 - 96	8	677
	IMGT	QQSHWYPFT	89 - 97	9	678
P9-22					
CDR-H1	Chothia	GFTFSSY---	26 - 32	7	679
	AbM	GFTFSSYYYYIH	26 - 35	10	680

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	Kabat	-----SYIIH	31 - 35	5	681
	Contact	-----SSYIIH	30 - 35	6	682
	IMGT	GFTFSSYY--	26 - 33	8	683
CDR-H2	Chothia	-----YPFSGG----- ---	52 - 57	6	684
	AbM	---AIYPFSGGTY----- ---	50 - 59	10	685
	Kabat	--- AIYPFSGGTYADSVKG	50 - 66	17	686
	Contact	WVAAIYPFSGGTY----- ---	47 - 59	13	687
	IMGT	-----IYPFSGGT----- ---	51 - 58	8	688
CDR-H3	Chothia	--GYVYVVMVDY	99 - 108	10	689
	AbM	--GYVYVVMVDY	99 - 108	10	690
	Kabat	--GYVYVVMVDY	99 - 108	10	691
	Contact	ARGYVYVVMVD-	97 - 107	11	692
	IMGT	ARGYVYVVMVDY	97 - 108	12	693
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	694
	AbM	RASQSVSSAVA--	24 - 34	11	695
	Kabat	RASQSVSSAVA--	24 - 34	11	696
	Contact	-----SSAVAWY	30 - 36	7	697
	IMGT	---QSVSSA----	27 - 32	6	698
CDR-L2	Chothia	----SASSLYS	50 - 56	7	699
	AbM	----SASSLYS	50 - 56	7	700
	Kabat	----SASSLYS	50 - 56	7	701
	Contact	LLIYSASSLY-	46 - 55	10	702
	IMGT	----SA-----	50 - 51	2	703
CDR-L3	Chothia	QQYKSSPWT	89 - 97	9	704
	AbM	QQYKSSPWT	89 - 97	9	705
	Kabat	QQYKSSPWT	89 - 97	9	706
	Contact	QQYKSSPW-	89 - 96	8	707
	IMGT	QQYKSSPWT	89 - 97	9	708
P9-27					
CDR-H1	Chothia	GFTFWGY---	26 - 32	7	709
	AbM	GFTFWGYGIH	26 - 35	10	710
	Kabat	-----GYGIH	31 - 35	5	711
	Contact	----WGYGIH	30 - 35	6	712
	IMGT	GFTFWGYG--	26 - 33	8	713
CDR-H2	Chothia	-----YPYGGs----- ---	52 - 57	6	714

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	AbM	---AIYPYGGSTY--- ---	50 - 59	10	715
	Kabat	--- AIYPYGGSTYYADSVKG	50 - 66	17	716
	Contact	WVAAIYPYGGSTY--- ---	47 - 59	13	717
	IMGT	-----IYPYGGST----- ---	51 - 58	8	718
CDR-H3	Chothia	--LSDIYHSFSGMDY	99 - 111	13	719
	AbM	--LSDIYHSFSGMDY	99 - 111	13	720
	Kabat	--LSDIYHSFSGMDY	99 - 111	13	721
	Contact	ARLSDIYHSFSGMD-	97 - 110	14	722
	IMGT	ARLSDIYHSFSGMDY	97 - 111	15	723
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	724
	AbM	RASQSVSSAVA--	24 - 34	11	725
	Kabat	RASQSVSSAVA--	24 - 34	11	726
	Contact	-----SSAVAWY	30 - 36	7	727
	IMGT	---QSVSSA----	27 - 32	6	728
CDR-L2	Chothia	----SASSLYS	50 - 56	7	729
	AbM	----SASSLYS	50 - 56	7	730
	Kabat	----SASSLYS	50 - 56	7	731
	Contact	LLIYSASSLY-	46 - 55	10	732
	IMGT	----SA-----	50 - 51	2	733
CDR-L3	Chothia	QQRYSTPVT	89 - 97	9	734
	AbM	QQRYSTPVT	89 - 97	9	735
	Kabat	QQRYSTPVT	89 - 97	9	736
	Contact	QQRYSTPV-	89 - 96	8	737
	IMGT	QQRYSTPVT	89 - 97	9	738
P9-28					
CDR-H1	Chothia	GFTFGFY---	26 - 32	7	739
	AbM	GFTFGFYIIH	26 - 35	10	740
	Kabat	-----FYIIH	31 - 35	5	741
	Contact	----GFYIIH	30 - 35	6	742
	IMGT	GFTFGFY--	26 - 33	8	743
CDR-H2	Chothia	-----DPHGG----- ---	52 - 57	6	744
	AbM	---FIDPHGGSTY--- ---	50 - 59	10	745
	Kabat	--- FIDPHGGSTYYADSVKG	50 - 66	17	746

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	Contact	WVAFIDPHGGSTY---- ---	47 - 59	13	747
	IMGT	-----IDPHGGST----- ---	51 - 58	8	748
CDR-H3	Chothia	--LSYPGVLDY	99 - 107	9	749
	AbM	--LSYPGVLDY	99 - 107	9	750
	Kabat	--LSYPGVLDY	99 - 107	9	751
	Contact	ARLSYPGVLD-	97 - 106	10	752
	IMGT	ARLSYPGVLDY	97 - 107	11	753
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	754
	AbM	RASQSVSSAVA--	24 - 34	11	755
	Kabat	RASQSVSSAVA--	24 - 34	11	756
	Contact	-----SSAVAWY	30 - 36	7	757
	IMGT	---QSVSSA----	27 - 32	6	758
CDR-L2	Chothia	----SASSLYS	50 - 56	7	759
	AbM	----SASSLYS	50 - 56	7	760
	Kabat	----SASSLYS	50 - 56	7	761
	Contact	LLIYSASSLY-	46 - 55	10	762
	IMGT	----SA-----	50 - 51	2	763
CDR-L3	Chothia	QQGYSTLVT	89 - 97	9	764
	AbM	QQGYSTLVT	89 - 97	9	765
	Kabat	QQGYSTLVT	89 - 97	9	766
	Contact	QQGYSTLV-	89 - 96	8	767
	IMGT	QQGYSTLVT	89 - 97	9	768
P9-31					
CDR-H1	Chothia	GFTFSQY---	26 - 32	7	769
	AbM	GFTFSQYAIH	26 - 35	10	770
	Kabat	-----QYAIH	31 - 35	5	771
	Contact	----SQYAIH	30 - 35	6	772
	IMGT	GFTFSQYA--	26 - 33	8	773
CDR-H2	Chothia	-----YPDSGY----- ---	52 - 57	6	774
	AbM	---RIYPDSGYTY---- ---	50 - 59	10	775
	Kabat	--- RIYPDSGYTYADSVKG	50 - 66	17	776
	Contact	WVARIYPDSGYTY---- ---	47 - 59	13	777
	IMGT	----IYPDSGYT----- ---	51 - 58	8	778

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
CDR-H3	Chothia	--PYHQYAEGMDY	99 - 109	11	779
	AbM	--PYHQYAEGMDY	99 - 109	11	800
	Kabat	--PYHQYAEGMDY	99 - 109	11	801
	Contact	ARPYHQYAEGMD-	97 - 108	12	802
	IMGT	ARPYHQYAEGMDY	97 - 109	13	803
CDR-L1	Chothia	RASQSVSRAVA--	24 - 34	11	804
	AbM	RASQSVSRAVA--	24 - 34	11	805
	Kabat	RASQSVSRAVA--	24 - 34	11	806
	Contact	-----SRAVAWY	30 - 36	7	807
	IMGT	---QSVSRA----	27 - 32	6	808
CDR-L2	Chothia	----SASSLYS	50 - 56	7	809
	AbM	----SASSLYS	50 - 56	7	810
	Kabat	----SASSLYS	50 - 56	7	811
	Contact	LLIYSASSLY-	46 - 55	10	812
	IMGT	----SA-----	50 - 51	2	813
CDR-L3	Chothia	QQYYSPLLT	89 - 97	9	814
	AbM	QQYYSPLLT	89 - 97	9	815
	Kabat	QQYYSPLLT	89 - 97	9	816
	Contact	QQYYSPLL-	89 - 96	8	817
	IMGT	QQYYSPLLT	89 - 97	9	818
P9-32					
CDR-H1	Chothia	GFTFSAY---	26 - 32	7	819
	AbM	GFTFSAYWIH	26 - 35	10	820
	Kabat	-----AYWIH	31 - 35	5	821
	Contact	----SAYWIH	30 - 35	6	822
	IMGT	GFTFSAYW--	26 - 33	8	823
CDR-H2	Chothia	-----GPDGGY----- ---	52 - 57	6	824
	AbM	---LIGPDGGYTY---- ---	50 - 59	10	825
	Kabat	--- LIGPDGGYTYADSVKG	50 - 66	17	826
	Contact	WVALIGPDGGYTY---- ---	47 - 59	13	827
	IMGT	----IGPDGGYT----- ---	51 - 58	8	828
CDR-H3	Chothia	--QASRGLDY	99 - 106	8	829
	AbM	--QASRGLDY	99 - 106	8	830
	Kabat	--QASRGLDY	99 - 106	8	831
	Contact	ARQASRGLD-	97 - 105	9	832

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	IMGT	ARQASRGLDY	97 - 106	10	833
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	834
	AbM	RASQSVSSAVA--	24 - 34	11	835
	Kabat	RASQSVSSAVA--	24 - 34	11	836
	Contact	-----SSAVAWY	30 - 36	7	837
	IMGT	---QSVSSA----	27 - 32	6	838
CDR-L2	Chothia	----SASSLYS	50 - 56	7	839
	AbM	----SASSLYS	50 - 56	7	840
	Kabat	----SASSLYS	50 - 56	7	841
	Contact	LLIYSASSLY-	46 - 55	10	842
	IMGT	----SA-----	50 - 51	2	843
CDR-L3	Chothia	QQWSSPLHT	89 - 97	9	844
	AbM	QQWSSPLHT	89 - 97	9	845
	Kabat	QQWSSPLHT	89 - 97	9	846
	Contact	QQWSSPLH-	89 - 96	8	847
	IMGT	QQWSSPLHT	89 - 97	9	848
P9-36					
CDR-H1	Chothia	GFTFGTY---	26 - 32	7	849
	AbM	GFTFGTYYYIH	26 - 35	10	850
	Kabat	-----TYYIH	31 - 35	5	851
	Contact	----GTYYIH	30 - 35	6	852
	IMGT	GFTFGTY--	26 - 33	8	853
CDR-H2	Chothia	-----LSGGGY----- ---	52 - 57	6	854
	AbM	---SILSGGGYTV----- ---	50 - 59	10	855
	Kabat	--- SILSGGGYTVYADSVKG	50 - 66	17	856
	Contact	WVASILSGGGYTV----- ---	47 - 59	13	857
	IMGT	-----ILSGGGYT----- ---	51 - 58	8	858
CDR-H3	Chothia	--RVYPGFDY	99 - 106	8	859
	AbM	--RVYPGFDY	99 - 106	8	860
	Kabat	--RVYPGFDY	99 - 106	8	861
	Contact	ARRVYPGFD-	97 - 105	9	862
	IMGT	ARRVYPGFDY	97 - 106	10	863
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	864
	AbM	RASQSVSSAVA--	24 - 34	11	865
	Kabat	RASQSVSSAVA--	24 - 34	11	866

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	Contact	-----SSAVAWY	30 - 36	7	867
	IMGT	---QSVSSA----	27 - 32	6	868
CDR-L2	Chothia	----SASSLYS	50 - 56	7	869
	AbM	----SASSLYS	50 - 56	7	870
	Kabat	----SASSLYS	50 - 56	7	871
	Contact	LLIYSASSLY-	46 - 55	10	872
	IMGT	----SA-----	50 - 51	2	873
CDR-L3	Chothia	QQDSWGLWT	89 - 97	9	874
	AbM	QQDSWGLWT	89 - 97	9	875
	Kabat	QQDSWGLWT	89 - 97	9	876
	Contact	QQDSWGLW-	89 - 96	8	877
	IMGT	QQDSWGLWT	89 - 97	9	878
P9-39					
CDR-H1	Chothia	GFTFSFY---	26 - 32	7	879
	AbM	GFTFSFYGIH	26 - 35	10	880
	Kabat	-----FYGIH	31 - 35	5	881
	Contact	----SFYGIH	30 - 35	6	882
	IMGT	GFTFSFYG--	26 - 33	8	883
CDR-H2	Chothia	-----YPYGGF----- ---	52 - 57	6	884
	AbM	---WIYPYGGFTD----- ---	50 - 59	10	885
	Kabat	--- WIYPYGGFTDYADSVKG	50 - 66	17	886
	Contact	WVAWIYPYGGFTD----- ---	47 - 59	13	887
	IMGT	-----IYPYGGFT----- ---	51 - 58	8	888
CDR-H3	Chothia	--SGFFAFDY	99 - 106	8	889
	AbM	--SGFFAFDY	99 - 106	8	890
	Kabat	--SGFFAFDY	99 - 106	8	891
	Contact	ARSGFFAFD-	97 - 105	9	892
	IMGT	ARSGFFAFDY	97 - 106	10	893
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	894
	AbM	RASQSVSSAVA--	24 - 34	11	895
	Kabat	RASQSVSSAVA--	24 - 34	11	896
	Contact	-----SSAVAWY	30 - 36	7	897
	IMGT	---QSVSSA----	27 - 32	6	898
CDR-L2	Chothia	----SASSLYS	50 - 56	7	899
	AbM	----SASSLYS	50 - 56	7	900

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	Kabat	----SASSLYS	50 - 56	7	901
	Contact	LLIYSASSLY-	46 - 55	10	902
	IMGT	----SA-----	50 - 51	2	903
CDR-L3	Chothia	QQVQTSLAT	89 - 97	9	904
	AbM	QQVQTSLAT	89 - 97	9	905
	Kabat	QQVQTSLAT	89 - 97	9	906
	Contact	QQVQTSLA-	89 - 96	8	907
	IMGT	QQVQTSLAT	89 - 97	9	908
P9-49					
CDR-H1	Chothia	GFTFSWY---	26 - 32	7	939
	AbM	GFTFSWYEIH	26 - 35	10	940
	Kabat	-----WYEIH	31 - 35	5	941
	Contact	-----SWYEIH	30 - 35	6	942
	IMGT	GFTFSWYE--	26 - 33	8	943
CDR-H2	Chothia	-----GPYSSY----- ---	52 - 57	6	944
	AbM	---RIGPYSSYTY---- ---	50 - 59	10	945
	Kabat	--- RIGPYSSYTYADSVKG	50 - 66	17	946
	Contact	WVARIGPYSSYTY---- ---	47 - 59	13	947
	IMGT	-----IGPYSSYT----- ---	51 - 58	8	948
CDR-H3	Chothia	--TYYPSYGMDY	99 - 108	10	949
	AbM	--TYYPSYGMDY	99 - 108	10	950
	Kabat	--TYYPSYGMDY	99 - 108	10	951
	Contact	ARTYYPSYGMD-	97 - 107	11	952
	IMGT	ARTYYPSYGMDY	97 - 108	12	953
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	954
	AbM	RASQSVSSAVA--	24 - 34	11	955
	Kabat	RASQSVSSAVA--	24 - 34	11	956
	Contact	-----SSAVAWY	30 - 36	7	957
	IMGT	---QSVSSA----	27 - 32	6	958
CDR-L2	Chothia	----SASSLYS	50 - 56	7	959
	AbM	----SASSLYS	50 - 56	7	960
	Kabat	----SASSLYS	50 - 56	7	961
	Contact	LLIYSASSLY-	46 - 55	10	962
	IMGT	----SA-----	50 - 51	2	963
CDR-L3	Chothia	QQSFSSPVT	89 - 97	9	964

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	AbM	QQSFSSPVT	89 - 97	9	965
	Kabat	QQSFSSPVT	89 - 97	9	966
	Contact	QQSFSSPV-	89 - 96	8	967
	IMGT	QQSFSSPVT	89 - 97	9	968
P9-54					
CDR-H1	Chothia	GFTFSTY---	26 - 32	7	969
	AbM	GFTFSTYFIH	26 - 35	10	970
	Kabat	-----TYFIH	31 - 35	5	971
	Contact	----STYFIH	30 - 35	6	972
	IMGT	GFTFSTYF--	26 - 33	8	973
CDR-H2	Chothia	-----SPSGSH----- ---	52 - 57	6	974
	AbM	---WISPSGSHTG---- ---	50 - 59	10	975
	Kabat	--- WISPSGSHTGYADSVKG	50 - 66	17	976
	Contact	WVAWISPSGSHTG---- ---	47 - 59	13	977
	IMGT	-----ISPSGSHT----- ---	51 - 58	8	978
CDR-H3	Chothia	--VRYPGVMDY	99 - 107	9	979
	AbM	--VRYPGVMDY	99 - 107	9	980
	Kabat	--VRYPGVMDY	99 - 107	9	981
	Contact	ARVRYPGVMD-	97 - 106	10	982
	IMGT	ARVRYPGVMDY	97 - 107	11	983
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	984
	AbM	RASQSVSSAVA--	24 - 34	11	985
	Kabat	RASQSVSSAVA--	24 - 34	11	986
	Contact	-----SSAVAWY	30 - 36	7	987
	IMGT	---QSVSSA----	27 - 32	6	988
CDR-L2	Chothia	----SASSLYS	50 - 56	7	989
	AbM	----SASSLYS	50 - 56	7	990
	Kabat	----SASSLYS	50 - 56	7	991
	Contact	LLIYSASSLY-	46 - 55	10	992
	IMGT	----SA-----	50 - 51	2	993
CDR-L3	Chothia	QQWYPSLIT	89 - 97	9	994
	AbM	QQWYPSLIT	89 - 97	9	995
	Kabat	QQWYPSLIT	89 - 97	9	996
	Contact	QQWYPSLI-	89 - 96	8	997

Table 5
CDR definitions

Region	Definition	Sequence	Residues	Length	SEQ ID NO:
	IMGT	QQWYPSLIT	89 - 97	9	998
P9-58					
CDR-H1	Chothia	GFTFSRY---	26 - 32	7	999
	AbM	GFTFSRYYYIH	26 - 35	10	1000
	Kabat	-----RYYIH	31 - 35	5	1001
	Contact	----SRYYYIH	30 - 35	6	1002
	IMGT	GFTFSRYY--	26 - 33	8	1003
CDR-H2	Chothia	-----SSDSGY----- ---	52 - 57	6	1004
	AbM	---FISSDSGYTQ---- ---	50 - 59	10	1005
	Kabat	--- FISSDSGYTQYADSVKG	50 - 66	17	1006
	Contact	WVAFISSDSGYTQ---- ---	47 - 59	13	1007
	IMGT	-----ISSDSGYT----- ---	51 - 58	8	1008
CDR-H3	Chothia	--TMSYSALDY	99 - 107	9	1009
	AbM	--TMSYSALDY	99 - 107	9	1010
	Kabat	--TMSYSALDY	99 - 107	9	1011
	Contact	ARTMSYSALD-	97 - 106	10	1012
	IMGT	ARTMSYSALDY	97 - 107	11	1013
CDR-L1	Chothia	RASQSVSSAVA--	24 - 34	11	1014
	AbM	RASQSVSSAVA--	24 - 34	11	1015
	Kabat	RASQSVSSAVA--	24 - 34	11	1016
	Contact	-----SSAVAWY	30 - 36	7	1017
	IMGT	---QSVSSA----	27 - 32	6	1018
CDR-L2	Chothia	----SASSLYS	50 - 56	7	1019
	AbM	----SASSLYS	50 - 56	7	1020
	Kabat	----SASSLYS	50 - 56	7	1021
	Contact	LLIYSASSLY-	46 - 55	10	1022
	IMGT	----SA-----	50 - 51	2	1023
CDR-L3	Chothia	QQGFGFLVT	89 - 97	9	1024
	AbM	QQGFGFLVT	89 - 97	9	1025
	Kabat	QQGFGFLVT	89 - 97	9	1026
	Contact	QQGFGFLV-	89 - 96	8	1027
	IMGT	QQGFGFLVT	89 - 97	9	1028

[0266] Table 6 presents full immunoglobulin heavy chain and full immunoglobulin light chain sequences, and the VH and VL sequences, of various ABS candidates formatted into a bivalent monospecific human full-length IgG1 architecture.

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-02B	EVQLVESGGGLVQPGGSLRLSCAASGFTFSGYFIHWVRQAPGKGLEWVARISSYGGHTDYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARAQYVPGLDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC SVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1029)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQSYPTLGTFGQGTKVEIKRTVAAPSVFIFPPSDSQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 1030)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSGYFIHWVRQAPGKGLEWVARISSYGGHTDYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARAQYVPGLDYWGQGTLVTVSS (SEQ ID NO: 1031)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQSYPTLGTFGQGTKVEIKRTV (SEQ ID NO: 1032)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-04	EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYFIHWVRQAPGKGLEWVA YISPTWGYTTYADSVKGRFTISADTSKNTAYLQ MNSLRAEDTAVYYCARAWFGFAFDYWQGGL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1033)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSG VPSRFSGSRSGTDFLTIS LQPEDFATYYCQQVYSSPL TFGQGTKVEIKRTVAAPSV FIFPPSDSQLKSGTASVCL LNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSF NRGEC (SEQ ID NO: 1034)	EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYFI HWVRQAPGKGLEWVA YISPTWGYTTYADSVKGRFTISADTSKN TAYLQMNSLRAEDTAVYYCAR AWFGFAFDYW QGGLTVTVSS (SEQ ID NO: 1035)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSGV PSRFSGSRSGTDFLTISLQ PEDFATYYC QQVYSSPLTF GQGTKVEIKR TV (SEQ ID NO: 1036)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-05	EVQLVESGGGLVQPGGSLRLSCAASGFTFTSYIHWVRQAPGKGLEWVAWIWPIGGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARDAGSGFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1037)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISSLQPEDFATYYCQYYYSLLTFGQGTKVEIKRTVAAPSVFIFPPSDSQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 1038)	EVQLVESGGGLVQPGGSLRLSCAASGFTFTSYIHWVRQAPGKGLEWVAWIWPIGGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARDAGSGFDYWGQGTLVTVSS (SEQ ID NO: 1039)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISSLQPEDFATYYCQYYYSLLTFGQGTKVEIKRTV (SEQ ID NO: 1040)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-08	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYA IHWVRQAPGKGLEWV AAIYSPTGYTDYADSV KGRFTISADTSKNTAYL QMNSLRAEDTAVYYC AREGYIGMDYWGGGT LVTVSSASTKGPSVFPL APSSKSTSGGTAALGCL VKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSG LYSLSSVTVPSSSLGT QTYICNVNHKPSNTKV DKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWY VDGVEVHNAKTKPREE QYNSTYRVVSVLTVLH QDWLNGKEYKCKVSN KALPAPIEKTISKAKGQ PREPQVYTLPPSRDEL KNQVSLTCLVKGFYPS DIAVEWESNGQPENNY KTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFS CSVMHEALHNHYTQKS LSLSPGK (SEQ ID NO: 1041)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSG VPSRFSGSRSGTDFLT LQPEDFATYYCQQSYSA LYTFGQGTKVEIKRTVA APSVFIFPPSDSQLKSGT ASVVLNNFYPREAKVQW KVDNALQSGNSQESVTEQ DSKDS TYSLSSTLTLSKADYEH KHK VYACEVTHQGLSSPVTK SF NRGEC (SEQ ID NO: 1042)	EVQLVESGGGLVQPGGSLRLSC AASGFTFSRYAI HWVRQAPGKGL EWVAAIYSPT GYTDYADSVK GRFTISADTSKN TAYLQMNSLRA EDTAVYYCARE GYIGMDYWGG GTLVTVSS (SEQ ID NO: 1043)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSGV PSRFSGSRSGTDFLT TISSLQPEDFATYYC QQSYSA LYTFGQGTKVEIKR TV (SEQ ID NO: 1044)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-09	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAWIYSSGGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARFTPDLGYGLDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKLSLSLSPGK (SEQ ID NO: 1045)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQADPSLVTFGQGTKVEIKRTVAAPSVFIFPPSDSQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 1046)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAWIYSSGGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARFTPDLGYGLDYWGQGLTVTVSS (SEQ ID NO: 1047)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQADPSLVTFGQGTKVEIKRTV (SEQ ID NO: 1048)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-10	EVQLVESGGGLVQPGGSLRLSCAASGFTFDYYV IHWVRQAPGKGLEWV AAIDSYWGDYYADSV KGRFTISADTSKNTAYL QMNSLRAEDTAVYYC ARFYFYGFDYWQGTL VTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQ TYICNVNHKPSNTKVD KKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWY VDGVEVHNAKTKPREE QYNSTYRVVSVLTVLH QDWLNGKEYKCKVSN KALPAPIEKTISKAKGQ PREPQVYTLPPSRDEL KNQVSLTCLVKGFYPS DIAVEWESNGQPENNY KTPPVLDSGDSFFLYS KLTVDKSRWQQGNVFS CSVMHEALHNHYTQKS LSLSPGK (SEQ ID NO: 1049)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSG VPSRFSGSRSGTDFTLT LQPEDFATYYCQYQSWSL WTFGQGTKVEIKRTVAAPS VFIFPPSDSQLKSGTASVVC LLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHK VYACEVTHQGLSPVTKSF NRGEC (SEQ ID NO: 1050)	EVQLVESGGGLVQPGGSLRLSC AASGFTFDYYV IHWVRQAPGKGL LEWVAAIDSY WGDYYADSV KGRFTISADTSK NTAYLQMNSLR AEDTAVYYCA RFYFYGFDYW QGQTLVTVSS (SEQ ID NO: 1051)	DIQMTQSPSSLSASVGDRV LSASVGDRV TITCRASQSV SSAVAWYQQ KPGKAPKLLI YSASSLYSGV PSRFSGSRSG TDFTLTSSLQ PEDFATYYC QYQSWSLWT FGQGTKVEIK RTV (SEQ ID NO: 1052)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-15	EVQLVESGGGLVQPGGSLRLSCAASGFTFRYYWIHWVRQAPGKGLEWVAAIFPSGGITTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGWPGWGLDYWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1053)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQRDRSPYTFGQGTKVEIKRTVAAPSVFIFPPSDSQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 1054)	EVQLVESGGGLVQPGGSLRLSCAASGFTFRYYWIHWVRQAPGKGLEWVAAIFPSGGITTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGWPGWGLDYWGQGT LVTVSS (SEQ ID NO: 1055)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQRDRSPYTFGQGTKVEIKRTV (SEQ ID NO: 1056)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs

ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-16	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYYW IHWVRQAPGKGLEWV ADIYPSSGYTTYADSV KGRFTISADTSKNTAYL QMNSLRAEDTAVYYC ARGWYAAYGMDYWG QGTLVTVSSASTKGPSV FPLAPSSKSTSGGTAAL GCLVKDYFPEPTVSW NSGALTSGVHTFPAVL QSSGLYSLSSVTVPS SLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVK FNWYVDGVEVHNAKT KPREEQYNSTYRVVSV LTVLHQDWLNGKEYK CKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPS RDELTKNQVSLTCLVK GFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSF FLYSKLTVDKSRWQQG NVFSCSVMEALHNHY TQKSLSLSPGK (SEQ ID NO: 1057)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSG VPSRFSGSRSGTDFLTSS LQPEDFATYYCQYALRPL TFGQGTKVEIKRTVAAPSV FIFPPSDSQLKSGTASVCL LNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHK VYACEVTHQGLSPVTKSF NRGEC (SEQ ID NO: 1058)	EVQLVESGGGLVQPGGSLRLSC AASGFTFSYYW IHWVRQAPGKGLEWV ADIYPSSGYTTYADSV KGRFTISADTSKNTAYL QMNSLRAEDTAVYYC ARGWYAAYGMDYWG QGTLVTVSSASTKGPSV FPLAPSSKSTSGGTAAL GCLVKDYFPEPTVSW NSGALTSGVHTFPAVL QSSGLYSLSSVTVPS SLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVK FNWYVDGVEVHNAKT KPREEQYNSTYRVVSV LTVLHQDWLNGKEYK CKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPS RDELTKNQVSLTCLVK GFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSF FLYSKLTVDKSRWQQG NVFSCSVMEALHNHY TQKSLSLSPGK (SEQ ID NO: 1059)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSGV PSRFSGSRSGTDFLTSS LQPEDFATYYCQYALRPLTF GGGTKVEIKRTV (SEQ ID NO: 1060)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-18	EVQLVESGGGLVQPGG SLRLSCAASGFTFSWYV IHWVRQAPGKGLEWV AAIYPYHSKTTYADSV KGRFTISADTSKNTAYL QMNSLRAEDTAVYYC ARGYWYGMDYWGQG TLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTK VDKKVEPKSCDKTHTC PPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNW YVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVL HQDWLNGKEYKCKVS NKALPAIEKTISKAKG QPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYP SDIAVEWESNGQPENN YKTTTPVLDSGDSFFLY SKLTVDKSRWQQGNVF SCSVMHEALHNHYTQK SLSLSPGK (SEQ ID NO: 1061)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSG VPSRFSGSRSGTDFLTSS LQPEDFATYYCQYTDAP WTFGQGTKVEIKRTVAAPS VFIFPPSDSQLKSGTASVVC LLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSF NRGEC (SEQ ID NO: 1062)	EVQLVESGGGL VQPGGSLRLSC AASGFTFSWYV IHWVRQAPGKG LEWVAAIYPYH SKTTYADSVKG RFTISADTSKNT AYLQMNSLRAE DTAVYYCARG YWYGMDYWG QGTTLTVSS (SEQ ID NO: 1063)	DIQMTQSPSS LSASVGDRV TITCRASQSV SSAVAWYQQ KPGKAPKLLI YSASSLYSGV PSRFSGSRSG TDFLTSSSLQ PEDFATYYC QYTDAPWT FGQGKVEIK RTV (SEQ ID NO: 1064)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs

ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-19	EVQLVESGGGLVQPGGSLRLSCAASGFTFGYYYIHWVRQAPGKGLEWVAWISPGSVTAYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGYGGWGM DYWGQGT LVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1065)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQYDTSPTFGQGTKVEIKRTVAAPSVFIFPPSDSQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 1066)	EVQLVESGGGLVQPGGSLRLSCAASGFTFGYYYIHWVRQAPGKGLEWVAWISPGSVTAYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARGYGGWGM DYWGQGT LVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1067)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQYDTSPTFGQGTKVEIKRTVAAPSVFIFPPSDSQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 1068)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-20	EVQLVESGGGLVQPGG SLRLSCAASGFTFRYYY IHWVRQAPGKGLEWV AGIYPYGGYTSYADSV KGRFTISADTSKNTAYL QMNSLRAEDTAVYYC ARGYYVEGVLDYWGQ GTLTVSSASTKGPSVF PLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQS SGLYSLSSVTVPSSSL GTQTYICNVNHKPSNT KVDKKVEPKSCDKTHT CPPCPAPELLGGPSVFL FPXKPKDTLMISRTPEV TCFVVDVSHEDPEVKF NWYVDGVEVHNAKTK PREEQYNSTYRVVSVL TVLHQDWLNGKEYKC KVSNNKALPAPIEKTISK AKGQPREPQVYTLPPSR DELTKNQVSLTCLVKG FYPSDIAVEWESNGQPE NNYKTTTPVLDSDGSFF LYSKLTVDKSRWQQGN VFSCSVMHEALHNHYT QKSLSLSPGK (SEQ ID NO: 1069)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSG VPSRFSGSRSGTDFLTISS LQPEDFATYYCQYYGSL ATFGQGTKVEIKRTVAAPS VFIFPPSDSQLKSGTASVVC LLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHK VYACEVTHQGLSPVTKSF NRGEC (SEQ ID NO: 1070)	EVQLVESGGGL VQPGGSLRLSC AASGFTFRYYY IHWVRQAPGKG LEWVAGIYPYG GYTSYADSVKG RFTISADTSKNT AYLQMNSLRAE DTAVYYCARG YYVEGVLDYW GQGTLLTVSS (SEQ ID NO: 1071)	DIQMTQSPSS LSASVGDRV TITCRASQSV SSAVAWYQQ KPGKAPKLLI YSASSLYSGV PSRFSGSRSG TDFLTISSLQ PEDFATYYC QYYGSLAT FGQGTKVEIK RTV (SEQ ID NO: 1072)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-21	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYYYIHWVRQAPGKGLEWVARIHPPSGYTDYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR GYYVFGVMDYWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1073)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQSHWYPFTFGQGTKVEIKRTVAAPS VFIFPPSDSQLKSGTASVVC LLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDS TYLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 1074)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYYYIHWVRQAPGKGLEWVARIHPPSGYTDYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR GYYVFGVMDYWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1075)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQSHWYPFTFGQGTKVEIKRTV (SEQ ID NO: 1076)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-22	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVA AIYPFSGGTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR GYYVYVVM DYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKLSLSLSPGK (SEQ ID NO: 1077)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQYKSSP WFTGQGTKVEIKRTVAAPS VFIFPPSDSQLKSGTASVVC LLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDS TYSLSSLTLSKADYEKHK VYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 1078)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVA AIYPFSGGTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR GYYVYVVM DYWGQGLTVTVSS (SEQ ID NO: 1079)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQYKSSPWT FGQGTKVEIKRTV (SEQ ID NO: 1080)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-27	EVQLVESGGGLVQPGGSLRLSCAASGFTFWGYGIHWVRQAPGKGLEWVAAIYPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARLSDIYHSFSGMDYWGGQTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVS WNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO: 1081)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQRYPSTVTFGQGTKVEIKRTVAAPSVFIFPPSDSQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 1082)	EVQLVESGGGLVQPGGSLRLSCAASGFTFWGYGIHWVRQAPGKGLEWVAAIYPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARLSDIYHSFSGMDYWGQGTTLTVSS (SEQ ID NO: 1083)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQRYPSTVTFGQGTKVEIKR TV (SEQ ID NO: 1084)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-28	EVQLVESGGGLVQPGGSLRLSCAASGFTFGFYI IHWVRQAPGKGLEWV AFIDPHGGSTYYADSV KGRFTISADTSKNTAYL QMNSLRAEDTAVYYC ARLSYPGVLDYWGQGT LVTVSSASTKGPSVFPL APSSKSTSGGTAALGCL VKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSG LYSLSSVTVPSSSLGT QYICNVNHKPSNTKVD KKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWY VDGVEVHNAKTKPREE QYNSTYRVVSVLTVLH QDWLNGKEYKCKVSN KALPAPIEKTISKAKGQ PREPQVYTLPPSRDEL KNQVSLTCLVKGFYPS DIAVEWESNGQPENNY KTPPVLDSGDSFFLYS KLTVDKSRWQQGNVFS CSVMHEALHNHYTQKS LSLSPGK (SEQ ID NO: 1085)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSG VPSRFSGSRSGTDFLT LQPEDFATYYCQGGYSTL VTFGQGTKVEIKRTVAAPS VFIFPPSDSQLKSGTASVVC LLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHK VYACEVTHQGLSPVTKSF NRGEC (SEQ ID NO: 1086)	EVQLVESGGGLVQPGGSLRLSC AASGFTFGFYI HWVRQAPGKGLEWVAFIDPHG STYYADSVKGRFTISADTSKNT AYLQMNSLRAEDTAVYYCARLS YPGVLDYWGQGTSLVTVSS (SEQ ID NO: 1087)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSGV PSRFSGSRSGTDFLT LQPEDFATYYCQGGYSTLVT FGQGTKVEIKRTV (SEQ ID NO: 1088)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-31	EVQLVESGGGLVQPGGSLRLSCAASGFTFSQYA IHWVRQAPGKGLEWV ARIYPDSGYTTYADSV KGRFTISADTSKNTAYL QMNSLRAEDTAVYYC ARPYHQYAEGMDYWG QGTLVTVSSASTKGPSV FPLAPSSKSTSGGTAAL GCLVKDYFPEPTVSW NSGALTSGVHTFPAVL QSSGLYSLSSVTVPS SLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVK FNWYVDGVEVHNAKT KPREEQYNSTYRVVSV LTVLHQDWLNGKEYK CKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPS RDELTKNQVSLTCLVK GFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSF FLYSKLTVDKSRWQQG NVFSCSVMEALHNHY TQKSLSLSPGK (SEQ ID NO: 1089)	DIQMTQSPSSLSASVGDRV TITCRASQSVSRAVAWYQ QKPGKAPKLLIYSASSLYS GVPSRFSGSRSGTDFLTIS SLQPEDFATYYCQQYYSP LTFGQGTKVEIKRTVAAPS VFIFPPSDSQLKSGTASVVC LLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHK VYACEVTHQGLSPVTKSF NRGEC (SEQ ID NO: 1090)	EVQLVESGGGLVQPGGSLRLSC AASGFTFSQYAI HWVRQAPGKGL LEWVARIYPDS GYTTYADSVK GRFTISADTSKN TAYLQMNSLRA EDTAVYYCARP YHQYAEGMDY WGQGTSLTVSS (SEQ ID NO: 1091)	DIQMTQSPSSLSASVGDRV TITCRASQSV SRAVAWYQQ KPGKAPKLLI YSASSLYSGV PSRFSGSRSG TDFLTISLQ PEDFATYYC QQYYSPLLTF GQGTKVEIKR TV (SEQ ID NO: 1092)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-32	EVQLVESGGGLVQPGGSLRLSCAASGFTFSAYWIHWVRQAPGKGLEWVALIGPDGGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARQASRGLDYWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1093)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQWSSPLHTFGQGTKVEIKRTVAAPSVFIFPPSDSQLKSGTASVVC LLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDS TYSLSSLTTLISKADYEKHK VYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 1094)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSAYWIHWVRQAPGKGLEWVALIGPDG GYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARQASRGLDYWGQGT LVTVSS (SEQ ID NO: 1095)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQWSSPLHTFGQGTKVEIKRTV (SEQ ID NO: 1096)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-36	EVQLVESGGGLVQPGGSLRLSCAASGFTFGTYI IHWVRQAPGKGLEWV ASILSGGGYTVYADSV KGRFTISADTSKNTAYL QMNSLRAEDTAVYYC ARRVYPGFDYWGGQT LVTVSSASTKGPSVFPL APSSKSTSGGTAALGCL VKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSG LYSLSSVTVPSSSLGT QTYICNVNHKPSNTKV DKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWY VDGVEVHNAKTKPREE QYNSTYRVVSVLTVLH QDWLNGKEYKCKVSN KALPAPIEKTISKAKGQ PREPQVYTLPPSRDEL KNQVSLTCLVKGFYPS DIAVEWESNGQPENNY KTPPVLDSGDGSFFLYS KLTVDKSRWQQGNVFS CSVMHEALHNHYTQKS LSLSPGK (SEQ ID NO: 1097)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSG VPSRFSGSRSGTDFLT LQPEDFATYYCQDQSWGL WTFGQGTKVEIKRTVAAPS VFIFPPSDSQLKSGTASVVC LLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHK VYACEVTHQGLSPVTKSF NRGEC (SEQ ID NO: 1098)	EVQLVESGGGLVQPGGSLRLSC AASGFTFGTYI HWVRQAPGKGL LEWVASILSGG GYTVYADSVK GRFTISADTSKN TAYLQMNSLRA EDTAVYYCARR VYPGFDYWGG GTLVTVSS (SEQ ID NO: 1099)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSGV PSRFSGSRSGTDFLT LQPEDFATYYCQDQSWGLWT FGQGTKVEIKRTV (SEQ ID NO: 1100)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-39	EVQLVESGGGLVQPGGSLRLSCAASGFTFSFYGIHWVRQAPGKGLEWVAWIYPYGGFTDYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSGFFAFDYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1101)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQVQTSLATFGQGTKVEIKRTVAAPSVFIFPPSDSQLKSGTASVVC LLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDS TYLSSTLTLSKADYEKHK VYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 1102)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSFYGIHWVRQAPGKGLEWVAWIYPYGGFTDYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARSGFFAFDYWGQGLTVTVSS (SEQ ID NO: 1103)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQVQTSLATFGQGTKVEIKRTV (SEQ ID NO: 1104)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-49	EVQLVESGGGLVQPGGSLRLSCAASGFTFSWYIEHWVRQAPGKGLEWVARIGPYSSYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR TYPPSYGMDYWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1105)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQSFSSPVTFGQGTKVEIKRTVAAPSVFIFPPSDSQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 1106)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSWYIEHWVRQAPGKGLEWVARIGPYSSYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR TYPPSYGMDYWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1107)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQSFSSPVTFGQGTKVEIKRTVAAPSVFIFPPSDSQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 1108)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-54	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYFIHWVRQAPGKGLEWVAWISPSGSHTGYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARVRYPGVMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1109)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQWYPSLITFGQGTKVEIKRTVAAPSVFIFPPSDSQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 1110)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYFIHWVRQAPGKGLEWVAWISPSGSHTGYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARVRYPGVMDYWGQGTLVTVSS (SEQ ID NO: 1111)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYCQQWYPSLITFGQGTKVEIKR TV (SEQ ID NO: 1112)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-55 NEG. CON.	EVQLVESGGGLVQPGGSLRLSCAASGFTFATYYIHWRQAPGKGLEWVAYIDSESGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARVSRGSSGTHVMDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 1113)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLLQPEDFATYYCQQRYSSLLTFGQGTKVEIKRTVAAPSVFIFPPSDSQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 1114)	EVQLVESGGGLVQPGGSLRLSCAASGFTFATYYIHWRQAPGKGLEWVAYIDSESGYTYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARVSRGSSGTHVMDYWGQGTLLTVSS (SEQ ID NO: 1115)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFLTISLLQPEDFATYYCQQRYSSLLTFGQGTKVEIKRTV (SEQ ID NO: 1116)

Table 6: full chain sequences and VH/VL sequences of candidate GAL9 ABS clones and IgG formatted antibodies comprising GAL9 ABSs				
ABS clone	Full IgG Heavy Chain	Full IgG Light Chain	VH sequence	VL sequence
P9-58	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYYIHVVRQAPGKGLEWVAFISSDSGYTQYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCAR TMSYSALDYWGQGTLT VTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGL YSLSSVTVPSSSLGTQ TYICNVNHKPSNTKVD KKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWY VDGVEVHNAKTKPREE QYNSTYRVVSVLTVLH QDWLNGKEYKCKVSN KALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNY KTTTPVLDSGDSFFLYS KLTVDKSRWQQGNVFS CSVMHEALHNHYTQKS LSLSPGK (SEQ ID NO: 1117)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSG VPSRFSGSRSGTDFLTIS LQPEDFATYYCQQGFGFLV TFGQGTKVEIKRTVAAPSV FIFPPSDSQLKSGTASVVCL LNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDS TYLSSTLTLSKADYEKHK VYACEVTHQGLSPVTKSF NRGEC (SEQ ID NO: 1118)	EVQLVESGGGLVQPGGSLRLSC AASGFTFSRYYIHWVRQAPGKGLWVAFISSDSGYTQYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARTMSYSALDYWGQGTLT VTS (SEQ ID NO: 1119)	DIQMTQSPSSLSASVGDRV TITCRASQSVSSAVAWYQQ KPGKAPKLLIYSASSLYSGV PSRFSGSRSGTDFLTISLQ PEDFATYYCQQGFGFLVTF GQGTKVEIKR TV (SEQ ID NO: 1120)

[0267] Select GAL9 binding candidates were analyzed for binding properties: cross-reactive binding with murine GAL9, qualitative binding, epitope binning (Bin 2 - candidates bin with Commercial antibody Clone ECA8 from LS Bio [LS-C179448], Bin 3 - candidates bin with Commercial antibody Clone ECA42 from LS Bio [LS-C179449], which is the “tool antibody” referenced in **FIG. 3**), and monovalent affinity binding. Analysis results are presented in **Table 7**.

Table 7: Candidate hGAL9 Binding Properties				
ABS	Mouse Cross-reactivity	Binding Off-Rate (++ = moderate, +++ = slow)	Bin	Calculated K _D (M)
P9-02B	Y	+++	1	
P9-04		+++	3	
P9-05	Y	+++	2	
P9-08		++	3	

Table 7: Candidate hGAL9 Binding Properties				
ABS	Mouse Cross-reactivity	Binding Off-Rate (++ = moderate, +++ = slow)	Bin	Calculated K _D (M)
P9-09	Y	+++	1	
P9-10		++	2	5.77x10 ⁻⁹
P9-15		++	1	2.208x10 ⁻⁹
P9-16	Y	+++	1	2.87x10 ⁻⁹
P9-18	Y	+++	3	6.243x10 ⁻⁹
P9-19		++	1	
P9-20	Y	+++	1	
P9-21	Y	+++	3	2.749x10 ⁻⁹
P9-22	Y	+++	1	4.85x10 ⁻⁹
P9-27		+++	2	
P9-28		+++	1	3.358x10 ⁻⁹
P9-31		+++	3	
P9-32	Y	+++	1	1.083x10 ⁻⁹
P9-36		++	1	
P9-39		++	1	
P9-49		+++	1	
P9-54		+++	1	
P9-55		Negative Control (NEG)		
P9-58		++	1	

[0268] Select GAL9 binding candidates were further analyzed for sequence motifs that could adversely affect antibody properties that are relevant to clinical development, such as stability, mutability, and immunogenicity. Computational analysis was performed according to Kumar and Singh (*Developability of biotherapeutics: computational approaches*. Boca Raton: CRC Press, Taylor & Francis Group, 2016). Analysis results are presented in **Table 8**, and demonstrate a limited number of adverse sequence motifs are present in the listed clones, indicating the potential for further clinical development.

Table 8: Candidate anti-human GAL9 Antibody Properties

ABS	CDR3 Loop Length	Yield (ug/mL)	Mol Weight (kDa)	Isoelectric Point	Number Deamidation Sites ¹	Number Isomerization Sites ²	Number Fragmentation Sites ³	Number N-linked Glycosylation Sites ⁴	Cys in CDR	Number Other Sites ⁵	Number T-cell Epitopes ⁶
P9-05	11	10	1.444x10 ⁵	8.32	0	1	1	0	No	0	0
P9-10	11	33	1.450x10 ⁵	8.08	0	2	2	0	No	0	0
P9-15	11	100	1.444x10 ⁵	8.59	0	1	1	0	No	0	1
P9-16	13	180.9	1.450x10 ⁵	8.42	0	1	1	0	No	0	1
P9-18	11	189.5	1.448x10 ⁵	8.42	0	1	3	0	No	0	0
P9-21	13	162.5	1.451x10 ⁵	8.42	0	1	2	0	No	0	2
P9-22	13	53.7	1.448x10 ⁵	8.50	0	1	1	0	No	0	1
P9-28	12	85	1.440x10 ⁵	8.33	0	2	1	0	No	0	1
P9-32	11	322.5	1.438x10 ⁵	8.43	0	2	1	0	No	0	1
P9-55			1.452x10 ⁵	8.42	0	2	1	0	No	0	0

¹ (NG, NS, NA, NH, ND)² (DG, DP, DS)³ (DP, DY, HS, KT, HXS, SXH)⁴ (NXS/T)⁵ (LLQG (SEQ ID NO: 1121), HPQ, FHENSP (SEQ ID NO: 1122), LPRWG (SEQ ID NO: 1123), HHH)⁶ 3% in at least 2 of DRB1_0101,DRB1_0301,DRB1_0401,DRB1_0701,DRB1_1101,DRB1_1301,DRB1_1501,DRB1_0801

6.11.3. Example 2: Treatment with Anti-GAL9 Candidates Increases Cytokine Production by Human PBMCs

[0269] Candidate GAL9 ABSs were formatted into a bivalent monospecific native human full-length IgG1 heavy chain and light chain architectures (SEQ ID NO:5 and SEQ ID NO:3, respectively) and were tested for their effect on cytokine production by PBMCs following peptide stimulation. PBMCs were stimulated essentially as described in **Section 6.11.1** above. Briefly, PBMCs were harvested from human donors known to be responsive to human CMV virus (HCMV), placed in culture, and stimulated with HCMV PepMix to prime an antigen specific response, and treated with one of: control IgG, a comparator tool activating mAb (clone ECA42), α -PD1 (Nivolumab), or candidate anti-GAL9 antibodies. Cytokine secretion was measured at 24 and 72 hrs post-treatment by bead cytokine array. Results for INF- γ and TNF- α are depicted in **FIGs. 3A** and **3B**, respectively. The data shown in **FIGS. 3A-3B** is described in more detail in the **Tables 9 and 10** provided below.

Table 9 INF-γ 72hr						
		Average/ donor				
		Donor 5	Donor 19	Donor 20	Average	as %
IgG	pg/ml	446	607	760		
P9-15	pg/ml	822	922	1114		
	Fold change	1.85	1.52	1.47	1.61	61%
P9-18	pg/ml	808	795	845		
	Fold change	1.81	1.31	1.11	1.41	41%
P9-21	pg/ml	938	1006	1089		
	Fold change	2.10	1.66	1.43	1.73	73%
P9-28	pg/ml	873	951	1054		
	Fold change	1.96	1.57	1.39	1.64	64%

Table 10 TNF-α 72hr						
		Average/ donor				
		Donor 5	Donor 19	Donor 20	Average	as %
IgG	pg/ml	2	111	189		
P9-15	pg/ml	992	987	950		
	Fold change	566.81	8.91	5.02	193.58	19258%
P9-18	pg/ml	774	693	747		
	Fold change	442.30	6.26	3.95	150.83	14983%

Table 10						
TNF-α 72hr						
		Average/ donor			Average	as %
		Donor 5	Donor 19	Donor 20		
P9-21	pg/ml	546	520	612		
	Fold change	311.97	4.70	3.24	106.63	10563%
P9-28	pg/ml	455	570	673		
	Fold change	259.74	5.14	3.56	89.48	8848%

[0270] Notably, PBMCs treated with candidates P9-15, P9-18, P9-21, and P9-28 demonstrated improved IFN- γ and TNF- α secretion following stimulation relative to both IgG control and the GAL9 comparator Tool antibody (clone ECA42). In addition, PBMCs treated with candidates P9-15, P9-18, P9-21, and P9-28 notably also demonstrated improved TNF- α production following stimulation relative to treatment with a commercial α -PD1 antibody. Thus, treatment of PBMCs with select anti-GAL9 candidates was able to improve cytokine secretion following peptide stimulation. Treatment with P9-54 resulted in a neutral response, with no significant difference in TNF- α and IFN- γ secretion (data not shown).

6.11.4. Example 3: Treatment with Anti-GAL9 Candidates Increases TNF- α Production by Natural Killer (NK) Cells

[0271] Candidate GAL9 ABSs were formatted into a bivalent monospecific native human full-length IgG1 heavy chain and light chains architectures (SEQ ID NO:5 and SEQ ID NO:3, respectively) and were tested for their effect on TNF- α secretion by NK Cells (lineage, CD56⁺) following 72 hours of peptide stimulation. NK Cells were treated with Control Antibody Clone 55, GAL9 antibody candidate P9-15 (Clone 15), or GAL9 antibody candidate P9-18 (Clone 18), at a dosage of 5 μ g or 20 μ g. After treatment, cells were assessed for levels of TNF- α secretion by flow cytometry. Representative data for the percentage of NK cells (CD56⁺) that secreted TNF- α are presented in **FIG. 6**.

[0272] Treatment with either GAL9 antibody candidate P9-18 or candidate P9-15 increased the percentages of NK cells that stained positive for TNF- α following stimulation, relative to the Clone P9-55, a negative control. In a population of NK cells treated with 5 μ g of control antibody, 7.75% of such NK cells (CD56⁺) were TNF- α positive. By contrast, in a population of NK cells treated with 5 μ g of P9-18, 12.0% of such NK cells were TNF- α positive. And NK cells treated with 5 μ g of P9-15, 22.5% of such NK cells were TNF- α positive. See **FIG. 6**.

[0273] In a population of NK cells treated with 20 µg of control antibody, 10.3% of such NK cells (CD56⁺) were TNF-α positive. By contrast, in a population of NK cells treated with 20 µg of P9-18, 16.9% of such NK cells were TNF-α positive. And NK cells treated with 20 µg of P9-15, 28.5% of such NK cells were TNF-α positive. See **FIG. 6**.

[0274] Thus, treatment with select anti-GAL9 candidates was able to increase TNF-α production by NK cells following stimulation. See **FIG. 6**.

6.11.5. Example 4: Treatment with Anti-GAL9 Candidates Increases IL-12 Production by Dendritic Cells

[0275] Candidate GAL9 ABSs were formatted into a bivalent monospecific native human full-length IgG1 heavy chain and light chains architectures (SEQ ID NO:5 and SEQ ID NO:3, respectively) and were tested for their effect on IL-12 secretion by dendritic cells (lineage negative, class II⁺, CD11c⁺) following peptide stimulation. PBMCs, which include the population of dendritic cells (DCs), were treated as described in **Example 2** then assessed for levels of IL-12 secretion using an IL-12 Secretion Assay-Detection Kit (PE), Human (Cat. No. 130-092-124, Miltenyi Biotec) as per the manufacturers protocol. Representative data for the percentage of DCs that secreted IL-12 are presented in **FIG. 5**.

[0276] Notably, treatment with the GAL9 antibody candidate P9-18 increased the percentages of DCs that stained positive for IL-12 following stimulation, relative to the IgG control. In a population of DCs treated with control IgG, 0.26% of such DCs were IL-12 positive. By contrast, in a population of DCs treated with P9-18, 7.74% of such DCs were IL-12 positive, a 28-fold increase in IL-12 positive DCs relative to the IgG control-treated population. Thus, treatment of PBMCs with select anti-GAL9 candidates was able to increase IL-12 production by DCs following stimulation.

6.11.6. Example 5: Treatment with Anti-GAL9 Candidates Increases Surface Expression of Co-Stimulatory Molecules on CD8⁺ T Cells

[0277] Candidate GAL9 ABSs that had been formatted into a bivalent monospecific native human full-length IgG1 heavy chain and light chain architectures (SEQ ID NO:5 and SEQ ID NO:3, respectively) were tested for their effect on immune stimulatory surface marker expression by CD8⁺ T-cells following peptide stimulation. PBMCs, which include the population of CD8⁺ T-cells, were treated as described in **Example 2**, stained with marker

antibodies as described herein, then harvested for flow cytometry. Levels of the immune stimulatory surface markers CD27, CD40L, ICOS, 4-1BB, and OX40 were assessed on CD8⁺ T-cells. Data are shown in **FIG. 4**. “% value” represents the % of CD8⁺ T cells with detectable levels of the relevant marker. **FIG. 4** indicates that treatment with the α GAL9 antibody candidates P9-15, P9-18, P9-21, and P9-28 increased the immune stimulatory surface markers CD27, CD40L, ICOS, 4-1BB, and OX40 in CD8⁺ T cells, as compared to an Ig control antibody clone ECA42.

[0278] Representative data for the percentage of CD8⁺ T-cells that stained positive for immune stimulatory surface marker are presented in **Table 11** below.

Table 11: Percent CD8⁺ cells positive for selected costimulatory molecules					
Antibody	4-1BB	CD27	CD40L	ICOS	OX40
IgG control	8.13	34.8	5.21	8.03	8.68
Comparator Tool mAb (clone ECA42)	11.2	35.2	5.28	11.3	7.77
α-PD-1 (Nivolumab)	8.46	34.6	5	8.45	7.81
α-GAL9 (P9-15)	16.5 (2.1x)	50 (1.4x)	24.8 (4.8x)	11.4 (1.4x)	30.5 (3.5x)
α-GAL9 (P9-18)	14.7 (1.8x)	42.3 (1.2x)	14.7 (2.7x)	10.7 (1.3x)	19 (2.2x)
α-GAL9 (P9-21)	13.4 (1.6x)	43.9 (1.3x)	16.7 (3.2x)	9.35 (1.2x)	21.7 (2.5x)
α-GAL9 (P9-22)	12.1 (1.5x)	37.2 (1.1x)	10.3 (2.0x)	11.1 (1.4x)	15.3 (1.8x)
α-GAL9 (P9-28)	13.3 (1.6x)	44.5 (1.3x)	26.1 (5x)	9.8 (1.2x)	22.4 (2.6x)

[0279] Notably, PBMCs treated with candidates P9-18 or P9-21 demonstrated increased percentages of CD8⁺ T-cells that stained positive for the various immune stimulatory surface markers following stimulation relative to the IgG control, the GAL9 comparator Tool antibody (clone ECA42), and α -PD1, including a greater than 2-fold increase in the percentage of CD8⁺ T-cells that stained positive for CD40L and OX40. Thus, treatment of PBMCs with select anti-

GAL9 candidates was able to improve immune stimulatory surface marker expression by CD8⁺ T cells following stimulation. The same immune stimulatory response was observed with low responder PBMC cells, donor 5 (data not shown).

6.11.7. Example 6: Treatment with Anti-GAL9 Candidates Alters PD-L1 and PD-L2 Cell Surface Expression on Dendritic Cells (DCs)

[0280] Candidate GAL9 ABSs were formatted into a bivalent monospecific native human full-length IgG1 heavy chain and light chains architecture (SEQ ID NO:5 and SEQ ID NO:3, respectively) and were tested for their effect on PD-L1 and PD-L2 cell surface expression on dendritic cells (lineage negative, class II, CD11c⁺) following peptide stimulation. PBMCs, which include the population of dendritic cells (DCs), were treated as described in **Example 2** then harvested for flow cytometry and the levels of PD-L1 and PD-L2 were assessed on DCs. Representative data for the percentage of DCs that stained positive for PD-L1 and PD-L2, as well as the geometric mean fluorescent intensity (GMI), are presented in **Table 12** below.

Table 12: Percent DCs positive for surface PD-L1 and PD-L2, amount of PD-L1/PD-L2 (GMI)					
Antibody	% of cells with marker			GMI	
	PD-L1	PD-L2		PD-L1	PD-L2
IgG control	84.7	1.15		37,215	3,273
Comparator Tool mAb (clone ECA42)	88.2	1.58		50,395	3,616
α -GAL9 (P9-18)	75.2	7.35		27,122	3,345
α -GAL (P9-21)	69.8	1.38		20,090	2,551

[0281] Notably, PBMCs treated with candidate P9-18 demonstrated increased percentages of DCs that stained positive for PD-L2 following stimulation relative to the IgG control and the GAL9 comparator Tool antibody (ECA42). Both P9-18 and P9-21 also demonstrated a decreased percentage of PD-L1, as well as decreased Geometric Mean Fluorescence (GMI) of PD-L1 on DCs. Thus, treatment of PBMCs with select anti-GAL9 candidates was able to alter PD-L1 and PD-L2 surface expression by DCs following stimulation.

6.11.8. Example 7: Treatment with Anti-GAL9 Candidates Leads to Clustering of GAL9 and PD-L2 on the Cell Surface of Dendritic Cells

[0282] Candidate GAL9 ABSs were formatted into a bivalent monospecific native human full-length IgG1 heavy chain and light chains architecture (SEQ ID NO:5 and SEQ ID NO:3, respectively) and were tested for their effect on clustering of GAL9, PD-L1, and PD-L2 on the cell-surface of dendritic cells (“DCs”).

[0283] PBMCs, which include the population of dendritic cells (DCs), were treated as described in **Example 2** then fixed for confocal imaging analysis to assess GAL9, CD11c, and PD-L2 distribution on dendritic cells.

[0284] Results/Conclusion

[0285] Confocal images of dendritic cells treated with IgG control (**FIG. 8A**), P9-18 (**FIG. 8B**), and P9-21 (**FIG. 8C**) are shown. The blue staining shows DNA (DAPI), the red staining shows PD-L2, the green staining shows CD11c, and the yellow staining shows GAL9. Non-labeled images are bright field; rendered in gray scale in the attached figures.

[0286] Treatment with candidate P9-18 or P9-21 demonstrated co-localization and clustering of GAL9 and PD-L2 on DCs (**FIGs. 8B-8C**), as compared to IgG control. Thus, treatment with P9-18 or P9-21 can induce co-localization and clustering of GAL9 and PD-L2 on the cell surface of DCs following stimulation.

6.11.9. Example 8: Treatment with Anti-GAL9 P9-18 Retains PD-L2 and PD-L1 Expression on Tumor Cells

[0287] Anti-GAL9 candidate P9-18 was tested for its effect on cellular retention and distribution of PD-L2 and PD-L1 in tumor cells.

[0288] Antibodies

[0289] Candidate GAL9 ABSs were formatted into a bivalent monospecific native human full-length IgG1 heavy chain and light chain architecture (SEQ ID NO:5 and SEQ ID NO:3, respectively). Anti-PD-L2 clone TY25 and anti-PD-L1 clone 10F.9G2 were obtained from BioXcell (Lebanon, NH).

[0290] Cell Culture and Immunostaining

[0291] CT26 tumor cells were cultured and treated with either anti-GAL9 candidate P9-18 or IgG control. Cells were fixed and stained with DAPI, anti-PD-L2, and anti-PD-L1 for confocal imaging analysis.

[0292] Results/Conclusion

[0293] **FIGs. 9A and 9B** show representative confocal images of CT26 tumor cells after treatment with P9-18 or IgG control. The blue staining shows DNA (DAPI), red shows PD-L2, and green shows PD-L1; rendered in gray scale in the attached figures. The imaging demonstrated that PD-L2 and PD-L1 are retained on the surface of CT26 tumor cells after treatment with P9-18 compared to IgG control. See **FIGS. 9A and 9B**. The speckles in **FIG. 9B** highlight increased expression of PD-L2 and PD-L1 proteins.

**6.11.10. Example 9: Treatment with anti-GAL9 P9-18 or P9-21
Inhibits Tumor Growth in Colon and Melanoma Tumor
Models**

[0294] This study was conducted to determine if anti-GAL9 candidates P9-18 and P9-21 can inhibit tumor growth in a colon and melanoma tumor models.

[0295] Antibodies

[0296] Candidate GAL9 ABSs were formatted into a bivalent monospecific formatted on a mouse IgG2a backbone.

[0297] Animals and Treatment

[0298] BALB/c mice were implanted subcutaneously with CT26 tumor line and treated with anti-GAL9 candidates P9-18, P9-21, or IgG control. Treatments were intraperitoneal (I.P.), 200 µg, on days 7, 11, 15, and 19 with ten mice per treatment group. Tumor growth was assessed by measuring tumor volume. Mice were euthanized if tumors reached a volume of ~1000 mm³.

[0299] C57BL/6 mice were implanted intradermally with a B16.F0 tumor line and treated with anti-GAL9 candidates P9-18, P9-21, or IgG control. Treatments were administered I.P., at 200 µg, on days 3, 7, 11, and 15, with ten mice per treatment group.

[0300] Results/Conclusion

[0301] Mice treated with P9-18 or P9-21 demonstrated a complete regression of CT26 tumors, while mice treated with the IgG control demonstrated continued tumor growth. See **FIG. 1**. Mice treated with P9-18 or P9-21 demonstrated reduced B16.F0 tumor growth compared to mice

treated with IgG control. See **FIG. 2**. Thus, P9-18 or P9-21 can inhibit tumor growth in colon and melanoma tumor models, including complete regression in some cases.

6.11.11. Example 10: Treatment with Anti-GAL9 P9-15 Results in fewer Epstein-Barr virus (EBV)-induced tumors and Reduces Viral Load

[0302] This study was conducted to determine the effect of anti-GAL9 P9-15 candidate on Epstein-Barr virus (EBV)-induced tumors in a humanized mouse model.

[0303] Epstein-Barr Virus (EBV) is a γ -herpes virus that infects human B cells. However, many human viruses do not infect mice. Therefore, to test the effect of anti-GAL9 P9-15 on EBV-induced tumor, we used a humanized mouse engrafted with human CD34⁺ hematopoietic stem cells to make a mouse model reconstituted with human immune system cells.

[0304] Infection of Humanized Mice and Treatment

[0305] **FIG. 10A** shows a schematic of the overall treatment schedule used for the study. Briefly, immunodeficient mice were intravenously injected with CD34⁺ human stem cells and allowed to graft over the next 12 weeks. Humanized mice were then infected with EBV and incubated for 3 weeks to allow infection to occur. At the end of the infection period, the mice were treated with two dosages of anti-GAL9 P9-15 or IgG control on day 22 and day 26. Ten days post-treatment, living mice were euthanized and analyzed.

[0306] Generation of Humanized NRG Mice (hu-NRG)

[0307] Five female NRG (NOD-Rag1^{null} IL2rg^{null}, NOD rag gamma) were used for each treatment group. The Rag1^{null} mutation renders the mice B and T cell deficient and the IL2rg^{null} mutation prevents cytokine signaling through multiple receptors, leading to a deficiency in functional NK cells. NRG mice are therefore extremely immunodeficient, allowing for engraftment of human CD34⁺ hematopoietic stem cells.

[0308] The mice were irradiated twice, 3-4 hours apart, with 275cGy per dose (total of 550cGy), injected intravenously with 5×10^4 CD34⁺ human stem cells, and then allowed to engraft for three weeks to produce the humanized NRG ("hu-NRG") mice. The hu-NRG mice were weighed bi-weekly for 12 weeks to assess their health. In addition, tail bleeds were performed on week 4, week 8, and week 12 after administration of human CD34⁺ stem cells to monitor and confirm

stable engraftment in the mice by flow cytometric analysis for detection of human CD45⁺ cells including total mononuclear cells (CD45⁺), T cells (CD3⁺) and B cells (CD19⁺).

[0309] Spleen tumors

[0310] Following euthanasia, the spleens were excised and examined to determine the number of macroscopically visible tumors, cell number, and weight, except where the mice died or were euthanized for ethical reasons.

[0311] Assessment of EBV viral load

[0312] EBV loads in the spleen and blood were measured using real-time PCR.

[0313] Statistical Analyses

[0314] *Mann-Whitney U* test based on 2-sided tail was conducted using GraphPad Prism 7 Software (San Diego, CA).

[0315] Results/Conclusion

[0316] The spleens from mice treated with anti-GAL9 P9-15 mice showed fewer macroscopically visible tumors than spleens from mice treated with IgG control. See **FIG. 10B**. P9-15 treated mice had lighter spleen weight (average 0.100 g per spleen) compared to the IgG control treated mice (average 0.224 g per spleen, p -value < 0.0079), as well as significantly fewer spleen cells (22.14×10^6 in P9-15 treated mice compared to 51.04×10^6 in IgG treated control, p -value < 0.0159). See **FIGs. 10C-10D**. Data are shown as the mean; error bars are $\pm$ SEM.

[0317] In addition, treatment with P9-15 controlled the viral load by 88%. P9-15 treated mice had an average of 0.32×10^6 copies/ μ g of EBV, while the IgG treated mice had an average of 2×10^6 copies/ μ g of EBV (p -value < 0.0079). See **FIG. 10E**. Data are shown as the mean; error bars are $\pm$ SEM. These results demonstrate that treatment with P9-15 can reduce the development of EBV-induced tumors as well as control viral load.

6.11.12. Example 11: Treatment with Anti-GAL9 P9-28 Results in fewer Epstein-Barr virus (EBV)-induced tumors

[0318] This study was conducted to determine the effect of GAL9 P9-28 candidate on Epstein-Barr virus (EBV)-induced tumors in a humanized mouse model.

[0319] Animals, Infection of Humanized Mice and Treatment

[0320] This study was conducted as described in Example 10 above.

[0321] Results/Conclusion

[0322] Anti-GAL9 P9-28 treated mice showed no visible macroscopic tumors on the spleens compared to IgG control. See **FIG. 11**. The anti-GAL9 P9-23 treated mice were unlikely to have tumors within the spleen, as inferred from the small spleen size with low cell numbers. These results demonstrate that treatment with P9-28 can reduce EBV-induced tumor development.

6.11.13. Example 12: Anti-GAL9 silent Fc P9-18 (sFcP9-18) has an Antitumor Effect; sFcP9-18 and P9-18 can establish Antitumor Immune Memory

[0323] This study was conducted to test the contribution of the Fc region to the antitumor effect of the immune-activating anti-Gal9 antibodies. In addition, a re-challenge study was conducted to determine if P9-18 or sFcP9-18 can establish antitumor immune memory.

[0324] Antibodies

[0325] P9-18 antigen-binding sites were formatted on either a murine IgG1 backbone, murine IgG2a backbone, or on a murine IgG2a backbone with Fc receptor-binding null mutations (sFc). The silent Fc (sFc) P9-18 antibody was made by making key point mutations that abrogate binding of the Fc to Fc receptors.

[0326] CT26 cells

[0327] CT26 tumor cells were cultured in RPMI medium in a humidified incubator at 37 °C, in an atmosphere of 5% CO₂ and 95% air.

[0328] Mice and Treatment Schedule

[0329] Seven to ten mice were implanted subcutaneously with 1×10^5 CT26 tumor cells, and then treated with either control IgG (mouse IgG2a), P9-18-IgG1 (murine IgG1 backbone), FcR-silent sFcP9-18 (murine IgG2a backbone with Fc-receptor binding null mutations), or P9-18 (murine IgG2a backbone) I.P., at 200 µg on days 7, 11, 15, and 19.

[0330] Tumor Volume Growth

[0331] Mice were monitored for up to 143 days and tumors measured every 1-3 days by calipers. Tumor volume (mm^3) was calculated according to the formula: tumor length x tumor width x 2/2.

[0332] Complete Regression Response (CR)

[0333] Complete regression for the study was defined as a tumor volume 0 mm^3 for 20 consecutive measurements during the study. Animals were scored every 1-3 days during the study for a complete regression (CR) event.

[0334] Re-challenge of CT26 tumors

[0335] Tumor-free mice surviving the original initial tumor clearance study were allowed to rest for 65-70 days after tumors cleared. On day 107, the animals were re-implanted with 1×10^5 CT26 tumor cells with no additional treatment. New control mice were given a treatment with IgG2a control on day 113. Tumors were then allowed to grow for an additional 36 days. Tumor volume was determined as described above for days 107-143.

[0336] Results/Conclusion

[0337] The results from the tumor growth study are shown in **FIG. 12A**. In mice administered the IgG (IgG2a) control antibody (■), tumors reached 900-1000 mm^3 over the initial 50-day period. In contrast, treatment with P9-18-IgG2a (○) had 77% (7/9) CR, and treatment with sFcP9-18-IgG2a (▽) had 70% (7/10) CR. These results demonstrate that the Fc region of the P9-18 antibody is not required for its antitumor effect.

[0338] The P9-18 ABS reformatted into an IgG1 backbone (◇) did not inhibit tumor growth, showing similar tumor growth to the control.

[0339] The results from the re-challenge study are shown in **FIG. 12B**. Mice originally treated with P9-18-IgG2a had 100% (7/7) CR to new tumors, without additional treatment. Likewise, mice originally treated with sFcP9-18-IgG2a had 100% (7/7) CR to new tumors, without additional treatment. Treatment with the control IgG (IgG2a) antibody (◆) showed similar tumor growth as in the initial tumor clearance study. These data demonstrate that mice treated

with P9-18 or sFcP9-18 have established anti-tumor immune memory to CT26 tumor cells following initial treatment with P9-18-IgG2a or sFc9-18-IgG2a.

6.11.14. Example 13: Treatment with Anti-GAL9 P9-18 Increases PD-L2 Expression on Tumor-associated Dendritic Cells and Tumor Cells

[0340] Anti-GAL9 P9-18 was tested for its effect on PD-L1 and PD-L2 cell surface expression on tumor-associated dendritic cells and tumor cells.

[0341] Animals and Treatment

[0342] Three to five BALB/c mice were implanted subcutaneously with CT26 tumor cells and treated with P9-18 ABS formatted on a mouse IgG2a backbone or with mouse IgG2a control. All treatments were administered (I.P.), at 200 µg, on days 7 and 11.

[0343] Flow cytometry

[0344] Tumors were dissected on day 13, digested, and dissociated. Next, a CD45.1⁺ cell population, which includes immune and tumor cells, was isolated using anti-CD45.1 magnetic beads (Miltenyi Biotec, Germany). The CD45.1⁺ cell population was labelled and analyzed by flow cytometry for PD-L1 and PD-L2 cell surface expression on tumor-associated dendritic cells (CD11c⁺) and tumor cells. The reagents used are shown in **Table 13** below.

Table 13 Reagents			
Reagents	Fluorophore	Clone	Supplier
CD45.1	APC	A20	eBioscience
CD11c	BV421	N418	BD Horizon
PD-L1	BV605	10F.9G2	Biolegend
PD-L2*	AF488	TY25	BioXcell
CD45.1 Micro-Beads			Milteny Biotec
Lightning-Link Rapid DyLight 488 Labelling			Bionovus Life Sciences

*labelled using Lightning-Link Rapid DyLight 488 labelling kit.

[0345] Statistical Analyses

[0346] Unpaired t test with Welch's correction was conducted using GraphPad Prism 7 Software (San Diego, CA).

[0347] Results/Conclusion

[0348] FIG. 13 shows the mean percentage of PD-L1⁺ or PD-L2⁺ tumor-associated dendritic cells (CD11c⁺) and the mean cell surface expression level (GMI) of PD-L1 or PD-L2 on tumor-associated dendritic cells (CD11c⁺) after treatment with P9-18 (murine IgG2a backbone) or control. Treatment with P9-18 significantly increased the percentage of PDL2⁺ tumor-associated dendritic cells. The amount of PD-L1 and PD-L2 expression (GMI) was also significantly increased on tumor-associated dendritic cells compared to control. See **FIG. 13**. Data are shown as the mean; error bars are $\pm$ SEM.

[0349] FIG. 14 shows the mean percentage of PD-L1⁺ or PD-L2⁺ tumor cells and the mean cell surface expression level (GMI) of PD-L1 or PD-L2 on tumor cells after treatment with P9-18 (murine IgG2a backbone) or IgG control. Treatment with P9-18 significantly increased the amount (GMI) of PD-L2 cell surface expression on tumor cells but not PD-L1 cell surface expression. See **FIG. 14**. Data are shown as the mean; error bars are $\pm$ SEM. Without wishing to be bound by any theory, we hypothesize that PD-L2⁺ tumor cells may inhibit PD-L1 binding to PD-1 on tumors.

7. EQUIVALENTS

[0350] While various specific embodiments have been illustrated and described, the above specification is not restrictive. It will be appreciated that various changes can be made without departing from the spirit and scope of the invention(s). Many variations will become apparent to those skilled in the art upon review of this specification.

CLAIMS

What is claimed is:

1. A Galectin-9 (GAL9) antigen binding molecule, comprising: a first antigen binding site (ABS) specific for a first epitope of a first GAL9 antigen, wherein the first antigen binding site comprises all three VH CDRs from any one of the ABS clones selected from P9-28, P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.
2. A Galectin-9 (GAL9) antigen binding molecule, comprising a first antigen binding site (ABS) specific for a first epitope of a first GAL9 antigen, wherein the first antigen binding site comprises all three VL CDRs from any one of the ABS clones selected from P9-28, P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.
3. A Galectin-9 (GAL9) antigen binding molecule, comprising a first antigen binding site (ABS) specific for a first epitope of a first GAL9 antigen, wherein the first antigen binding site comprises all three VH CDRs and all three VL CDRs from any one of the ABS clones selected from P9-28, P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.
4. A Galectin-9 (GAL9) antigen binding molecule, comprising a first antigen binding site (ABS) specific for a first epitope of a first GAL9 antigen, wherein the first antigen binding site comprises the VL sequence and the VH sequence from any one of the ABS clones selected from P9-28, P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.
5. The GAL9 antigen binding molecule of claim 4, wherein the first antigen binding site (ABS) further comprises a first IgG heavy chain polypeptide and a first IgG light chain polypeptide.
6. The GAL9 antigen binding molecule of any one of claims 1-5, wherein the GAL9 antigen is a human GAL9 antigen.

7. The GAL9 antigen binding molecule of any of claims 1-6, wherein the GAL9 antigen binding molecule further comprises a second antigen binding site (ABS).
8. The GAL9 antigen binding molecule of claim 7, wherein the second ABS is specific for a GAL9 antigen.
9. The GAL9 antigen binding molecule of claim 7, wherein the second ABS is specific for a second epitope of the first GAL9 antigen.
10. The GAL9 antigen binding molecule of claim 7, wherein the second ABS is specific for the first epitope of the first GAL9 antigen and is identical to the first ABS.
11. The GAL9 antigen binding molecule of any one of claims 7-10, wherein the second ABS comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from another ABS clone selected from P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.
12. The GAL9 antigen binding molecule of claim 11, wherein the second antigen binding site comprises the VL sequence and the VH sequence from the other ABS clone.
13. The GAL9 antigen binding molecule of claim 12, wherein the second antigen binding site comprises a full immunoglobulin heavy chain sequence comprising the VH sequence and a full immunoglobulin light chain sequence comprising the VL sequence from the other ABS clone.
14. The GAL9 antigen binding molecule of claim 7, wherein the second antigen binding site is specific for an antigen other than the first GAL9 antigen.
15. The GAL9 antigen binding molecule of any one of the preceding claims, wherein the first antigen binding site comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from any one of the ABS clones selected from: P9-18, P9-15, P9-21, P9-22, P9-28, and P9-32.
16. The GAL9 antigen binding molecule of any of claims 1-14, wherein the first antigen binding site comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from any one of the ABS clones selected from: P9-18, P9-15, P9-21, and P9-28.

17. The GAL9 antigen binding molecule of any of claims 1-14, wherein the first antigen binding site comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from ABS clone P9-15.
18. The GAL9 antigen binding molecule of any of claims 1-14, wherein the first antigen binding site comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from ABS clone P9-18.
19. The GAL9 antigen binding molecule of any of claims 1-14, wherein the first antigen binding site comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from ABS clone P9-21.
20. The GAL9 antigen binding molecule of any of claims 1-14, wherein the first antigen binding site comprises all three VH CDRs, all three VL CDRs, or all three VH CDRs and all three VL CDRs from ABS clone P9-28.
21. The GAL9 antigen binding molecule of any of claims 1-20, wherein the GAL9 antigen binding molecule comprises an antibody format selected from the group consisting of: full-length antibodies, Fab fragments, Fvs, scFvs, tandem scFvs, Diabodies, scDiabodies, DARTs, tandAbs, minibodies, and B-bodies.
22. The GAL9 antigen binding molecule of any of claims 1-21, wherein the GAL9 antigen binding molecule increases TNF- α secretion by activated immune cells upon contact, wherein the increase is greater than an 80-fold increase relative to activated immune cells treated with a control agent.
23. The GAL9 antigen binding molecule of any of claims 1-22, wherein the GAL9 antigen binding molecule increases IFN- γ secretion by activated immune cells upon contact, wherein the increase is greater than a 1.2-fold increase relative to activated immune cells treated with a control agent.
24. The GAL9 antigen binding molecule of any of claims 1-23, wherein the GAL9 antigen binding molecule increases CD40L surface expression of activated CD8⁺ T-cells upon contact, wherein the increase is greater than a 2-fold increase relative to activated CD8⁺ T-cells treated with a control agent.

25. The GAL9 antigen binding molecule of any of claims 1-24, wherein the GAL9 antigen binding molecule increases OX40 surface expression of activated CD8⁺ T-cells upon contact, wherein the increase is greater than a 2-fold increase relative to activated CD8⁺ T-cells treated with a control agent.
26. The GAL9 antigen binding molecule of any of claims 1-25, wherein the GAL9 antigen binding molecule increases IL-12 production of activated dendritic cells (DCs) upon contact, wherein the increase is greater than an 20-fold increase relative to activated DCs treated with a control agent.
27. The GAL9 antigen binding molecule of any of claims 1-26, wherein the GAL9 antigen binding molecule increases PD-L2 surface expression on activated dendritic cells (DCs) upon contact, wherein the increase is greater than an 4-fold increase relative to activated DCs treated with a control agent.
28. The GAL9 antigen binding molecule of any of claims 22-27, wherein the control agent is a negative control agent or positive control agent.
29. The GAL9 antigen binding molecule of claim 28, wherein the control agent is a control antibody.
30. The GAL9 antigen binding molecule of claim 29, wherein the control antibody is selected from the group consisting of: an ECA42 clone anti-GAL9 antibody, an RG9.1 clone anti-GAL9 antibody, an RG9.35 clone anti-GAL9 antibody, an anti-PD1 antibody, and a non-GAL9 binding isotype control antibody.
31. The GAL9 antigen binding molecule of any one of claims 22-30, wherein the activated immune cells, activated CD8⁺ T-cells, or activated DCs were activated by peptide stimulation, e.g., by a peptide or plurality of peptides known to induce an immune response.
32. A GAL9 antigen binding molecule, wherein the GAL9 antigen binding molecule increases TNF- α secretion by activated immune cells, wherein the increase is greater than an 80-fold increase relative to activated immune cells treated with a control agent.

33. A GAL9 antigen binding molecule, wherein the GAL9 antigen binding molecule increases IFN γ secretion by activated immune cells, wherein the increase is greater than an 1.2-fold increase relative to activated immune cells treated with a control agent.
34. A GAL9 antigen binding molecule, wherein the GAL9 antigen binding molecule increases CD40L surface expression of Activated CD8 $^{+}$ T-cells, wherein the increase is greater than a 2-fold increase relative to activated CD8 $^{+}$ T-cells treated with a control agent.
35. A GAL9 antigen binding molecule, wherein the GAL9 antigen binding molecule increases OX40 surface expression of Activated CD8 $^{+}$ T-cells, wherein the increase is greater than a 2-fold increase relative to activated CD8 $^{+}$ T-cells treated with a control agent.
36. A GAL9 antigen binding molecule, wherein the GAL9 antigen binding molecule increases IL-12 production of activated dendritic cells (DCs), wherein the increase is greater than an 20-fold increase relative to activated DCs treated with a control agent.
37. A GAL9 antigen binding molecule, wherein the GAL9 antigen binding molecule increases PD-L2 surface expression on activated dendritic cells (DCs), wherein the increase is greater than an 4-fold increase relative to activated DCs treated with a control agent.
38. A GAL9 antigen binding molecule, wherein the GAL9 antigen binding molecule demonstrates one or more of the following properties:
 - A) increases TNF- α secretion by activated immune cells, wherein the increase is greater than an 80-fold increase relative to activated immune cells treated with a control agent,
 - B) increases IFN- γ secretion by activated immune cells, wherein the increase is greater than an 1.2-fold increase relative to activated immune cells treated with a control agent,
 - C) increases CD40L surface expression of activated CD8 $^{+}$ T-cells, wherein the increase is greater than a 2-fold increase relative to activated CD8 $^{+}$ T-cells treated with a control agent,

D) increases OX40 surface expression of activated CD8⁺ T-cells, wherein the increase is greater than a 2-fold increase relative to activated CD8⁺ T-cells treated with a control agent,

E) increases IL-12 production of activated dendritic cells (DCs), wherein the increase is greater than an 20-fold increase relative to activated DCs treated with a control agent,

F) increases PD-L2 surface expression on activated dendritic cells (DCs), wherein the increase is greater than an 4-fold increase relative to activated DCs treated with a control agent.

39. The GAL9 antigen binding molecule of any one of claims 32-38, wherein the control agent is a negative control agent or positive control agent.
40. The GAL9 antigen binding molecule of claim 39, wherein the control agent is a control antibody.
41. The GAL9 antigen binding molecule of claim 40, wherein the control antibody is selected from the group consisting of: an ECA42 clone anti-GAL9 antibody, an RG9.1 clone anti-GAL9 antibody, an RG9.35 clone anti-GAL9 antibody, an anti-PD1 antibody, and a non-GAL9 binding isotype control antibody.
42. The GAL9 antigen binding molecule of any one of claims 32-41, wherein the activated immune cells, activated CD8⁺ T-cells, or activated DCs were activated by peptide stimulation, *e.g.*, by a peptide or plurality of peptides known to induce an immune response.
43. The GAL9 antigen binding molecule of any of claims 32-39, comprising a first antigen binding site specific for a first epitope of a first GAL9 antigen, wherein the first antigen binding site comprises all three VH CDRs and all three VL CDRs from any one of the ABS clones selected from, P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.
44. The GAL9 antigen binding molecule of claim 43, comprising the VL sequence and the VH sequence from any one of the ABS clones selected from P9-02B, P9-04, P9-05, P9-

08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.

45. The GAL9 antigen binding molecule of claim 44, comprising a full immunoglobulin heavy chain sequence comprising the VH sequence and a full immunoglobulin light chain sequence comprising the VL sequence, wherein the VH sequence and the VL sequence are from any one of the ABS clones selected from, P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.
46. The GAL9 antigen binding molecule of any one of claims 32-45, wherein the GAL9 antigen is a human GAL9 antigen.
47. The GAL9 antigen binding molecule of any of claims 32-46, wherein the GAL9 antigen binding molecule further comprises a second antigen binding site.
48. The GAL9 antigen binding molecule of claim 47, wherein the second antigen binding site is specific for the GAL9 antigen.
49. The GAL9 antigen binding molecule of claim 48, wherein the second antigen binding site is identical to the first antigen binding site.
50. The GAL9 antigen binding molecule of claim 47, wherein the second antigen binding site is specific for a second epitope of the first GAL9 antigen.
51. The GAL9 antigen binding molecule of claim 50, wherein the second antigen binding site comprises all three VH CDRs and all three VL CDRs from another ABS clone selected from, P9-02B, P9-04, P9-05, P9-08, P9-09, P9-10, P9-15, P9-16, P9-18, P9-19, P9-20, P9-21, P9-22, P9-27, P9-28, P9-31, P9-32, P9-36, P9-39, P9-49, P9-54, and P9-58.
52. The GAL9 antigen binding molecule of claim 51, wherein the second antigen binding site comprises the VL sequence and the VH sequence from the other ABS clone.
53. The GAL9 antigen binding molecule of claim 52, wherein the second antigen binding site comprises a full immunoglobulin heavy chain sequence comprising the VH sequence and a full immunoglobulin light chain sequence comprising the VL sequence from the other ABS clone.

54. The GAL9 antigen binding molecule of claim 47, wherein the second antigen binding site is specific for an antigen other than the first GAL9 antigen.
55. The GAL9 antigen binding molecule of any of claims 43-54, wherein the first antigen binding site comprises all three VH CDRs and all three VL CDRs from any one of the ABS clones selected from: P9-15, P9-18, P9-21, P9-22, P9-28, and P9-32.
56. The GAL9 antigen binding molecule of any of claims 43-54, wherein the first antigen binding site comprises all three VH CDRs and all three VL CDRs from any one of the ABS clones selected from: P9-15, P9-18, P9-21, and P9-28.
57. The GAL9 antigen binding molecule of any of claims 43-54, wherein the first antigen binding site comprises all three VH CDRs and all three VL CDRs from ABS clone P9-15.
58. The GAL9 antigen binding molecule of any of claims 43-54, wherein the first antigen binding site comprises all three VH CDRs and all three VL CDRs from ABS clone P9-18.
59. The GAL9 antigen binding molecule of any of claims 43-54, wherein the first antigen binding site comprises all three VH CDRs and all three VL CDRs from ABS clone P9-21.
60. The GAL9 antigen binding molecule of any of claims 43-54, wherein the first antigen binding site comprises all three VH CDRs and all three VL CDRs from ABS clone P9-28.
61. The GAL9 antigen binding molecule of any of claims 32-60, wherein the GAL9 antigen binding molecule comprises an antibody format selected from the group consisting of: full-length antibodies, Fab fragments, Fvs, scFvs, tandem scFvs, Diabodies, scDiabodies, DARTs, tandAbs, minibodies, and B-bodies.
62. A GAL9 antigen binding molecule which binds to the same epitope as a GAL9 antigen binding molecule of any one of the preceding claims.
63. A GAL9 antigen binding molecule which competes for binding with a GAL9 antigen binding molecule of any one of the preceding claims.

64. The GAL9 antigen binding molecule of any one of the preceding claims, which is purified.
65. A pharmaceutical composition comprising the GAL9 antigen binding molecule of any one of the preceding claims and a pharmaceutically acceptable diluent.
66. A method for treating a subject with cancer, the method comprising administering a therapeutically effective amount of the pharmaceutical composition of claim 65 to the subject.
67. The method of claim 66, wherein the cancer is selected from the group consisting of: pancreatic cancer, ovarian cancer, breast cancer, lung cancer, gastric cancer, melanoma, Ewing sarcoma, chronic lymphocytic leukemia, mantle cell lymphoma, B-ALL, hematological cancer, head and neck squamous cell carcinoma, prostate cancer, colon cancer, renal cancer, uterine cancer, and viral-induced cancer.

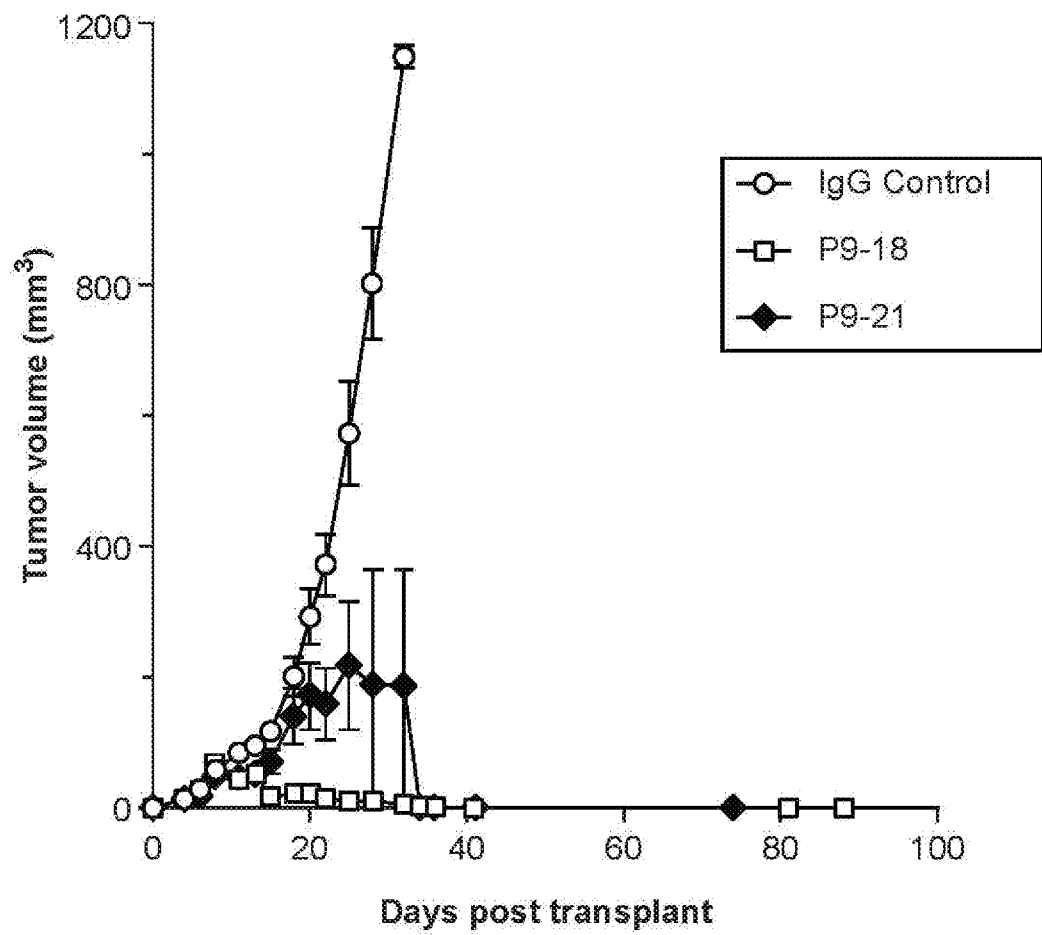


FIG. 1

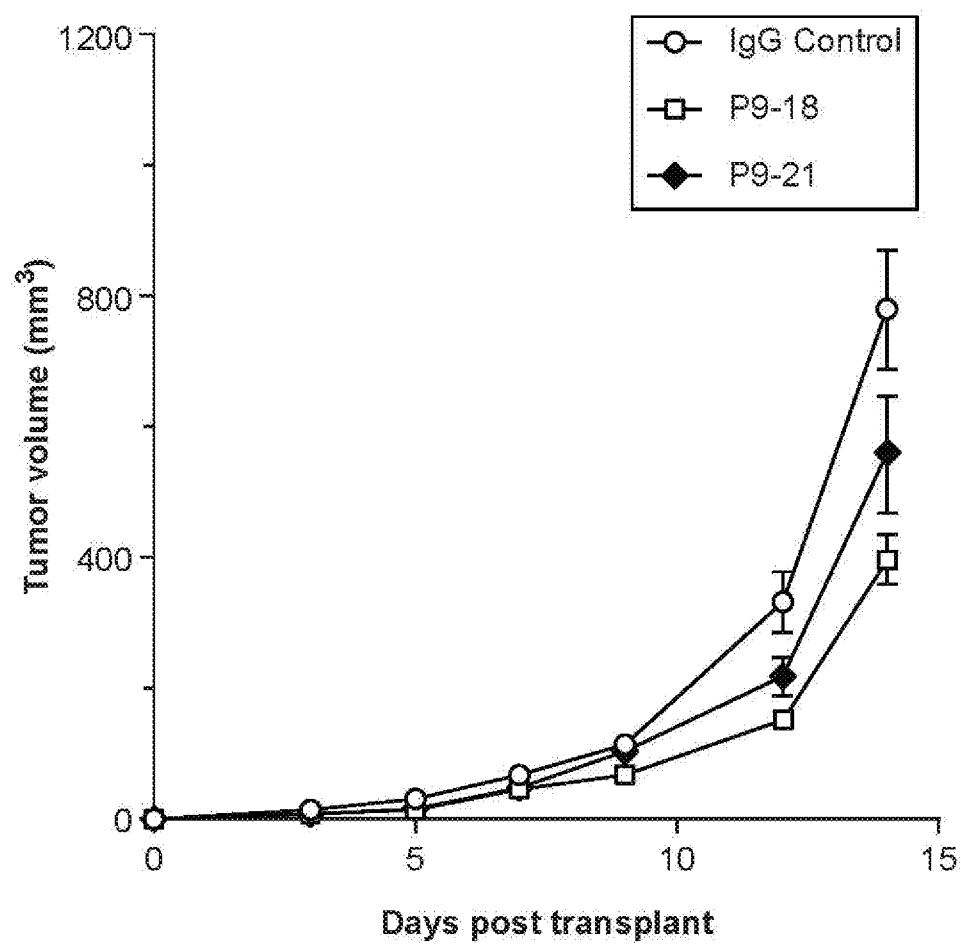


FIG. 2

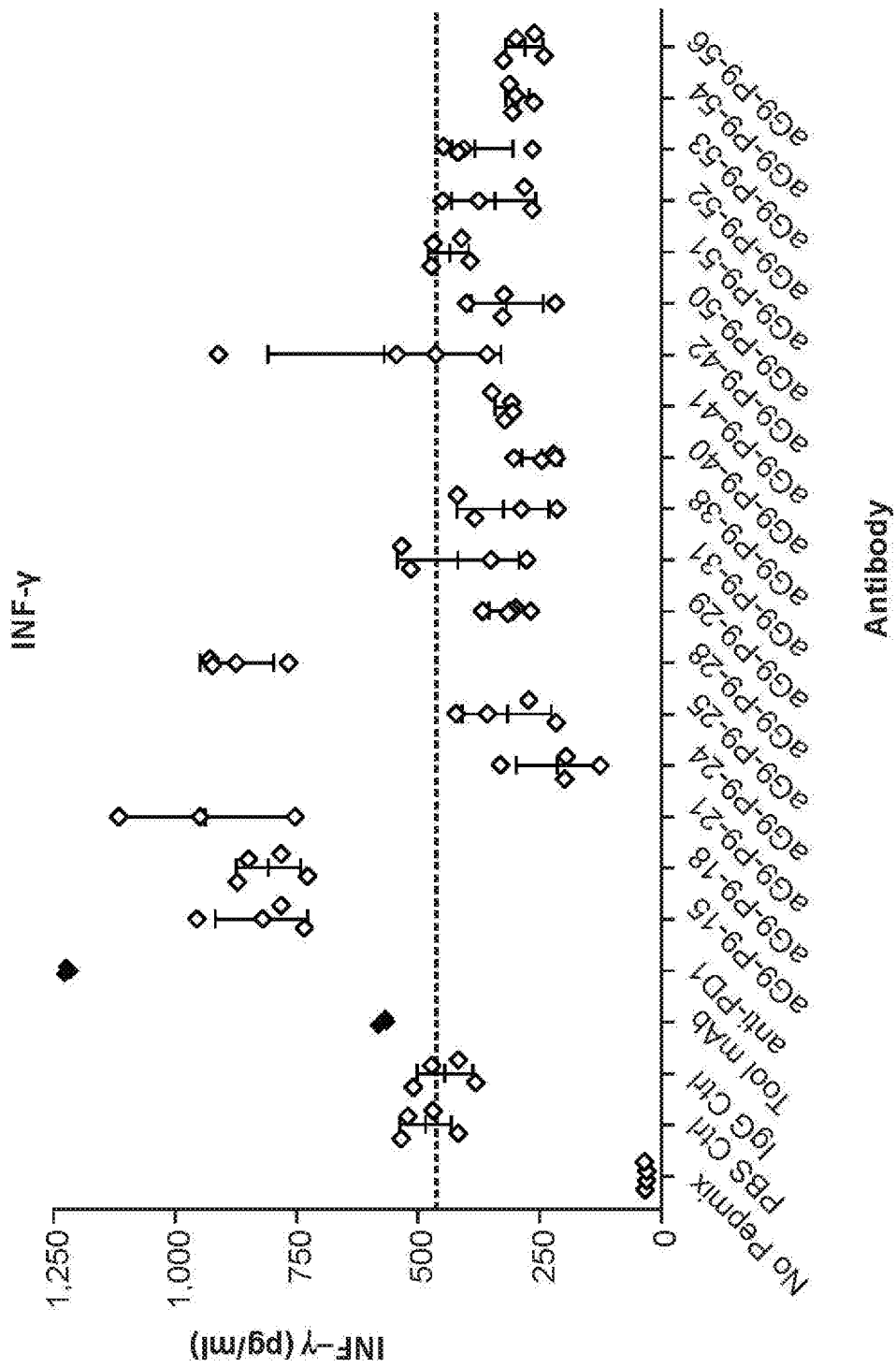


FIG. 3A

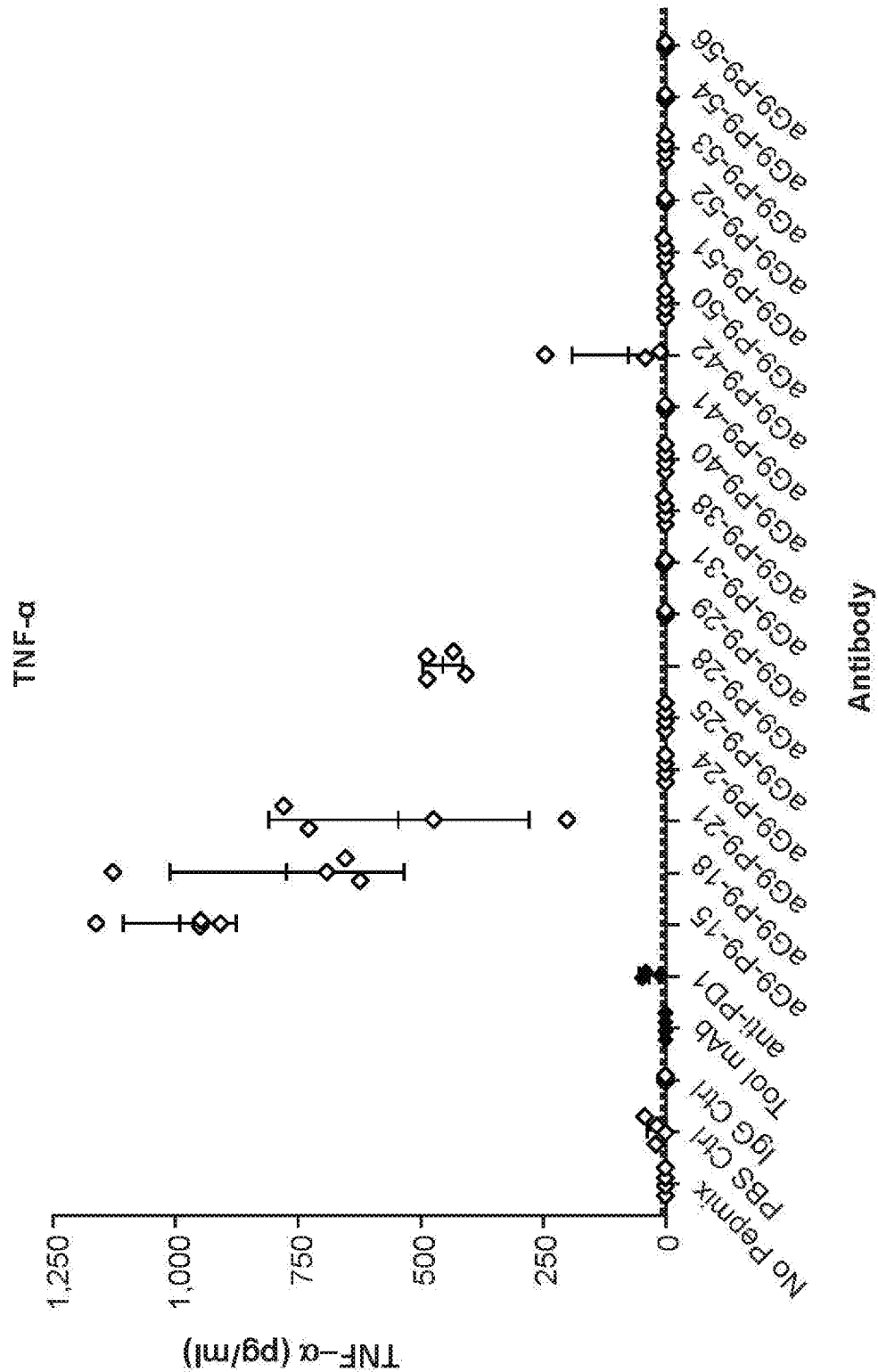


FIG. 3B

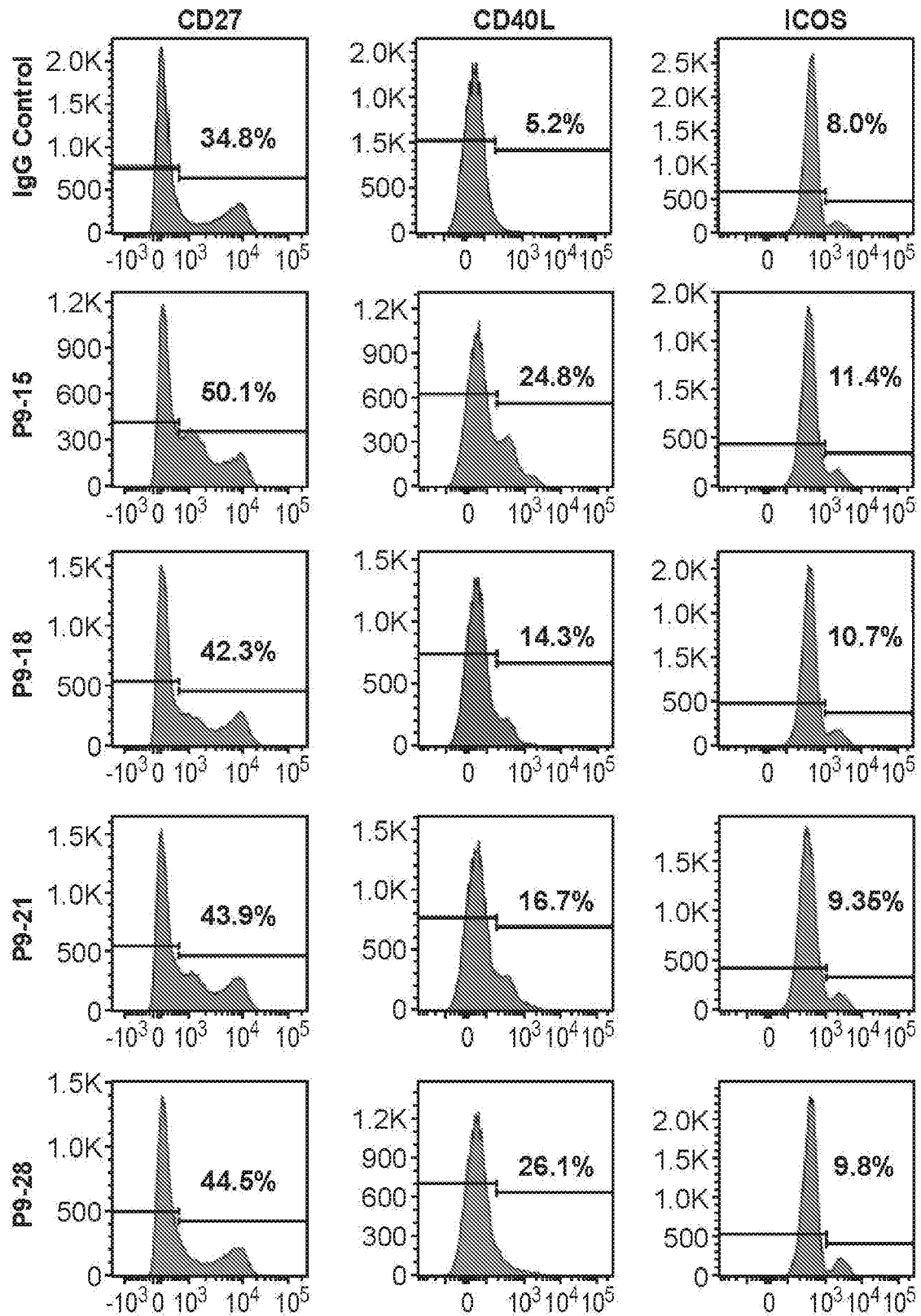


FIG. 4

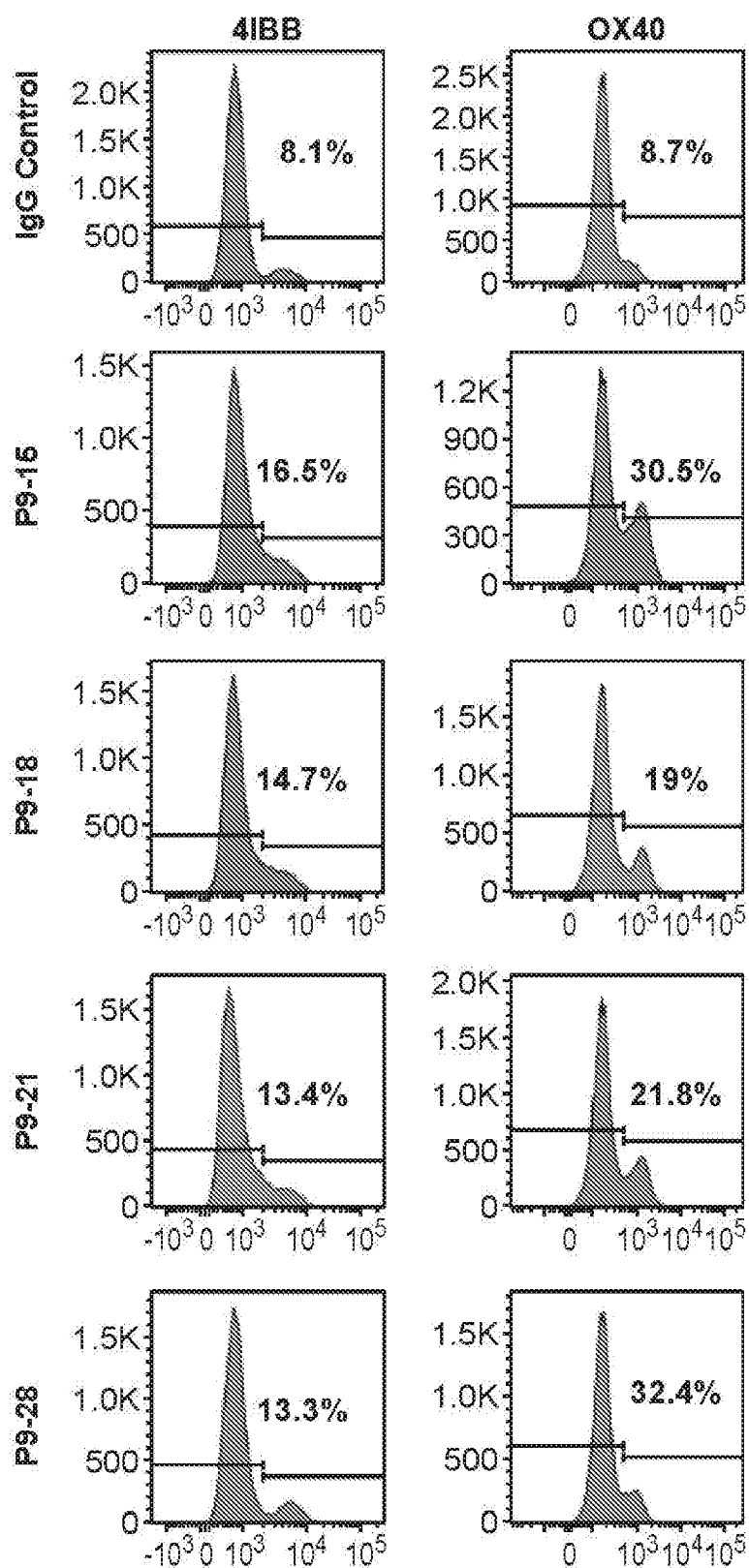


FIG. 4 (Cont.)

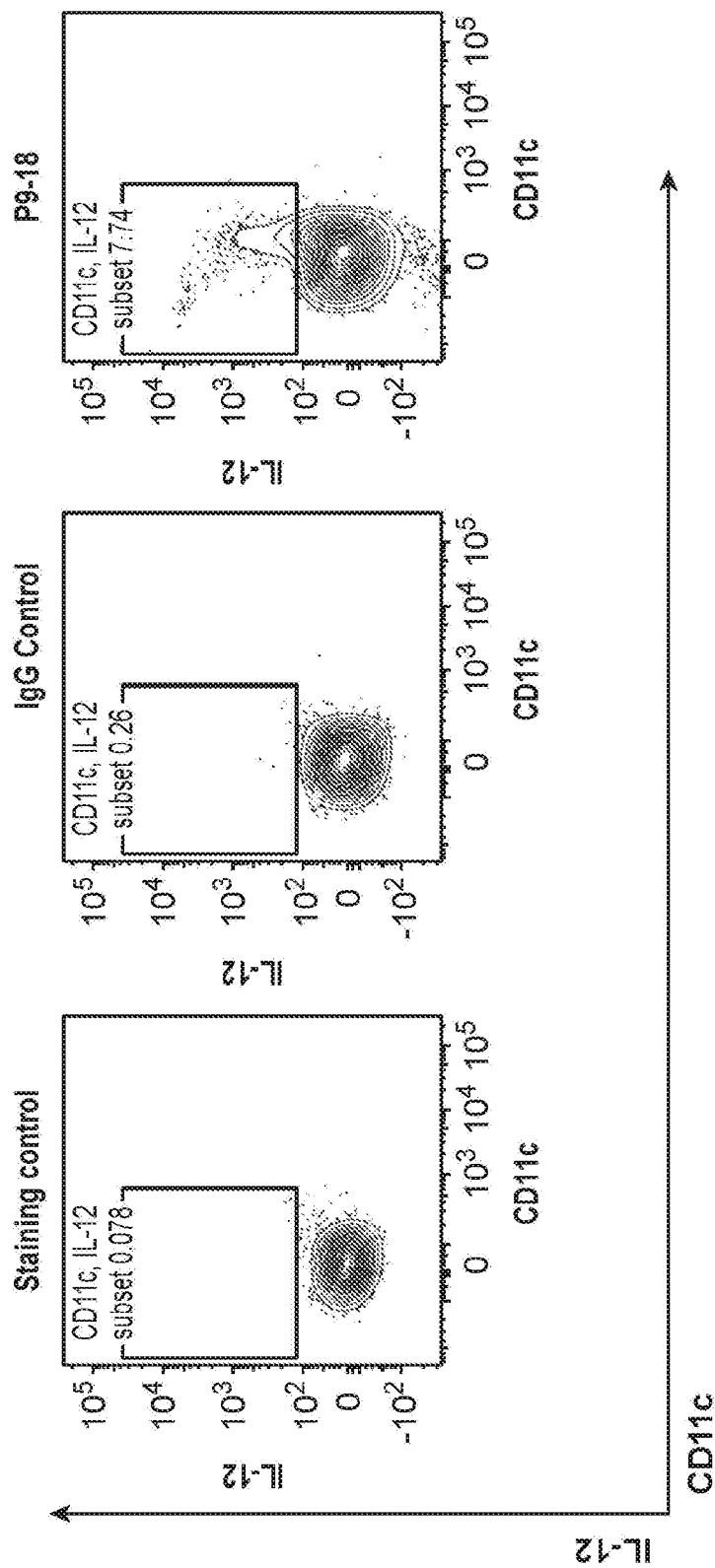


FIG. 5

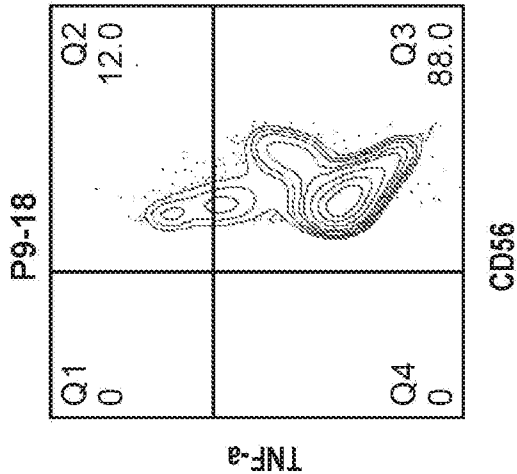
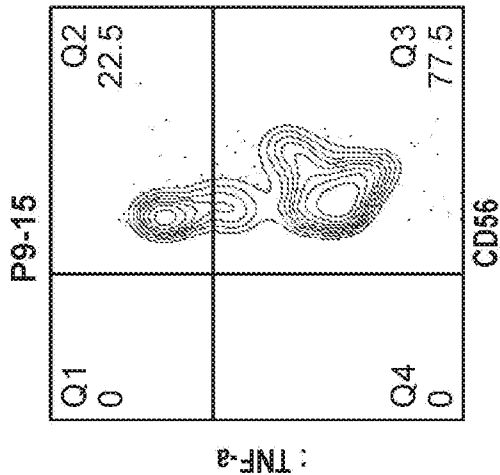
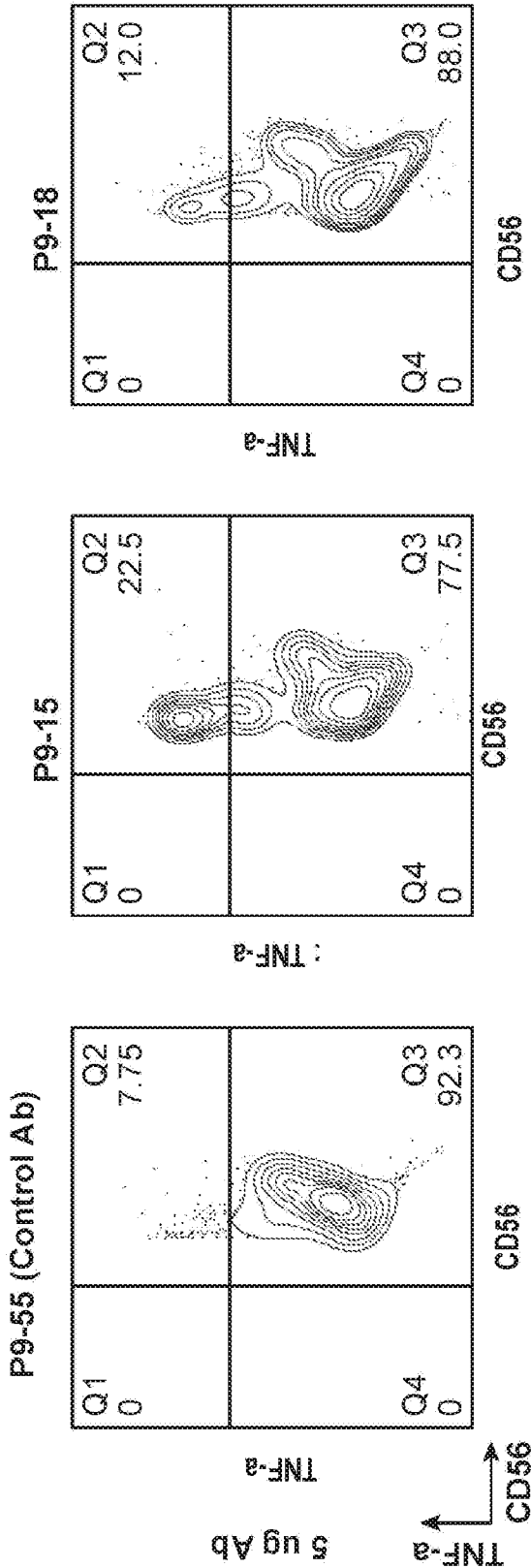


FIG. 6A

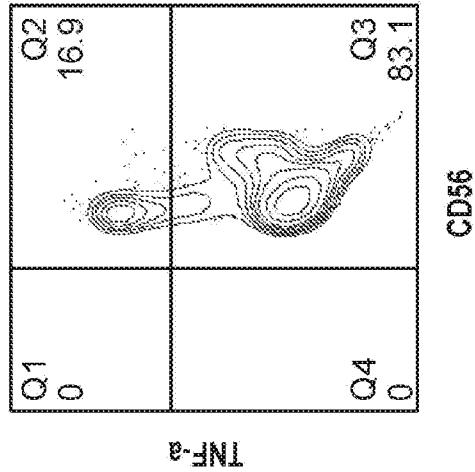
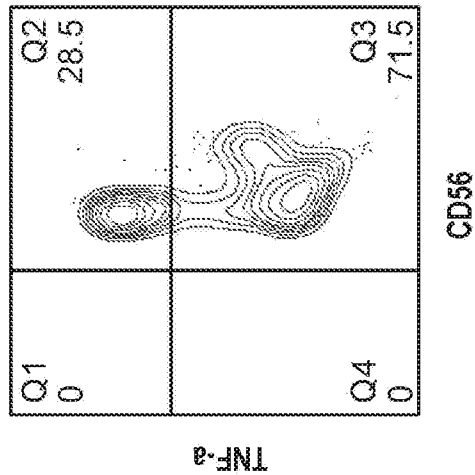
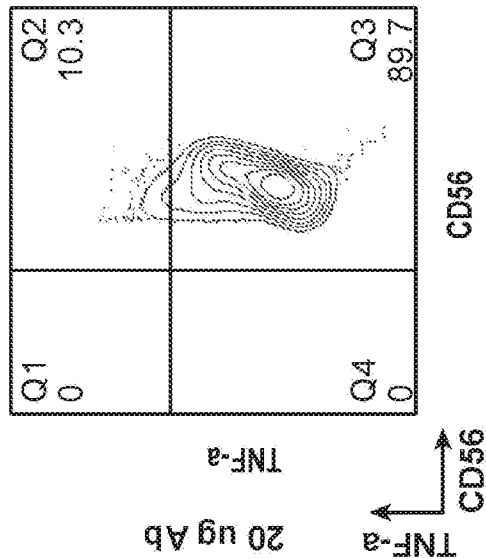


FIG. 6B

P9-28 IgG Heavy Chain – Martin Numbering / Chothia CDRs

	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	F	T	F	G	F	Y	Y	I
Martin	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23	H24	H25	H26	H27	H28	H29	H30	H31	H32	H33	H34
Frequency	39%	85%	79%	95%	33%	57%	84%	95%	40%	52%	71%	71%	31%	95%	79%	31%	79%	63%	35%	73%	74%	100%	36%	63%	94%	92%	45%	56%	70%	3%	<1%	73%	22%	19%
Chothia	HFR1																CDR-H1																HFR2	

↑

↓

	H	W	V	R	Q	A	P	G	K	G	L	E	W	V	A	F	I	D	P	H	G	G	S	T	Y	Y	A	D	S	V	K	G	R	F
Martin	H35	H36	H37	H38	H39	H40	H41	H42	H43	H44	H45	H46	H47	H48	H49	H50	H51	H52	H52A	H53	H54	H55	H56	H57	H58	H59	H60	H61	H62	H63	H64	H65	H66	H67
Frequency	31%	100%	75%	81%	97%	50%	92%	91%	63%	86%	96%	96%	94%	36%	20%	2%	85%	13%	42%	4%	35%	80%	27%	64%	32%	93%	45%	32%	55%	39%	71%	62%	76%	41%
Chothia	HFR2																CDR-H2																HFR3	

⊞

⊞

	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	Y	C	A	R	L	S	Y	P
Martin	H68	H69	H70	H71	H72	H72A	H72B	H72C	H73	H74	H75	H76	H77	H78	H79	H80	H81	H82	H83	H84	H85	H86	H87	H88	H89	H90	H91	H92	H93	H94	H95	H96	H97	H98
Frequency	85%	65%	59%	12%	93%	32%	71%	50%	53%	63%	34%	67%	68%	58%	42%	39%	83%	80%	39%	38%	67%	99%	78%	97%	68%	99%	86%	100%	85%	68%	4%	7%	17%	3%
Chothia	HFR3																CDR-H3																CDR-H3	

↓

↑

	G	V	L	D	Y	W	G	Q	G	T	L	V	T	V	S	S
Martin	H99	H100	H100A	H101	H102	H103	H104	H105	H106	H107	H108	H109	H110	H111	H112	H113
Frequency	16%	5%	5%	77%	49%	99%	99%	78%	100%	89%	48%	83%	91%	97%	95%	89%
Chothia	CDR-H3								HFR4							

FIG. 7A

P9-28 IgG Light Chain - Martin Numbering / Chothia CDRs

	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	S	V	S	S	A	V	A
Martin	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	L25	L26	L27	L28	L29	L30	L31	L32	L33	L34
Frequency	36%	45%	15%	34%	94%	98%	78%	89%	28%	70%	50%	80%	39%	66%	21%	98%	24%	43%	63%	71%	71%	41%	99%	40%	43%	79%	47%	60%	24%	29%	24%	3%	29%	29%
Chothia	LFR1										CDR-L1										CDR-L1													

C										H										S														
	W	Y	Q	Q	K	P	G	K	A	P	K	L	L	I	Y	S	A	S	S	L	Y	S	G	V	P	S	R	F	S	G	S	R	S	G
Martin	L35	L36	L37	L38	L39	L40	L41	L42	L43	L44	L45	L46	L47	L48	L49	L50	L51	L52	L53	L54	L55	L56	L57	L58	L59	L60	L61	L62	L63	L64	L65	L66	L67	L68
Frequency	100%	88%	89%	90%	67%	87%	92%	23%	57%	95%	57%	75%	78%	89%	85%	7%	40%	67%	15%	32%	2%	75%	95%	64%	93%	26%	99%	99%	89%	97%	96%	3%	92%	93%
Chothia	LFR2											CDR-L2										LFR3												

	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	Q	G	Y	S	T	L	V	T	F	G	Q	G	T
Martin	L69	L70	L71	L72	L73	L74	L75	L76	L77	L78	L79	L80	L81	L82	L83	L84	L85	L86	L87	L88	L89	L90	L91	L92	L93	L94	L95	L96	L97	L98	L99	L100	L101	L102
Frequency	71%	43%	50%	66%	96%	71%	97%	71%	34%	59%	60%	28%	78%	99%	26%	85%	26%	99%	85%	100%	52%	50%	8%	17%	43%	11%	17%	7%	54%	99%	22%	99%	98%	
Chothia	LFR3											CDR-L3										LFR4												

	K	V	E	I	K	R	T	V
Martin	L103	L104	L105	L106	L107	L108	L109	L110
Frequency	80%	38%	51%	49%	69%	60%	24%	52%
Chothia	LFR4							

FIG. 7A (Cont.)

P9-28 IgG Light Chain - Martin Numbering / Kabat CDRs

	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	S	V	S	S	A	V	A
Martin	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	L25	L26	L27	L28	L29	L30	L31	L32	L33	L34
Frequency	36%	45%	15%	34%	94%	98%	78%	89%	28%	70%	50%	80%	39%	66%	21%	98%	24%	43%	63%	71%	71%	41%	99%	40%	43%	79%	47%	60%	24%	29%	3%	29%	29%	
Kabat	LFR1														CDR-L1																			

CDR-L2																																LFR2						LFR3					
	W	Y	Q	Q	K	P	G	K	A	P	K	L	L	I	Y	S	A	S	S	L	Y	S	G	V	P	S	R	F	S	G	S	R	S	G									
Martin	L35	L36	L37	L38	L39	L40	L41	L42	L43	L44	L45	L46	L47	L48	L49	L50	L51	L52	L53	L54	L55	L56	L57	L58	L59	L60	L61	L62	L63	L64	L65	L66	L67	L68									
Frequency	100%	86%	89%	90%	67%	87%	92%	23%	57%	95%	57%	75%	76%	89%	85%	7%	40%	67%	15%	32%	2%	75%	95%	64%	93%	26%	99%	99%	89%	97%	96%	3%	92%	95%									
Kabat	LFR2														CDR-L2														LFR3														

	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	Q	G	Y	S	T	L	V	T	F	G	Q	G	T
Martin	L69	L70	L71	L72	L73	L74	L75	L76	L77	L78	L79	L80	L81	L82	L83	L84	L85	L86	L87	L88	L89	L90	L91	L92	L93	L94	L95	L96	L97	L98	L99	L100	L101	L102
Frequency	71%	43%	50%	66%	96%	71%	97%	71%	34%	59%	60%	28%	78%	99%	26%	85%	26%	99%	85%	100%	52%	50%	8%	17%	43%	11%	17%	7%	54%	99%	22%	99%	98%	
Kabat	LFR3														CDR-L3														LFR4					

	K	V	E	I	K	R	T	V
Martin	L103	L104	L105	L106	L107	L108	L109	L110
Frequency	80%	38%	51%	48%	69%	60%	24%	52%
Kabat	LFR4							

FIG. 7B (Cont.)

P9-28 IgG Heavy Chain – Martin Numbering / AbM CDRs

	E	V	Q	L	V	E	S	G	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	F	T	F	G	F	Y	Y	I
Martin	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23	H24	H25	H26	H27	H28	H29	H30	H31	H32	H33	H34	
Frequency	39%	85%	79%	95%	38%	57%	84%	95%	40%	52%	71%	71%	31%	95%	79%	31%	79%	63%	35%	73%	74%	100%	36%	63%	94%	92%	45%	56%	70%	3%	<1%	73%	22%	19%	
AbM	HFR1																CDR-H1												CDR-H1						
	H	W	V	R	Q	A	P	G	K	G	L	E	W	V	A	F	I	D	P	H	G	G	S	T	Y	Y	A	D	S	V	K	G	R	F	
Martin	H35	H36	H37	H38	H39	H40	H41	H42	H43	H44	H45	H46	H47	H48	H49	H50	H51	H52	H52A	H53	H54	H55	H56	H57	H58	H59	H60	H61	H62	H63	H64	H65	H66	H67	
Frequency	31%	100%	75%	81%	97%	50%	92%	91%	63%	88%	96%	96%	94%	36%	20%	2%	85%	13%	42%	4%	35%	60%	27%	64%	32%	93%	45%	32%	55%	39%	71%	62%	76%	41%	
AbM	HFR2																CDR-H2												HFR3						
	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	Y	C	A	R	L	S	Y	P	
Martin	H68	H69	H70	H71	H72	H72A	H72B	H72C	H73	H74	H75	H76	H77	H78	H79	H80	H81	H82	H83	H84	H85	H86	H87	H88	H89	H90	H91	H92	H93	H94	H95	H96	H97	H98	
Frequency	85%	65%	59%	12%	93%	32%	71%	50%	53%	63%	34%	67%	69%	58%	42%	39%	83%	80%	39%	38%	67%	99%	78%	97%	68%	99%	86%	100%	85%	68%	4%	7%	17%	3%	
AbM	HFR3																CDR-H3												CDR-H3						
	G	V	L	D	Y	W	G	Q	G	T	L	V	T	V	S	S	HFR4												CDR-H3						
Martin	H99	H100	H100A	H101	H102	H103	H104	H105	H106	H107	H108	H109	H110	H111	H112	H113																			
Frequency	16%	5%	5%	77%	49%	99%	99%	78%	100%	89%	48%	83%	91%	97%	95%	89%																			
AbM	CDR-H3																HFR4																		

FIG. 7C

P9-28 IgG Light Chain - Martin Numbering / AbM CDRs

	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	S	V	S	A	V	A	
Martin	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	L25	L26	L27	L28	L29	L30	L31	L32	L33	L34
Frequency	36%	45%	15%	34%	94%	98%	78%	89%	28%	70%	50%	80%	39%	66%	21%	88%	24%	43%	63%	71%	71%	41%	99%	40%	43%	79%	47%	60%	24%	28%	24%	3%	29%	29%
AbM	LFR1																CDR-L1																	

C										S										A																
	W	Y	Q	Q	K	P	G	K	A	P	K	L	L	L	I	Y	S	A	S	S	L	Y	S	G	V	P	S	R	F	S	G	S	R	S	G	
Martin	L35	L36	L37	L38	L39	L40	L41	L42	L43	L44	L45	L46	L47	L48	L49	L50	L51	L52	L53	L54	L55	L56	L57	L58	L59	L60	L61	L62	L63	L64	L65	L66	L67	L68		
Frequency	100%	86%	89%	90%	67%	87%	92%	23%	57%	95%	57%	75%	76%	89%	85%	7%	40%	67%	15%	32%	2%	75%	95%	64%	93%	26%	99%	99%	89%	97%	96%	3%	92%	93%		
AbM	LFR2																CDR-L2														LFR3					

		T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	Q	G	G	Y	S	T	L	V	T	F	G	Q	G	T	
	Martin	L69	L70	L71	L72	L73	L74	L75	L76	L77	L78	L79	L80	L81	L82	L83	L84	L85	L86	L87	L88	L89	L90	L91	L92	L93	L94	L95	L96	L97	L98	L99	L100	L101	L102		
	Frequency	71%	43%	50%	66%	90%	71%	97%	71%	34%	59%	60%	28%	78%	99%	20%	85%	26%	99%	85%	100%	52%	50%	8%	17%	43%	11%	17%	7%	54%	99%	99%	22%	99%	98%		
	AbM	LFR3																CDR-L3														LFR4					

	K	V	E	I	K	R	T	V
Martin	L103	L104	L105	L106	L107	L108	L109	L110
Frequency	80%	38%	51%	48%	69%	60%	24%	52%
AbM	LFR4							

FIG. 7C (Cont.)

P9-28 IgG Heavy Chain – Martin Numbering / Contact CDRs

	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	F	T	F	G	F	Y	Y	I
Martin	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23	H24	H25	H26	H27	H28	H29	H30	H31	H32	H33	H34
Frequency	39%	85%	79%	95%	38%	57%	84%	95%	40%	52%	71%	71%	31%	95%	79%	31%	79%	63%	35%	73%	74%	100%	36%	63%	94%	92%	45%	56%	70%	3%	<1%	73%	22%	19%
Contact	CDR-H1																																	

	H	W	V	R	Q	A	P	G	K	G	L	E	W	V	A	F	I	D	P	H	G	G	S	T	Y	Y	A	D	S	V	K	G	R	F
Martin	H35	H36	H37	H38	H39	H40	H41	H42	H43	H44	H45	H46	H47	H48	H49	H50	H51	H52	H52A	H53	H54	H55	H56	H57	H58	H59	H60	H61	H62	H63	H64	H65	H66	H67
Frequency	31%	100%	75%	81%	97%	50%	92%	91%	63%	88%	96%	96%	94%	35%	20%	2%	85%	13%	42%	4%	35%	60%	27%	64%	32%	93%	45%	32%	55%	39%	71%	62%	76%	41%
Contact	CDR-H2																																	

CDR-H1		CDR-H3																												CDR-H3				
	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	Y	C	A	R	L	S	Y	P
Martin	H68	H69	H70	H71	H72	H72A	H72B	H72C	H73	H74	H75	H76	H77	H78	H79	H80	H81	H82	H83	H84	H85	H86	H87	H88	H89	H90	H91	H92	H93	H94	H95	H96	H97	H98
Frequency	85%	85%	59%	12%	93%	32%	71%	50%	53%	63%	34%	67%	69%	58%	42%	39%	83%	80%	39%	38%	67%	99%	78%	97%	68%	99%	86%	100%	85%	68%	4%	7%	17%	3%
Contact	HFR3																												CDR-H3					

	G	V	L	D	Y	W	G	Q	G	T	L	V	T	V	S	S
Martin	H99	H100	H100A	H101	H102	H103	H104	H105	H106	H107	H108	H109	H110	H111	H112	H113
Frequency	16%	5%	5%	77%	49%	99%	99%	98%	78%	100%	89%	48%	83%	91%	97%	89%
Contact	CDR-H3															

FIG. 7D

P9-28 IgG Light Chain – Martin Numbering / Contact CDRs

	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q	S	V	S	A	V	A					
Martin	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	L25	L26	L27	L28	L29	L30	L31	L32	L33	L34				
Frequency	36%	45%	15%	34%	94%	98%	78%	89%	28%	70%	50%	80%	39%	66%	21%	98%	24%	43%	63%	71%	71%	41%	99%	40%	43%	79%	47%	60%	24%	29%	24%	3%	29%	29%				
Contact	LFR1																														CDR-L1							

C										S										P															
	W	Y	Q	Q	K	P	G	K	A	P	K	L	L	L	I	Y	S	A	S	S	L	Y	S	G	V	P	S	R	F	S	G	S	R	S	G
Martin	L35	L36	L37	L38	L39	L40	L41	L42	L43	L44	L45	L46	L47	L48	L49	L50	L51	L52	L53	L54	L55	L56	L57	L58	L59	L60	L61	L62	L63	L64	L65	L66	L67	L68	
Frequency	100%	88%	89%	90%	67%	87%	92%	23%	57%	95%	57%	75%	76%	88%	85%	7%	40%	67%	15%	32%	2%	75%	95%	64%	93%	26%	99%	99%	89%	97%	96%	3%	92%	93%	
Contact	LFR2														CDR-L2														LFR3						

★																																		
	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	Q	G	Y	S	T	L	V	T	F	G	Q	G	T
Martin	L69	L70	L71	L72	L73	L74	L75	L76	L77	L78	L79	L80	L81	L82	L83	L84	L85	L86	L87	L88	L89	L90	L91	L92	L93	L94	L95	L96	L97	L98	L99	L100	L101	L102
Frequency	71%	43%	50%	66%	96%	71%	97%	71%	34%	59%	60%	28%	78%	99%	26%	85%	26%	99%	85%	100%	52%	50%	8%	17%	43%	11%	17%	7%	54%	99%	99%	22%	99%	98%
Contact	LFR3														CDR-L3														LFR4					

★

	K	V	E	I	K	R	T	V
Martin	L103	L104	L105	L106	L107	L108	L109	L110
Frequency	80%	36%	51%	48%	69%	60%	24%	52%
Contact	LFR4							

FIG. 7D (Cont.)

P9-28 IgG Heavy Chain – Martin Numbering / IMGT CDRs

	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	F	T	F	G	F	Y	Y	I
Martin	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23	H24	H25	H26	H27	H28	H29	H30	H31	H32	H33	H34
Sequency	39%	85%	79%	95%	38%	57%	84%	95%	40%	52%	71%	71%	31%	95%	79%	31%	79%	63%	35%	73%	74%	100%	36%	63%	94%	92%	45%	56%	70%	3%	<1%	73%	22%	19%
IMGT	HFR1																														CDR-H1		HFR2	

↑

	H	W	V	R	Q	A	P	G	K	G	L	E	W	V	A	F	I	D	P	H	G	G	S	T	Y	Y	A	D	S	V	K	G	R	F				
Martin	H35	H36	H37	H38	H39	H40	H41	H42	H43	H44	H45	H46	H47	H48	H49	H50	H51	H52	H52A	H53	H54	H55	H56	H57	H58	H59	H60	H61	H62	H63	H64	H65	H66	H67				
Sequency	31%	100%	75%	81%	97%	50%	92%	91%	63%	86%	96%	96%	94%	36%	20%	2%	85%	13%	42%	4%	35%	60%	27%	64%	32%	93%	45%	32%	55%	39%	71%	62%	76%	41%				
IMGT	HFR2														CDR-H2														HFR3									

III

● →

	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	Y	C	A	R	L	S	Y	P
Martin	H68	H70	H71	H71	H72	H72A	H72B	H72C	H73	H74	H75	H76	H77	H78	H79	H80	H81	H82	H83	H84	H85	H86	H87	H88	H89	H90	H91	H92	H93	H94	H95	H96	H97	H98
Sequency	85%	65%	59%	12%	93%	32%	71%	50%	53%	63%	34%	67%	69%	58%	42%	39%	83%	80%	39%	39%	67%	99%	78%	97%	68%	99%	86%	100%	35%	68%	4%	7%	17%	3%
IMGT	HFR3														CDR-H3																			

v

● →

● →

● →

	G	V	L	D	Y	W	G	Q	G	T	L	V	T	V	S	S
Martin	H99	H100	H100A	H101	H102	H103	H104	H105	H106	H107	H108	H109	H110	H111	H112	H113
Sequency	16%	5%	5%	77%	49%	99%	99%	78%	100%	89%	48%	83%	91%	97%	95%	89%
IMGT	CDR-H3							HFR4								

FIG. 7E

P9-28 IgG Light Chain – Martin Numbering / IMGT CDRs

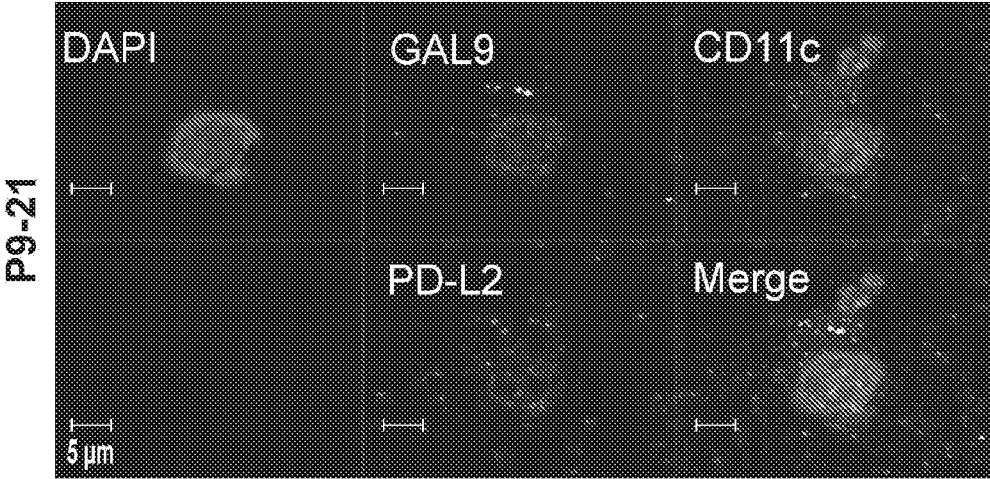
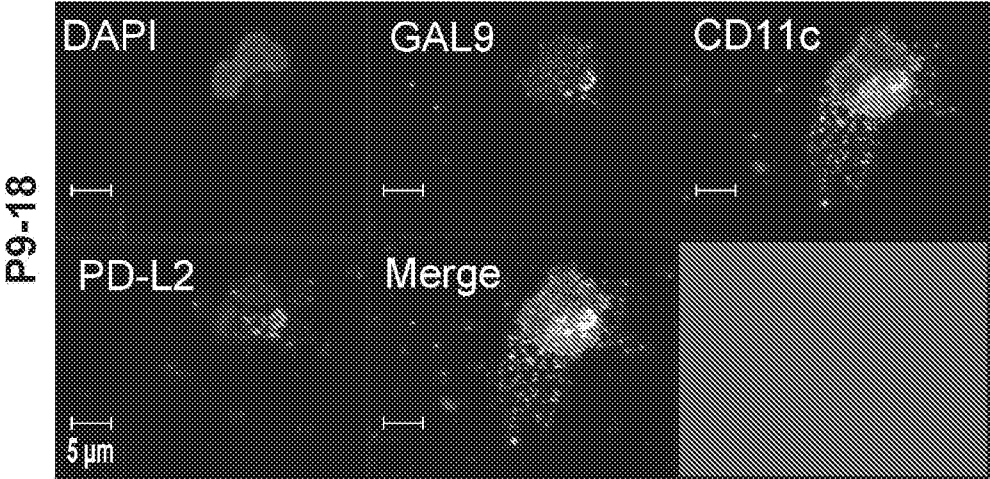
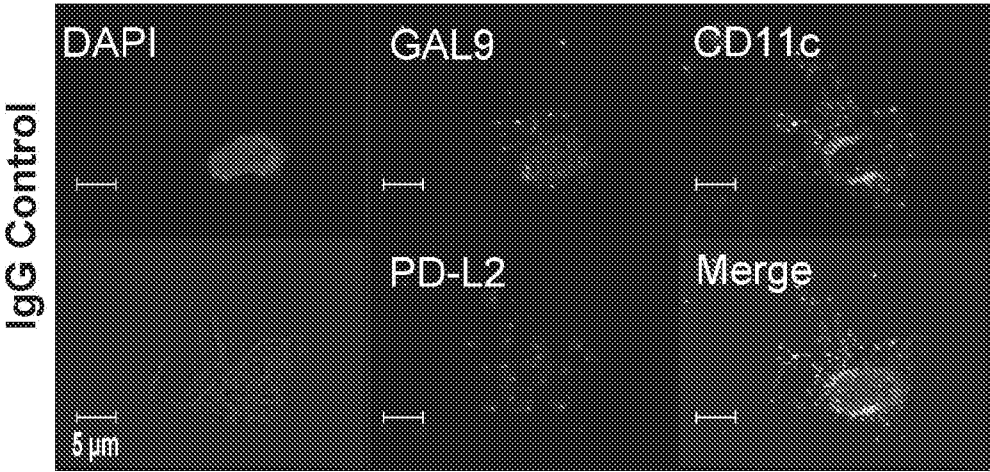
	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	C	R	A	S	Q	S	V	S	A	V	A				
Marlin	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	L25	L26	L27	L28	L29	L30	L31	L32	L33	L34			
Sequence	36%	45%	15%	34%	94%	95%	76%	89%	28%	70%	50%	83%	66%	21%	88%	24%	43%	63%	71%	71%	41%	99%	40%	43%	79%	47%	60%	24%	29%	24%	3%	29%	29%			
INGT	LFR1																CDR-L1														LFR2					

[illegible]

	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	Q	G	Y	S	T	L	V	T	F	G	Q	G	T
Martin	L69	L70	L71	L72	L73	L74	L75	L76	L77	L78	L79	L80	L81	L82	L83	L84	L85	L86	L87	L88	L89	L90	L91	L92	L93	L94	L95	L96	L97	L98	L99	L100	L101	L102
Sequency	71%	43%	50%	66%	96%	71%	97%	71%	34%	59%	60%	28%	78%	99%	26%	85%	26%	99%	85%	100%	52%	50%	8%	17%	43%	11%	17%	7%	54%	99%	99%	22%	99%	98%
IMGT	LFR3										CDR-L3										LFR4								22					

[illegible]

FIG. 7E (Cont.)



A. IgG Control

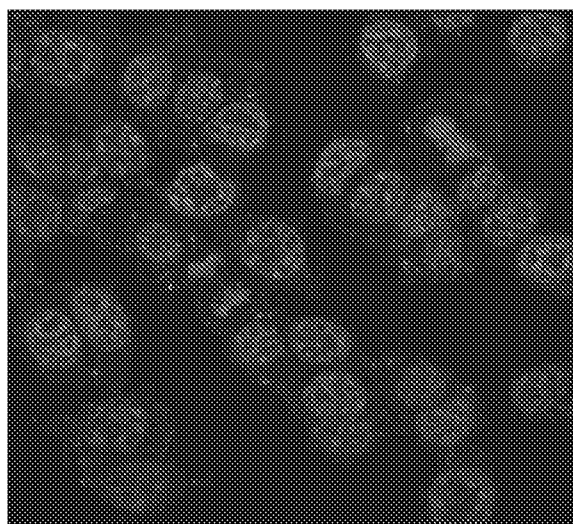


FIG. 9A

B. P9-18

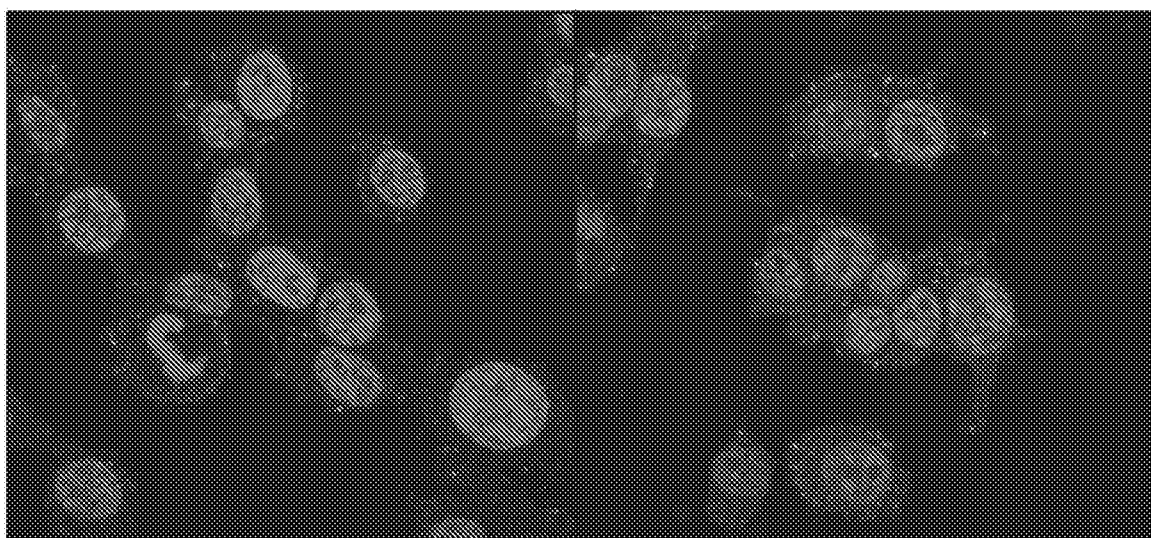


FIG. 9B

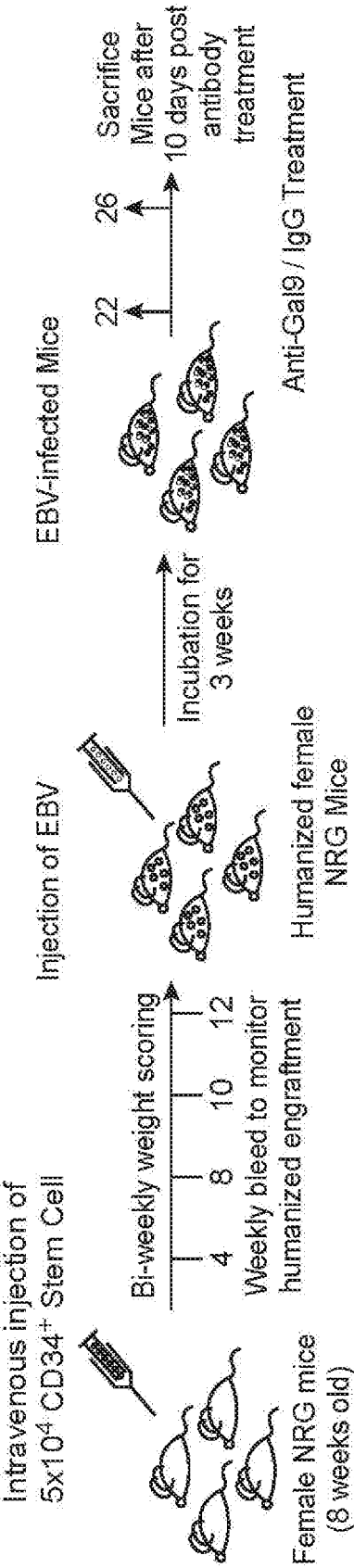


FIG. 10A

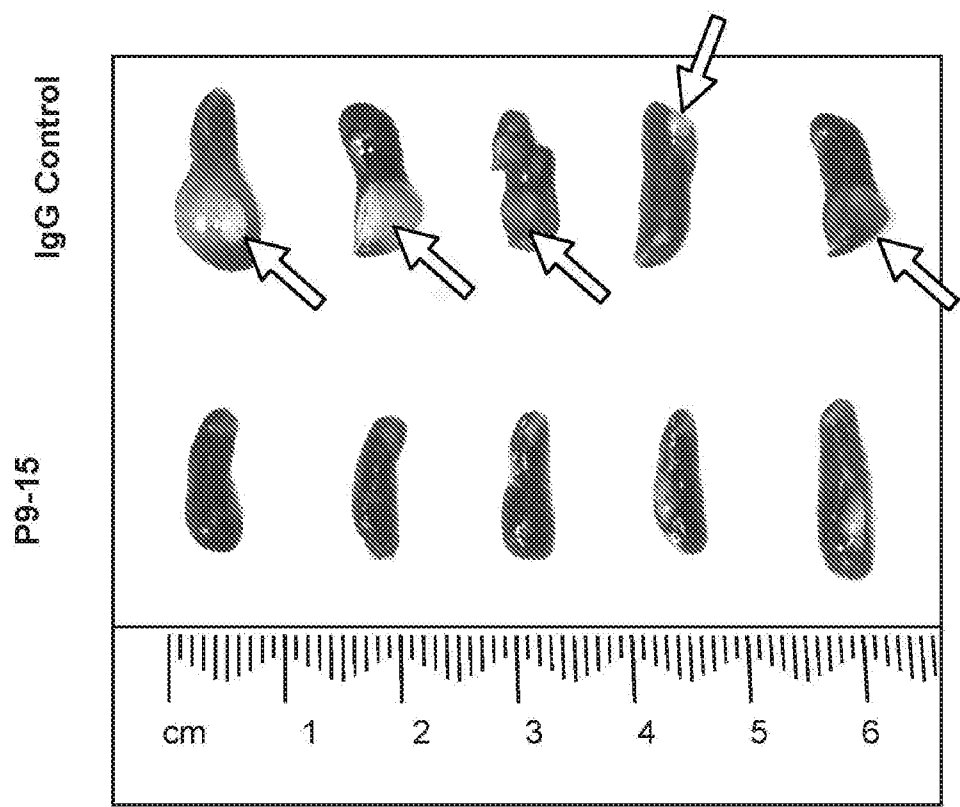


FIG. 10B

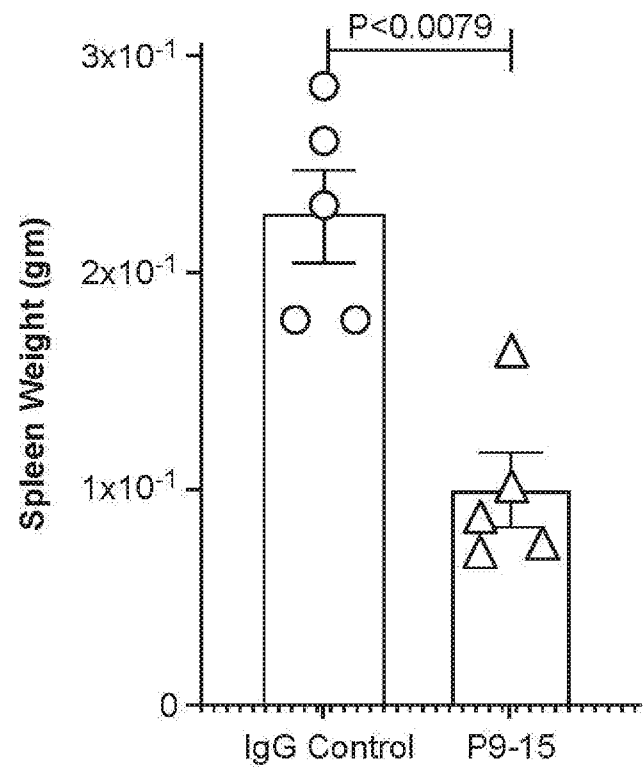


FIG. 10C

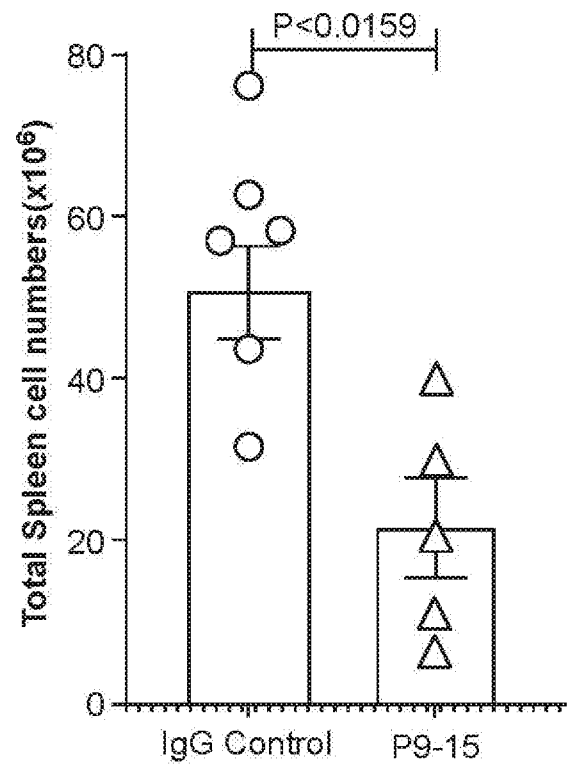


FIG. 10D

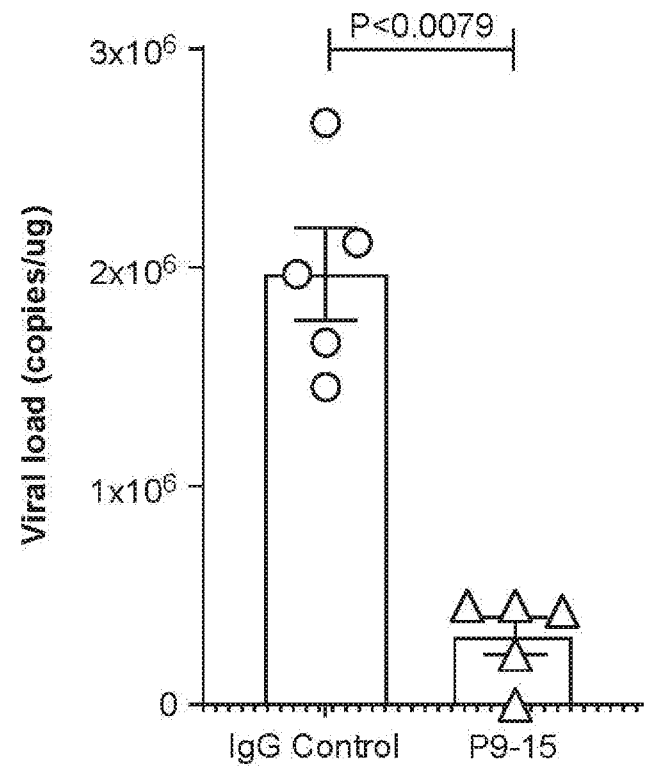


FIG. 10E



FIG. 11

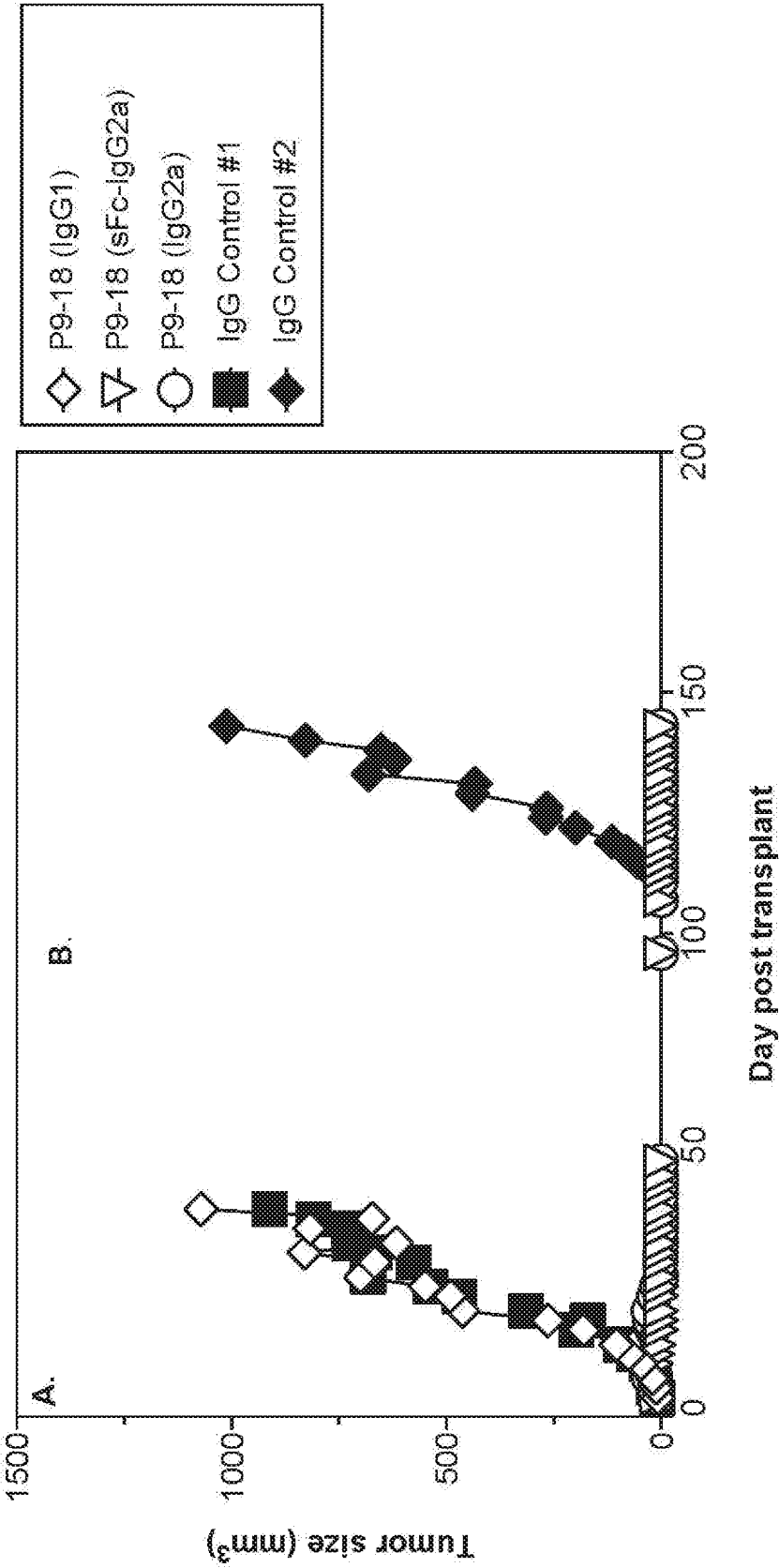


FIG. 12

28/29

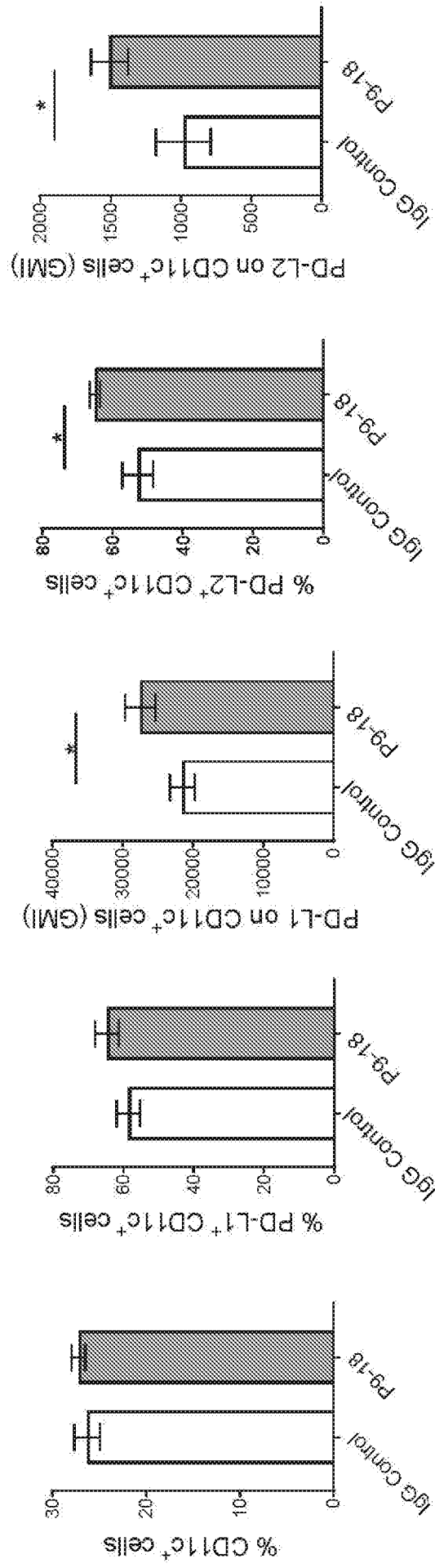


FIG. 13

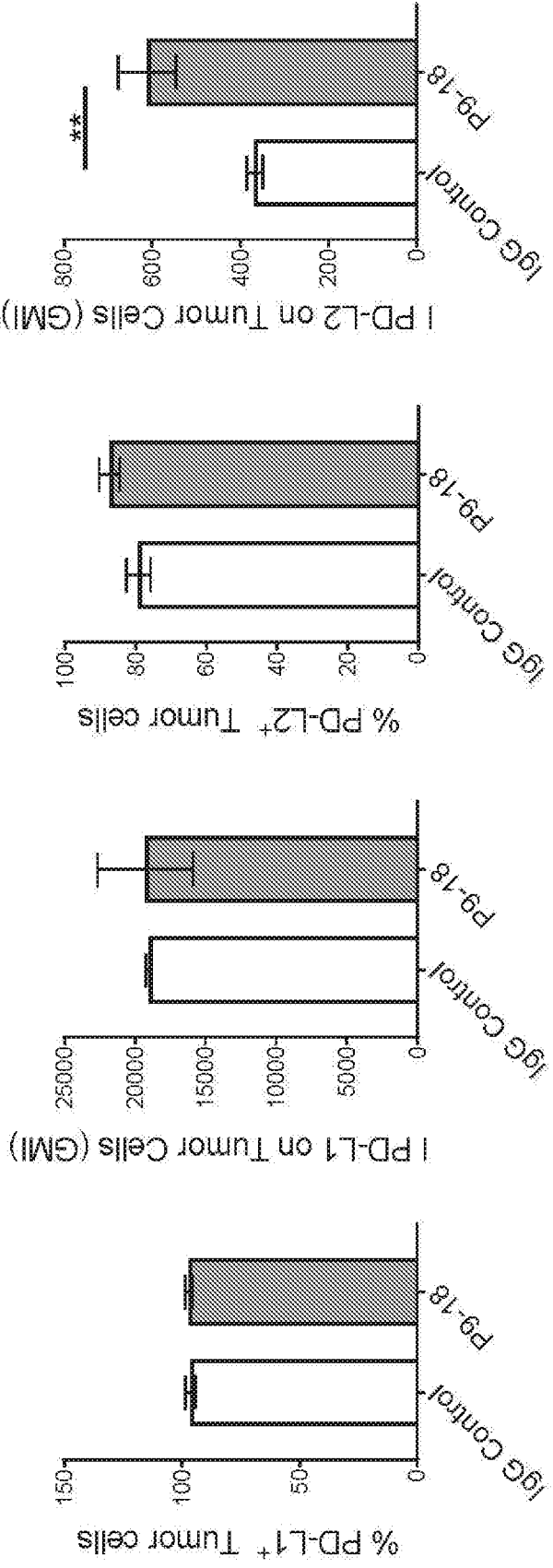


FIG. 14

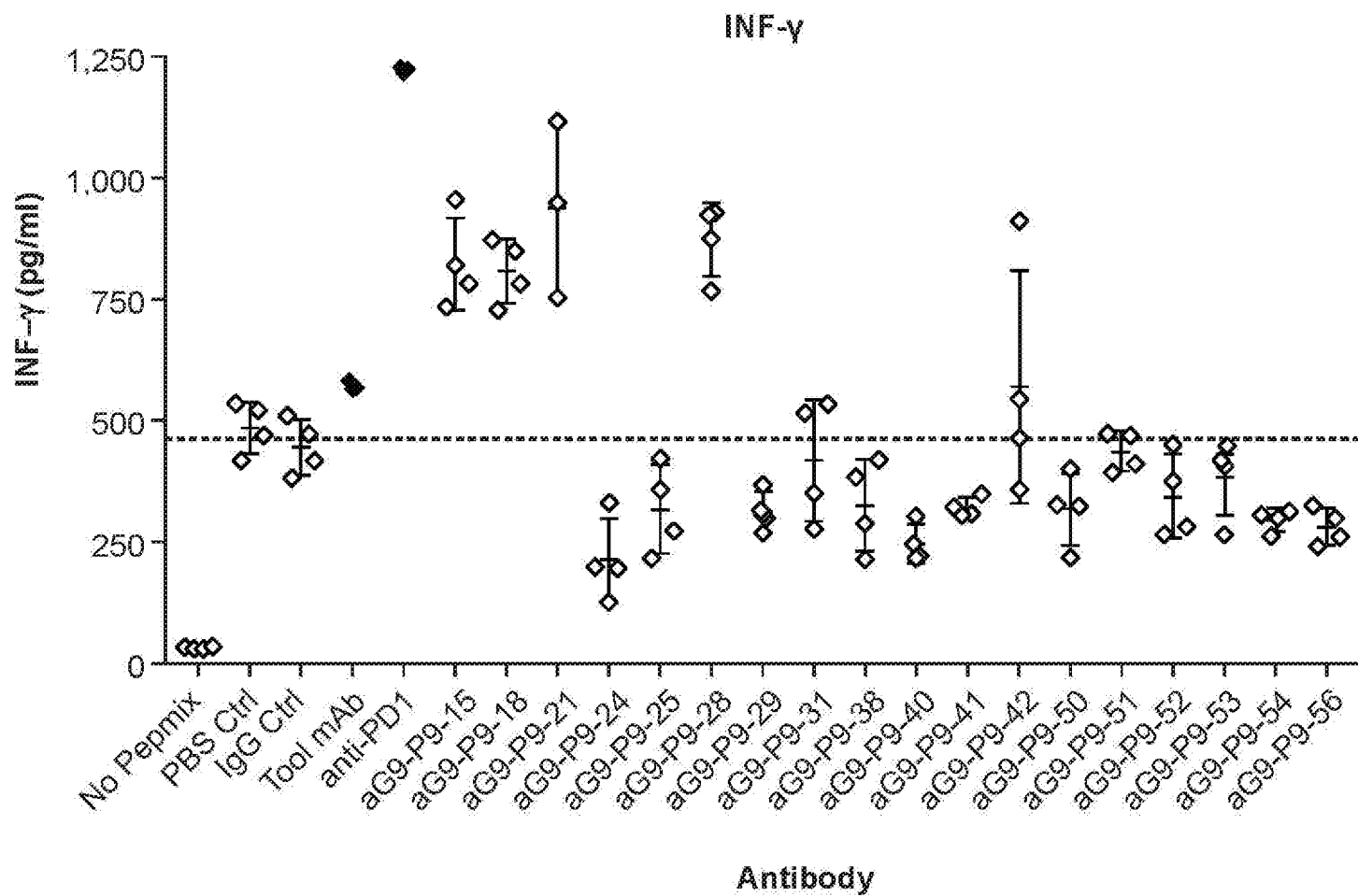


FIG. 3A