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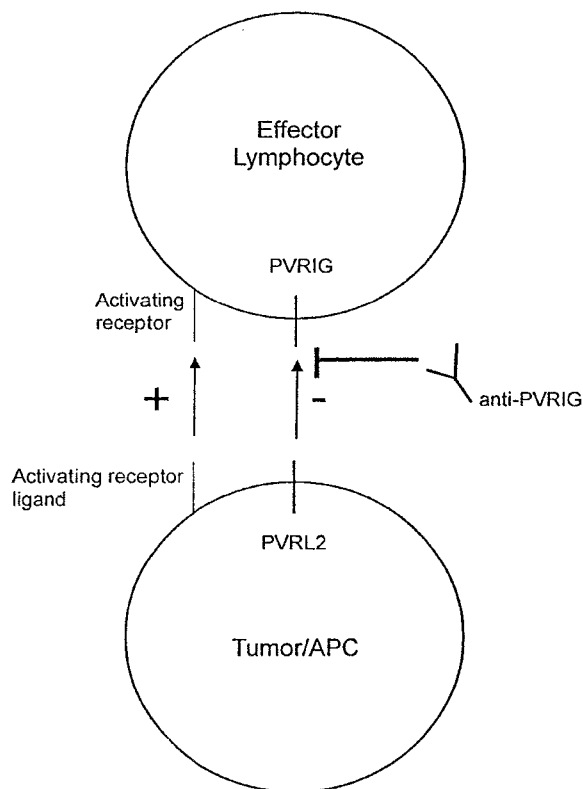
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[Continued on next page]

(54) Title: PVRIG POLYPEPTIDES AND METHODS OF TREATMENT

Figure 1



(57) Abstract: The present invention is directed to PVRIG polypeptides and their uses.





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PVRIG POLYPEPTIDES AND METHODS OF TREATMENT

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. §119 to USSN 62/118,235, filed February 19, 2015, and to USSN 62/141,168, filed March 31, 2015, both of which are
5 expressly incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

[0002] Naïve T cells must receive two independent signals from antigen-presenting cells (APC) in order to become productively activated. The first, Signal 1, is antigen-specific and occurs when T cell antigen receptors encounter the appropriate antigen-MHC complex on the
10 APC. The fate of the immune response is determined by a second, antigen-independent signal (Signal 2) which is delivered through a T cell costimulatory molecule that engages its APC-expressed ligand. This second signal could be either stimulatory (positive costimulation) or inhibitory (negative costimulation or coinhibition). In the absence of a costimulatory signal, or in the presence of a coinhibitory signal, T-cell activation is impaired or aborted, which
15 may lead to a state of antigen-specific unresponsiveness (known as T-cell anergy), or may result in T-cell apoptotic death.

[0003] Costimulatory molecule pairs usually consist of ligands expressed on APCs and their cognate receptors expressed on T cells. The prototype ligand/receptor pairs of costimulatory molecules are B7/CD28 and CD40/CD40L. The B7 family consists of structurally related,
20 cell-surface protein ligands, which may provide stimulatory or inhibitory input to an immune response. Members of the B7 family are structurally related, with the extracellular domain containing at least one variable or constant immunoglobulin domain.

[0004] Both positive and negative costimulatory signals play critical roles in the regulation of cell-mediated immune responses, and molecules that mediate these signals have proven to be
25 effective targets for immunomodulation. Based on this knowledge, several therapeutic approaches that involve targeting of costimulatory molecules have been developed, and were shown to be useful for prevention and treatment of cancer by turning on, or preventing the turning off, of immune responses in cancer patients and for prevention and treatment of autoimmune diseases and inflammatory diseases, as well as rejection of allogenic
30 transplantation, each by turning off uncontrolled immune responses, or by induction of “off

signal” by negative costimulation (or coinhibition) in subjects with these pathological conditions.

[0005] Manipulation of the signals delivered by B7 ligands has shown potential in the treatment of autoimmunity, inflammatory diseases, and transplant rejection. Therapeutic strategies include blocking of costimulation using monoclonal antibodies to the ligand or to the receptor of a costimulatory pair, or using soluble fusion proteins composed of the costimulatory receptor that may bind and block its appropriate ligand. Another approach is induction of co-inhibition using soluble fusion protein of an inhibitory ligand. These approaches rely, at least partially, on the eventual deletion of auto- or allo-reactive T cells (which are responsible for the pathogenic processes in autoimmune diseases or transplantation, respectively), presumably because in the absence of costimulation (which induces cell survival genes) T cells become highly susceptible to induction of apoptosis. Thus, novel agents that are capable of modulating costimulatory signals, without compromising the immune system’s ability to defend against pathogens, are highly advantageous for treatment and prevention of such pathological conditions.

[0006] Costimulatory pathways play an important role in tumor development. Interestingly, tumors have been shown to evade immune destruction by impeding T cell activation through inhibition of co-stimulatory factors in the B7-CD28 and TNF families, as well as by attracting regulatory T cells, which inhibit anti-tumor T cell responses (see Wang (2006), “Immune Suppression by Tumor Specific CD4⁺ Regulatory T cells in Cancer”, *Semin. Cancer. Biol.* 16:73-79; Greenwald, et al. (2005), “The B7 Family Revisited”, *Ann. Rev. Immunol.* 23:515-48; Watts (2005), “TNF/TNFR Family Members in Co-stimulation of T Cell Responses”, *Ann. Rev. Immunol.* 23:23-68; Sadum, et al., (2007) “Immune Signatures of Murine and Human Cancers Reveal Unique Mechanisms of Tumor Escape and New Targets for Cancer Immunotherapy”, *Clin. Canc. Res.* 13(13): 4016-4025). Such tumor expressed co-stimulatory molecules have become attractive cancer biomarkers and may serve as tumor-associated antigens (TAAs). Furthermore, costimulatory pathways have been identified as immunologic checkpoints that attenuate T cell dependent immune responses, both at the level of initiation and effector function within tumor metastases. As engineered cancer vaccines continue to improve, it is becoming clear that such immunologic checkpoints are a major barrier to the vaccines’ ability to induce therapeutic anti-tumor responses. In that regard, costimulatory molecules can serve as adjuvants for active (vaccination) and passive

(antibody-mediated) cancer immunotherapy, providing strategies to thwart immune tolerance and stimulate the immune system.

[0007] Over the past decade, agonists and/or antagonists to various costimulatory proteins have been developed for treating autoimmune diseases, graft rejection, allergy and cancer.

5 For example, CTLA4-Ig (Abatacept, Orencia®) is approved for treatment of RA, mutated CTLA4-Ig (Belatacept, Nulojix®) for prevention of acute kidney transplant rejection and by the anti-CTLA4 antibody (Ipilimumab, Yervoy®), recently approved for the treatment of melanoma.

[0008] Accordingly, there is a need for additional agonists and antagonists of immune
10 checkpoint pathways.

BRIEF SUMMARY OF THE INVENTION

[0009] The present invention provides methods of screening for inhibitors of the binding association of PVRIG polypeptide with PVLR2, the method comprising: a) providing a surface comprising a first ligand protein comprising one of PVRIG polypeptide or PVLR2
15 polypeptide; b) contacting said surface with a candidate agent under physiological conditions, wherein if said candidate agent binds to said first ligand protein it forms a first binding complex; c) contacting said surface with a second ligand protein comprising the other of PVRIG polypeptide or PVLR2; and d) determining whether said PVRIG polypeptide and said PVLR2 are bound as an indication of whether said candidate agent inhibits said binding
20 association.

[0010] In a further aspect, the present invention provides methods of screening for inhibitors of the binding association of PVRIG polypeptide with PVLR2, said method comprising: a) providing a cell comprising an exogenous recombinant nucleic acid encoding a human PVRIG polypeptide, wherein said cell expresses said human PVRIG polypeptide; b)
25 contacting said cell with a candidate agent and a labeled PVLR2 polypeptide; c) determining whether said PVRIG polypeptide binds to PVLR2 as an indication of whether said candidate agent inhibits the binding of PVRIG polypeptide with PVLR2.

[0011] In an additional aspect, the present invention provides methods of screening for inhibitors of the binding association of PVRIG polypeptide with PVLR2, said method
30 comprising: a) providing a cell comprising an exogenous recombinant nucleic acid encoding a human PVLR2 polypeptide, wherein said cell expresses said human PVLR2 polypeptide; b)

contacting said cell with a candidate agent and a labeled PVRIG polypeptide; c) determining whether said PVLR2 polypeptide binds to PVRIG as an indication of whether said candidate agent inhibits the binding of PVRIG polypeptide with PVLR2.

[0012] In a further aspect, the invention provides methods of screening for inhibitors of the binding association of PVRIG polypeptide with PVLR2, said method comprising: a) providing a test solution comprising: i) a PVRIG polypeptide comprising a first FRET label; ii) a PVLR2 polypeptide comprising a second FRET label; c) providing a candidate agent; and d) detecting a FRET signal between said first and second label, wherein a difference in said FRET signal in the presence or absence of said candidate agent indicates that the candidate agent inhibits said binding association.

[0013] In an additional aspect, the invention provides methods as above wherein a plurality and/or a library of candidate agents are tested. The candidate agents can be proteins, including anti-PVRIG antibodies and/or proteins comprising a wild type or variant extracellular domain (ECD) of PVRIG. In some methods the proteins are fusion proteins comprising the ECD and a fusion partner, and the fusion partner can be a human IgG Fc domain or a human serum albumin (HSA).

[0014] In a further aspect, the methods above can further comprise contacting a candidate agent that has the first activity with a population of cytotoxic T cells (CTLs) under conditions wherein said CTLs would normally be activated; and b) determining the effect of said agent on said activation.

[0015] In a further aspect, the methods above can further comprise contacting a candidate agent that has the first activity with a population of cytotoxic T cells (CTLs); and b) determining the effect of said agent on IFN γ production.

[0016] In an addition aspect, the methods above can further comprise contacting the candidate agent with a population of $\gamma\delta$ T cells under conditions wherein said $\gamma\delta$ T cells would normally be activated; and b) determining the effect of said agent on said activation.

[0017] In a further aspect, the methods above can further comprise contacting the candidate agent that has the first activity with a population of Th1 cells under conditions wherein said Th1 cells would normally be activated; and b) determining the effect of said agent on said activation.

[0018] In an additional aspect, the methods above can further comprise contacting said candidate agent with a population of regulatory T cells (Tregs) under conditions and determining the effect of said agent on Treg cell number or activity.

5 [0019] In a further aspect, the methods above determine efficacy by measuring the presence or absence of increased expression of a protein selected from the group consisting of IFN γ , TNF α , GM-CSF, CD25, CD137, CD69, PD1, CD107A, HLA-DR, IL-2, IL-6, IL-4, IL-5, IL-10 and IL-13, wherein increased expression is an indication of activation.

[0020] The invention further provides methods of treating a disorder associated with the interaction of PVRIG and PVLR2 comprising administering to a patient a composition
10 comprising a stimulator of PVRIG to effect treatment. The treatment that occurs can be one or more of a decrease in immune response, a decrease in activation of $\alpha\beta$ and/or $\gamma\delta$ T cells, a decrease in cytotoxic T cell activity, a decrease in NK and/or NKT cell activity, a decrease of $\alpha\beta$ and/or $\gamma\delta$ T-cell activity, a decrease in pro-inflammatory cytokine secretion; a decrease in IL-2 secretion, a decrease in interferon- γ production, a decrease in Th1 response, a increase
15 in Th2 response, an increase in inhibition of T cell activity, an increase in inhibition of CTL activity, an increase in inhibition of NK cell activity, an increase in $\alpha\beta$ and/or $\gamma\delta$ T cell exhaustion, a decrease in $\alpha\beta$ and/or $\gamma\delta$ T cell response, a decrease in activity of cytotoxic cells, a reduction in antigen-specific memory responses, an inhibition of apoptosis or lysis of cells, a decrease in cytotoxic or cytostatic effect on cells, a reduction in direct killing of cells,
20 a decreases in Th17 activity, an reduction of complement dependent cytotoxicity and/or antibody dependent cell-mediated cytotoxicity.

[0021] In a further aspect in the methods above, the stimulator can be a protein or a nucleic acid. In some cases, the protein comprises an extracellular domain (ECD) of PVRIG. In some cases, the protein is a fusion protein comprising said ECD and a fusion partner. The
25 fusion partner can be selected from the group consisting of a human IgG Fc domain and a human serum albumin (HSA).

[0022] In an additional aspect, the patient to whom the stimulator is administered has an immune disorder, which can be selected from the group consisting of an autoimmune disease, organ transplant rejection and inflammation. The autoimmune disease is selected from the
30 group consisting of rheumatoid arthritis, lupus, Inflammatory bowel disease, psoriasis, multiple sclerosis and diabetes type I.

[0023] In further aspects, the invention provides composition comprising an isolated PVRIG polypeptide consisting of a PVRIG polypeptide ECD domain having at least 95% identity to the ECD domain of an amino acid sequence selected from the group consisting of the sequences depicted in Figure 67, Figure 91 and Figures 92A to 92AT. In some cases, the isolated PVRIG polypeptide has at least 99% identity to an amino acid sequence selected from the group consisting of the sequences depicted in Figure 67, Figure 91 and Figures 92A to 92AT. In some embodiments they have 100% identity.

[0024] In a further aspect, the invention provides compositions comprising a PVRIG fusion polypeptide comprising: a) an ECD from a PVRIG polypeptide; and b) a covalently attached fusion partner moiety. The fusion partner can be selected from the group consisting of a human IgG Fc domain, a human serum albumin (HSA) and a polyethylene glycol (PEG). In some cases, the ECD from the fusion partner can have 95%-99%-100% identity to the sequences depicted in Figure 67, Figure 91 and Figures 92A to 92AT.

[0025] In an additional aspect, the PVRIG polypeptide and the fusion partner moiety are directly covalently attached. In some cases, the fusion partner moiety is a polyethylene glycol (PEG) moiety.

[0026] In a further aspect, the PVRIG polypeptide and said fusion partner moiety are covalently attached using an exogeneous linker. In some cases, the exogeneous linker is selected from the group consisting of those depicted in Figure 93 or has the formula (GGGS)_n, wherein n is from 1 to 5.

[0027] In a further aspect, the fusion partner moiety is either a human serum albumin (HSA) or an Fc domain. In the latter case, the Fc domain is a human IgG Fc domain, selected from the group consisting of the Fc domain of human IgG1, the Fc domain of human IgG2, the Fc domain of human IgG3 and the Fc domain of human IgG4. In some cases, the Fc domain is a variant human Fc domain from IgG1 or IgG2.

[0028] In an additional aspect, the compositions further comprise a pharmaceutically acceptable carrier.

[0029] In a further aspect, the invention provides methods of treatment using the compositions above to a patient, such that said patient's immune response is suppressed as a result of treatment. In some cases, the patient has an immune disorder. In some cases, the immune disorder is selected from the group consisting of an autoimmune disease, and organ transplant rejection. In some cases, the autoimmune disease is selected from the group

consisting of rheumatoid arthritis, lupus, Inflammatory bowel disease, psoriasis, multiple sclerosis, Diabetes type I.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] Figure 1 Schematic presentation of the mechanisms of action of the invention.

5 [0031] Figure 2 presents mRNA Expression of PVRIG in various normal human tissues.

[0032] Figure 3 presents mRNA expression of PVRIG in various immune population derived from peripheral blood and bone marrow (based on GSE49910).

[0033] Figure 4 presents mRNA expression of PVRIG in various CD3+ lymphocyte population (based on GSE47855).

10 [0034] Figure 5 A, 5B and 5C presents mRNA expression of PVRIG in specific cell populations. Figure 5A presents mRNA expression of PVRIG in specific cell populations obtained by laser capture microscopy (based on GSE39397). Figure 5B presents mRNA expression of PVRIG in CD4 T-cells from normal and cancer patient as well as expression from CD4 T-cell expression from draining lymph nodes and TILs from breast cancer patients
15 (based on GSE36765). Figure 5C presents mRNA expression of PVRIG from CD8 and CD4 T-cells derived from follicular lymphoma tumor and tonsil (based on GSE27928).

[0035] Figure 6 presents PVRIG expression in normal tissues based on GTEx. Expression levels are shown in log₂(RPKM) values (fragments identified per million reads per kilobase). Values above 1 are considered high expression. Tissues are ranked from top to bottom by the median expression. Each dot on the plot represent a single sample.
20

[0036] Figure 7 presents PVRIG expression in cancerous tissues based on TCGA. Expression levels are shown in log₂(RPKM) values (fragments identified per million reads per kilobase). Values above 1 are considered high expression. Tissues are ranked from top to bottom by the median expression. Each dot on the plot represent a single sample

25 [0037] Figure 8 shows a heatmap representation of the enrichment analysis results in three categories: protein interactions, pathways and disease associations. Results are ranked from top to bottom by average p-value per row. Only the top 10 results from each category are shown. Gray squares indicate p-values<0.05. Each column in the heatmap corresponds to a normal or cancer tissue from which a list of highly correlated genes was derived ($r>0.55$
30 using at least 50 samples). As shown in the heatmap, PVRIG correlates with a T cell gene

expression signature which is strongly associated with the immune response and immune diseases.

[0038] Figure 9 presents PVRIG expression in normal skin vs. melanoma (GTEx and TCGA analysis). Such over-expression was observed in additional solid tumors and results from infiltrating lymphocytes and NK cells in the tumor microenvironment. In normal conditions, no infiltrating immune cells are present and therefore PVRIG expression levels are very low.

[0039] Figure 10 presents the correlations of PVRIG and PD1 in melanoma from TCGA samples, with several T cell makers in lung adenocarcinoma, colon adenocarcinoma and melanoma. The marker CD3 is a general markers for T cells and is also expressed on NKT cells. CD4 and CD8 markers are used to characterized subpopulation of T cells.

[0040] Figure 11 shows expression of PVRIG on human PBLs. Human PBLs derived from two donors were evaluated for PVRIG expression. Both donor 61 and donor 40 showed significant staining with anti-PVRIG specific Ab.

[0041] Figure 12 shows PVRIG-Ig exhibits strong binding to all four human melanoma cell lines MEL-23, Mel-624 and Mel-624.38 and mel-888 tested. Binding is not affected by co-culture with engineered melanoma specific T cells. Grey line corresponds to isotype control, solid black line corresponds to PVRIG-ECD-Ig.

[0042] Figure 13 Correlation of PVRIG with T cells and subpopulations of T cells. CD3G is component of the T cell receptor complex, CD4 is a maker for T helper cells and CD8A is component of CD8 protein used to identify cytotoxic T cells. PVRIG highly correlated with T cells in many types of tumors including lung adenocarcinoma, colon adenocarcinoma and melanoma which are shown here.

[0043] Figure 14 presents representative images from the Confirmation/Specificity screen. All hits from the Primary screen, and EGFR-expressing vector (negative control), were re-arrayed/expressed in duplicate and probed with PVRIG at 20ug/ml. A specific hit with strong intensity is shown in green (PVRL2). Non-specific hits are shown in black. Another weak hit (MAG) was later shown to bind also other ligands, thus suggesting that it is not specific.

[0044] Figure 15A-15E presents effect of various PVRIG-ECD-Ig M:M proteins on mouse CD4 T cell activation. Plates were coated with anti-CD3 mAb (2µg/mL) in the presence of 10µg/ml PVRIG-ECD Ig (batch #198) or control mIgG2a as described in materials and methods. Wells were plated with 1×10^5 CD4+CD25- mouse T cells per well in the presence

of 2ug/ml of soluble anti-CD28. (A) The expression of CD69 was analyzed by flow cytometry at 48h post-stimulation, representative histograms are shown. Each bar is the mean of duplicate cultures, the error bars indicating the standard deviation. (B-C) Culture supernatants were collected at 48 h post-stimulation and mouse IL-2 and IFN γ levels were analyzed by ELISA. Results are shown as Mean $\pm$ Standard errors of duplicate samples. (D) Dose response effect of immobilized PVRIG-ECD Ig (Figure 92BB on surface CD69 (D) and IFN γ secretion (E) is presented. Each bar is the mean of triplicate cultures, the error bars indicating the standard errors.

[0045] Figure 16 presents FACS analysis on PVRIG transduced PBLs using a specific antibody. The percent of cells staining positive (relative to empty vector transduced) for the protein is provided.

[0046] Figure 17 presents FACS analysis on PVRIG (either co-expressed with F4 TCR or in a bi-cystronic vector with F4 TCR and NGFR transduced PBLs using a specific antibody. The percent of cells staining positive (relative to empty vector transduced) for the protein is provided.

[0047] Figure 18A-18B presents FACS analysis performed on TCR transduced stimulated PBLs for experiment 1 (**Error! Reference source not found.A**) and in experiment 2 (**Error! Reference source not found.B**) using a specific monoclonal antibody that recognizes the extra-cellular domain of the beta-chain from the transduced specific TCR. The percentage of cells staining positive is provided.

[0048] Figure 19 shows expression of PVRIG on F4 expressing PBLs causes a reduction of IFN γ secretion upon co-culture with SK-MEL23, MEL-624 and MEL-624.38 in comparison to expression of an empty vector.

[0049] Figure 20A-20B shows expression of PVRIG and F4 in PBLs by co-transduction (**Error! Reference source not found.A**) does not affect IFN γ secretion in co-culture with melanoma cell lines. Expression of PVRIG and F4 in PBLs using a bi-cystronic vector (**Error! Reference source not found.B**) causes a reduction of IFN γ secretion upon co-culture with SK-MEL23, MEL-624 and MEL-624.38 in comparison to expression of an empty vector.

[0050] Figure 21 shows expression of PVRIG and F4 in PBLs using a bi-cystronic vector causes a reduction in T cell mediated cytotoxicity upon co-culture with melanoma cell lines.

[0051] Figure 22 shows PVRIG expression in 3 subgroups of low, no change and high levels of exhausted T cells. Exhausted T cells were selected based on high level expression of 4 markers: CD8A, PD-1, TIM-3 and TIGIT. Low expressing samples are not shown since none had any detectable levels of PVRIG.

5 [0052] Figure 23A-23B: Western blot analysis of ectopically expressed human PVRIG protein. Whole cell extracts of HEK293 cell pools, previously transfected with expression construct encoding human PVRIG-flag (lane 2) or with empty vector (lane 1) were analyzed by WB using an anti-flag antibody (23A) or anti-PVRIG antibodies (23B).

10 [0053] Figure 24: Cell surface expression of HEK293 cells ectopically expressed human PVRIG-flag protein by FACS analysis. Anti-PVRIG pAb (Abnova) was used to analyze HEK293 cells stably expressing the human PVRIG-flag protein. Cells expressing the empty vector were used as negative control. Detection was carried out by Goat Anti-mouse PE-conjugated secondary Ab and analyzed by FACS.

15 [0054] Figure 25 depicts the full length sequence of human PVRIG (showing two different methionine starting points) and the PVRIG Fc fusion protein used in the Examples. The signal peptide is underlined, the ECD is double underlined, and the Fc domain is the dotted underlining.

20 [0055] Figure 26 depicts the sequence of the human Poliovirus receptor-related 2 protein (PVR2, also known as nectin-2, CD112 or herpesvirus entry mediator B, (HVEB)), the binding partner of PVRIG as shown in **Example 5**. PVR2 is a human plasma membrane glycoprotein.

25 [0056] Figure 27 PVRIG antibody specificity towards HEK cells engineered to overexpress PVRIG. Data shows absolute geometric MFI (gMFI) measurements as a function of increasing antibody concentration. The broken black line with squares shows staining of HEK hPVRIG cells with a representative anti-human PVRIG antibody (CPA.7.021), and the solid black line with circles shows staining of HEK parental cells with the same antibody.

[0057] Figure 28 PVRIG RNA was assessed in various cancer cell lines by qPCR. Data shown is relative expression of PVRIG RNA in cell lines as fold change over levels in expi cells as assessed by the $2^{(-\Delta\Delta Ct)}$ method.

30 [0058] Figure 29 PVRIG RNA was assessed in sorted PBMC subsets by qPCR. Data shown is relative expression of PVRIG RNA in each subset as fold change over levels in HEK GFP

cells as assessed by the $2^{(-\Delta\Delta Ct)}$ method. D47-D49 denote three individual donors. CD4 denotes CD4 T cells, CD8 denotes CD8 T cells, CD14 denotes monocytes, and CD56 denotes NK cells.

[0059] Figure 30A-30B. Figure 30A: PVRIG RNA was assessed in sorted CD4 T cells (CD4) and NK cells (NK) under naïve and activated conditions by qPCR. CD4 T cells were stimulated with human T cell stimulator dynabeads and 50U/ml IL-2 for 3 days. NK cells were stimulated in 50U/ml IL-2 for 3 days. Data shown is relative expression of PVRIG RNA in each subset as fold change over levels in expi cells as assessed by the $2^{(-\Delta\Delta Ct)}$ method. Jurkat is included as a positive control. D47-D49 denote three individual donors. Figure 30B PVRIG RNA was assessed in sorted CD8 T cells under naïve and activated conditions by qPCR. CD8 T cells were stimulated with human T cell stimulator dynabeads and 100U/ml IL-2 for 3 days. Data shown is relative expression of PVRIG RNA in each subset as fold change over levels in expi cells as assessed by the $2^{(-\Delta\Delta Ct)}$ method. Jurkat is included as a positive control. D49, 70, and 71 indicate three individual donors.

[0060] Figure 31A-31B PVRIG binding characteristics to HEK hPVRIG engineered cell lines, HEK parental cells, CA46 cells, and Jurkat cells. HEK OE denotes HEK hPVRIG cells, HEK par denotes HEK parental cells. For Jurkat and CA46 data, gMFIr indicates the fold difference in geometric MFI of PVRIG antibody staining relative to their controls. Concentration indicates that at which the gMFIr was calculated. Not reliable fit indicates antibody binding characteristics do not meet appropriate mathematical fitting requirements. Some antibodies were not tested in some conditions due to poor binding characteristics, specificity, or manufacturability.

[0061] Figure 32A-32B PVRIG binding characteristics to primary human PBMC, cyno transient over-expressing cells, and cyno primary PBMC. Expi cyno OE denotes expi cells transiently transfected with cPVRIG, expi par denotes expi parental cells. gMFIr indicates the fold difference in geometric MFI of PVRIG antibody staining relative to their controls. Concentration indicates that at which the gMFIr was calculated. Some antibodies were not tested in some conditions due to poor binding characteristics, specificity, or manufacturability as in **Error! Reference source not found.** Additionally, select antibodies were triaged for screening on cyno PBMC subsets based on their ability to bind cPVRIG transient cells or functionality. Expression of PVRIG on CD4 T cells is similar to that described in the table for CD8 T cells.

[0062] Figure 33 PVRIG antibody specificity towards CA46 and Jurkat cells. Data shows absolute geometric MFI (gMFI) measurements by FACS as a function of increasing antibody concentration. The solid black line with triangles shows staining of CA46 cells with anti-human PVRIG antibody (CPA.7.021) and the solid black line with squares shows staining of Jurkat cells. OV-90 (broken line with upside down triangles) and NCI-H4411 (broken line with diamonds) are shown as negative controls.

[0063] Figure 34A-34D PVRIG antibody cross-reactivity towards cPVRIG transient cells. Data shows an example of an antibody that is a negative binder (a-b, CPA.7.021) and a positive binder (c-d, CPA.7.024) on cPVRIG transient cells. Solid grey histograms indicate control antibody, open black histograms indicate the antibody of interest. Cells were stained with each antibody at a concentration of 5ug/ml.

[0064] Figure 35 cPVRIG RNA was assessed in sorted cyno PBMC subsets by qPCR. Data shown is the average Ct values from three cyno donors as detected by two primer sets directed at two distinct areas of the cPVRIG gene.

[0065] Figure 36A-36C cPVRIG protein was assessed on a) CD16+ lymphocytes (NK cells), b) CD14+ CD56+ myeloid cells (monocytes), and c) CD3+ lymphocytes (T cells) by FACS. Data is shown as absolute geometric MFI, with the solid black line indicating background fluorescence levels. Data is representative of a sample of our panel of anti-human PVRIG antibodies tested in three cyno donors.

[0066] Figure 37A-37B shows the CDR sequences for Fabs that were determined to successfully block interaction of the PVRIG with its counterpart PVRL2, as described in **Example 5**.

[0067] Figure 38A-38AA shows the amino acid sequences of the variable heavy and light domains, the full length heavy and light chains, and the variable heavy and variable light CDRs for the enumerated human CPA anti-PVRIG sequences of the invention that both bind PVRIG and block binding of PVRIG and PVLR2.

[0068] Figure 39A-39H depicts the amino acid sequences of the variable heavy and light domains, the full length heavy and light chains, and the variable heavy and variable light CDRs for eight human CPA anti-PVRIG sequences of the invention that bind PVRIG and but do not block binding of PVRIG and PVLR2.

[0069] Figure 40A-40D depicts the CDRs for all CPA anti-PVRIG antibody sequences that were generated that bind PVRIG, including those that do not block binding of PVRIG and PVLR2.

[0070] Figure 41A to 41DD depicts the variable heavy and light chains as well as the
 5 vhCDR1, vhCDR2, vhCDR3, vlCDR1, vlCDR2 and vlCDR3 sequences of each of the enumerated CHA antibodies of the invention, CHA.7.502, CHA.7.503, CHA.7.506, CHA.7.508, CHA.7.510, CHA.7.512, CHA.7.514, CHA.7.516, CHA.7.518, CHA.7.520.1, CHA.7.520.2, CHA.7.522, CHA.7.524, CHA.7.526, CHA.7.527, CHA.7.528, CHA.7.530, CHA.7.534, CHA.7.535, CHA.7.537, CHA.7.538.1, CHA.7.538.2, CHA.7.543,
 10 CHA.7.544, CHA.7.545, CHA.7.546, CHA.7.547, CHA.7.548, CHA.7.549 and CHA.7.550 (these include the variable heavy and light sequences from mouse sequences (from Hybridomas).

[0071] Figure 42 depicts the binning results from **Example 11**. Not binned: CPA.7.029 and CPA.7.026 (no binding to the antigen).

[0072] Figure 43 Binary matrix of pair-wise blocking ("0", red box) or sandwiching ("1", green box) of antigen for 35 anti-PVRIG mAbs. MAbs listed vertically on the left of the matrix are mAbs covalently immobilized to the ProteOn array. MABs listed horizontally across the top of the matrix were analytes injected with pre-mixed antigen. Clone CPA.7.041 was studied only as an analyte. The black boxes outline four epitope bins according to the
 20 vertical blocking patterns of the mAbs.

[0073] Figure 44 Hierarchical clustering dendrogram of the vertical binding patterns of each mAb in the binary matrix in **Error! Reference source not found.** There are four bins of mAbs with identical epitope blocking patterns within each group. The only difference between bins 1 and 2 is mAbs in bin 1 block antigen binding to clone CPA.7.039 while mAbs
 25 in bin 2 can sandwich the antigen with CPA.7.039. Clone CPA.7.050 can sandwich the antigen with all other clones.

[0074] Figure 45A-45JJ Sensorgrams indicating the antigen blocking pattern for CPA.7.036 with all other immobilized mAbs, which are representative data for Bin #1. Each panel represents a different ProteOn chip array spot having a different immobilized mAb. Blue responses are antigen-only controls. Black responses are pre-mixed solutions of CPA.7.036
 30 in molar excess of antigen. Gray responses are mAb-only control injections. CPA.7.36 blocks antigen binding to all other mAbs except for CPA.7.050 (JJ).

[0075] Figure 46A-46JJ Sensorgrams indicating the antigen blocking pattern for CPA.7.034 with all other immobilized mAbs, which are representative data for Bin #2. Each panel represents a different ProteOn chip array spot having a different immobilized mAb. Blue responses are antigen-only controls. Black responses are pre-mixed solutions of CPA.7.34 in molar excess of antigen. Gray responses are mAb-only control injections. CPA.7.34 blocks antigen binding to all other mAbs except for CPA.7.039 (DD) and CPA.7.050 (JJ).

[0076] Figure 47A-47JJ Sensorgrams indicating the antigen blocking pattern for CPA.7.039 with all other immobilized mAbs. CPA.7.039 is the only mAb in Bin #3. Each panel represents a different ProteOn chip array spot having a different immobilized mAb. Blue responses are antigen-only controls. Black responses are pre-mixed solutions of CPA.7.039 in molar excess of antigen. Gray responses are mAb-only control injections. Panels C, F, H, J, L, N, R, S, Z, EE, GG, HH, II, and JJ show sandwiching of the antigen.

[0077] Figure 48A-48JJ Sensorgrams indicating the antigen blocking pattern for CPA.7.050 with all other immobilized mAbs. CPA.7.050 is the only mAb in Bin #4. Each panel represents a different ProteOn chip array spot having a different immobilized mAb. Blue responses are antigen-only controls. Black responses are pre-mixed solutions of CPA.7.50 in molar excess of antigen. Gray responses are mAb-only control injections. Only panel JJ shows antigen blocking which is where CPA.7.050 was injected w/antigen over itself.

[0078] Figure 49 show the results of the SPR experiments of **Example 12**.

[0079] Figure 50A-50Q SPR sensorgram data of multiple concentrations of anti PVRIG fabs in supernatant injected over captured human PVRIG fusion protein (black lines). The red lines show the 1:1 global kinetic fit to multiple concentrations of the fabs to estimate the k_a and k_d of the interactions. Letters indicate the clone listed in Table 1, which also lists the resulting rate constants and calculated K_D

[0080] Figure 51A-51C SPR sensorgrams for clones CPA.7.009 (A), CPA.7.003 (B), and CPA.7.014 (C) binding to captured human PVRIG fusion protein. These are examples where the sensorgrams showed complex, multi-phasic kinetics and therefore the rate constants could not be reliably estimated.

[0081] Figure 52A-52B shows the results of the blocking studies from “Additional Validation Study 4” in **Example 5**.

[0082] Figure 53 shows that following allo-activation, the expression of PVRIG was upregulated on CD4⁺ T cells as well as on CD8⁺ T cells and double negative gamma delta T cells. This upregulation was observed in PBMCs of one out of two donors tested.

[0083] Figure 54 shows the human cell lines tested in Specifically incorporated by reference herein is USSN 62/141,168, filed 31 March 2015, and USSN 62/118,235, filed 19 February 2015, in their entirety, and in particular for the Examples therein, and for the associated Figures and Legends.

[0084] **Example 1G.**

[0085] Figure 55 shows the mouse cell lines tested in Specifically incorporated by reference herein is USSN 62/141,168, filed 31 March 2015, and USSN 62/118,235, filed 19 February 2015, in their entirety, and in particular for the Examples therein, and for the associated Figures and Legends.

[0086] **Example 1G.**

[0087] Figure 56A-56C. Transcript expression of human PVRIG in various Human cancer cell lines. Verification of the human transcript in several cell lines was performed by qRT-PCR using TaqMan probe. Column diagram represents data observed using TaqMan probe Hs04189293_g1. Ct values are detailed in the table. Analysis indicating high transcript in Jurkat, HUT78 and HL60, and lower levels in THP1 and RPMI8226 cell lines.

[0088] Figure 57A-57B Transcript expression of mouse PVRIG in various mouse cell lines. Verification of the mouse transcript in several cell lines was performed by qRT-PCR using TaqMan probe. Column diagram represents data observed using TaqMan probe CC70L8H. Ct values are detailed in the table. Analysis indicating high transcript in NIH/3T3, Renca, SaI/N and J774A.1, and lower levels in CT26 and B104-1-1 cell lines.

[0089] Figure 58 Endogenous expression of PVRIG protein was analyzed by WB with the commercial anti-human PVRIG rabbit polyclonal antibody (Sigma, cat# HPA047497), using whole cell extracts of various cell lines. Extracts of HEK293 cells ectopically over-expressing human PVRIG (lane 2) or cells transfected with empty vector (lane 1), were used as positive and negative controls, respectively.

[0090] Figure 59 qRT-PCR analysis of human PVRIG transcript in Jurkat cell line transfected with PVRIG siRNA. Jurkat human cancer cell line, transfected with human PVRIG siRNA or with scrambled siRNA were analyzed by qRT-PCR using human PVRIG

TaqMan probe # Hs04189293_g1, and was normalized with geo-mean of two housekeeping genes indicated in table above. Ct values are detailed in the table. Standard deviation of technical triplicates of the PCR reaction are indicated.

[0091] Figure 60 Membrane expression of human PVRIG protein in Jurkat human cell line transfected with human PVRIG siRNA. Jurkat cells transfected with Human PVRIG siRNA were stained with monoclonal anti-PVRIG Ab Inc, CPA.7.021 (left panel, green line) or with IgG2 isotype control antibody (left panel, blue line) and with Sigma Ab (right panel, red line) or with IgG (right panel, blue line). Cells transfected with Scrambled siRNA were stained with the same anti-PVRIG (orange) or isotype control (left panel red line for mAb staining; right panel green line for Sigma Ab). Following cell washing, PE-Goat anti-mouse secondary conjugated Ab was added to Sigma Ab only.

[0092] Figure 61 indicates the summary of the findings described in this report, highlighting the cell lines showing correlation between qPCR and FACS, confirmed by knock down, HSKG- housekeeping gene, +- Positive, NT-Not Tested, X-negative, KD-knockdown.

[0093] Figure 62 indicates the summary of the findings described in this report, highlighting the cell lines showing correlation between qPCR and FACS, confirmed by knock down. HSKG- housekeeping gene, +- Positive, NT-Not Tested, X-negative, KD-knockdown.

[0094] Figure 63A-63D depicts the vhCDR1, vhCDR2, vhCDR3, vlCDR1, vlCDR2 and vlCDR3 sequences of each of the enumerated CPA antibodies of the invention, CPA.7.001 to CPA.7.050 are human sequences (from Phage display).

[0095] Figure 64A-64B shows the results of the screening in Example 1B.

[0096] Figure 65 Antibodies specifics and staining concentration used in Specifically incorporated by reference herein is USSN 62/141,168, filed 31 March 2015, and USSN 62/118,235, filed 19 February 2015, in their entirety, and in particular for the Examples therein, and for the associated Figures and Legends.

[0097] **Example 1I.**

[0098] Figure 66A-66C depicts the sequences of human IgG1, IgG2, IgG3 and IgG4.

[0099] Figure 67 depicts a number of human PVRIG ECD fragments.

[00100] Figure 68 depicts the binding curve for CPA.7.021 as shown in EXAMPLE

[00101] Figure 69A-69C Detection of CD137 and PD-1 surface expression. CD8+ T cells, CD4+ T cells and TILs were activated and monitored over time at 4 time-points as described in M&M. Resting or activated cells were first gated for lymphocytes (FSC-A vs. SSC-A), followed by live cells gate, further gated for singlets (FSC-H vs. FSC-A), CD4/CD8 positive cells and further gated for CD137 and PD1. Surface expression of PD-1 (left) and CD137 (right) on **(A)** CD8+ T cells **(B)** CD4+ T cells and **(C)** TILs at different time-points normalized to isotype control over the time course of activation.

[00102] Figure 70A-70C PVRIG expression on resting and activated CD4+ T and CD8+ T cells. CD4+ and CD8+ T cells were activated and monitored over time at 4 time-points as described in M&M. Cells were stained with viability dye, then incubated with anti-PVRIG and isotype control (7.5 μ g/ml), and evaluated by flow cytometry. **(A)** Expression on CD4+ T cells. Expression of PVRIG on live resting (time 0) and activated CD4+ cells following singlet gating for 24, 48, 72h and 144h compared to isotype control. **(B)** Expression on CD8+ T cells. Expression of PVRIG on live resting (time 0) and activated CD8+ cells following singlet gating for 24, 48, 72h and 144h compared to isotype control. Shown are the Geometric Mean of the fluorescent intensity values obtained. **(C)** Normalization of fold induction staining with anti-PVRIG-CPA.7.021 ab compared to human IgG2 isotype over the time course of activation.

[00103] Figure 71A-71C PVRIG expression on resting and activated TILs. TILs Mart1 and 209 were activated and monitored over time at 4 time-points as described in M&M. Cells were stained with viability dye, then incubated with anti-PVRIG and isotype control (7.5 μ g/ml), and evaluated by flow cytometry. **(A)** Expression on TIL Mart1. Expression of PVRIG on live resting (time 0) and activated TIL following singlet gating for 24, 48, 72h and 144h compared to isotype control. **(B)** Expression on TIL 209. Expression of PVRIG on live resting (time 0) and activated TIL following singlet gating for 24, 48, 72h and 144h compared to isotype control. Shown are the Geometric Mean of the fluorescent intensity values obtained. **(C)** Normalization of fold induction staining with anti PVRIG-CPA.7.021 ab compared with human IgG2 isotype control over the time course of activation.

[00104] Figure 72 Expression of PVRL2 on monocyte-derived DC. PVRL2 expression (triangles with broken line) as a function of time (days) relative to isotype control (circles with solid line) is shown. Day after differentiation indicates time after addition of GM-CSF and IL-4 to monocytes.

[00105] Figure 73A-73B Expression of PVRIG on CD4 and CD8 T cells in the MLR. The expression of PVRIG on proliferating (CFSE low) and non-proliferating T cells (CFSE high) is shown. Data is derived from three individual CD3 T cell donors and from a range of PVRIG antibodies. CFSE is measured on the X axis and PVRIG expression is measured on the Y axis. The top 3 series of scatter plots indicates PVRIG expression on CD4 T cells, and the bottom 3 series indicates expression on CD8 T cells.

[00106] Figure 74A-74B Normalised expression of PVRIG on CD4 and CD8 T cells in the MLR. The expression of PVRIG relative to mIgG1 isotype control is shown from three individual CD3 T cell donors across all antibodies analysed.

10 [00107] Figure 75A-75B PVRIG antibodies increase T cell proliferation in the MLR. The percentages of CFSE low cells are shown from MLR assays treated with the indicated PVRIG antibodies. Each graph represents one individual CD3 T cell donor.

[00108] Figure 76 FACS-based epitope analysis of PVRIG antibodies on T cells. The level of binding of conjugated CPA.7.021 (derived from phage campaign) is indicated after pre-incubation of T cells with unconjugated PVRIG antibodies derived from our hybridoma campaign, as well as relevant controls. Analysis was performed on CFSE low T cells derived from the MLR.

[00109] Figure 77 PVRIG antibody specificity towards HEK cells engineered to overexpress PVRIG. Data shows absolute geometric MFI (gMFI) measurements as a function of increasing antibody concentration. The broken black line with squares shows staining of HEK hPVRIG cells with a representative anti-human PVRIG antibody (CHA.7.518), and the solid black line with circles shows staining of HEK parental cells with the same antibody.

[00110] Figure 78 PVRIG antibodies show specificity towards Jurkat cells. Data shows absolute geometric MFI (gMFI) measurements by FACS as a function of increasing antibody concentration. The broken black line with squares shows staining of Jurkat cells with anti-human PVRIG antibody (CHA.7.518) and the solid black line with circles shows staining with an mIgG1 control antibody.

[00111] Figure 79A-79B PVRIG hybridoma antibody binding characteristics to HEK hPVRIG engineered cell lines, HEK parental cells, and Jurkat cells. HEK OE denotes HEK hPVRIG cells, HEK par denotes HEK parental cells. For Jurkat data, gMFIr indicates the fold difference in geometric MFI of PVRIG antibody staining relative to their controls. Concentration indicates that at which the gMFIr was calculated. No binding indicates

antibody does not bind to the tested cell line. Highlighted antibodies are the ‘top four’ antibodies of interest.

[00112] Figure 80A-80B PVRIG hybridoma antibody binding characteristics to primary human PBMC, cyno over-expressing cells, and cyno primary PBMC. Expi cyno OE denotes expi cells transiently transfected with cPVRIG, expi par denotes expi parental cells. gMFIr indicates the fold difference in geometric MFI of PVRIG antibody staining relative to their controls. Concentrations indicate that at which the gMFIr was calculated. Not tested indicates antibodies that were not tested due to an absence of binding to human HEK hPVRIG, expi cPVRIG cells, or not meeting binding requirements to PBMC subsets.

Highlighted antibodies are the ‘top four’ antibodies of interest.

[00113] Figure 81A-81B Summary of blocking capacity of PVRIG antibodies in the FACS-based competition assay. The IC₅₀ of inhibition is indicated. No IC₅₀ indicates that these antibodies are non-blockers. Highlighted antibodies are the ‘top four’ antibodies of interest.

[00114] Figure 82 KD validation performed in TILs 24hr post-electroporation with siRNA. TILs were stained with anti PVRIG or anti PD-1 analyzed by FACS. Percentage of the KD population is calculated relative to SCR stained with the relevant Ab.

[00115] Figure 83A-83C KD TILs (MART-1 specific) were co-cultured with melanoma cells 624 in 1:1 E:T for 18hr and stained with anti CD8a antibody as well as anti CD137 antibody and analyzed by FACS. Geometric mean fluorescence intensity are plotted (A). Co-culture supernatant was collected as well and tested in Th1 Th2 Th17 cytometric bead array assay to detect secreted cytokines. IFN γ and TNF levels were detected (B,C). The percentage effect of a treatment is calculated by comparing each treatment to SCR control. The figure shows representative data of 2 independent experiments. Treatments were compared by Student's t-test (*P $\leq$ 0.05, **P $\leq$ 0.01) of triplicate samples.

[00116] Figure 84A-84B KD TILs (F4 gp100 specific) were co-cultured with melanoma cells 624 in 1:3 E:T for 18hr and stained with anti CD8a antibody as well as anti CD137 antibody and analyzed by FACS. Geometric mean fluorescence intensity are plotted (A). Co-culture supernatant was collected as well and tested in Th1 Th2 Th17 cytometric bead array assay to detect secreted cytokines. IFN γ levels were detected (B). Percentage of the effect a treatment has is calculated by comparing each treatment to SCR control. Figure

shows representative data of 2 independent experiments. Treatments were compared by Student's t-test (* $P \leq 0.05$, ** $P \leq 0.01$) of triplicate samples.

[00117] Figure 85A-85B TILs from were co-cultured with melanoma cells 624 at 1:1 E:T for 18hr in the presence of anti-PVRIG Ab (CPA.7.021; 10ug/ml) , anti-TIGIT (10A7 clone; 10ug/ml) or in combination. Supernatant was collected and tested in Th1 Th2 Th17 cytometric bead array assay to detect secreted cytokines. IFN γ (**A**) and TNF (**B**) levels were detected. Treatments were compared by Student's t-test (* $P \leq 0.05$, ** $P \leq 0.01$) of triplicate samples.

[00118] Figure 86A-86F MART-1 or 209 TILs were co-cultured with melanoma cells 624 at 1:1 E:T for 18hr in the presence of anti-PVRIG Ab (CPA.7.021; 10ug/ml) , anti-DNAM1 (DX11 clone; 10ug/ml) or in in combination. Supernatant was collected and tested in Th1 Th2 Th17 cytometric bead array assay to detect secreted cytokines. IFN γ (**A,D**) and TNF (**B,E**) levels were detected. TILs were stained for surface expression of CD137 (**C,F**).

[00119] Figure 87A-87B TILs (F4) were co-cultured with melanoma cells 624 at 1:3 E:T for 18hr in the presence of anti-PVRIG Ab (CPA.7.021; 10ug/ml) , anti-TIGIT (10A7 clone; 10ug/ml), anti-PD1 (mAb 1B8, Merck; 10ug/ml) or in combination. Supernatant was collected and tested in Th1 Th2 Th17 cytometric bead array assay to detect secreted cytokines. IFN γ (**A**) and TNF (**B**) levels were detected.

[00120] Figures 88A-88I I depict four humanized sequences for each of CHA.7.518, CHA.7.524, CHA.7.530, CHA.7.538_1 and CHA.7.538_2. Note that the light chain for CHA.7.538_2 is the same as for CHA.7.538_1. The “H1” of each is a “CDR swap” with no changes to the human framework. Subsequent sequences alter framework changes shown in larger bold font. CDR sequences are noted in bold. CDR definitions are AbM from website www.bioinf.org.uk/abs/. Human germline and joining sequences from IMGT® the international ImMunoGeneTics® information system www.imgt.org (founder and director: Marie-Paule Lefranc, Montpellier, France). Residue numbering shown as sequential (seq) or according to Chothia from website www.bioinf.org.uk/abs/ (AbM). “b” notes buried sidechain; “p” notes partially buried; “i” notes sidechain at interface between VH and VL domains. Sequence differences between human and murine germlines noted by asterisk (*). Potential additional mutations in frameworks are noted below sequence. Potential changes in CDR sequences noted below each CDR sequence as noted on the figure (# deamidation substitutions: Q/S/A; these may prevent asparagine (N) deamidation. @ tryptophan oxidation

substitutions: Y/F/H; these may prevent tryptophan oxidation; @ methionine oxidation substitutions: L/F/A).

[00121] Figures 89A-E depicts a collation of the humanized sequences of five CHA antibodies.

5 **[00122]** Figure 90 depicts schemes for combining the humanized VH and VL CHA antibodies of Figure 88 and Figure 89. The “chimVH” and “chimVL” are the mouse variable heavy and light sequences attached to a human IgG constant domain.

[00123] Figure 91 PVRIG hybridoma antibody binding characteristics to primary human PBMC, cyno over-expressing cells, and cyno primary PBMC. Expi cyno OE denotes
 10 expi cells transiently transfected with cPVRIG, expi par denotes expi parental cells. gMFIr indicates the fold difference in geometric MFI of PVRIG antibody staining relative to their controls. Concentrations indicate that at which the gMFIr was calculated. Not tested indicates antibodies that were not tested due to an absence of binding to human HEK hPVRIG, expi cPVRIG cells, or not meeting binding requirements to PBMC subsets. Highlighted antibodies
 15 are four antibodies for which humanization was done (See Figure 90).

[00124] Figure 92 Summary of blocking capacity of PVRIG antibodies in the FACS-based competition assay. The IC₅₀ of inhibition is indicated. No IC₅₀ indicates that these antibodies are non-blockers. Highlighted antibodies are four antibodies for which humanization was done (See Figure 90).

20 **[00125]** Figure 93A-93C Effect of PVRIG antibodies in blocking the interaction between PVRIG and PVRL2. (a-b) Data shows changes in absolute gMFI representing changes in binding of soluble PVRIG to HEK cells when four PVRIG antibodies are added to disrupt the interaction. Also indicated are the IC₅₀ values of each antibody in each assay. A) Data shows disruption of soluble PVRIG with HEK cells when the antibodies are pre-
 25 incubated with antigen. B) Data shows disruption of soluble PVRIG with HEK cells when the antibodies are added concomitantly with antigen. C) Data shows changes in absolute gMFI representing changes in binding of soluble PVRL2 Fc to HEK hPVRIG cells when four PVRIG antibodies are added to disrupt the interaction. IC₅₀ values of each antibody are indicated. ND denotes not determined.

30 **[00126]** Figure 94A-94H NK cell receptor and ligand expression on Reh cells. Expression of NK cell receptors such as a) PVRIG, b) DNAM-1, c) TIGIT are shown. Expression of NK receptor ligands such as d) PVR, e) PVRL2, f) ULBP2/5/6, g) ULBP3, and

h) MICA/B are shown. Solid grey histograms represent isotype controls and open black histograms represent the antibody of interest.

[00127] Figure 95 Effect of PVRIG antibodies on enhancing NK cell-mediated cytotoxicity against Reh cells. The effect of 5ug/ml CPA.7.002 (a), CPA.7.005 (b), CPA.7.021 (a-c), and CPA.7.050 (c) was examined in NK cell cytotoxicity assays against Reh cells where the number of NK cells was titrated against a constant number of Reh cells. d) The effect of varying the concentration of CPA.7.002 and CPA.7.021 on NK cell-mediated cytotoxicity with a constant number of NK to Reh cells (5:1) was examined. DNAM-1 (e) and TIGIT (f) were examined in assays with conditions as outlined in panels a-c.

[00128] Figure 96A-96H NK cell receptor and ligand expression on MOLM-13 cells. Expression of NK cell receptors such as a) PVRIG, b) DNAM-1, c) TIGIT are shown. Expression of NK receptor ligands such as d) PVR, e) PVRL2, f) ULBP2/5/6, g) ULBP3, and h) MICA/B are shown. Solid grey histograms represent isotype controls and open black histograms represent the antibody of interest.

[00129] Figure 97A-97B Effect of PVRIG antibodies on enhancing NK cell-mediated cytotoxicity against MOLM-13 cells. a) The effect of 5ug/ml CPA.7.002, CPA.7.005, and CPA.7.021 was examined in NK cell cytotoxicity assays against MOLM-13 cells where the number of NK cells was titrated against a constant number of MOLM-13 cells. b) TIGIT was examined similar to panel a.

[00130] Figure 98 Summary of blocking capacity of PVRIG antibodies in the cellular biochemical assay. Assay permutation and orientation, and the IC₅₀ of inhibition are indicated. (P) indicates the assay permutation where PVRIG antibodies are pre-incubated with PVRIG antigen prior to addition to HEK cells. (NP) indicates the concomitant addition of PVRIG antibodies and PVRIG antigen to HEK cells. Increased binding indicates that PVRL2 Fc binding to HEK hPVRIG cells was enhanced, rather than inhibited.

[00131] Figure 99: Summary of the activity of select PVRIG antibodies in NK cell cytotoxicity assays against Reh and MOLM-13 cells. Fold change in cytotoxicity relative to control was calculated by dividing the absolute level of killing (%) in the condition with PVRIG antibody, by the absolute level of killing (%) with control antibody. Fold change is calculated from the 5:1 effector to target ratio.

[00132] Figure 100 Sequence alignment of PVRIG orthologs. Aligned sequences of the human, cynomolgus, marmoset, and rhesus PVRIG extra-cellular domain. The differences between human and cynomolgus are highlighted in yellow.

[00133] Figure 101 Binding of anti human PVRIG antibodies to cyno, human, cyno/human hybrid PVRIG variants. Binding of antibodies to wild type cyno PVRIG (●), H61R cyno PVRIG (■), P67S cyno PVRIG (▲), L95R/T97I cyno PVRIG (▼), and wild type human PVRIG (◆) are shown. The ELISA signals are plotted as a function of antibody concentration.

[00134] Figure 102 Correlation of epitope group and cyno cross-reactivity of anti-human PVRIG antibodies.

[00135] Figure 103A-103BX shows a number of sequences of use in the invention.

[00136] Figure 104A and 104B shows the alignment of the PVRIG protein isoforms as splice variants. P0 is SEQ ID NO:XX, P1 is SEQ ID NO:XX, P6 is SEQ ID NO:XX, P8 is SEQ ID NO:XX, P4 is SEQ ID NO:XX, P7 is SEQ ID NO:XX, P14 is SEQ ID NO:XX, P3 is SEQ ID NO:XX, P10 is SEQ ID NO:XX and P13 is SEQ ID NO:XX.

[00137] Figure 105 depicts a number of linker sequences that can find use in the present invention.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[00138] Cancer can be considered as an inability of the patient to recognize and eliminate cancerous cells. In many instances, these transformed (e.g. cancerous) cells counteract immunosurveillance. There are natural control mechanisms that limit T-cell activation in the body to prevent unrestrained T-cell activity, which can be exploited by cancerous cells to evade or suppress the immune response. Restoring the capacity of immune effector cells—especially T cells—to recognize and eliminate cancer is the goal of immunotherapy. The field of immuno-oncology, sometimes referred to as “immunotherapy” is rapidly evolving, with several recent approvals of T cell checkpoint inhibitory antibodies such as Yervoy, Keytruda and Opdivo. These antibodies are generally referred to as “checkpoint inhibitors” because they block normally negative regulators of T cell immunity. It is generally understood that a variety of immunomodulatory signals, both costimulatory and coinhibitory, can be used to orchestrate an optimal antigen-specific immune response.

Generally, these antibodies bind to checkpoint inhibitor proteins such as CTLA-4 and PD-1, which under normal circumstances prevent or suppress activation of cytotoxic T cells (CTLs). By inhibiting the checkpoint protein, for example through the use of antibodies that bind these proteins, an increased T cell response against tumors can be achieved. That is, these cancer checkpoint proteins suppress the immune response; when the proteins are blocked, for example using antibodies to the checkpoint protein, the immune system is activated, leading to immune stimulation, resulting in treatment of conditions such as cancer and infectious disease.

[00139] Thus, without wishing to be limited by a single hypothesis, PVRIG shows inhibitory effects on the following immune functions: T cell activation and proliferation, cytotoxic T lymphocyte (CTL) immunity and CTL-directed killing of target cells, e.g., cancer cells, CD4⁺ T cell immunity, antigen-specific CD4⁺ T cell immunity, natural killer (NK) cell mediated killing of target cells, and the secretion of certain cytokines such as IL-2, INFN- γ and TNF- α by T cells.

[00140] Again without wishing to be limited by a single hypothesis, PVRIG shows potentiating effects on the following immune functions: induction or differentiation and proliferation of inducible T regulatory or suppressor cells (iTregs). These cells are known to be involved in eliciting tolerance to self-antigens and to suppress anti-tumor immunity.

[00141] Again without wishing to be limited by a single hypothesis, PVRIG contributes to a non-functional phenotype of CD8 T cell from the tumor environment, also known as T cell exhaustion.

[00142] The flip side of immuno-oncology is the suppression of T cell activation in conditions where the immune system is too active, or is launching an immune response to an auto-antigen, etc. Thus, by providing PVRIG proteins (for example as fusion proteins, as discussed below), treatment of immune conditions such as auto-immune disease, inflammation and allergic diseases can be treated. That is, as PVRIG has an inhibitory effect on specific immune cells such as CD4⁺ T cells, CD8⁺ T cells or CTLs, and NK cells, which cells are known to be involved in the pathology of certain immune conditions such as autoimmune and inflammatory disorders, as well as eliciting a potentiating effect on Tregs or MDSCs, PVRIG polypeptides which potentiate or agonize the effects of PVRIG on immunity may optionally be used for treating conditions wherein the suppression of T cell or NK mediated immunity and/or the induction of immune tolerance or prolonged suppression of

antigen-specific immunity is therapeutically desirable, e.g., the treatment of autoimmune, inflammatory or allergic conditions, and/or the suppression of undesired immune responses such as to cell or gene therapy, adverse immune responses during pregnancy, and adverse immune responses to transplanted heterologous, allogeneic or xenogeneic cells, organs and tissues and for inhibiting or preventing the onset of graft versus host disease (GVHD) after transplant.

[00143] Therefore, in one embodiment the present invention broadly relates to the development of novel “immunomodulatory proteins” wherein this includes PVRIG polypeptides that antagonize or block the effects of PVRIG on immunity and particularly the effects of PVRIG, on specific types of immune cells and cytokine production (i. e., “immunostimulatory PVRIG polypeptides or fusion proteins and/or immunostimulatory PVRIG antibodies”).

[00144] Additionally, the invention relates to PVRIG polypeptides that agonize or mimic the effects of PVRIG on immunity and particularly the effects of PVRIG on specific types of immune cells and cytokine production (i.e., “immunoinhibitory PVRIG polypeptides or fusion proteins”).

II. PVRIG Mechanism of Action

[00145] Accordingly, as discussed herein, PVRIG is a immune checkpoint protein, sometimes referred to as “an immuno-oncology protein”. As has been shown for PD-1 and CTLA-4, among others, immune checkpoint proteins can be exploited in several ways, to either immunopotentiate the immune system to increase immune activity, such as through the activation of T cells for treatments of diseases such as cancer and infectious disease, or through immunoinhibition, where immunosuppression is desired, for example in allergic reactions, autoimmune diseases and inflammation. PVRIG as shown herein exhibits negative signaling on the immune system, by suppressing T cell activation and other pathways as outlined herein. Thus, by reducing the activity of PVRIG, for example by inhibiting its binding ability to its ligand such as PVLR2, the suppression is decreased and the immune system can be activated or stimulated to treat cancer, for example. Conversely, by increasing the activity of PVRIG (“stimulating” the activity with a “stimulator”), for example by adding additional PVRIG (a “stimulator of PVRIG”) to a host in the form of a soluble ECD (and optionally a fusion protein), the suppression is increased and the immune system is suppressed, allowing for treatment of diseases associated with increased immune function such as autoimmune diseases and others outlined herein.

[00146] As shown in the Example section, the expression of PVRIG has been positively correlated to expression of PD-1, a known immune checkpoint protein. Additionally, introduction of PVRIG (as over expressed on PBLs or as an Fc-fusion protein) was shown to inhibit the activation of T cells, as shown in the Examples, and KD of PVRIG in T cells was shown to increase the activation of T cells, and anti-PVRIG antibodies have
5 been shown to activate T cells and NK cells.

[00147] Accordingly, the present invention is directed to compounds that either suppress the signaling pathway triggered by the binding interaction of PVRIG and PVLR2 (leading to increased T cell and NK cell activation, among other things, leading to treatment
10 of diseases such as cancer and pathogen infection), or activate the signaling pathway triggered by the binding interaction of PVRIG and PVLR2 (leading to decreased T cell and NK cell activation, among other things), leading to treatment of diseases such as autoimmune diseases and inflammation.

[00148] Thus, specific mechanisms of action are provided for the immunostimulatory
15 actions of, for example, anti-PVRIG antibodies, that are useful for increasing immune function, for example for the treatment of cancer. These include, but are not limited to, (i) increases in immune response, (ii) increases in activation of $\alpha\beta$ and/or $\gamma\delta$ T cells, (iii) increases in cytotoxic T cell activity, (iv) increases in NK and/or NKT cell activity, (v) alleviation of $\alpha\beta$ and/or $\gamma\delta$ T-cell suppression, (vi) increases in pro-inflammatory cytokine
20 secretion, (vii) increases in IL-2 secretion; (viii) increases in interferon- γ production, (ix) increases in Th1 response, (x) decreases in Th2 response, and (xi) decreases or eliminates cell number and/or activity of at least one of regulatory T cells (Tregs).

[00149] Furthermore, in some embodiments a PVRIG ECD (for example, in the form of an Fc fusion, for example) binds PVRL2 and interrupts one or more inhibitory signals (via
25 PVRIG) and thus acts in an immunoinhibitory manner. These include, but are not limited to, (i) increases in immune response, (ii) increases in activation of $\alpha\beta$ and/or $\gamma\delta$ T cells, (iii) increases in cytotoxic T cell activity, (iv) increases in NK and/or NKT cell activity, (v) alleviation of $\alpha\beta$ and/or $\gamma\delta$ T-cell suppression, (vi) increases in pro-inflammatory cytokine secretion, (vii) increases in IL-2 secretion; (viii) increases in interferon- γ production, (ix)
30 increases in Th1 response, (x) decreases in Th2 response, (xi) decreases or eliminates cell number and/or activity of at least one of regulatory T cells (Tregs), (xii) reduces regulatory cell activity, and/or the activity of one or more of myeloid derived suppressor cells (MDSCs),

iMCs, mesenchymal stromal cells, TIE2-expressing monocytes, (xiii) decreases or eliminates M2 macrophages, (xiv) reduces M2 macrophage pro-tumorigenic activity, (xv) decreases or eliminates N2 neutrophils, (xvi) reduces N2 neutrophils pro-tumorigenic activity, (xvii) reduces inhibition of T cell activation, (xviii) reduces inhibition of CTL activation, (xix) reduces inhibition of NK and/or NKT cell activation, (xx) reverses $\alpha\beta$ and/or $\gamma\delta$ T cell exhaustion, (xxi) increases $\alpha\beta$ and/or $\gamma\delta$ T cell response, (xxii) increases activity of cytotoxic cells, (xxiii) stimulates antigen-specific memory responses, (xxiv) elicits apoptosis or lysis of cancer cells, (xxv) stimulates cytotoxic or cytostatic effect on cancer cells, (xxvi) induces direct killing of cancer cells, (xxvii) increases Th17 activity and/or (xxviii) induces complement dependent cytotoxicity and/or antibody dependent cell-mediated cytotoxicity.

[00150] Additionally, specific mechanisms of action are provided for the immunoinhibitory actions of, for example, PVRIG ECD domains (including fusions, as are outlined herein), that are useful for decreasing immune function, for example for the treatment of autoimmune disease and inflammation. These include, but are not limited to, (i) decreases immune response, (ii) decreases $\alpha\beta$ and/or $\gamma\delta$ T cell activation, (iii) decreases cytotoxic T cell activity, (iv) decreases natural killer (NK) and/or NKT cell activity, (v) decreases $\alpha\beta$ and/or $\gamma\delta$ T-cell activity, (vi) decreases pro-inflammatory cytokine secretion, (vii) decreases IL-2 secretion; (viii) decreases interferon- γ production, (ix) decreases Th1 response, (x) decreases increases Th2 response, (xvii) increases inhibition of T cell activation, (xviii) increases inhibition of CTL activation, (xix) increases inhibition of NK cell activation, (xx) increases $\alpha\beta$ and/or $\gamma\delta$ T cell exhaustion, (xxi) decreases $\alpha\beta$ and/or $\gamma\delta$ T cell response, (xxii) decreases activity of cytotoxic cells, (xxiii) reduces antigen-specific memory responses, (xxiv) inhibits apoptosis or lysis of cells, (xxv) decreases cytotoxic or cytostatic effect on cells, (xxvi) reduces direct killing of cells, (xxvii) decreases Th17 activity, and/or (xxviii) reduces complement dependent cytotoxicity and/or antibody dependent cell-mediated cytotoxicity.

III. Methods of Screening

[00151] Accordingly, the present invention provides methods of screening for modulators of the PVRIG-PVLR2 interaction, which either leads to immunostimulation or immunoinhibition, as outlined herein. For example, compounds that inhibit the interaction of PVRIG and PVLR2, which normally leads to the suppression of T cell and/or NK cell activation and thus increase the immune response to allow for the ultimate administration to patients for the treatment of cancer and pathogen infections, for example. Conversely,

compounds that increase the signaling due to the interaction of PVRIG and PVLR2, lead to increased suppression of T cell and/or NK cell activation, thus resulting in decreased immune responses, to allow for the ultimate administration to patients for the treatment of diseases associated with increased immune function such as autoimmune diseases and inflammation.

5 In this latter case, the increase of signaling is termed “stimulation of binding”, which can be effected, for example, by adding a compound (a “stimulator”) such as the ECD of PVRIG, resulting in stimulated binding of the ECD to endogenous PVLR2 and triggering the signaling pathway.

A. Cell Based Assays

10 **[00152]** Accordingly, in one embodiment, the invention provides assays to screen for inhibitors of the binding association of PVRIG and PVLR2.

[00153] In one embodiment, the methods of screening provide cells that comprise an exogenous recombinant nucleic acid encoding a human PVRIG protein, generally the full length protein including the transmembrane domain, such that the PVRIG protein is
15 expressed in the correct orientation, resulting in the extracellular domain (ECD) being on the surface of the cell. By “exogenous” in this context herein is meant that the gene (and any required expression vector sequences) is not endogenous (naturally occurring in the genome) to the cell. In the case of non-human cell lines to be used in the assays herein, this means that the non-human cell line has a human gene transfected into the cell. In the case
20 where human cell lines are used (preferable in most instances), and thus contain an endogenous PVRIG gene, the cells contain at least an additional, recombinant human gene, if not additional copies as well.

[00154] In this embodiment, cells expressing exogenous PVRIG are contacted with candidate agent(s) as is more fully outlined below, and a labeled PVLR2 protein (generally
25 the ECD domain). By comparing the results to a reference standard not including the candidate agent, where binding is known to occur, the lack of bound label means the candidate agent binds to the PVRIG in such a way as to prevent PVLR2 binding.

[00155] In one embodiment, the methods are reversed, and thus use cells that comprise an exogenous recombinant nucleic acid encoding a human PVLR2 protein, generally the full
30 length protein including the transmembrane domain, such that the PVLR2 protein is expressed in the correct orientation, resulting in the extracellular domain (ECD) being on the surface of the cell.

[00156] In this embodiment, cells expressing exogenous PVLR2 are contacted with candidate agent(s) as is more fully outlined below, and a labeled PVRIG protein (generally the ECD domain). By comparing the results to a reference standard not including the candidate agent, where binding is known to occur, the lack of bound label means the
5 candidate agent binds to the PVLR2 in such a way as to prevent PVRIG binding.

[00157] As will be appreciated by those in the art, these assays can be done on surfaces such as in microtiter plates.

B. Support Based Assays

[00158] In one embodiment, the screening assay is a solid support assay, where one or
10 the other of PVRIG and PVLR2 is attached, for example to a microtiter plate. Candidate agents and labeled proteins, e.g. the “other” of the PVRIG and PVLR2 is added. If the candidate agent blocks binding, this can be determined using the read out. For example, in one embodiment, PVRIG is attached to the solid support, generally at discrete locations. A candidate agent and PVLR2 is added, for example that is either directly labeled (for example
15 with a fluorophore as outlined below), or indirectly labeled (for example using a labeled anti-PVLR2 antibody). After allowing a sufficient period of time and after washing, if the candidate agent blocks the interaction of PVRIG and PVLR2, no signal will be seen. If the agent does not, the signal will be generated and bound to the support. Similarly, this can be done using attachment of the PVLR2 to the solid support and adding labeled PVRIG and
20 candidate agents.

C. FRET Based Assays

[00159] In some embodiments, this binding assay can be done using fluorescent resonance energy transfer (FRET) assays, as is well known in the art, where one of the receptor-ligand pair of PVRIG and PVLR2 has a FRET donor and the other has a FRET
25 acceptor. Upon binding of the two, FRET occurs. If the candidate agent prevents binding, the FRET signal will be lost. This is also useful in competition assays, to determine whether the binding of the agent to PVRIG is stronger than the binding of PVLR2.

D. Functional Assays

[00160] These identified candidate agents that bind and block the interaction of the
30 PVRIG and PVLR2 can then be further tested to see their effect on the signaling pathway. That is, the binding/blocking agents can be run in assays that measure T cell or NK cell activation, for example, to determine whether the blocking agents are immunostimulatory

(increasing immune function such that diseases such as cancer can be treated) or immunoinhibitory (decreasing immune function to treat autoimmune diseases and inflammation).

5 [00161] In addition, the assays below can also be used to assess treatment efficacy, as is more further outlined below.

[00162] In some embodiments, the functional assay uses CTLs. The CTLs express a T cell receptor (TCR) recognizing a specific antigen (Ag) presented on an MHC molecule. Upon TCR Ag engagement, CTLs undergo activation as manifested by cell proliferation, up-regulation of activation markers (e.g. CD25, CD137 etc.), and cytokine secretion (e.g. 10 interferon gamma, IL2, TNF α etc.) and cytotoxic activity. Upon contact with PVRL2 expressed on cancer cells or antigen presenting cells, PVRIG mediates a negative signal to CTLs thereby causing down-regulation of CTL activation as manifested by the above readouts. Thus, contacting candidate agents that have shown binding and/or inhibition of receptor-ligand binding with CTLs will to interrupt the PVRIG-PVRL2 interaction and 15 thereby release the negative signal mediated by PVRIG and enhances antigen specific CTL activation as manifested by cell proliferation, up-regulation of activation markers (e.g. CD25, CD137 etc.), and cytokine secretion (e.g. interferon gamma, IL2, TNF alfa etc.).

[00163] Similarly, in some embodiments, the functional assay uses NK cells. The NK cells express various activating and inhibitory receptors. The execution of NK cytotoxic 20 activity is determined by the balance between the activatory and inhibitory signals derived from these receptors. Upon engagement of NK cells with certain target cells, NK cells undergo activation as manifested by cell proliferation, cytokine secretion (e.g. interferon gamma, IL2, TNF alfa etc.) and cytotoxic activity. Upon contact with PVRL2 expressed on cancer target cells, PVRIG mediates a negative signal to NK cells thereby causing down- 25 regulation of NK cell activation as manifested by the above readouts. Contacting of candidate agents with NK cells will interrupt the PVRIG-PVRL2 interaction and thereby release the negative signal mediated by PVRIG and enhances NK cell activation as manifested by cell proliferation, cytokine secretion (e.g. interferon gamma, IL2, TNF alfa etc.) and cytotoxic activity.

30 [00164] In one embodiment, the signaling pathway assay measures increases or decreases in immune response as measured for an example by phosphorylation or de-phosphorylation of different factors, or by measuring other post translational modifications.

An increase in activity indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

5 [00165] In one embodiment, the signaling pathway assay measures increases or decreases in activation of $\alpha\beta$ and/or $\gamma\delta$ T cells as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

10 [00166] In one embodiment, the signaling pathway assay measures increases or decreases in cytotoxic T cell activity as measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

15 [00167] In one embodiment, the signaling pathway assay measures increases or decreases in NK and/or NKT cell activity as measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by changes in expression of activation markers like for an example CD107a, etc. An increase in activity indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

20 [00168] In one embodiment, the signaling pathway assay measures increases or decreases in $\alpha\beta$ and/or $\gamma\delta$ T-cell suppression. as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

25 [00169] In one embodiment, the signaling pathway assay measures increases or decreases in pro-inflammatory cytokine secretion as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

[00170] In one embodiment, the signaling pathway assay measures increases or decreases in IL-2 secretion as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity.

[00171] In one embodiment, the signaling pathway assay measures increases or decreases in interferon- γ production as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

[00172] In one embodiment, the signaling pathway assay measures increases or decreases in Th1 response as measured for an example by cytokine secretion or by changes in expression of activation markers. An increase in response indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

[00173] In one embodiment, the signaling pathway assay measures increases or decreases in Th2 response as measured for an example by cytokine secretion or by changes in expression of activation markers. An increase in response indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

[00174] In one embodiment, the signaling pathway assay measures increases or decreases cell number and/or activity of at least one of regulatory T cells (Tregs), as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity and an increase indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

[00175] In one embodiment, the signaling pathway assay measures increases or decreases in M2 macrophages cell numbers, as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity and a increase indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

[00176] In one embodiment, the signaling pathway assay measures increases or decreases in M2 macrophage pro-tumorigenic activity, as measured for an example by cytokine secretion or by changes in expression of activation markers. A decrease in response indicates immunostimulatory activity and a increase indicates immunoinhibitory activity.

5 [00177] In one embodiment, the signaling pathway assay measures increases or decreases in N2 neutrophils increase, as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity and a increase indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

10 [00178] In one embodiment, the signaling pathway assay measures increases or decreases in N2 neutrophils pro-tumorigenic activity, as measured for an example by cytokine secretion or by changes in expression of activation markers. A decrease in response indicates immunostimulatory activity and a increase indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

15 [00179] In one embodiment, the signaling pathway assay measures increases or decreases in inhibition of T cell activation, as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in response indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity
20 are outlined below.

[00180] In one embodiment, the signaling pathway assay measures increases or decreases in inhibition of CTL activation as measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc.

25 An increase in response indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

[00181] In one embodiment, the signaling pathway assay measures increases or decreases in $\alpha\beta$ and/or $\gamma\delta$ T cell exhaustion as measured for an example by changes in expression of activation markers. A decrease in response indicates immunostimulatory
30 activity and a increase indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

[00182] In one embodiment, the signaling pathway assay measures increases or decreases $\alpha\beta$ and/or $\gamma\delta$ T cell response as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity and
5 a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

[00183] In one embodiment, the signaling pathway assay measures increases or decreases in stimulation of antigen-specific memory responses as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers
10 like for an example CD45RA, CCR7 etc. An increase in activity indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

[00184] In one embodiment, the signaling pathway assay measures increases or decreases in apoptosis or lysis of cancer cells as measured for an example by cytotoxicity
15 assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

[00185] In one embodiment, the signaling pathway assay measures increases or
20 decreases in stimulation of cytotoxic or cytostatic effect on cancer cells. as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

[00186] In one embodiment, the signaling pathway assay measures increases or
25 decreases direct killing of cancer cells as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity.
30 Appropriate increases or decreases in activity are outlined below.

[00187] In one embodiment, the signaling pathway assay measures increases or decreases Th17 activity as measured for an example by cytokine secretion or by proliferation

or by changes in expression of activation markers. An increase in activity indicates immunoinhibitory activity and a decrease indicates immunostimulatory activity. Appropriate increases or decreases in activity are outlined below.

[00188] In one embodiment, the signaling pathway assay measures increases or decreases in induction of complement dependent cytotoxicity and/or antibody dependent cell-mediated cytotoxicity, as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity and a decrease indicates immunoinhibitory activity. Appropriate increases or decreases in activity are outlined below.

[00189] In one embodiment, the assay measures increases or decreases in cell proliferation as a function of activation or inhibition, using well known methodologies such as thymidine incorporation and CFSE dilution.

[00190] Appropriate increases in activity or response (or decreases, as appropriate as outlined above), are increases of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 98 to 99% percent over the signal in either a reference sample or in control samples, for example test samples that do not contain an anti-PVRIG antibody of the invention. Similarly, increases of at least one-, two-, three-, four- or five-fold as compared to reference or control samples show efficacy.

E. Candidate Agents

[00191] The assays are run by contacting candidate agents with the PVRIG and PVLR2 proteins. By "candidate agent", "candidate bioactive agent" or "candidate drugs" or grammatical equivalents herein is meant any molecule, e.g. proteins (which herein includes proteins, polypeptides, and peptides), small organic or inorganic molecules, polysaccharides, polynucleotides, etc. which are to be tested for binding to PVRIG, inhibition of the PVRIG-PVLR2 interaction, or activation of PVRIG. Candidate agents encompass numerous chemical classes. In a preferred embodiment, the candidate agents are organic molecules, particularly small organic molecules, comprising functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more chemical functional groups.

5 [00192] Candidate agents are obtained from a wide variety of sources, as will be appreciated by those in the art, including libraries of synthetic or natural compounds. As will be appreciated by those in the art, the present invention provides a rapid and easy method for screening any library of candidate agents, including the wide variety of known combinatorial chemistry-type libraries.

[00193] In a preferred embodiment, candidate agents are synthetic compounds. Any number of techniques are available for the random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides.

10 [00194] Alternatively, a preferred embodiment utilizes libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts that are available or readily produced.

[00195] Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means. Known pharmacological agents may be subjected to directed or random chemical modifications, 15 including enzymatic modifications, to produce structural analogs.

[00196] In a preferred embodiment, candidate bioactive agents include proteins, nucleic acids, and chemical moieties.

[00197] In a preferred embodiment, the candidate bioactive agents are proteins. By "protein" herein is meant at least two covalently attached amino acids, which includes 20 proteins, polypeptides, oligopeptides and peptides. The protein may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures. Thus "amino acid", or "peptide residue", as used herein means both naturally occurring and synthetic amino acids. For example, homo-phenylalanine, citrulline and noreleucine are considered amino acids for the purposes of the invention. "Amino acid" also includes imino 25 acid residues such as proline and hydroxyproline. The side chains may be in either the (R) or the (S) configuration. In the preferred embodiment, the amino acids are in the (S) or L-configuration. If non-naturally occurring side chains are used, non-amino acid substituents may be used, for example to prevent or retard in vivo degradations.

[00198] In a preferred embodiment, the candidate bioactive agents are naturally 30 occurring proteins or fragments of naturally occurring proteins. Thus, for example, cellular extracts containing proteins, or random or directed digests of proteinaceous cellular extracts, may be attached to beads as is more fully described below. In this way libraries of

procaryotic and eucaryotic proteins may be made for screening against any number of targets. Particularly preferred in this embodiment are libraries of bacterial, fungal, viral, and mammalian proteins, with the latter being preferred, and human proteins being especially preferred.

5 **[00199]** In many embodiments, the candidate agents are antibodies to PVRIG, generated as is known in the art and outlined herein.

[00200] In many embodiments, the candidate agents are ECDs of PVRIG, including fusion proteins and variants, as is known in the art and more fully outlined herein.

10 **[00201]** In a preferred embodiment, the candidate bioactive agents are peptides of from about 2 to about 50 amino acids, with from about 5 to about 30 amino acids being preferred, and from about 8 to about 20 being particularly preferred. The peptides may be digests of naturally occurring proteins as is outlined above, random peptides, or "biased" random peptides. By "randomized" or grammatical equivalents herein is meant that each nucleic acid and peptide consists of essentially random nucleotides and amino acids, respectively. Since
15 generally these random peptides (or nucleic acids, discussed below) are chemically synthesized, they may incorporate any nucleotide or amino acid at any position. The synthetic process can be designed to generate randomized proteins or nucleic acids, to allow the formation of all or most of the possible combinations over the length of the sequence, thus forming a library of randomized candidate bioactive proteinaceous agents.

20 **[00202]** The library should provide a sufficiently structurally diverse population of randomized agents to effect a probabilistically sufficient range of diversity to allow binding to a particular target. Accordingly, an interaction library must be large enough so that at least one of its members will have a structure that gives it affinity for the target. Although it is difficult to gauge the required absolute size of an interaction library, nature provides a hint
25 with the immune response: a diversity of 10^{10} different antibodies provides at least one combination with sufficient affinity to interact with most potential antigens faced by an organism. Published in vitro selection techniques have also shown that a library size of 10^{10} to 10^{11} is sufficient to find structures with affinity for the target. A library of all combinations of a peptide 7 to 20 amino acids in length, such as generally proposed herein,
30 has the potential to code for 20^{10} to 20^{20} . Thus, with libraries of 10^{10} to 10^{11} different molecules the present methods allow a "working" subset of a theoretically complete interaction library for 7 amino acids, and a subset of shapes for the

20.sup.20 library. Thus, in a preferred embodiment, at least 10.sup.6, preferably at least 10.sup.7, more preferably at least 10.sup.8 and most preferably at least 10.sup.9 different sequences are simultaneously analyzed in the subject methods. Preferred methods maximize library size and diversity.

5 [00203] In one embodiment, the library is fully randomized, with no sequence preferences or constants at any position. In a preferred embodiment, the library is biased. That is, some positions within the sequence are either held constant, or are selected from a limited number of possibilities. For example, in a preferred embodiment, the nucleotides or amino acid residues are randomized within a defined class, for example, of hydrophobic
10 amino acids, hydrophilic residues, sterically biased (either small or large) residues, towards the creation of cysteines, for cross-linking, prolines for SH-3 domains, serines, threonines, tyrosines or histidines for phosphorylation sites, etc., or to purines, etc.

IV. PVRIG Proteins

[00204] The present invention is directed to the use of human Poliovirus Receptor
15 Related Immunoglobulin Domain Containing Protein, or “PVRIG”, sometimes also referred to herein as “PV protein”, including fragments (including ECDs) as outlined below. The present invention provides PVRIG proteins. “Protein” in this context is used interchangeably with “polypeptide”, and includes peptides as well. PVRIG is a transmembrane domain protein of 326 amino acids in length, with a signal peptide (spanning from amino acid 1 to
20 40), an extracellular domain (spanning from amino acid 41 to 171), a transmembrane domain (spanning from amino acid 172 to 190) and a cytoplasmic domain (spanning from amino acid 191 to 326). The full length human PVRIG protein is shown in Figure 25. There are two methionines that can be start codons, but the mature proteins are identical.

[00205] The full length PVRIG protein is shown in Figure 25, and the wild type has a
25 variety of putative splice variants, some of which are shown in Figure 104.

[00206] Accordingly, as used herein, the term “PVRIG” or “PVRIG protein” or “PVRIG polypeptide” may optionally include any such protein, or variants, conjugates, or fragments thereof, including but not limited to known or wild type PVRIG, as described herein, including but not limited to those depicted in Figure 104. In addition, there are some
30 PVRIG ECD amino acid variants shown in Figure 103, that also find use in the present invention.

[00207] Included in the invention are the sequences shown in Figures 67, Figure 91 and Figure 92, as well as variants thereof possessing at least 80% sequence identity, more preferably at least 90% sequence identity therewith and even more preferably at least 95, 96, 97, 98 or 99% sequence identity therewith, and/or fusions and or conjugates thereof, and/or polynucleotides encoding same.

[00208] The term “soluble” form of PVRIG is also used interchangeably with the terms “soluble ectodomain (ECD)” or “ectodomain” or “extracellular domain (ECD)” as well as “fragments of PVRIG polypeptides”, which may refer broadly to one or more of the sequences shown in Figure 67, Figure 91 and Figure 92.

10 [00209] In particular, the fragments of the extracellular domain of PVRIG can include any sequence corresponding to any portion of or comprising the Ig domain of the extracellular domain of PVRIG, having any sequence corresponding to residues of PVRIG (shown in Figure 26) starting from any position between 21 and 25 and ending at any position between 124 and 128.

15 [00210] The PVRIG proteins contain an immunoglobulin (Ig) domain within the extracellular domain, which is a PVR-like Ig fold domain. The PVR-like Ig fold domain may be responsible for functional counterpart binding, by analogy to the other B7 family members. The PVR-like Ig fold domain of the extracellular domain includes one disulfide bond formed between intra domain cysteine residues, as is typical for this fold and may be
20 important for structure-function. In SEQ ID NO: 3 these cysteines are located at residues 22 and 93 (or 94). In one embodiment, there is provided a soluble fragment of PVRIG; as described in greater detail below with regard to the section on fusion proteins, such a soluble fragment may optionally be described as a first fusion partner. Useful fragments are those that alone or when comprised in fusion proteins or multimerized retain the ability to bind to
25 their natural molecular partner or partners, e.g., expressed on antigen presenting, T and NK cells, and/or which modulate (inhibit or promote) T cell and/or NK cell activation. A PVRIG polypeptide that is a fragment of full-length PVRIG typically has at least 20 percent, 30 percent, 40 percent, 50 percent, 60 percent, 70 percent, 80 percent, 90 percent, 95 percent, 98 percent, 99 percent, 100 percent, or even more than 100 percent of the ability to bind its
30 natural molecular partner(s) and/or the modulation (agonism or antagonism) of one or more of the functional effects of PVRIG on immunity and on specific immune cells as compared to full-length PVRIG. Soluble PVRIG polypeptide fragments are fragments of PVRIG

polypeptides that may be shed, secreted or otherwise extracted from the producing cells. In other embodiments, the soluble fragments of PVRIG polypeptides include fragments of the PVRIG extracellular domain that retain PVRIG biological activity, such as fragments that retain the ability to bind to their natural functional counterpart(s) and/or which modulate (inhibit or promote) T or NK cell activation. The extracellular domain can include 1, 2, 3, 4, or 5 contiguous amino acids from the transmembrane domain, and/or 1, 2, 3, 4, or 5 contiguous amino acids from the signal sequence. Alternatively, the extracellular domain can have 1, 2, 3, 4, 5 or more amino acids removed from the C-terminus, N-terminus, or both.

[00211] In some embodiments the PVRIG extracellular domain polypeptide comprises the amino acid sequence of the PVR-like Ig fold domain as set forth in Figure 104, or fragments or variants thereof. In other embodiments the PVRIG extracellular domain polypeptide consists essentially of the amino acid sequence of the PVR-like Ig fold domain as set forth in Figure 104.

[00212] Optionally, the PVRIG fragments refer also to any one of the polypeptide sequences listed in Figure 67 and Figure 92, which are reasonably expected to comprise functional regions of the PVRIG protein. This expectation is based on a systematic analysis of a set of protein complexes with solved 3d structures, which contained complexes of Ig proteins (for example PDB id 1i85 which describe the complex of CTLA4 and CD86). The intermolecular contact residues from each "co-structure" from each PDB were collected and projected on the sequence of PVRIG. Several regions with clusters of interacting residues supported by several contact maps were identified and synthesized as a series of peptides and are reasonably expected to mimic the structure of the intact full length protein and thereby modulate one or more of the effects of PVRIG on immunity and on specific immune cell types. According to at least some embodiments of the invention, the the PVRIG ECD fragments represented by polypeptide sequences listed in Figure 67, are located as follows (as compared to human PVRIG ECD of Figure 25, counting from the first amino acid of the ECD): PVRIG Fragment A is located at positions 46 to 66; PVRIG Fragment B is located at positions 46 to 79; PVRIG Fragment C is located at positions 63 to 79; PVRIG Fragment D is located at positions 91 to 106; PVRIG Fragment E is located at positions 91 to 114; PVRIG Fragment F is located at positions 11 to 25; PVRIG Fragment G is located at positions 3 to 24; PVRIG Fragment H is located at positions 18 to 36; PVRIG Fragment I is located at positions 29 to 52; PVRIG Fragment J is located at positions 73-98.

[00213] Generally, the PVRIG polypeptide fragments are expressed from nucleic acids that include sequences that encode a signal sequence. The signal sequence is generally cleaved from the immature polypeptide to produce the mature polypeptide lacking the signal sequence. The signal sequence of PVRIG can be replaced by the signal sequence of another polypeptide using standard molecule biology techniques to affect the expression levels, secretion, solubility, or other property of the polypeptide. The signal peptide sequence that is used to replace the PVRIG signal peptide sequence can be any known in the art.

V. PVRIG Fusion Proteins

[00214] In some embodiments, the invention uses PVRIG polypeptides in the form of fusion proteins, wherein the PVRIG polypeptide (generally an ECD) is fused, recombinantly in frame to a fusion partner.

A. Fusion Partners

[00215] In many embodiments, the PVRIG polypeptide is fused to a “fusion partner” (also referred to herein as a “fusion partner moiety”), either directly or indirectly through the use of a linker as is more fully described below. As will be appreciated by those in the art, the fusion partner can be any moiety that is fused to the PVRIG polypeptide for any number of biochemical and/or biological reasons. In some embodiments, the fusion partner moiety increases the half life of the PVRIG fusion protein as is described below. In some embodiments, the fusion partner moiety adds an additional biologic or biochemical function to the PVRIG polypeptide.

[00216] In some embodiments, the fusion partner is generally linked at either the N-terminus or the C-terminus of the PVRIG polypeptide, optionally using a linker as described herein, such that the fusion protein has a formula selected from the group consisting of NH₂-PVRIG polypeptide-fusion partner-COOH, PVRIG polypeptide, NH₂-fusion partner-L-PVRIG polypeptide-COOH, and NH₂-fusion partner-PVRIG polypeptide-COOH.

1. HSA

[00217] In some embodiments, the PVRIG fusion partner is a human serum albumin (HSA), as is known in the art. In particular, fusions to HSA are known to increase serum half life of the fusion protein, as compared to the protein itself. These can include standard flexible linkers such as described herein and shown in Figure 107.

2. Fc Domains

[00218] In some embodiments, the PVRIG polypeptide is fused to a fusion partner that is an Fc domain. By "Fc domain" herein is meant the CH2-CH3 domains of an antibody, as is known in the art, optionally including some or all of the hinge region residues. The Fc domain is generally derived from a human IgG protein, generally IgG1, IgG2, IgG3 or IgG4, the sequences of which are shown in **Error! Reference source not found.** The choice of Fc domain may depend on whether effector function is desired (e.g. such that IgG1 is chosen), or not (IgG2 and IgG4, for example).

[00219] In addition, there are a number of Fc domain variants that can be optionally and independently included as amino acid substitutions. By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with a different amino acid. In particular, in some embodiments, the substitution is to an amino acid that is not naturally occurring at the particular position, either not naturally occurring within the organism or in any organism. For example, the substitution E272Y refers to a variant polypeptide, in this case an Fc variant, in which the glutamic acid at position 272 is replaced with tyrosine. For clarity, a protein which has been engineered to change the nucleic acid coding sequence but not change the starting amino acid (for example exchanging CGG (encoding arginine) to CGA (still encoding arginine) to increase host organism expression levels) is not an "amino acid substitution"; that is, despite the creation of a new gene encoding the same protein, if the protein has the same amino acid at the particular position that it started with, it is not an amino acid substitution.

[00220] In some embodiments, amino acid substitutions can be made in the Fc region, in general for altering binding to FcγR receptors. By "Fc gamma receptor", "FcγR" or "FcγgammaR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an FcγR gene. In humans this family includes but is not limited to FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIIb-NA1 and FcγRIIIb-NA2) (Jefferis et al., 2002, Immunol Lett 82:57-65, entirely incorporated by reference), as well as any undiscovered human FcγRs or FcγR isoforms or allotypes. An FcγR may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. Mouse FcγRs include but are not limited to FcγRI (CD64), FcγRII (CD32),

FcγRIII-1 (CD16), and FcγRIII-2 (CD16-2), as well as any undiscovered mouse FcγRs or FcγR isoforms or allotypes.

[00221] There are a number of useful Fc substitutions that can be made to alter binding to one or more of the FcγR receptors. Substitutions that result in increased binding as well as
5 decreased binding can be useful. For example, it is known that increased binding to FcγRIIIa generally results in increased ADCC (antibody dependent cell-mediated cytotoxicity; the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell. Similarly, decreased binding to FcγRIIb (an inhibitory receptor) can be beneficial as well in some circumstances.

10 Amino acid substitutions that find use in the present invention include those listed in U.S. Ser. Nos. 11/124,620 (particularly FIG. 41) and U.S. Patent No. 6,737,056, both of which are expressly incorporated herein by reference in their entirety and specifically for the variants disclosed therein. Particular variants that find use include, but are not limited to, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E,
15 239D/332E/330Y, 239D, 332E/330L, 299T and 297N.

[00222] In addition, the antibodies of the invention are modified to increase its biological half-life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Pat. No. 6,277,375 to Ward. Alternatively, to increase the biological half-life, the antibody can be
20 altered within the C_{H1} or C_L region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Pat. Nos. 5,869,046 and 6,121,022 by Presta et al. Additional mutations to increase serum half life are disclosed in U.S. Patent Nos. 8,883,973, 6,737,056 and 7,371,826, and include 428L, 434A, 434S, and 428L/434S.

25 [00223] In yet other embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector functions of the antibody. For example, one or more amino acids selected from amino acid residues 234, 235, 236, 237, 297, 318, 320 and 322 can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding
30 ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Pat. Nos. 5,624,821 and 5,648,260, both by Winter et al.

[00224] In another example, one or more amino acids selected from amino acid residues 329, 331 and 322 can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Pat. Nos. 6,194,551
5 by Idusogie et al.

[00225] In another example, one or more amino acid residues within amino acid positions 231 and 239 are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in PCT Publication WO 94/29351 by Bodmer et al.

10 [00226] In yet another example, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids at the following positions: 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 301, 303, 305,
15 307, 309, 312, 315, 320, 322, 324, 326, 327, 329, 330, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438 or 439. This approach is described further in PCT Publication WO 00/42072 by Presta. Moreover, the binding sites on human IgG1 for FcγRI, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields, R. L. et al. (2001) *J. Biol.*
20 *Chem.* 276:6591-6604). Specific mutations at positions 256, 290, 298, 333, 334 and 339 are shown to improve binding to FcγRIII. Additionally, the following combination mutants are shown to improve FcγRIII binding: T256A/S298A, S298A/E333A, S298A/K224A and S298A/E333A/K334A. Furthermore, mutations such as M252Y/S254T/T256E or M428L/N434S improve binding to FcRn and increase antibody circulation half-life (see Chan
25 CA and Carter PJ (2010) *Nature Rev Immunol* 10:301-316).

[00227] In still another embodiment, the glycosylation of an Fc domain is modified. For example, an aglycosylated Fc domain can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen or reduce effector function such as ADCC. Such carbohydrate
30 modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence, for example N297. For example, one or more

amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site.

[00228] Additionally or alternatively, an Fc domain can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an Fc domain having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies according to at least some embodiments of the invention to thereby produce an antibody with altered glycosylation. For example, the cell lines Ms704, Ms705, and Ms709 lack the fucosyltransferase gene, FUT8 (α (1,6) fucosyltransferase), such that antibodies expressed in the Ms704, Ms705, and Ms709 cell lines lack fucose on their carbohydrates. The Ms704, Ms705, and Ms709 FUT8 cell lines are created by the targeted disruption of the FUT8 gene in CHO/DG44 cells using two replacement vectors (see U.S. Patent Publication No. 20040110704 by Yamane et al. and Yamane-Ohnuki et al. (2004) *Biotechnol Bioeng* 87:614-22). As another example, EP 1,176,195 by Hanai et al. describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation by reducing or eliminating the α 1,6 bond-related enzyme. Hanai et al. also describe cell lines which have a low enzyme activity for adding fucose to the N-acetylglucosamine that binds to the Fc region of the antibody or does not have the enzyme activity, for example the rat myeloma cell line YB2/0 (ATCC CRL 1662). PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields, R. L. et al. (2002) *J. Biol. Chem.* 277:26733-26740). PCT Publication WO 99/54342 by Umana et al. describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (e.g., β (1,4)-N-acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana et al. (1999) *Nat. Biotech.* 17:176-180). Alternatively, the fucose residues of the antibody may be cleaved off using a fucosidase enzyme. For example, the fucosidase α -L-fucosidase removes fucosyl residues from antibodies (Tarentino, A. L. et al. (1975) *Biochem.* 14:5516-23).

3. Polyethylene Glycol Fusion Partners

[00229] In some embodiments, the fusion partner moiety is one or more polyethylene glycol (PEG) moieties. As is well known in the art, the modification of therapeutic protein drugs, such as erythropoietin, GM-CSF, interferon alpha and beta and human growth hormone, is frequently done to increase to alter a number of pharmacological properties, including, but not limited to, increased solubility, extended serum half-life, decreased dosage frequency, increased stability, decreased immunogenicity and enhanced protection from proteases.

[00230] As is known in the art, generally a number of PEG molecules are “loaded” onto each protein, depending on a number of factors, and the PEG molecules may be of varying length.

[00231] In general, the PEG moieties are covalently attached directly to the amino acid side chains of the PVRIG polypeptide, using activated PEG derivatives as is well known in the art. That is, generally no additional linkers are used, e.g. there are no extra atoms between the PEG and the amino acid side chain. In other embodiments, linkers such as those outlined below are used.

4. Additional Heterologous Fusion Partners

[00232] In addition to half life extension fusion partner moieties, PVRIG polypeptides can be fused (generally but optionally using linkers as outlined herein), with heterologous polypeptide that give additional biochemical functionalities to the PVRIG polypeptides. These heterologous fusion partner moieties including, but are not limited to, receptors, hormones, cytokines, antigens, B-cell targets, NK cell targets, T cell targets, TNF receptor superfamily members, Hedgehog family members, a receptor tyrosine kinases, a proteoglycan-related molecules, a TGF- β superfamily members, Wnt-related molecules, receptor ligands, dendritic cell targets, myeloid cell targets, monocyte/macrophage cell targets or angiogenesis targets.

[00233] In some embodiments, the fusion partner is a T cell target selected from the group consisting of 2B4/SLAMF4, IL-2 R α , 4-1BB/TNFRSF9, IL-2R β , ALCAM, B7-1/CD80, IL-4R, B7-H3, BLAME/SLAMF8, BTLA, IL-6R, CCR3, IL-7 R α , CCR4, CXCR1/IL-8 RA, CCR5, CCR6, IL-10 R α , CCR7, IL-10 R β , CCR8, IL-12 R β 1, CCR9, IL-12 R β 2, CD2, IL-13R α 1, IL-13, CD3, CD4, ILT2/CD85j, ILT3/CD85k, ILT4/CD85d, ILT5/CD85a, Integrin α 4/CD49d, CD5, Integrin α E/CD103, CD6, Integrin α M/CD11b,

CD8, Integrin α X/CD11c, Integrin β 2/CD18, KIR/CD158, CD27/TNFRSF7, KIR2DL1, CD28, KIR2DL3, CD30/TNFRSF8, KIR2DL4/CD158d, CD31/PECAM-1, KIR2DS4, CD40 Ligand/TNFSF5, LAG-3, CD43, LAIR1, CD45, LAIR2, CD83, Leukotriene B4 R1, CD84/SLAMF5, NCAM-L1, CD94, NKG2A, CD97, NKG2C, CD229/SLAMF3, NKG2D, CD2F-10/SLAMF9, NT-4, CD69, NTB-A/SLAMF6, Common γ Chain/IL-2 R γ , Osteopontin, CRACC/SLAMF7, PD-1, CRTAM, PSGL-1, CTLA-4, RANK/TNFRSF11A, CX3CR1, CX3CL1, L-Selectin, CXCR3, SIRP β 1, CXCR4, SLAM, CXCR6, TCCR/WSX-1, DNAM-1, Thymopoietin, EMMPRIN/CD147, TIM-1, EphB6, TIM-2, Fas/TNFRSF6, TIM-3, Fas Ligand/TNFSF6, TIM-4, Fc γ RIII/CD16, TIM-6, GITR/TNFRSF18, TNF R1/TNFRSF1A, Granulysin, TNF R11/TNFRSF1B, HVEM/TNFRSF14, TRAIL R1/TNFRSF10A, ICAM-1/CD54, TRAIL R2/TNFRSF10B, ICAM-2/CD102, TRAIL R3/TNFRSF10C, IFN- γ R1, TRAIL R4/TNFRSF10D, IFN- γ R2, TSLP, IL-1 RI and TSLP R.

[00234] In some embodiments, the heterologous fusion partner moiety is a T cell target protein. These proteins include, but are not limited to, 2B4/SLAMF4, IL-2 R α , 4-1BB/TNFRSF9, IL-2R β , ALCAM, B7-1/CD80, IL-4R, B7-H3, BLAME/SLAMF8, BTLA, IL-6R, CCR3, IL-7 R α , CCR4, CXCR1/IL-8 RA, CCR5, CCR6, IL-10 R α , CCR7, IL-10 R β , CCR8, IL-12 R β 1, CCR9, IL-12 R β 2, CD2, IL-13R α 1, IL-13, CD3, CD4, ILT2/CD85j, ILT3/CD85k, ILT4/CD85d, ILT5/CD85a, Integrin α 4/CD49d, CD5, Integrin α E/CD103, CD6, Integrin α M/CD11b, CD8, Integrin α X/CD11c, Integrin β 2/CD18, KIR/CD158, CD27/TNFRSF7, KIR2DL1, CD28, KIR2DL3, CD30/TNFRSF8, KIR2DL4/CD158d, CD31/PECAM-1, KIR2DS4, CD40 Ligand/TNFSF5, LAG-3, CD43, LAIR1, CD45, LAIR2, CD83, Leukotriene B4 R1, CD84/SLAMF5, NCAM-L1, CD94, NKG2A, CD97, NKG2C, CD229/SLAMF3, NKG2D, CD2F-10/SLAMF9, NT-4, CD69, NTB-A/SLAMF6, Common γ Chain/IL-2 R γ , Osteopontin, CRACC/SLAMF7, PD-1, CRTAM, PSGL-1, CTLA-4, RANK/TNFRSF11A, CX3CR1, CX3CL1, L-Selectin, CXCR3, SIRP β 1, CXCR4, SLAM, CXCR6, TCCR/WSX-1, DNAM-1, Thymopoietin, EMMPRIN/CD147, TIM-1, EphB6, TIM-2, Fas/TNFRSF6, TIM-3, Fas Ligand/TNFSF6, TIM-4, Fc γ RIII/CD16, TIM-6, GITR/TNFRSF18, TNF R1/TNFRSF1A, Granulysin, TNF R11/TNFRSF1B, HVEM/TNFRSF14, TRAIL R1/TNFRSF10A, ICAM-1/CD54, TRAIL R2/TNFRSF10B, ICAM-2/CD102, TRAIL R3/TNFRSF10C, IFN- γ R1, TRAIL R4/TNFRSF10D, IFN- γ R2, TSLP, IL-1 RI and TSLP R.

[00235] In some embodiments, the heterologous fusion partner moiety is a monocyte/macrophage cell target. These proteins include, but are not limited to, B7-1/CD80,

ILT4/CD85d, B7-H1, ILT5/CD85a, Common β Chain, Integrin α 4/CD49d,
 BLAME/SLAMF8, Integrin α X/CD11c, CCL6/C10, Integrin β 2/CD18, CD155/PVR,
 Integrin β 3/CD61, CD31/PECAM-1, Latexin, CD36/SR-B3, Leukotriene B4 R1,
 CD40/TNFRSF5, LIMP2/SR-B2, CD43, LMIR1/CD300A, CD45, LMIR2/CD300c, CD68,
 5 LMIR3/CD300LF, CD84/SLAMF5, LMIR5/CD300LB, CD97, LMIR6/CD300LE, CD163,
 LRP-1, CD2F-10/SLAMF9, MARCO, CRACC/SLAMF7, MD-1, ECF-L, MD-2,
 EMMPRIN/CD147, MGL2, Endoglin/CD105, Osteoactivin/GPNMB, Fc γ R1/CD64,
 Osteopontin, Fc γ RIIB/CD32b, PD-L2, Fc γ RIIC/CD32c, Siglec-3/CD33, Fc γ RIIA/CD32a,
 SIGNR1/CD209, Fc γ RIII/CD16, SLAM, GM-CSF R α , TCCR/WSX-1, ICAM-2/CD102,
 10 TLR3, IFN- γ R1, TLR4, IFN- γ R2, TREM-1, IL-1 RII, TREM-2, ILT2/CD85j, TREM-3,
 ILT3/CD85k, TREML1/TLT-1, 2B4/SLAMF4, IL-10 R α , ALCAM, IL-10 R β ,
 Aminopeptidase N/ANPEP, ILT2/CD85j, Common β Chain, ILT3/CD85k, C1q R1/CD93,
 ILT4/CD85d, CCR1, ILT5/CD85a, CCR2, Integrin α 4/CD49d, CCR5, Integrin α M/CD11b,
 CCR8, Integrin α X/CD11c, CD155/PVR, Integrin β 2/CD18, CD14, Integrin β 3/CD61,
 15 CD36/SR-B3, LAIR1, CD43, LAIR2, CD45, Leukotriene B4 R1, CD68, LIMP2/SR-B2,
 CD84/SLAMF5, LMIR1/CD300A, CD97, LMIR2/CD300c, CD163, LMIR3/CD300LF,
 Coagulation Factor III/Tissue Factor, LMIR5/CD300LB, CX3CR1, CX3CL1,
 LMIR6/CD300LE, CXCR4, LRP-1, CXCR6, M-CSF R, DEP-1/CD148, MD-1, DNAM-1,
 MD-2, EMMPRIN/CD147, MMR, Endoglin/CD105, NCAM-L1, Fc γ R1/CD64, PSGL-1, Fc
 20 γ RIII/CD16, RP105, G-CSF R, L-Selectin, GM-CSF R α , Siglec-3/CD33,
 HVEM/TNFRSF14, SLAM, ICAM-1/CD54, TCCR/WSX-1, ICAM-2/CD102, TREM-1, IL-
 6 R, TREM-2, CXCR1/IL-8 RA, TREM-3 and TREML1/TLT-1.

[00236] In some embodiments, the heterologous fusion partner moiety is a Dendritic
 cell target, including, but not limited to, CD36/SR-B3, LOX-1/SR-E1, CD68, MARCO,
 25 CD163, SR-AI/MSR, CD5L, SREC-I, CL-P1/COLEC12, SREC-II, LIMP2/SR-B2, RP105,
 TLR4, TLR1, TLR5, TLR2, TLR6, TLR3, TLR9, 4-1BB Ligand/TNFSF9, IL-12/IL-23 p40,
 4-Amino-1,8-naphthalimide, ILT2/CD85j, CCL21/6CKine, ILT3/CD85k, 8-oxo-dG,
 ILT4/CD85d, 8D6A, ILT5/CD85a, A2B5, Integrin α 4/CD49d, Aag, Integrin β 2/CD18,
 AMICA, Langerin, B7-2/CD86, Leukotriene B4 R1, B7-H3, LMIR1/CD300A,
 30 BLAME/SLAMF8, LMIR2/CD300c, C1q R1/CD93, LMIR3/CD300LF, CCR6,
 LMIR5/CD300LB, CCR7, LMIR6/CD300LE, CD40/TNFRSF5, MAG/Siglec-4a, CD43,
 MCAM, CD45, MD-1, CD68, MD-2, CD83, MDL-1/CLEC5A, CD84/SLAMF5, MMR,
 CD97, NCAM-L1, CD2F-10/SLAMF9, Osteoactivin/GPNMB, Chem 23, PD-L2, CLEC-1,

RP105, CLEC-2, Siglec-2/CD22, CRACC/SLAMF7, Siglec-3/CD33, DC-SIGN, Siglec-5, DC-SIGNR/CD299, Siglec-6, DCAR, Siglec-7, DCIR/CLEC4A, Siglec-9, DEC-205, Siglec-10, Dectin-1/CLEC7A, Siglec-F, Dectin-2/CLEC6A, SIGNR1/CD209, DEP-1/CD148, SIGNR4, DLEC, SLAM, EMMPRIN/CD147, TCCR/WSX-1, Fc γ R1/CD64, TLR3, Fc γ RIIB/CD32b, TREM-1, Fc γ RIIC/CD32c, TREM-2, Fc γ RIIA/CD32a, TREM-3, Fc γ RIII/CD16, TREML1/TLT-1, ICAM-2/CD102 and Vanilloid R1.

[00237] In some embodiments, the heterologous fusion partner moiety is a TNF receptor superfamily member, including, but not limited to, 4-1BB/TNFRSF9, NGF R/TNFRSF16, BAFF R/TNFRSF13C, Osteoprotegerin/TNFRSF11B, BCMA/TNFRSF17, OX40/TNFRSF4, CD27/TNFRSF7, RANK/TNFRSF11A, CD30/TNFRSF8, RELT/TNFRSF19L, CD40/TNFRSF5, TACI/TNFRSF13B, DcR3/TNFRSF6B, TNF RI/TNFRSF1A, DcTRAIL R1/TNFRSF23, TNF RII/TNFRSF1B, DcTRAIL R2/TNFRSF22, TRAIL R1/TNFRSF10A, DR3/TNFRSF25, TRAIL R2/TNFRSF10B, DR6/TNFRSF21, TRAIL R3/TNFRSF10C, EDAR, TRAIL R4/TNFRSF10D, Fas/TNFRSF6, TROY/TNFRSF19, GITR/TNFRSF18, TWEAK R/TNFRSF12, HVEM/TNFRSF14, XEDAR, Lymphotoxin β R/TNFRSF3, 4-1BB Ligand/TNFRSF9, Lymphotoxin, APRIL/TNFRSF13, Lymphotoxin β /TNFRSF3, BAFF/TNFRSF13C, OX40 Ligand/TNFRSF4, CD27 Ligand/TNFRSF7, TL1A/TNFRSF15, CD30 Ligand/TNFRSF8, TNF- α /TNFRSF1A, CD40 Ligand/TNFRSF5, TNF- β /TNFRSF1B, EDA-A2, TRAIL/TNFRSF10, Fas Ligand/TNFRSF6, TRANCE/TNFRSF11, GITR Ligand/TNFRSF18, TWEAK/TNFRSF12 and LIGHT/TNFRSF14.

[00238] In some embodiments, the heterologous fusion partner moiety is a Hedgehog family member selected from the group consisting of Patched and Smoothened.

[00239] In some embodiments, the heterologous fusion partner moiety is a receptor tyrosine kinase including, but not limited to, Ax1, FGF R4, C1q R1/CD93, FGF R5, DDR1, Flt-3, DDR2, HGF R, Dtk, IGF-I R, EGF R, IGF-II R, Eph, INSR, EphA1, Insulin R/CD220, EphA2, M-CSF R, EphA3, Mer, EphA4, MSP R/Ron, EphA5, MuSK, EphA6, PDGF R α , EphA7, PDGF R β , EphA8, Ret, EphB1, ROR1, EphB2, ROR2, EphB3, SCF R/c-kit, EphB4, Tie-1, EphB6, Tie-2, ErbB2, TrkA, ErbB3, TrkB, ErbB4, TrkC, FGF R1, VEGF R1/Flt-1, FGF R2, VEGF R2/Flk-1, FGF R3 and VEGF R3/Flt-4.

[00240] In some embodiments, the heterologous fusion partner moiety is a Transforming Growth Factor (TGF)- β superfamily member selected from the group consisting of Activin RIA/ALK-2, GFR α -1, Activin RIB/ALK-4, GFR α 2, Activin RHA,

GFR α -3, Activin RIIB, GFR α -4, ALK-1, MIS RII, ALK-7, Ret, BMPR-IA/ALK-3, TGF- β R1/ALK-5, BMPR-IB/ALK-6, TGF- β RII, BMPR-II, TGF- β RIIB, Endoglin/CD 105 and TGF- β RIII.

[00241] In some embodiments, the heterologous fusion partner moiety is a Wnt-related molecule selected from the group consisting of Frizzled-1, Frizzled-8, Frizzled-2, Frizzled-9, Frizzled-3, sFRP-1, Frizzled-4, sFRP-2, Frizzled-5, sFRP-3, Frizzled-6, sFRP-4, Frizzled-7, MFRP, LRP 5, LRP 6, Wnt-1, Wnt-8a, Wnt-3a, Wnt-10b, Wnt-4, Wnt-11, Wnt-5a, Wnt-9a and Wnt-7a.

[00242] In some embodiments, the heterologous fusion partner moiety is a receptor ligand selected from the group consisting of 4-1BB Ligand/TNFSF9, Lymphotoxin, APRIL/TNFSF13, Lymphotoxin β /TNFSF3, BAFF/TNFSF13C, OX40 Ligand/TNFSF4, CD27 Ligand/TNFSF7, TL1A/TNFSF15, CD30 Ligand/TNFSF8, TNF- α /TNFSF1A, CD40 Ligand/TNFSF5, TNF- β /TNFSF1B, EDA-A2, TRAIL/TNFSF10, Fas Ligand/TNFSF6, TRANCE/TNFSF11, GITR Ligand/TNFSF18, TWEAK/TNFSF12, LIGHT/TNFSF14, Amphiregulin, NRG1 isoform GGF2, Betacellulin, NRG1 Isoform SMDF, EGF, NRG1- α /HRG1- α , Epigen, NRG1- β 1/HRG1- β 1, Epregrulin, TGF- α , HB-EGF, TMEFF1/Tomoregulin-1, Neuregulin-3, TMEFF2, IGF-I, IGF-II, Insulin, Activin A, Activin B, Activin AB, Activin C, BMP-2, BMP-7, BMP-3, BMP-8, BMP-3b/GDF-10, BMP-9, BMP-4, BMP-15, BMP-5, Decapentaplegic, BMP-6, GDF-1, GDF-8, GDF-3, GDF-9, GDF-5, GDF-11, GDF-6, GDF-15, GDF-7, Artemin, Neurturin, GDNF, Persephin, TGF- β , TGF- β 2, TGF- β 1, TGF- β 3, LAP (TGF- β 1), TGF- β 5, Latent TGF- β 1, Latent TGF- β bp1, TGF- β 1.2, Lefty, Nodal, MIS/AMH, FGF acidic, FGF-12, FGF basic, FGF-13, FGF-3, FGF-16, FGF-4, FGF-17, FGF-5, FGF-19, FGF-6, FGF-20, FGF-8, FGF-21, FGF-9, FGF-23, FGF-10, KGF/FGF-7, FGF-11, Neuropilin-1, P1GF, Neuropilin-2, P1GF-2, PDGF, PDGF-A, VEGF, PDGF-B, VEGF-B, PDGF-C, VEGF-C, PDGF-D, VEGF-D and PDGF-AB.

[00243] In some embodiments, the heterologous fusion partner moiety is a tumor antigen selected from the group consisting of Squamous Cell Carcinoma Antigen 1 (SCCA-1), (PROTEIN T4-A), Squamous Cell Carcinoma Antigen 2 (SCCA-2), Ovarian carcinoma antigen CA125 (1A1-3B; KIAA0049), MUCIN 1 (TUMOR-ASSOCIATED MUCIN; Carcinoma-Associated Mucin; Polymorphic Epithelial Mucin; PEM; PEMT; EPISIALIN; Tumor-Associated Epithelial Membrane Antigen; EMA; H23AG; Peanut-Reactive Urinary Mucin; PUM; and Breast Carcinoma-Associated Antigen DF3), CTCL tumor antigen sel-1,

CTCL tumor antigen se14-3, CTCL tumor antigen se20-4, CTCL tumor antigen se20-9, CTCL tumor antigen se33-1, CTCL tumor antigen se37-2, CTCL tumor antigen se57-1, CTCL tumor antigen se89-1, Prostate-specific membrane antigen, 5T4 oncofetal trophoblast glycoprotein, Orf73 Kaposi's sarcoma-associated herpesvirus, MAGE-C1 (cancer/testis antigen CT7), MAGE-B1 ANTIGEN (MAGE-XP Antigen; DAM10), MAGE-B2 Antigen (DAM6), MAGE-2 ANTIGEN, MAGE-4a antigen, MAGE-4b antigen, Colon cancer antigen NY-CO-45, Lung cancer antigen NY-LU-12 variant A, Cancer associated surface antigen, Adenocarcinoma antigen ART1, Paraneoplastic associated brain-testis-cancer antigen (onconeural antigen MA2; paraneoplastic neuronal antigen), Neuro-oncological ventral antigen 2 (NOVA2), Hepatocellular carcinoma antigen gene 520, Tumor-Associated Antigen CO-029, Tumor-associated antigen MAGE-X2, Synovial sarcoma, X breakpoint 2, Squamous cell carcinoma antigen recognized by T cell, Serologically defined colon cancer antigen 1, Serologically defined breast cancer antigen NY-BR-15, Serologically defined breast cancer antigen NY-BR-16, Chromogranin A, parathyroid secretory protein 1, DUPAN-2, CA 19-9, CA 72-4, CA 195 and L6.

[00244] In some embodiments, the heterologous fusion partner moiety is a B cell target selected from the group consisting of CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD38, CD39, CD40, CD72, CD73, CD74, CDw75, CDw76, CD77, CD78, CD79a/b, CD80, CD81, CD82, CD83, CD84, CD85, CD86, CD89, CD98, CD126, CD127, CDw130, CD138 and CDw150.

[00245] In some embodiments, the heterologous fusion partner moiety is an angiogenesis target selected from the group consisting of Angiopoietin-1, Angiopoietin-like 2, Angiopoietin-2, Angiopoietin-like 3, Angiopoietin-3, Angiopoietin-like 7/CDT6, Angiopoietin-4, Tie-1, Angiopoietin-like 1, Tie-2, Angiogenin, iNOS, Coagulation Factor III/Tissue Factor, nNOS, CTGF/CCN2, NOV/CCN3, DANCE, OSM, EDG-1, Plfr, EG-VEGF/PK1, Proliferin, Endostatin, ROBO4, Erythropoietin, Thrombospondin-1, Kininostatin, Thrombospondin-2, MFG-E8, Thrombospondin-4, Nitric Oxide, VG5Q, eNOS, EphA1, EphA5, EphA2, EphA6, EphA3, EphA7, EphA4, EphA8, EphB1, EphB4, EphB2, EphB6, EphB3, Ephrin-A1, Ephrin-A4, Ephrin-A2, Ephrin-A5, Ephrin-A3, Ephrin-B1, Ephrin-B3, Ephrin-B2, FGF acidic, FGF-12, FGF basic, FGF-13, FGF-3, FGF-16, FGF-4, FGF-17, FGF-5, FGF-19, FGF-6, FGF-20, FGF-8, FGF-21, FGF-9, FGF-23, FGF-10, KGF/FGF-7, FGF-11, FGF R1, FGF R4, FGF R2, FGF R5, FGF R3, Neuropilin-1, Neuropilin-2, Semaphorin 3A, Semaphorin 6B, Semaphorin 3C, Semaphorin 6C, Semaphorin

3E, Semaphorin 6D, Semaphorin 6A, Semaphorin 7A, MMP, MMP-11, MMP-1, MMP-12, MMP-2, MMP-13, MMP-3, MMP-14, MMP-7, MMP-15, MMP-8, MMP-16/MT3-MMP, MMP-9, MMP-24/MT5-MMP, MMP-10, MMP-25/MT6-MMP, TIMP-1, TIMP-3, TIMP-2, TIMP-4, ACE, IL-13 R α 1, IL-13, C1q R1/CD93, Integrin α 4/CD49d, VE-Cadherin, Integrin β 2/CD18, CD31/PECAM-1, KLF4, CD36/SR-B3, LYVE-1, CD151, MCAM, CL-P1/COLEC12, Nectin-2/CD112, Coagulation Factor III/Tissue Factor, E-Selectin, D6, P-Selectin, DC-SIGNR/CD299, SLAM, EMMPRIN/CD147, Tie-2, Endoglin/CD105, TNF RI/TNFRSF1A, EPCR, TNF RII/TNFRSF1B, Erythropoietin R, TRAIL R1/TNFRSF10A, ESAM, TRAIL R2/TNFRSF10B, FABP5, VCAM-1, ICAM-1/CD54, VEGF R2/Flk-1, ICAM-2/CD102, VEGF R3/Flt-4, IL-1 RI and VG5Q.

B. Linkers

[00246] In many embodiments of fusion proteins comprising a PVRIG polypeptide and a fusion partner moiety, optional flexible linkers are used to join the sequences in frame. A "flexible linker" herein refers to a peptide or polypeptide containing two or more amino acid residues joined by peptide bond(s) that provides increased rotational freedom for two polypeptides linked thereby than the two linked polypeptides would have in the absence of the flexible linker. Such rotational freedom allows each component of the fusion protein to interact with its intended target without hindrance. Generally these linkers are mixtures of glycine and serine, such as $-(GGGS)_n-$, where n is from 1, 2, 3, 4, or 5.

[00247] Exemplary flexible peptides/polypeptides include, but are not limited to, the amino acid sequences shown in Figure 105. Additional flexible peptide/polypeptide sequences are well known in the art. Other suitable peptide linker domains optionally include the TEV linker ENLYFQG, a linear epitope recognized by the Tobacco Etch Virus protease. Exemplary peptides/polypeptides include, but are not limited to, GSENLVYFQGSG (SEQ ID NO:84). Other suitable peptide linker domains include helix forming linkers such as Ala-(Glu-Ala-Ala-Ala-Lys) $_n$ -Ala ($n=1-5$). Additional helix forming peptide/polypeptide sequences are well known in the art.

C. Particular Constructs

[00248] In one embodiment, the linker domain contains the hinge region of an immunoglobulin. In a further embodiment, the hinge region is derived from a human immunoglobulin. Suitable human immunoglobulins that the hinge can be derived from include IgG, IgD and IgA. In a further embodiment, the hinge region is derived from human IgG. Amino acid sequences of immunoglobulin hinge regions and other domains are well

known in the art. In one embodiment, PVRIG fusion polypeptides contain the hinge, CH2 and CH3 regions of a human immunoglobulin C γ 1 chain, optionally with the Cys at position 220 (according to full length human IgG1, position 5 in SEQ ID NO:64) replaced with a Ser (

[00249] BF) having at least 85%, 90%, 95%, 99% or 100% sequence homology to amino acid sequence set forth in SEQ ID NO:XX:

[00250] EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00251] The hinge can be further shortened to remove amino acids 1, 2, 3, 4, 5, or combinations thereof of any one of

[00252] BE to BG and. In one embodiment, amino acids 1-5 of any one of

[00253] BE to BG are deleted. Exemplary PVRIG fusion polypeptides comprised of the hinge, CH2 and CH3 regions of a human immunoglobulin C γ 1 chain with the Cys at position 220 replaced with a Ser are set forth in

[00254] AZ.

[00255] In another embodiment, the PVRIG fusion polypeptides contain the CH2 and CH3 regions of human immunoglobulin C γ 1 chain having N297A mutation (

[00256] BG) or the human Fc carrying the C220S, C226 and C229S mutations (

[00257] BL).

[00258] In another embodiment, PVRIG fusion polypeptides contain the CH2 and CH3 regions of a human immunoglobulin C γ 1 chain having at least 85%, 90%, 95%, 99% or 100% sequence homology to amino acid sequence set forth in

[00259] BH:

[00260] APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

K

[00261] In another embodiment, the PVRIG fusion polypeptides contain the hinge, CH2 and CH3 regions of a murine immunoglobulin C γ 2a chain at least 85%, 90%, 95%, 99% or 100% sequence homology to amino acid sequence set forth in

[00262] BI:

5 [00263] EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMSLSPIVTCVVVD
VSEDDPDVQISWVFNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKC
KVNNKDLPAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIY
VEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLH
NHHTTKSFSRTPGK.

10 [00264] In another embodiment, the PVRIG fusion polypeptides contain the CH2 and CH3 regions of a murine immunoglobulin C γ 2a chain having N297A mutation (

[00265] BJ) or the murine Fc without the Hinge (

[00266] BK).

[00267] In another embodiment, the linker domain optionally contains a hinge region
15 of an immunoglobulin as described above, and further includes one or more additional immunoglobulin domains.

VI. Nucleic Acid Compositions

[00268] Nucleic acid compositions encoding the PVRIG polypeptides of the invention are also provided, as well as expression vectors containing the nucleic acids and host cells
20 transformed with the nucleic acid and/or expression vector compositions.

[00269] The nucleic acid compositions that encode the PVRIG polypeptides are generally put into a single expression vector as is known in the art, transformed into host cells, where they are expressed to form the PVRIG proteins (or fusion proteins) of the invention. The nucleic acids can be put into expression vectors that contain the appropriate
25 transcriptional and translational control sequences, including, but not limited to, signal and secretion sequences, regulatory sequences, promoters, origins of replication, selection genes, etc.

[00270] For example, to express the protein DNA, DNAs can be obtained by standard molecular biology techniques (e.g., PCR amplification or gene synthesis) and the DNAs can
30 be inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively

linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The protein genes are inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the gene fragment and vector, or blunt end ligation if no restriction sites are present). Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the protein (including fusion proteins) from a host cell. The gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

[00271] In addition to the protein genes, the recombinant expression vectors according to at least some embodiments of the invention carry regulatory sequences that control the expression of the genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the genes. Such regulatory sequences are described, for example, in Goeddel ("Gene Expression Technology", *Methods in Enzymology* 185, Academic Press, San Diego, Calif. (1990)). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences, may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV), Simian Virus 40 (SV40), adenovirus, (e.g., the adenovirus major late promoter (AdMLP) and polyoma. Alternatively, nonviral regulatory sequences may be used, such as the ubiquitin promoter or β -globin promoter. Still further, regulatory elements composed of sequences from different sources, such as the SR α promoter system, which contains sequences from the SV40 early promoter and the long terminal repeat of human T cell leukemia virus type 1 (Takebe, Y. et al. (1988) *Mol. Cell. Biol.* 8:466-472).

[00272] In addition to the protein genes and regulatory sequences, the recombinant expression vectors according to at least some embodiments of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells

(e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, e.g., U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel et al.). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

[00273] For expression of the proteins of the invention, an expression vector encoding the protein is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the proteins according to at least some embodiments of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred.

[00274] Preferred mammalian host cells for expressing the recombinant proteins according to at least some embodiments of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, e.g., as described in R. J. Kaufman and P. A. Sharp (1982) *Mol. Biol.* 159:601-621), NSO myeloma cells, COS cells and SP2 cells. In particular, for use with NSO myeloma cells, another preferred expression system is the GS gene expression system disclosed in WO 87/04462, WO 89/01036 and EP 338,841. When recombinant expression vectors encoding protein genes are introduced into mammalian host cells, the proteins are produced by culturing the host cells for a period of time sufficient to allow for expression of the protein in the host cells or, more preferably, secretion of the protein into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

[00275] PVRIG protein coding sequences of interest include those encoded by native sequences, as well as nucleic acids that, by virtue of the degeneracy of the genetic code, are not identical in sequence to the disclosed nucleic acids, and variants thereof. Variant polypeptides can include amino acid substitutions as discussed herein. Techniques for in

vitro mutagenesis of cloned genes are known. Also included in the subject invention are polypeptides that have been modified using ordinary molecular biological techniques so as to improve their resistance to proteolytic degradation or to optimize solubility properties or to render them more suitable as a therapeutic agent.

5 [00276] The invention further provides nucleic acids which encode a PVRIG protein according to the invention, or a fragment or conjugate thereof. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, e.g., other cellular nucleic acids or proteins, by
10 standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art. See, F. Ausubel, et al., ed. (1987) *Current Protocols in Molecular Biology*, Greene Publishing and Wiley Interscience, New York. A nucleic acid according to at least some embodiments of the invention can be, for example, DNA or RNA and may or may not contain intronic sequences.

15 VII. **Formulations**

[00277] The therapeutic compositions used in the practice of the foregoing methods can be formulated into pharmaceutical compositions comprising a carrier suitable for the desired delivery method. Suitable carriers include any material that when combined with the therapeutic composition retains the anti-tumor function of the therapeutic composition and is
20 generally non-reactive with the patient's immune system. Examples include, but are not limited to, any of a number of standard pharmaceutical carriers such as sterile phosphate buffered saline solutions, bacteriostatic water, and the like (see, generally, Remington's *Pharmaceutical Sciences* 16th Edition, A. Osal., Ed., 1980). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include
25 buffers such as phosphate, citrate, acetate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues)
30 polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as

sucrose, mannitol, trehalose or sorbitol; sweeteners and other flavoring agents; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; additives; coloring agents; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN.TM., PLURONICS.TM. or polyethylene glycol (PEG).

[00278] In a preferred embodiment, the pharmaceutical composition that comprises the antibodies of the invention may be in a water-soluble form, such as being present as pharmaceutically acceptable salts, which is meant to include both acid and base addition salts. "Pharmaceutically acceptable acid addition salt" refers to those salts that retain the biological effectiveness of the free bases and that are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like. "Pharmaceutically acceptable base addition salts" include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. The formulations to be used for in vivo administration are preferably sterile. This is readily accomplished by filtration through sterile filtration membranes or other methods.

[00279] Administration of the pharmaceutical composition comprising antibodies of the present invention, preferably in the form of a sterile aqueous solution, may be done in a variety of ways, including, but not limited to subcutaneously and intravenously. Subcutaneous administration may be preferable in some circumstances because the patient may self-administer the pharmaceutical composition. Many protein therapeutics are not sufficiently potent to allow for formulation of a therapeutically effective dose in the maximum acceptable volume for subcutaneous administration. This problem may be addressed in part by the use of protein formulations comprising arginine-HCl, histidine, and

polysorbate (see WO 04091658). Fc polypeptides of the present invention may be more amenable to subcutaneous administration due to, for example, increased potency, improved serum half-life, or enhanced solubility.

[00280] As is known in the art, protein therapeutics are often delivered by IV infusion or bolus. The antibodies of the present invention may also be delivered using such methods. For example, administration may venious be by intravenous infusion with 0.9% sodium chloride as an infusion vehicle.

[00281] In addition, any of a number of delivery systems are known in the art and may be used to administer the Fc variants of the present invention. Examples include, but are not limited to, encapsulation in liposomes, microparticles, microspheres (eg. PLA/PGA microspheres), and the like. Alternatively, an implant of a porous, non-porous, or gelatinous material, including membranes or fibers, may be used. Sustained release systems may comprise a polymeric material or matrix such as polyesters, hydrogels, poly(vinylalcohol), polylactides, copolymers of L-glutamic acid and ethyl-L-gutamate, ethylene-vinyl acetate, lactic acid-glycolic acid copolymers such as the LUPRON DEPOT.RTM., and poly-D-(-)-3-hydroxybutyric acid. The antibodies disclosed herein may also be formulated as immunoliposomes. A liposome is a small vesicle comprising various types of lipids, phospholipids and/or surfactant that is useful for delivery of a therapeutic agent to a mammal. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., 1985, Proc Natl Acad Sci USA, 82:3688; Hwang et al., 1980, Proc Natl Acad Sci USA, 77:4030; U.S. Pat. No. 4,485,045; U.S. Pat. No. 4,544,545; and PCT WO 97/38731. Liposomes with enhanced circulation time are disclosed in U.S. Pat. No. 5,013,556. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. A chemotherapeutic agent or other therapeutically active agent is optionally contained within the liposome (Gabizon et al., 1989, J National Cancer Inst 81:1484).

[00282] The antibodies may also be entrapped in microcapsules prepared by methods including but not limited to coacervation techniques, interfacial polymerization (for example using hydroxymethylcellulose or gelatin-microcapsules, or poly-(methylmethacrylate)

microcapsules), colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), and macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed., 1980. Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymer, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and gamma ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT.RTM. (which are injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), poly-D-(-)-3-hydroxybutyric acid, and ProLease.RTM. (commercially available from Alkermes), which is a microsphere-based delivery system composed of the desired bioactive molecule incorporated into a matrix of poly-DL-lactide-co-glycolide (PLG).

[00283] The dosing amounts and frequencies of administration are, in a preferred embodiment, selected to be therapeutically or prophylactically effective. As is known in the art, adjustments for protein degradation, systemic versus localized delivery, and rate of new protease synthesis, as well as the age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

[00284] The concentration of the antibody in the formulation may vary from about 0.1 to 100 weight %. In a preferred embodiment, the concentration of the Fc variant is in the range of 0.003 to 1.0 molar. In order to treat a patient, a therapeutically effective dose of the Fc variant of the present invention may be administered. By "therapeutically effective dose" herein is meant a dose that produces the effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. Dosages may range from 0.0001 to 100 mg/kg of body weight or greater, for example 0.1, 1, 10, or 50 mg/kg of body weight, with 1 to 10 mg/kg being preferred.

VIII. Methods of Using Compositions of the Invention in Treatment

[00285] As discussed herein, PVRIG is involved in the immuno-oncology pathway, which means that manipulating certain signaling pathways can have two different effects. On one hand, the PVRIG protein suppresses T cell activation and one or more of a number of

other pathways, through binding to its ligands, including PVLR2. Thus, by inhibiting the interaction of PVRIG and PVLR2, for example using antibodies to PVRIG, the suppression is alleviated, thereby increasing an immune response to allow treatment of conditions for which a stronger immune response is desired, such as cancer and pathogen infection. This is referred to as a “immuno-stimulatory” response. On the other hand, by increasing the amount of PVRIG in a patient, such as by adding PVRIG ECD polypeptides, the suppression is increased, thereby decreasing the immune response to allow treatment of conditions for which a decreased immune response is desired, such as autoimmune diseases and inflammation.

10 [00286] Accordingly, once made, the PVRIG proteins of the invention find use in a variety of applications, including using them in screening assays for additional immunomodulatory agents, as well as treatment of patients as is more fully outlined below.

[00287] With regard to the immuno-stimulatory treatments using anti-PVRIG antibodies, reference is made to USSN XX, filed February 19, 2016, entitled “Anti-PVRIG
15 Antibodies and Methods of Use, claiming priority to USSN 62/118,208, filed February 19, 2015, and to USSN 62/141,120, filed March 31, 2015, and to USSN 62/235,823, filed October 1, 2015, all of which is expressly incorporated by reference in its entirety herein.

A. Treatment

[00288] The present invention provides methods of treating a number of diseases
20 and/or conditions associated with an immune condition. An “immune condition” includes patients who would benefit from immunostimulatory action, such as cancer or pathogen infection, as well as patients who would benefit from immunoinhibitory action, such as autoimmune diseases and inflammation.

[00289] In some embodiments, the PVRIG polypeptides of the invention are used to
25 treat autoimmune diseases. Suitable autoimmune diseases include, but are not limited to, multiple sclerosis, including relapsing-remitting multiple sclerosis, primary progressive multiple sclerosis, and secondary progressive multiple sclerosis, progressive relapsing multiple sclerosis, chronic progressive multiple sclerosis, transitional/progressive multiple sclerosis, rapidly worsening multiple sclerosis, clinically-definite multiple sclerosis,
30 malignant multiple sclerosis, also known as Marburg's Variant, acute multiple sclerosis, conditions relating to multiple sclerosis, psoriasis, rheumatoid arthritis, psoriatic arthritis, gout and pseudo-gout, juvenile idiopathic arthritis, Still's disease, rheumatoid vasculitis,

conditions relating to rheumatoid arthritis, discoid lupus erythematosus, lupus arthritis, lupus pneumonitis, lupus nephritis, conditions relating to systemic lupus erythematosus include osteoarticular tuberculosis, antiphospholipid antibody syndrome, systemic lupus erythematosus (SLE); discoid lupus erythematosus, inflammatory bowel disease, ulcerative colitis, Crohn's disease, benign lymphocytic angiitis, thrombocytopenic purpura, idiopathic thrombocytopenia, idiopathic autoimmune hemolytic anemia, pure red cell aplasia, Sjögren's syndrome, rheumatic disease, connective tissue disease, inflammatory rheumatism, degenerative rheumatism, extra-articular rheumatism, juvenile rheumatoid arthritis, arthritis uratica, muscular rheumatism, chronic polyarthritis, cryoglobulinemic vasculitis, ANCA-associated vasculitis, antiphospholipid syndrome, myasthenia gravis, autoimmune hemolytic anaemia, Guillain-Barré syndrome, chronic immune polyneuropathy, autoimmune thyroiditis, insulin dependent diabetes mellitus, type I diabetes, Addison's disease, membranous glomerulonephropathy, Goodpasture's disease, autoimmune gastritis, autoimmune atrophic gastritis, pernicious anaemia, pemphigus, pemphigus vulgaris, cirrhosis, primary biliary cirrhosis, dermatomyositis, polymyositis, fibromyositis, myogelosis, celiac disease, immunoglobulin A nephropathy, Henoch-Schönlein purpura, Evans syndrome, Dermatitis, atopic dermatitis, psoriasis, psoriasis arthropathica, Graves' disease, Graves' ophthalmopathy, scleroderma, systemic scleroderma, progressive systemic scleroderma, asthma, allergy, primary biliary cirrhosis, Hashimoto's thyroiditis, primary myxedema, sympathetic ophthalmia, autoimmune uveitis, anterior uveitis (or iridocyclitis), intermediate uveitis (pars planitis), posterior uveitis (or chorioretinitis), panuveitic form, hepatitis, chronic action hepatitis, collagen diseases, ankylosing spondylitis, peri arthritis humeroscapularis, panarteritis nodosa, chondrocalcinosis, Wegener's granulomatosis, microscopic polyangiitis, chronic urticaria, bullous skin disorders, pemphigoid, bullous pemphigoid, cicatricial pemphigoid, vitiligo, atopic eczema, eczema, chronic urticaria, autoimmune urticaria, normocomplementemic urticarial vasculitis, hypocomplementemic urticarial vasculitis, autoimmune lymphoproliferative syndrome, Devic's disease, sarcoidosis, pernicious anemia, childhood autoimmune hemolytic anemia, idiopathic autoimmune hemolytic anemia, Refractory or chronic Autoimmune Cytopenias, Prevention of development of Autoimmune Anti-Factor VIII Antibodies in Acquired Hemophilia A, Cold Agglutinin Disease, Neuromyelitis Optica, Stiff Person Syndrome, gingivitis, periodontitis, pancreatitis, myocarditis, vasculitis, gastritis, gout, gouty arthritis, and inflammatory skin disorders, selected from the group consisting of psoriasis, atopic dermatitis, eczema, rosacea, urticaria,

and acne, normocomplementemic urticarial vasculitis, inflammation of various parts of the heart, such as pericarditis, myocarditis, and endocarditis, lung and pleura inflammation, pleuritis, pleural effusion, chronic diffuse interstitial lung disease, pulmonary hypertension, pulmonary emboli, pulmonary hemorrhage, and shrinking lung syndrome, lupus headache,

5 idiopathic pericarditis, myositis, demyelinating syndrome, mononeuropathy, mononeuritis multiplex, myelopathy, cranial neuropathy, polyneuropathy, , collagenous colitis, lymphocytic colitis, ischaemia colitis, diversion colitis, indeterminate colitis, idiopathic autoimmune hemolytic anemia, anti-synthetase syndrome, scleritis, macrophage activation syndrome, Behçet's Syndrome, PAPA Syndrome, Blau's Syndrome, gout, gouty arthritis,

10 inflammatory skin disorders selected from the group consisting of psoriasis, Non pustular Psoriasis including Psoriasis vulgaris and Psoriatic erythroderma (erythrodermic psoriasis), Pustular psoriasis including Generalized pustular psoriasis (pustular psoriasis of von Zumbusch), Pustulosis palmaris et plantaris (persistent palmoplantar pustulosis, pustular psoriasis of the Barber type, pustular psoriasis of the extremities), Annular pustular psoriasis,

15 Acrodermatitis continua, Impetigo herpetiformis, drug-induced psoriasis, Inverse psoriasis, Napkin psoriasis, Seborrheic-like psoriasis, Guttate psoriasis, Nail psoriasis, eczema, rosacea, urticaria, and acne, normocomplementemic urticarial vasculitis, adult and juvenile Still's disease, cryopyrinopathy, chronic immune polyneuropathy, autoimmune thyroiditis, idiopathic diabetes, juvenile type 1 diabetes, maturity onset diabetes of the young, latent

20 autoimmune diabetes in adults, gestational diabetes, conditions relating to type 1 diabetes, membranous glomerulonephropathy, autoimmune gastritis, Muckle-Wells syndrome, familial cold-induced auto-inflammatory syndrome, neonatal onset multisystemic inflammatory disease, familial Mediterranean fever, chronic infantile neurologic, cutaneous and articular syndrome, any rheumatic disease, polymyalgia rheumatica, mixed connective tissue disease,

25 inflammatory rheumatism, degenerative rheumatism, extra-articular rheumatism, juvenile arthritis, juvenile rheumatoid arthritis, systemic juvenile idiopathic arthritis, arthritis uratica, muscular rheumatism, chronic polyarthritis, reactive arthritis, Reiter's syndrome, rheumatic fever, relapsing polychondritis, Raynaud's phenomenon, vasculitis, cryoglobulinemic vasculitis, temporal arteritis, giant cell arteritis, Takayasu arteritis, Behcet's disease, chronic

30 inflammatory demyelinating polyneuropathy, autoimmune thyroiditis, insulin dependent diabetes mellitus, type I diabetes, Addison's disease, membranous glomerulonephropathy, polyglandular autoimmune syndromes, Goodpasture's disease, autoimmune gastritis, autoimmune atrophic gastritis, pernicious anaemia, pemphigus, pemphigus vulgaris,

cirrhosis, primary biliary cirrhosis, idiopathic pulmonary fibrosis, myositis, dermatomyositis, juvenile dermatomyositis, polymyositis, fibromyositis, myogelosis, celiac disease, celiac sprue dermatitis, immunoglobulin A nephropathy, Henoch-Schönlein purpura, Evans syndrome, atopic dermatitis, psoriasis, psoriasis vulgaris, psoriasis arthropathica, Graves' disease, Graves' ophthalmopathy, scleroderma, systemic scleroderma, progressive systemic scleroderma, diffuse scleroderma, localized scleroderma, Crest syndrome, asthma, allergic asthma, allergy, primary biliary cirrhosis, fibromyalgia, chronic fatigue and immune dysfunction syndrome (CFIDS), autoimmune inner ear disease, Hyper IgD syndrome, Schnitzler's syndrome, autoimmune retinopathy, age-related macular degeneration, atherosclerosis, chronic prostatitis, alopecia, alopecia areata, alopecia universalis, alopecia totalis, autoimmune thrombocytopenic purpura, idiopathic thrombocytopenic purpura, pure red cell aplasia, and TNF receptor-associated periodic syndrome (TRAPS).

[00290] Of particular interest in some embodiments is the treatment of rheumatoid arthritis, lupus, inflammatory bowel disease, psoriasis, multiple sclerosis and diabetes type I.

[00291] As described herein, PVRIG polypeptides which modulate immunity may optionally be used to treat or detect "immune related diseases (or disorders or conditions)". These phrases or terms are used interchangeably and encompass any disease, disorder or condition selected from the group including but not limited to autoimmune diseases, inflammatory disorders, allergic disorders, e.g., chronic allergic disorders such as asthma, and immune disorders associated with graft transplantation rejection, such as acute and chronic rejection of organ or tissue transplantation, allogenic stem cell transplantation, autologous stem cell transplantation, bone marrow transplantation, and graft versus host disease.

[00292] As further noted, the present PVRIG polypeptides which modulate immunity may be used to treat "inflammatory disorders" and/or "inflammation". These phrases or terms are used interchangeably herein and include e.g., inflammatory abnormalities characterized by dysregulated immune response to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammatory disorders underlie a vast variety of human diseases. Non-immune diseases with etiological origins in inflammatory processes include but are not limited to cancer, atherosclerosis, and ischemic heart disease. Examples of disorders associated with inflammation include but are not limited to: Chronic prostatitis, Glomerulonephritis, Hypersensitivities, Pelvic inflammatory disease, Reperfusion injury, Sarcoidosis, Vasculitis, Interstitial cystitis, normocomplementemic urticarial vasculitis, pericarditis, myositis, anti-

synthetase syndrome, scleritis, macrophage activation syndrome, Behçet's Syndrome, PAPA Syndrome, Blau's Syndrome, gout, adult and juvenile Still's disease, cryopyrinopathy, Muckle-Wells syndrome, familial cold-induced auto-inflammatory syndrome, neonatal onset multisystemic inflammatory disease, familial Mediterranean fever, chronic infantile

5 neurologic, cutaneous and articular syndrome, systemic juvenile idiopathic arthritis, Hyper IgD syndrome, Schnitzler's syndrome, TNF receptor-associated periodic syndrome (TRAPSP), gingivitis, periodontitis, hepatitis, cirrhosis, pancreatitis, myocarditis, vasculitis, gastritis, gout, gouty arthritis, and inflammatory skin disorders, selected from the group consisting of psoriasis, atopic dermatitis, eczema, rosacea, urticaria, and acne.

- 10 **[00293]** According to at least some embodiments, any one of the foregoing therapeutic agents according to the present invention can be used for adoptive immunotherapy. Immune tolerance or immunological tolerance or prolonged immunosuppression is the process by which the immune system does not attack an antigen. It can be either 'natural' or 'self-tolerance', where the body does not mount an immune response to self-antigens, or 'induced
- 15 tolerance', where tolerance to external antigens can be created by manipulating the immune system. It occurs in three forms: central tolerance, peripheral tolerance and acquired tolerance. Without wishing to be bound by a single theory, tolerance employs regulatory immune cells - including Tregs - or potentially other immunosuppressive cells such as MDSCs, iMSCs, monocytes, neutrophils, macrophages, that directly suppress autoreactive
- 20 cells, as well as several other immune cell subsets with immunoregulatory properties - including CD8⁺ T cells and other types of CD4⁺ T cells (Tr1, Th3), Th17 cells, in addition to natural killer (NK), NKT cells, dendritic cells (DC) and B cells.

- [00294]** Tolerance or prolonged immunosuppression can be induced by blocking costimulation or upon engagement of a co-inhibitory B7 with its counter receptor. Transfer of
- 25 tolerance involves isolation of the cells that have been induced for tolerance either *in vivo* (i. e. prior to cell isolation) or *ex-vivo*, enrichment and expansion of these cells *ex vivo*, followed by reinfusion of the expanded cells to the patient. This method can be used for treatment of autoimmune diseases as recited herein, immune related diseases as recited herein, transplantation and graft rejection. Thus, according to at least some embodiments, the
- 30 invention optionally provides methods for tolerance induction, comprising *in vivo* or *ex vivo* treatment administration of effective amount of any one of isolated soluble PVRIG polypeptide, or a polypeptide comprising the extracellular domain of PVRIG, or fragment thereof, or a fusion thereof to a heterologous sequence, to a patient or to leukocytes isolated

from the patient, in order to induce differentiation of tolerogenic regulatory cells, followed by *ex-vivo* enrichment and expansion of said cells and reinfusion of the tolerogenic regulatory cells to said patient.

1. Treating by inhibiting the Interaction of PVRIG and PVRL2

5 [00295] In some embodiments, the invention provides methods of treating subjects by inhibiting the interaction of PVRIG and PVRL2, its associated ligand, as outlined in the examples. As both PVRIG and PVLR2 contain transmembrane domain, the inhibition can be done by preventing the binding of the two, such as by using anti-PVRIG antibodies. Alternately, by administering a soluble PVRIG polypeptide, that will interact with the
10 transmembrane PVLR2 ligand, preventing it from binding to the membrane bound endogenous PVRIG, thus preventing signaling (either by the loss of signaling due to the lack of PVRIG signaling, or by the prevention of free PVLR2 binding to other of its signaling partners, such as DNAM1).

[00296] In some embodiments, the invention provides methods of treating patients by
15 modulating the interaction of PVRIG and PVRL2 by administering a PVRIG polypeptide as outlined herein.

[00297] In some embodiments, the invention provides methods of treating patients by inhibiting the Interaction of PVRIG and PVRL2 by inhibiting the binding of PVRIG to PVLR2 by administering an anti-PVRIG antibody.

20 [00298] According to at least some embodiments, PVRIG therapeutic agents and/or a pharmaceutical composition comprising same, as described herein, which function as PVRIG agonizing therapeutic agents, may optionally be used for treating an immune system related disease. In some instances the immune system related condition comprises an immune related condition, including but not limited to autoimmune, inflammatory or allergic diseases such as
25 recited herein, transplant rejection and graft versus host disease.

[00299] In some instances the immune condition is selected from autoimmune disease, inflammatory disease, allergic disease, transplant rejection, undesired gene or cell therapy immune responses, or graft versus host disease.

[00300] In some embodiments the treatment is combined with another moiety useful
30 for treating immune related condition. Non limiting examples thereof include immunosuppressants such as corticosteroids, cyclosporin, cyclophosphamide, prednisone, azathioprine, methotrexate, rapamycin, tacrolimus, leflunomide or an analog thereof;

mizoribine; mycophenolic acid; mycophenolate mofetil; 15-deoxyspergualine or an analog thereof; biological agents such as TNF- α blockers or antagonists, or any other biological agent targeting any inflammatory cytokine, nonsteroidal antiinflammatory drugs/Cox-2 inhibitors, hydroxychloroquine, sulphasalazopyrine, gold salts, etanercept, infliximab, mycophenolate mofetil, basiliximab, atacicept, rituximab, cytoxan, interferon β -1a, interferon β -1b, glatiramer acetate, mitoxantrone hydrochloride, anakinra and/or other biologics and/or intravenous immunoglobulin (IVIG), interferons such as IFN- β 1a (REBIF®, AVONEX® and CINNOVEX®) and IFN- β 1b (BETASERON®); EXTAVIA®, BETA FERON®, ZIFERON®; glatiramer acetate (COPAXONE®), a polypeptide; natalizumab (TYSABRI®), mitoxantrone (NOVANTRONE®), a cytotoxic agent, a calcineurin inhibitor, e.g. Cyclosporin A or FK506; an immunosuppressive macrolide, e.g. Rapamycin or a derivative thereof; e.g. 40-O-(2-hydroxy)ethyl-rapamycin, a lymphocyte homing agent, e.g. FTY720 or an analog thereof, corticosteroids; cyclophosphamide; azathioprine; methotrexate; leflunomide or an analog thereof; mizoribine; mycophenolic acid; mycophenolate mofetil; 15-deoxyspergualine or an analog thereof; immunosuppressive monoclonal antibodies, e.g., monoclonal antibodies to leukocyte receptors, e.g., MHC, CD2, CD3, CD4, CD11a/CD18, CD7, CD25, CD27, B7, CD40, CD45, CD58, CD137, ICOS, CD150 (SLAM), OX40, 4-1BB or their ligands; or other immunomodulatory compounds, e.g. CTLA4-Ig (abatacept, ORENCIA®, belatacept), CD28-Ig, B7-H4-Ig, or other costimulatory agents, or adhesion molecule inhibitors, e.g. mAbs or low molecular weight inhibitors including LFA-1 antagonists, Selectin antagonists and VLA-4 antagonists, or another immunomodulatory agent.

[00301] In particular, treatment of multiple sclerosis using PVRIG immunoinhibitory proteins according to the invention may e.g., be combined with, any therapeutic agent or method suitable for treating multiple sclerosis. Non-limiting examples of such known therapeutic agent or method for treating multiple sclerosis include interferon class, IFN- β -1a (REBIF®, AVONEX® and CINNOVEX®) and IFN- β -1b (BETASERON®, EXTAVIA®, BETA FERON®, ZIFERON®); glatiramer acetate (COPAXONE®), a polypeptide; natalizumab (TYSABRI®); and mitoxantrone (NOVANTRONE®), a cytotoxic agent, Fampridine (AMPYRA®). Other drugs include corticosteroids, methotrexate, cyclophosphamide, azathioprine, and intravenous immunoglobulin (IVIG), inosine, Ocrelizumab (R1594), Mylinax (Caldribine®), alemtuzumab (Campath®), daclizumab (Zenapax®), Panaclar/ dimethyl fumarate (BG-12), Teriflunomide (HMR1726), fingolimod

(FTY720), laquinimod (ABR216062), as well as Hematopoietic stem cell transplantation, NeuroVax®, Rituximab (Rituxan®) BCG vaccine, low dose naltrexone, helminthic therapy, angioplasty, venous stents, and alternative therapy, such as vitamin D, polyunsaturated fats, medical marijuana.

- 5 **[00302]** Similarly, treatment of rheumatoid arthritis, using PVRIG immunoinhibitory proteins according to the invention may be combined with, for example, any therapeutic agent or method suitable for treating rheumatoid arthritis. Non-limiting examples of such known therapeutic agents or methods for treating rheumatoid arthritis include glucocorticoids, nonsteroidal anti-inflammatory drug (NSAID) such as salicylates, or
- 10 cyclooxygenase-2 inhibitors, ibuprofen and naproxen, diclofenac, indomethacin, etodolac Disease-modifying antirheumatic drugs (DMARDs)- Oral DMARDs: Auranofin (Ridaura®), Azathioprine (Imuran®), Cyclosporine (Sandimmune®, Gengraf, Neoral, generic), D-Penicillamine (Cuprimine), Hydroxychloroquine (Plaquenil®), IM gold Gold sodium thiomalate (Myochrysine®) Aurothioglucose (Solganal®), Leflunomide (Arava®),
- 15 Methotrexate (Rheumatrex®), Minocycline (Minocin®), *Staphylococcal* protein A immunoabsorption (Prosorba column), Sulfasalazine (Azulfidine®). Biologic DMARDs: TNF- α blockers including Adalimumab (Humira®) Etanercept (Enbrel®), Infliximab (Remicade®), golimumab (Simponi®), certolizumab pegol (Cimzia®), and other biological DMARDs, such as Anakinra (Kineret®), Rituximab (Rituxan®), Tocilizumab (Actemra®),
- 20 CD28 inhibitor including Abatacept (Orencia®) and Belatacept.

- [00303]** Thus, treatment of IBD, using the agents according to at least some embodiments of the present invention may be combined with, for example, any known therapeutic agent or method for treating IBD. Non-limiting examples of such known therapeutic agents or methods for treating IBD include immunosuppression to control the
- 25 symptom, such as prednisone, Mesalazine (including Asacol®, Pentasa®, Lialda®, Aspiro®, azathioprine (Imuran®), methotrexate, or 6-mercaptopurine, steroids, Ondansetron®, TNF- α blockers (including infliximab, adalimumab golimumab, certolizumab pegol), Orencia® (abatacept), ustekinumab (Stelara®), Briakinumab (ABT-874), Certolizumab pegol (Cimzia®), ITF2357 (Givinostat®), Natalizumab (Tysabri®), Finategrast® (SB-683699),
- 30 Remicade® (infliximab), vedolizumab (MLN0002), other drugs including GSK1605786 CCX282-B (Traficet-EN®), AJM300, Stelara® (ustekinumab), Semapimod® (CNI-1493) tasocitinib (CP-690550), LMW Heparin MMX, Budesonide MMX, Simponi® (golimumab), MultiStem®, Gardasil® HPV vaccine, Epaxal® (viroosomal hepatitis A vaccine), surgery,

such as bowel resection, strictureplasty or a temporary or permanent colostomy or ileostomy; antifungal drugs such as nystatin (a broad spectrum gut antifungal) and either itraconazole (Sporanox) or fluconazole (Diflucan); alternative medicine, prebiotics and probiotics, cannabis, Helminthic therapy or ova of the *Trichuris suis* helminth.

5 **[00304]** Thus, treatment of psoriasis, using the agents according to at least some embodiments of the present invention may be combined with, for example, any known therapeutic agent or method for treating psoriasis. Non-limiting examples of such known therapeutics for treating psoriasis include topical agents, typically used for mild disease, phototherapy for moderate disease, and systemic agents for severe disease. Non-limiting
10 examples of topical agents: bath solutions and moisturizers, mineral oil, and petroleum jelly; ointment and creams containing coal tar, dithranol (anthralin), corticosteroids like desoximetasone (Topicort), Betamethasone, fluocinonide, vitamin D3 analogues (for example, calcipotriol), and retinoids. Non-limiting examples of phototherapy: sunlight; wavelengths of 311–313 nm, psoralen and ultraviolet A phototherapy (PUVA). Non-limiting
15 examples of systemic agents: biologics, such as interleukin antagonists, TNF- α blockers including antibodies such as infliximab (Remicade®), adalimumab (Humira®), golimumab, certolizumab pegol, and recombinant TNF- α decoy receptor, etanercept (Enbrel®); drugs that target T cells, such as efalizumab (Xannelim®/Raptiva®), alefacept (Ameviv®), dendritic cells such Efalizumab; monoclonal antibodies (MAbs) targeting cytokines, including anti- IL-
20 12/IL-23 (ustekinumab (Stelara®)) and anti-Interleukin-17; Briakinumab® (ABT-874); small molecules, including but not limited to ISA247; immunosuppressants, such as methotrexate, cyclosporine; vitamin A and retinoids (synthetic forms of vitamin A); and alternative therapy, such as changes in diet and lifestyle, fasting periods, low energy diets and vegetarian diets, diets supplemented with fish oil rich in vitamin A and vitamin D (such as cod liver oil), fish
25 oils rich in the two omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and contain vitamin E, ichthyotherapy, hypnotherapy, and cannabis.

[00305] Thus, treatment of type 1 diabetes, using the agents according to at least some embodiments of the present invention may be combined with, for example, any known therapeutic agent or method for treating type 1 diabetes. Non-limiting examples of such
30 known therapeutics for treating type 1 diabetes include insulin, insulin analogs, islet transplantation, stem cell therapy including PROCHYMAL®, non-insulin therapies such as il-1 β inhibitors including Anakinra (Kineret®), Abatacept (Orencia®), Diamyd, alefacept (Ameviv®), Otelixizumab, DiaPep277 (Hsp60 derived peptide), α 1-Antitrypsin, Prednisone,

azathioprine, and Cyclosporin, E1-INT (an injectable islet neogenesis therapy comprising an epidermal growth factor analog and a gastrin analog), statins including Zocor®, Simlup®, Simcard®, Simvacor®, and Sitagliptin® (dipeptidyl peptidase (DPP-4) inhibitor), anti-CD3 mAb (e.g., Teplizumab®); CTLA4-Ig (abatacept), anti-IL-1 β (Canakinumab), Anti-CD20 mAb (e.g., rituximab) and combinations thereof.

[00306] Thus, treatment of uveitis, using the agents according to at least some embodiments of the present invention may be combined with, for example, any known therapeutic agent or method for treating uveitis. Non-limiting examples of such known therapeutics for treating uveitis include corticosteroids, topical cycloplegics, such as atropine or homatropine, or injection of PSTTA (posterior subtenon triamcinolone acetate), antimetabolite medications, such as methotrexate, TNF- α blockers (including infliximab, adalimumab, etanercept, golimumab, and certolizumab pegol).

[00307] Thus, treatment for Sjögren's syndrome, using the agents according to at least some embodiments of the present invention may be combined with, for example, any known therapeutic agent or method for treating for Sjögren's syndrome. Non-limiting examples of such known therapeutics for treating for Sjögren's syndrome include Cyclosporine, pilocarpine (Salagen®) and cevimeline (Evoxac®), Hydroxychloroquine (Plaquenil), cortisone (prednisone and others) and/or azathioprine (Imuran®) or cyclophosphamide (Cytosan®), Dexamethasone, Thalidomide, Dehydroepiandrosterone, NGX267, Rebamipide®, FID 114657, Etanercept®, Raptiva®, Belimumab, MabThera® (rituximab); Anakinra®, intravenous immune globulin (IVIG), Allogeneic Mesenchymal Stem Cells (AlloMSC®), and Automatic neuro-electrostimulation by "Saliwell Crown".

[00308] Thus, treatment for systemic lupus erythematosus, using the agents according to at least some embodiments of the present invention may be combined with, for example, any known therapeutic agent or method for treating for systemic lupus erythematosus. Non-limiting examples of such known therapeutics for treating for systemic lupus erythematosus include corticosteroids and Disease-modifying antirheumatic drugs (DMARDs), commonly anti-malarial drugs such as plaquenil and immunosuppressants (e.g. methotrexate and azathioprine) Hydroxychloroquine, cytotoxic drugs (e.g., cyclophosphamide and mycophenolate), Hydroxychloroquine (HCQ), Benlysta® (belimumab), nonsteroidal anti-inflammatory drugs, Prednisone, Cellcept®, Prograf®, Atacicept®, Lupuzor®, Intravenous Immunoglobulins (IVIGs), CellCept® (mycophenolate mofetil), Orencia®, CTLA4-

IgG4m (RG2077), rituximab, Ocrelizumab, Epratuzumab, CNTO 136, Sifalimumab (MEDI-545), A-623 (formerly AMG 623), AMG 557, Rontalizumab, paquinimod (ABR-215757), LY2127399, CEP-33457, Dehydroepiandrosterone, Levothyroxine, abetimus sodium (LJP 394), Memantine®, Opiates, Rapamycin®, renal transplantation, stem cell transplantation and combinations of any of the foregoing.

[00309] The immunoinhibitory PVRIG therapeutic agents and/or a pharmaceutical composition comprising same, as recited herein, according to at least some embodiments of the invention, may be administered as the sole active ingredient or together with other drugs in immunomodulating regimens or other anti-inflammatory agents e.g. for the treatment or prevention of allo- or xenograft acute or chronic rejection or inflammatory or autoimmune disorders, or to induce tolerance.

EXAMPLES

[00310] Specifically incorporated by reference herein is USSN 62/141,168, filed 31 March 2015, and USSN 62/118,235, filed 19 February 2015, in their entirety, and in particular for the Examples therein, and for the associated Figures and Legends.

Example 1: Expression Analysis of PVRIG Proteins

[00311] Specifically incorporated by reference herein is USSN 62/141,168, filed 31 March 2015, and USSN 62/118,235, filed 19 February 2015, in their entirety, and in particular for the Examples therein, and for the associated Figures and Legends.

Example 1A:

[00313] The GDS3113 data set (<http://www.ncbi.nlm.nih.gov/sites/GDSbrowser?acc=GDS3113>) was analyzed to identify genes with a lymphoid organ specific pattern. PVRIG was identified as lymphocyte specific due to high expression in primary and secondary lymphoid organs, which include peripheral blood, bone marrow, spleen, lymph nodes, tonsil and thymus (Figure 2). Other tissue types were negative or showed expression at background levels. In order to investigate which specific cell types within the total population of immune cells express PVRIG, additional data sets from the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/GEO) were analyzed, as described in “methodology” section herein. The analysis was performed on immune cell populations derived from peripheral blood and bone marrow. PVRIG was expressed in lymphocytes both in the B-cell lineage and the T-cell lineage including CD8 T-cells naïve,

effector and memory (Figure 3). In addition, PVRIG was expressed in NK cells and had the highest expression in the iNKT population (Figure 4). The iNKT population of lymphocytes act as potent activators of antitumor immunity when stimulated with a synthetic agonist in experimental models. However, in some settings, iNKT cells can act as suppressors and regulators of antitumor immunity (Clin Dev Immunol. 2012;2012:720803). Furthermore, in early clinical trials of iNKT cell-based immunotherapy demonstrated that the infusion of ligand-pulsed antigen presenting cells treatment of and/or in vitro activated iNKT cells were safe and well tolerated in lung cancer and head and neck cancer (Clin Immunol. 2011 Aug;140(2):167-76.).

[00314] A key question in regards to PVRIG expression was whether Tumor Infiltrating Lymphocytes (TILs) retain expression of PVRIG in the tumor microenvironment. Analyzing expression data of TILs from follicular lymphoma, breast cancer and colon cancer showed clear expression of PVRIG in the TILs infiltrating the tumor. In the colon cancer example the specificity to the immune infiltrating cells was seen as the expression is found only in the CD45 positive population (leukocyte specific marker), and no expression is found in EPCAM positive population (epithelial specific marker) or in the CD45 negative EPCAM negative (stromal cell population). Although the CD45 is not a lymphocyte specific marker, the other expression description infers that it is expressed on the lymphocyte population (Figure 5A colon cancer, Figure 5B breast cancer and Figure 5C follicular lymphoma).

[00315] The mRNA expression data shown herein indicates that PVRIG is expressed in lymphocytes and in tumor infiltrating lymphocytes (TILs). These results together with PVRIG inhibitory activity propose an inhibitory role of the molecule in T-cells, suggesting that inhibitory antibodies to PVRIG elevates PVRIG's suppressive role on the TILs and thus enable the TILs to induce an immune response against cancer. As the proposed mechanism of action is directed to the TILs infiltrating the tumor, rather than direct effect on the tumor cells, any cancer with immune infiltration is candidate for treatment using PVRIG inhibitory antibodies.

[00316] Methodology: Raw data is downloaded from the GEO site in SOFT format. In cases where the raw data was in MAS5 format, the data was taken without manipulation. If the data was in Log MAS5 then the data was converted to linear data. If the data was in RMA format CEL files (raw data) were downloaded and re-analyzed using MAS5. If raw CEL files were not available the RMA format was used.

[00317] Data was then normalized by multiplicative according to the 95th percentile for Affy data. Datasets analyzed: GSE49910, GSE47855, GSE39397, GSE36765, GSE27928.

[00318] Specifically incorporated by reference herein is USSN 62/141,168, filed 31
5 March 2015, and USSN 62/118,235, filed 19 February 2015, in their entirety, and in particular for the Examples therein, and for the associated Figures and Legends.

[00319] **Example 1B**

[00320] A transcriptome reference was generated based on UCSC know genes models (<http://hgdownload.cse.ucsc.edu/goldenPath/hg19/database/knownGene.txt.gz>). All RNA
10 sequencing reads were aligned to the transcriptome sequences first. This alignment allowed for non-unique mapping because isoforms share many exons. Each read was then assigned genomic coordinates and exon junctions based on the transcriptome matching. The remaining unmapped reads were aligned directly to the genome by considering one or more exon junctions. Finally, read counts were normalized as described by Bo et al.
15 (Bioinformatics 2010, 26 (4): 493-500) and converted to gene expression values as described by Trapnell et al (Nat Biotechnol. 2010 May;28(5):511-5).

[00321] As shown in Figure6, based on Genotype-Tissue Expression (GTEx) data (<http://www.nature.com/ng/journal/v45/n6/full/ng.2653.html>;
<http://www.gtexportal.org/home/>), PVRIG is expressed mainly in blood cells and to lesser
20 extent in various normal tissues. The same results were observed in cancerous tissues from The Cancer Genome Atlas (TCGA) (<http://cancergenome.nih.gov/>) in which high expression are seen in blood cancers like B- cell lymphomas and AML (Figure 7). A gene expression signature was generated for a variety of cancers and normal tissues using GTEx and TCGA data by identifying genes with a highly correlated expression pattern to PVRIG.

25 [00322] The correlation analysis was conducted per tumor type and only correlations where both genes were expressed above 0 RPKM with at least 50 samples in the same tumor type, were considered. These gene expression signatures were tested for enrichment of interacting proteins, pathways and disease genes. Enrichment p-values were calculated for each tumor type and the mean $-\log(p\text{-value})$ was used to rank the scoring gene sets. A clear
30 signature of lymphocytes and T- cells was observed in a variety of cancers, as shown in . For instance, the top scoring gene in protein interaction was IL2, meaning that genes known to interact with IL2 are more correlated with PVRIG than expected by chance across most

cancers. Further analysis showed that PVRIG expression in cancer tissues are higher than normal. While in Figure 5 the median expression level of PVRIG is below 1 across most normal solid tissues, in Figure 6 it is clearly higher than 1 in many cancers. As an example, when compared side by side in Figure 7, melanoma PVRIG was expressed higher than normal skin (Figure 9). We further characterized the source of over-expression in cancer. PVRIG is highly expressed in T cells and is highly correlated to markers of T cells in cancer. In Figure 10, PVRIG correlation to CD3, CD4 and CD8 are shown as an example in three cancer types, namely, lung adenocarcinoma, colon adenocarcinoma and melanoma. In addition, PVRIG is highly correlated to PD1, a validated target for immunotherapy in cancer known to be expressed on T cells (Figure 10).

[00323] These gene expression signatures were tested for enrichment of interacting proteins, pathways and disease genes. A clear signature of lymphocytes and T- cells was observed in a variety of cancers, as shown in Figure 8. We further analyzed the correlation of PVRIG to PD1 and showed high correlation between their expression in various tumors including breast lung pancreas and kidney (Table 2). Both PD-1 and PVRIG are highly expressed on activated T cells. PVRIG showed high correlation with T cell markers in cancer, namely, CD8A, CD4 and CD3G (Figure 13). Taken together, these data demonstrate that cancer expression of PVRIG is associated with tumor infiltrating lymphocytes.

[00324]

[00325] Methods: Genes correlation: FPKM values were transformed to $\log_2$ (FPKM+0.1). Samples with value that fulfills $\log_2$ (FPKM+0.1) < $\log_2$ (0.1) for at least one of the genes, were omitted. Pearson Correlation Coefficient (PCC) and the Least Squared Estimators for the regression line were computed for the 2 lists (one list per gene). PCCs with lower value than 0.5 were omitted as well as PCCs that failed to show significant value when testing the linear correlation between the expression levels of the 2 genes.

[00326] Gene Enrichment analysis: Pathway, interaction and disease data were obtained from GeneGo Metacore (<https://portal.genego.com>), Reactome (<http://www.reactome.org>) and KEGG Pathways (<http://www.genome.jp/kegg>). To identify pathways and processes that were enriched within a given gene list, a hyper-geometric-based enrichment analysis was implemented. The hyper-geometric p-value was calculated using the R program (<http://www.R-project.org>) with the following command: `phyper(x - 1, m, n-m, k and lower.tail = FALSE)`, where x is the number of genes from the gene list that are members

of the pathway, m is the number of genes in the pathway, n is the total number of unique genes in all pathways, and k is the number of genes from the list that were present in at least one pathway. The resulting p-value is indicative of the likelihood of enriching for a specific pathway by chance given the size of the gene list. The same analytical procedure was applied to gene interactions where all genes interacting with a given gene were treated as a pathway; or genes associated with a disease where all associated genes were treated as a pathway. See Figure 64.

[00327] PVRIG expression was associated with exhausted T cells in cancer. Cancer samples from TCGA were chosen that have high (4th quartile) expression of the following 4 markers: CD8, PD-1, TIM-3 and TIGIT. Cancer samples were then divided to high, no change and low levels of the combined expression of the 4 markers. PVRIG was not detected in any of the low expressing markers (low or no exhausted T cells). The vast majority of tumors associated with high levels of exhausted T cells expressed high levels of PVRIG (Figure 22).

[00328] Specifically incorporated by reference herein is USSN 62/141,168, filed 31 March 2015, and USSN 62/118,235, filed 19 February 2015, in their entirety, and in particular for the Examples therein, and for the associated Figures and Legends.

[00329] **Example 1C:**

[00330] The expression of human and non-human primate PVRIG RNA and protein in cell lines and primary leukocytes was evaluated.

[00331] Protocols

[00332] **FACS analysis of engineered over-expressing cells:** The following cell lines were used to assess the specificity of anti-human PVRIG antibodies: HEK parental and HEK hPVRIG over-expressing cells. These cells were cultured in DMEM (Gibco) + 10% fetal calf serum (Gibco) + glutamax (Gibco). For the HEK hPVRIG over-expressing cells, 0.5ug/ml puromycin (Gibco) was also added to the media for positive selection. For FACS analysis, all cell lines were harvested in log phase growth and 50,000-100,000 cells per well were seeded in 96 well plates. Anti- human PVRIG antibodies (human IgG1, hIgG1) and their respective controls were added in single point dilutions (5ug/ml), or as an 8 point titration series starting at 30ug/ml on ice for 30 mins-1 hr. The titration series were conducted as either 1:3 or 1:3.3 fold serial dilutions. Data was acquired using a FACS Canto II (BD Biosciences) and analyzed using FlowJo (Treestar) and Prism (Graphpad) software.

[00333] FACS analysis of human cell lines: The following cell lines were used to assess the expression and specificity of anti-human PVRIG antibodies: Jurkat, CA46, NK-92, OV-90, HepG2, and NCI-H441. Jurkat, CA46, and NCI-H441 cells were cultured in RPMI media + 10% fetal calf serum, glutamax, non-essential amino acids (Gibco), sodium pyruvate (Gibco), and penicillin/streptomycin (Gibco). NK-92 cells were cultured in RPMI media + 25% fetal calf serum, glutamax, non-essential amino acids, sodium pyruvate, penicillin/streptomycin, and 500U/ml IL-2 (R&D systems). OV-90 cells were cultured in a 1:1 mixture of MCDB 105 media (Sigma) containing a final concentration of 1.5 g/L sodium bicarbonate (Life Technologies) and Media 199 (Sigma) containing a final concentration of 2.2 g/L sodium bicarbonate with a final concentration of 15% fetal calf serum. HepG2 cells were cultured in DMEM + 10% fetal calf serum + glutamax. For FACS analysis, all cell lines were harvested in log phase growth and 50,000-100,000 cells per well were seeded in 96 well plates. Anti- human PVRIG antibodies (hIgG1) and their respective controls were added in single point dilutions (5ug/ml), or as an 8 point titration series starting at 30ug/ml on ice for 30 mins-1 hr. The titration series were conducted as either 1:3 or 1:3.3 fold serial dilutions. Data was acquired using a FACS Canto II and analyzed using FlowJo and Prism software.

[00334] FACS analysis of naïve human primary leukocytes: Primary leukocytes were obtained by Ficoll (GE Healthcare) gradient isolation of peripheral blood (Stanford Blood Bank). Leukocytes as isolated peripheral blood mononuclear cells (PBMC) were frozen down in liquid nitrogen at a density between 1×10^7 and 5×10^7 cells/ml in a 10% DMSO (Sigma), 90% fetal calf serum mixture. To assess protein expression of PVRIG on PBMC, antibody cocktails towards major immune subsets were designed that included human anti-PVRIG antibodies. Anti- human PVRIG antibodies (hIgG1) and their respective controls were added in single point dilutions (5ug/ml), or in some cases, as an 8 point titration series starting at 10 or 30ug/ml on ice for 30 mins-1 hr.

[00335] Briefly, antibody cocktail mixtures were added to resuscitated PBMC that were seeded at $5 \times 10^5 - 1 \times 10^6$ cells/well upon prior Fc receptor blockade and live/dead staining (Aqua Live/Dead, Life Technologies). Antibody cocktails were incubated with PBMC for 30mins – 1hr on ice. PBMC were then washed and data was acquired by FACS using a FACS Canto II. Data was analysed using FlowJo and Prism software. Immune subsets that were analysed include CD56 dim NK cells, CD56 bright NK cells, CD4+ T cells, CD8+ T cells, non-conventional T cells (e.g. NKT cells and $\gamma\delta$ T cells), B cells, and monocytes.

[00336] FACS analysis of activated human effector lymphocytes: In some cases, expression of PVRIG was assessed on activated effector lymphocyte subsets either isolated from whole PBMC or in whole PBMC preparations. Effector lymphocytes were stimulated with combinations of cytokines, combinations of antibodies and cytokines, or pathogenic products. FACS analysis of PVRIG expression on activated cells was performed analogous to that described above for naïve primary leukocytes.

[00337] To study PVRIG expression on stimulated NK cells, CD56⁺ cells were isolated and cultured in various cocktails of cytokines for 1-3 days in NK cell media (RPMI + 10% fetal calf serum, glutamax, penicillin/streptomycin, non-essential amino acids, sodium pyruvate, and beta-mercaptoethanol [Gibco]). NK cells were sorted either using anti-human CD56⁺ microbeads (Miltenyi Biotec) or the human NK cell isolation kit (Miltenyi Biotec) according to the manufacturer's instructions. Cocktails of cytokines used to simulate NK cells included IL-2, IL-12, IL-15, IL-2/IL-12, IL-2/IL-15, IL-12/IL-15 (R&D systems).

[00338] To study PVRIG expression on stimulated T cells, CD4⁺ or CD8⁺ T cells were isolated using CD4⁺ or CD8⁺ microbeads (Miltenyi Biotec). The isolated cells were cultured for 3 days in the presence of various activating conditions in T cell media (RPMI + 10% fetal calf serum, glutamax, penicillin/streptomycin, non-essential amino acids, sodium pyruvate). Conditions used to stimulate isolated T cells include human dynabead stimulation (beads coupled to CD3/CD28 antibodies, Life Technologies) with IL-2 or cytokine cocktails that drive T cells to certain phenotypes (e.g. Th1, Th2, Th17, and T regulatory phenotypes). Th1 driving cytokines are recombinant IL-12 (R&D systems) and an anti-IL-4 neutralizing antibody (Biolegend). Th2 driving conditions are recombinant IL-4 (R&D systems) and an anti-IFN- γ neutralizing antibody (Biolegend). Th17 driving conditions are recombinant IL-6 (R&D systems), TGF- β (R&D systems), IL-23 (R&D systems), and anti-IL-4 and anti-IFN γ neutralizing antibodies. T regulatory driving conditions are recombinant TGF- β and IL-2, and anti-IL-4 and anti-IFN γ neutralizing antibodies.

[00339] Alternatively, activated T cells were also analyzed in whole stimulated PBMC cultures with staphylococcal enterotoxin B (SEB) antigen (List Biological Laboratories) for 3 days, or in a mixed lymphocyte reaction (MLR) where CD4⁺ T cells are co-cultured with allogeneic dendritic cells for 2 or 5 days.

[00340] FACS analysis of human polarized monocytes: PVRIG expression was assessed on dendritic cells derived from polarized monocytes. In this instance, CD14⁺ cells

were enriched using RosetteSep human monocyte enrichment according to manufacturer's instructions. After CD14+ cell enrichment, monocytes were polarized to dendritic cells upon culture with GM-CSF (R&D systems) and IL-4 (R&D systems) for 4 days in RPMI + 10% fetal calf serum, glutamax, penicillin/streptomycin, non-essential amino acids, sodium pyruvate, and beta-mercaptoethanol.

[00341] RNA expression analysis of human cell lines and leukocytes by qPCR: Cell lines that were assessed for RNA expression by qPCR were Jurkat, CA46, Daudi, Raji, and expi 293 cells. Jurkat, CA46, Raji, and Daudi cells were cultured in RPMI media + 10% fetal calf serum, glutamax, non-essential amino acids, sodium pyruvate, and penicillin/streptomycin. Expi 293 cells were cultured in DMEM + 10% FCS + glutamax. OV-90, HepG2, and NCI-H441 RNA was analysed by a bioinformatics screen of the cancer cell line atlas. For those cell lines that were assessed for RNA expression by qPCR, the cells were harvested in log phase growth and 1,000,000 cells were harvested, washed in PBS, and lysed in 350ul of RLT buffer (Qiagen). Lysed cells in RLT buffer were stored at -80oc until use.

[00342] Primary leukocytes that were assessed for RNA expression were CD56+ NK cells, CD4+ T cells, CD8+ T cells, and CD14+ monocytes. Cell populations were isolated using human CD56+, CD4+, CD8+, and CD14+ positive selection kits according to manufacturer's instructions (Miltenyi Biotec). After sorting, cells were lysed in 350ul of RLT buffer and stored at -80oc until use. In some instances, activated PBMC subsets (activation conditions outlined above) were harvested from culture and were lysed in 350ul of RLT buffer and stored at -80oc until use.

[00343] Upon day of use, RNA was generated from lysed cells using the Qiagen mini kit according to the manufacturer's instructions. cDNA was generated using Applied Biosystems high capacity cDNA reverse transcription kit. qPCR using cDNA was performed using Taqman primers (ThermoFisher) and Applied Biosystems Taqman fast advanced mastermix. The PVRIG primer set used was Taqman catalogue number: Hs04189293_g1. Beta-actin housekeeping primer set used was Taqman catalogue number: Hs01060665_g1. Expression of transcript was assessed by quantifying Ct values and relative expression was calculated by the $2(-\Delta\Delta Ct)$ method. Data was acquired on an Applied Biosystems Step One Plus instrument.

[00344] FACS analysis of cynomolgus PVRIG engineered over-expressing cells: The following cell lines were used to assess the cross-reactivity of anti-human PVRIG antibodies

with cynomolgus PVRIg (cPVRIg): expi parental and expi cPVRIg over-expressing cells. These cells were cultured in DMEM + 10% fetal calf serum + glutamax. expi cPVRIg transient over-expressing cells were generated by electroporating cPVRIg DNA into parental expi cells using the Neon transfection system. For FACS analysis, expi cPVRIg cells were used between 1-3 days post transfection. Parental expi cells were harvested from log growth phase. 50,000-100,000 cells of per well of each type were seeded in 96 well plates. Anti-human PVRIg antibodies (hIgG1) and their respective controls were added in single point dilutions (5ug/ml), or as an 8 point titration series starting at 100ug/ml on ice for 30 mins-1 hr. The titration series were conducted as either 1:3 or 1:3.3 fold serial dilutions. Data was acquired using a FACS Canto II and analyzed using FlowJo and Prism software.

[00345] FACS analysis of naïve primary cynomolgus monkey leukocytes: Primary cynomolgus monkey (cyno) leukocytes were obtained from fresh blood which was drawn no longer than 24 hours prior to expression analysis. Blood was sourced from Bioreclamation. To assess protein expression of PVRIg on cyno PBMC, antibody cocktails towards major immune subsets were designed that included human anti-PVRIg antibodies. Anti-human PVRIg antibodies (hIgG1) and their respective controls were added in single point dilutions (5ug/ml).

[00346] Briefly, antibody cocktail mixtures were added to PBMC that were seeded at $5 \times 10^5 - 1 \times 10^6$ cells/well upon prior Fc receptor blockade and live/dead staining. Antibody cocktails were incubated with PBMC for 30mins – 1hr on ice. PBMC were then washed and data was acquired by FACS using a FACS Canto II. Data was analysed using Prism software. Immune subsets that were analysed include CD16+ lymphocytes, CD14+/CD56+ monocytes/myeloid cells, and CD3+ T cells.

[00347] RNA expression analysis of primary cynomolgus monkey leukocytes: Primary leukocytes that were assessed for RNA expression were CD56+, CD16+, and CD56-/CD16- subsets. Cell populations were isolated using non-human primate CD56 and CD16 positive selection kits according to manufacturer's instructions (Miltenyi Biotec). After sorting, cells were lysed in 350ul of RLT buffer and stored at -80oc until use.

[00348] Upon day of use, RNA was generated from lysed cells using the Qiagen mini kit according to the manufacturer's instructions. cDNA was generated using Applied Biosystems high capacity cDNA reverse transcription kit. qPCR using cDNA was performed using Taqman primers and Applied Biosystems Taqman fast advanced mastermix. Two sets

of primers to detect cyno PVRIG were designed by Compugen USA, Inc and manufactured by Genscript. The sequence and primer codes are:

[00349] Primer set 1

[00350] Forward: CTTGTGTTTCACCACCTCTGG

5 [00351] Reverse: TGTTCATCGCAGGAGGTC

[00352] Primer set 2

[00353] Forward: TTGGCTGTGGATACCTCCTT

[00354] Reverse: ATAAGGGTCGTGGAGAGCAG

[00355] Beta-actin primers were used for housekeeping and the primer set used was

10 Taqman catalogue number: Mf04354341_g1. Expression of transcripts was assessed by quantifying Ct values and relative expression was calculated by the $2^{(-\Delta\Delta Ct)}$ method. Products generated with PVRIG primers and beta-actin primers were also size analysed by traditional RT-PCR using a 2.5% agarose gel. qPCR data was acquired using an Applied Biosystems Step One Plus instrument.

15 [00356] Results

[00357] PVRIG antibodies recognize PVRIG on overexpressing cells: To screen for antibodies that were specific for PVRIG, we assessed the ability of antibodies that were generated from a phage campaign to bind HEK cell lines that were engineered to overexpress PVRIG. The majority of antibodies from this campaign upon reformatting to human IgG1
20 bound to the HEK hPVRIG cells, albeit with varying affinity. Furthermore, the majority of these antibodies also showed low background binding to HEK parental cell lines indicating high specificity towards PVRIG. Figure 27 shows one example of the specificity of PVRIG antibodies. A summary of all binding characteristics of the antibodies towards HEK hPVRIG cells relative to control that were generated in this phage campaign are displayed in Figure
25 31.

[00358] Human PVRIG RNA is expressed in a range of cancer cell lines: To initially screen for cell lines that could be used to assess PVRIG protein expression by antibodies, we examined the cancer cell line atlas for cell lines that were high for PVRIG RNA as assessed by bioinformatics. We found four cell lines that were readily accessible commercially that

were high expressors for PVRIG RNA that we chose to validate by qPCR analysis. These cell lines were Jurkat, CA46, Raji, and Daudi.

[00359] When qPCR analysis was conducted, we detected PVRIG RNA in all four cell lines consistent with the bioinformatics analysis (Figure 28). As a negative control we included expi cells that had relatively low PVRIG RNA expression.

[00360] Human PVRIG RNA is expressed in T cells and NK cells: To initially screen PBMC for subsets likely to be positive for PVRIG protein as detected by our antibodies, we sorted major PBMC subsets and examined PVRIG RNA expression by qPCR. Levels of PVRIG RNA in CD56+ NK cells, CD4+ T cells, CD8+ T cells, and CD14+ monocytes were compared to those in Jurkat, HEK parental, and HEK hPVRIG cell lines. As shown in Figure 29, PVRIG RNA was detected most highly and up to 50 fold higher in CD4+ T cells, CD8+ T cells, and CD56+ NK cells when normalized to HEK GFP cells. Similar to Figure 28, Jurkat cells also showed positive expression. In contrast, CD14+ monocytes did not show higher PVRIG expression relative to HEK GFP cells indicating very low PVRIG RNA expression.

[00361] In addition to analyzing naïve PBMC, select populations (effector lymphocytes) were also activated under various stimulatory conditions and expression of PVRIG RNA was assessed. More specifically, NK cells were activated with various combinations of stimulatory cytokines, whereas T cells were polyclonally activated with human activator dynabeads or staphylococcus enterotoxin B (SEB) with or without polarizing cytokines (see protocol section for details). As shown in Figure30A and B, PVRIG RNA expression generally increased in both NK cells and T cells upon various stimulation conditions, the extent of which depended on the individual donor. More specifically, Figure 30a shows PVRIG RNA expression in naïve and activated CD4 T cells and NK cells. Figure 30b shows PVRIG RNA expression in naïve and activated CD8 T cells.

[00362] PVRIG antibodies recognize PVRIG protein on NK cells most prominently in naïve and activated primary immune subsets: Upon confirming the RNA expression pattern of PVRIG RNA expression in naïve and activated PBMC subsets, we used our panel of PVRIG antibodies to assess protein expression. We first assessed PVRIG expression in naïve PBMC subsets. The population which displayed the highest level of PVRIG was NK cells. CD4+ and CD8+ T cells showed low levels of PVRIG, while B cells and monocytes had no detectable expression. A summary of expression on NK cells and CD8+ T cells as detected

by our antibodies is shown in Figure 32. Other minor subsets also displayed PVRIG expression and included non-conventional T cells such as NKT cells and $\gamma\delta$ T cells. The expression pattern on PBMC subsets was very similar across all donors we sourced and analyzed.

5 **[00363]** When PVRIG protein was assessed after various stimulation conditions (including polyclonal stimulation, cytokine stimulation, and MLR), there was no robust up-regulation of PVRIG on any PBMC subsets, including NK cells and CD4⁺ and CD8⁺ T cells. Furthermore, monocytes which were polarized in vitro to dendritic cells with GM-CSF and IL-4 did not show detectable PVRIG expression consistent with that seen on non-polarized
10 monocytes.

[00364] PVRIG is detected on cell lines by a proportion of PVRIG antibodies: In addition to screening PBMC for PVRIG protein expression, we wanted to understand whether it was also expressed on cancer cell lines. Using the positive cell lines identified by RNA expression (Figure 28), we chose to screen our antibodies on Jurkat and CA46 cells as
15 they showed the lowest absolute Ct values relative to our housekeeping gene. We also chose a range of negative cell lines to further validate the specificity of our antibodies which included OV-90, NCI-H441, and HepG2. A proportion of our antibodies did detect PVRIG protein expression on Jurkat and CA46 cells (Figure 31), but not the negative cell lines. An example of PVRIG detection on Jurkat and CA46 is shown in Figure 33 with a representative
20 antibody, CPA.7.021. The expression on Jurkat and CA46 was completely in accordance with each other and the intensity of expression was similar across the two cell lines.

[00365] PVRIG antibodies detect cynomolgus PVRIG transiently expressed on expi cells: In order to assess the pre-clinical suitability of our anti-human PVRIG antibodies for pharmacological studies in cynomolgus monkey, we wanted to understand whether our
25 antibodies were able to cross-react with cynomolgus PVRIG (cPVRIG). A proportion of our antibodies were able to detect cPVRIG which was transiently transfected onto expi cells (Figure 29). An example of an antibody that yielded negative staining (CPA.7.021) and one that yielded positive staining (CPA.7.024) are shown in Figure 34.

[00366] PVRIG RNA is detected in cynomolgus PBMC: Prior to assessment of
30 PVRIG protein on cyno PBMC, we firstly wanted to determine the PVRIG RNA expression profile in cyno PBMC subsets. As no cPVRIG primers set existed, we designed two sets that were directed at two distinct sites on the cPVRIG gene. One primer set was specific for the

X2 variant of cPVRIG, while the other set was able to pick up both the X1 and X2 variant. As shown in Figure 35, both primer sets were able to detect cPVRIG RNA at a similar level when compared to each other. Furthermore, unlike human PBMC where there was a distinct PVRIG RNA signature in effector lymphocytes (NK and T cells) compared to monocytes, cPVRIG RNA was expressed at a similar level across all PBMC subsets from all donors assessed.

[00367] PVRIG protein expression on cynomolgus PBMC is very low or negative:

Having established a cPVRIG RNA profile for cyno PBMC, we screened for the presence of cPVRIG protein on cyno PBMC using a select panel of anti-human PVRIG antibodies. The antibodies chosen to screen PBMC were based on their ability to bind cPVRIG transient cells and/or functional activity. As shown in Figure 36, we were able to detect low level of expression of cPVRIG on the CD16⁺ lymphocyte subset (NK cells) from a range of antibodies, but not the CD3⁺ lymphocyte subset (T cells) nor the CD14⁺ CD56⁺ myeloid subset (monocytes). Despite this data, those antibodies that showed positive detection over control (as denoted by the solid black line) did not correlate to those that were able to bind the cPVRIG transient cells. For example, the level of staining by CPA.7.021 was more than CPA.7.024 despite the former not binding to cPVRIG transient cells (see Figure 36).

[00368] Summary and Conclusions: Using an antibody phage platform, we have been able to successfully generate monoclonal antibodies towards the human PVRIG antigen.

Using engineered over-expressing cells as well as a suite of cancer cell lines, we showed that our antibodies are highly specific to the PVRIG antigen, and are able to detect protein expression which correlated with RNA expression. Upon analysis of human PBMC subsets, we showed that the PVRIG protein is most highly expressed on NK cells, with low expression on conventional CD3⁺ T cells, and not detectable on B cells and myeloid cells. The expression did not robustly change upon exposing these cell types to various stimulation conditions. We also showed that a panel of our antibodies are cross-reactive with the cynomolgus monkey (cyno) PVRIG antigen through assessing their binding to over-expressing cells. However, the combination of the low level of binding of this panel of antibodies to cyno PBMC, the lack of protein correlation with RNA, and the discordance of their ability to bind to over-expressing cells (compared to PBMC) indicates that the PVRIG antigen on cyno PBMC may be very low/negative, or it is expressed in a different/more complex form compared to the over-expressing cells.

[00369] Specifically incorporated by reference herein is USSN 62/141,168, filed 31 March 2015, and USSN 62/118,235, filed 19 February 2015, in their entirety, and in particular for the Examples therein, and for the associated Figures and Legends.

[00370] **Example 1D:**

5 [00371] **Expression of PVRIG in PBMC subsets from healthy donors:** The expression of PVRIG in PBMC subsets from healthy donors was tested (gating strategy is shown in Figure 1a). In the tested samples, PVRIG was shown to express on CD8⁺ T cells (data not shown), CD8 α ⁺ $\gamma\delta$ T cell (data not shown), double-negative $\gamma\delta$ T cells (data not shown) and to a milder extent also on CD4⁺ T cells (data not shown) of healthy donors PBMCs (n=5).

10 [00372] Specifically incorporated by reference herein is USSN 62/141,168, filed 31 March 2015, and USSN 62/118,235, filed 19 February 2015, in their entirety, and in particular for the Examples therein, and for the associated Figures and Legends.

[00373] **Example 1E**

[00374] **Co-expression of PVRIG with PD1, TIGIT and HLA-DR in Ovarian Cancer**
 15 **ascites, PBLs of MSS, CRC, and in resting and allo-activated healthy PBMCs:** PVRIG is co-expressed with TIGIT on CD8⁺ T cells in ovarian cancer ascites (data not shown) . In this sample, a mixed level of PVRIG expression was observed, that overlapped with that of PD-1 expression. Low level of HLA-DR correlated with low level of PVRIG expression. Very low level of PVRIG was observed on CD4⁺ T cells in this specific sample, indicating no
 20 correlation with PD1, TIGIT and HLA-DR.

[00375] In PBLs of MSS CRC patients, PVRIG is co-expressed with TIGIT on CD8⁺ T cells (data not shown). Low expression levels of PVRIG were observed in this sample which was in correlation with the low levels of TIGIT and HLA-DR. TILs from this patient had small CD8⁺ population that stained positive for surface PVRIG, which was also positive
 25 for PD1 and TIGIT (data not shown). Intracellular stain revealed prominent PVRIG stain that mirrored the expression pattern of PD-1, showing two distinct populations that are PD1-PVRIG- and PD1+PVRIG+ (data not shown). Intracellular PVRIG⁺ CD8⁺ T cells seem to better correlate with the HLA-DR⁺ and TIGIT⁺. PVRIG was not detectable on the surface of CD4⁺ T cells and only minority of the CD4⁺ cells showed positive intracellular PVRIG stain
 30 in the PD1⁺ population. Due to the very small intracellular PVRIG⁺ population, it is difficult to determine if PVRIG is co-expressed with TIGIT and HLA-DR.

[00376] In healthy PBMCs, PVRIG stain on CD8 T cells mirrored the expression pattern of PD-1 and TIGIT, showing distinct PD1-PVRIG- and PD1+PVRIG+ populations and distinct TIGIT-PVRIG- and TIGIT+PVRIG+ populations (data not shown). PVRIG was not detected on CD4+ cells. Interestingly, following allo- activation, co-expression of PVRIG and PD-1 was observed on CD4+ (but no on CD8+) (data not shown).

[00377] In summary, PVRIG was shown to co-express with TIGIT in CD8+ T cells from ovarian cancer ascites, MSS CRC patient's PBLs and with PD-1 healthy donor's PBMCs and with PD1 in CD4+ T cells of allo activated PBMCs from healthy donor.

[00378] Specifically incorporated by reference herein is USSN 62/141,168, filed 31 March 2015, and USSN 62/118,235, filed 19 February 2015, in their entirety, and in particular for the Examples therein, and for the associated Figures and Legends.

[00379] **Example 1F**

[00380] Expression of PVRIG on lymphocyte populations from Healthy PBMCs Urachal cancer, colorectal cancer, ovarian cancer ascites and lung cancer: Results: The expression of PVRIG on CD4+ and CD8+ T cells, NK cells and on CD4+ and CD8+ NKT cells was analyzed in healthy donors' PBMCs and tonsils and in TILs from urachal cancer, colorectal cancer, ovarian cancer ascites, lung cancer and melanoma.

[00381] In healthy donors' PBMCs (n=5) and in ovarian cancer ascites TILs (n=1) high levels of PVRIG expression was detected on NK cells (data not shown) and CD8+NKT cells (data not shown) and to a lower extent also on CD8+ T cells (data not shown) and CD4+ NKT (data not shown). CD4+ T cells also stained positively for PVRIG in some of the PBMCs, however the level of expression was quite low (data not shown).

[00382] In addition, PVRIG expression was detected on CD4+ T cells from two out of 6 colorectal cancer TILs tested, and in lung cancer TILs (n=3) (data not shown) and on NK cells from urachal cancer TILs (n=1).

[00383] No PVRIG expression was detected in melanoma TILs due to absence of TILs in the tested sample.

[00384] Specifically incorporated by reference herein is USSN 62/141,168, filed 31 March 2015, and USSN 62/118,235, filed 19 February 2015, in their entirety, and in particular for the Examples therein, and for the associated Figures and Legends.

[00385] **Example 1G**

[00386] Additional evaluations were done to identify addition tissues that over express PVRIG in human and mouse cell lines.

[00387] Reagents: Human PVRIG TaqMan probes (Life technologies)

Hs04189293_g1, Cat. # 4331182, TaqMan probe for Housekeeping gene (HSKG) (Life

5 technologies) human RPL19 Mm 01577060_gH, human HPRT1 Hs02800695_m1, human SDHA Hs00417200_m1, human PBGD Hs00609296_g1, and human TATA Box

Hs00375874_g1. Mouse PVRIG TaqMan probes (Life technologies) CC70L8H, CC6RN19

Custom TaqMan probes. TaqMan probes for Housekeeping gene (HSKG) (Life technologies) mouse RPL19: Mm02601633_g1. ABI TaqMan Fast Advanced Master mix, part no.

10 4444557, Applied Biosystem. Commercial Human and Mouse cancer cell lines from American Type Culture Collection (ATCC) and CLS (Cell line service) are detailed in Table 1. RNA extraction from human and mouse cell lines was performed with RNAeasy Mini Kit (Qiagen cat # 74014). cDNA was produced using High Capacity cDNA Reverse

Transcription Kit (Applied Biosystems cat#4368814. Commercial mouse polyclonal Anti-

15 PVRIG Ab MaxPab (B01), Abnova, Cat#H00079037-B01, diluted 1:200. Mouse IgG1, Life Technologies, Cat#MG100, diluted 1:200. Commercial mouse polyclonal Anti-PVRIG Ab, Sigma, Cat#SAB1407935, 10ug/ml. Chrom pure Mouse IgG, whole molecule, Jackson, Cat#015-000-003, 10ug/ml. Goat Anti Mouse-PE, Jackson, Cat#115-116-146, diluted 1:100. Custom polyclonal Rat-Anti mouse PVRIG, Batch#20153456C.1, Aldevron, 10ug/ml.

20 Custom Rat total IgG, Batch#GV20884.1, Aldevron, 10ug/ml. Goat Anti Rat-PE, Jackson, cat# 112-116-143, diluted 1:100. Anti-human PVRIG-CPA.7.024 mIgG1 conjugated to AF647, 10ug/ml. Anti-human PVRIG-CPA.7.050 mIgG1 conjugated to AF647, 10ug/ml.

Anti-human PVRIG-CPA.7.005 mIgG1 conjugated to AF647, 10ug/ml. Anti-human PVRIG-CPA.7.002 mIgG1 conjugated to AF647, 10ug/ml. Synagis IgG1 conjugated to A647,

25 10ug/ml. Anti-human PVRIG-CPA.7.021 mIgG1 conjugated to AF647, 10ug/ml. Synagis IgG2 conjugated to A647, 10ug/ml. Rabbit polyclonal anti PVRIG Ab, Sigma,

Cat#HPA047497, diluted 1:300. Goat Anti Rabbit-HRP, Jackson, Cat# 111-035-003, diluted 1:100. VioBlue, Fixable viability stain 450, BD Bioscience, cat # 562247, diluted 1:1000.

Human Trustain FcX, Biolegend, Cat#422302. Rat anti mouse CD16/CD32 Fc block, BD,

30 Cat#553142. Ingenio Electroporation solution, Mirus, Cat#MIR50114. ON-TARGETplus Human PVRIG siRNA – SMARTpool, Dharmacon, Cat# L-032703-02. ON TARGET plus non targeting siRNA, Dharmacon, Cat# D-001810-01-05. The human cell lines used in the study are shown in Figure 54.

[00388] Transcript expression. Quantitative RT-PCR (qRT-PCR): RNA (1-5ug) extraction of human and mouse cell lines (detailed above in Tables 1 and 2) was preformed according to manufactures protocols. cDNA was prepared according to manufactures protocols (1ug RNA diluted in 20ul cDNA mix reaction). cDNA, prepared as described above, diluted 1:10 (representing 25ng RNA per reaction), was used as a template for qRT-PCR reactions, using a gene specific TaqMan probes (as above). Detection was performed using QuantStudio 12k device. The cycle in which the reactions achieved a threshold level of fluorescence (Ct= Threshold Cycle) was registered and was used to calculate the relative transcript quantity in the RT reactions. The absolute quantity was calculated by using the equation $Q=2^{-Ct}$. The resulting relative quantities were normalized to a relative quantities of housekeeping gene, mRPL19 or hRPL19.

[00389] Protein expression detection by Western Blot (WB): The expression of human PVRIG in human cell lines was analyzed by WB using whole cell extracts (45ug for the cancer cell lines, and 30ug for the over expressing cell line and negative control cell line). Commercial rabbit polyclonal anti-human PVRIG pAb, Sigma, cat # HPA047497, diluted 1:300 in 5% BSA/TBST followed by secondary Ab goat anti-Rabbit –Peroxidase conjugated (Jackson, cat # 111-035-003), diluted 1:20,000 in 5% milk TBST.

[00390] Protein expression analysis by Flow Cytometry (FACS): The cell surface expression of PVRIG protein was analyzed by FACS. Human or mouse cell lines were stained with VioBlue reagent diluted 1:1000 in PBS. Cells were incubated 15 min at R.T. and then washed once with PBS. Cell lines for endogenous protein analysis were pre-incubated with the Fc receptor blocking solutions listed above in material section (2.5 µl/reaction of human blocker and 1µl/reaction of mouse blocker was used according to the manufactures procedures). To detect the human PVRIG protein, cells were stained with a commercial polyclonal anti human PVRIG or by a custom monoclonal anti-human PVRIG mAbs (Inc production, detailed in materials & methods section above) diluted to a concentration of 10ug/ml or 1:200 (for Sigma Ab and for mAb or for Abnova Ab respectively) or IgG1 Isotype control at the same concentration followed by Goat anti mouse PE conjugated Ab.

[00391] To detect the mouse PVRIG protein, cells were stained with a Custom rat polyclonal anti-mouse PVRIG pAb (Aldevron,) diluted to a concentration of 10ug/ml or rat IgG whole molecule as isotypes control at the same concentration followed by Donkey anti Rat-PE conjugated Ab diluted 1:100.

[00392] PVRIG knock down: Knock down of endogenous human PVRIG was carried out by transient transfection of siRNA. Transfection of 100 pmol PVRIG siRNA pool or scrambled siRNA performed by electroporation using Amaxa nucleofector device and MIRUS Ingenio electroporation solution, as listed above in materials & methods and according to the manufacture procedure. 48 hours post transfection, cells were collected for further analysis by qRT-PCR and FACS.

[00393] Results: Endogenous expression of the PVRIG transcript in human and mouse cell lines by qRT-PCR

[00394] Human cell lines: In order to verify the presence of the PVRIG transcript in human cell lines (listed in Figure 54), qRT-PCR was performed using a specific TaqMan probe as describe above in Material & Methods. As shown in Figure 56, human PVRIG transcript is observed using TaqMan probe Hs04189293_g1 with relatively high levels in Jurkat (A, B), HUT78 (A, B) and HL60 (B) cell lines. Lower transcript level is observed in THP1, RPMI8226 (B) cell lines. All other cell lines show very low to no transcript.

[00395] Endogenous expression of the PVRIG transcript in mouse cell lines by qRT-PCR: In order to verify the presence of the PVRIG transcript in mouse cell lines (listed in Figure 55), qRT-PCR was performed using a specific TaqMan probe as describe above in Material & Methods. As shown in Figure 57 mouse PVRIG transcript is observed using TaqMan probe CC70L8H with relatively high levels in NIH/3T3, Renca, SaI/N and J774A.1 (A), cell lines. Lower transcript level is observed in CT26 (A) and B-104-1-1(B) cell lines. All other cell lines show very low transcript.

[00396] Endogenous expression of the PVRIG proteins in human cell lines by WB: WB analysis for endogenous expression of PVRIG protein was carried out on various human cancer cell lines lysates as detailed in Figure 54 using commercial anti human PVRIG pAb (Sigma, HPA047497) as described in Materials & Methods above. As a positive control, whole cell extract of stable HEK293 cell pool over- expressing PVRIG was used while cells transfected with an empty vector served as the negative control. As shown in Figure 58, a protein band corresponding to ~35kD was detected in the positive control HEK293 over expressing cells (lane 2), as well as in the Jurkat cell line (lane 3). No expression of human PVRIG was detected in the empty vector cells (lane 1) which served as a negative control nor in ZR75-1 human cell line (lane 4).

[00397] Endogenous expression of the PVRIG proteins in human and mouse cell lines by FACS:

[00398] Human cell line: To verify the cell-surface endogenous expression of human PVRIG, various human cell lines (detailed in Figure 54) were tested as described in Material & Methods above. The cell lines were stained with the commercial Ab (Abnova) or with Isotype control followed by a secondary goat anti mouse PE Ab. Analysis was performed by FACS. Binding of Abnova antibody was observed in Jurkat human cancer cell line as compared to isotype control binding. No binding of Abnova Ab was observed in the other tested cell lines: For Capan2 and ZR75-1 as compared to isotype control binding, additional FACS analysis was done using Sigma commercial Ab on a various human cell lines (Jurkat, HUT78, Karpas299 and NK-YTS), binding was observed in Jurkat cells only but no binding was observed to other cell lines (data not shown).

[00399] Further analysis for endogenous confirmation of human PVRIG in Jurkat cell line, was done by testing binding of various monoclonal antibodies of the invention. Jurkat cell line was stained with five anti-human PVRIG custom mAbs (CPA.7.024, CPA.7.050, CPA.7.005, CPA.7.002 and CPA.7.021) conjugated to AF647 or with relative Isotype control Ab conjugated to AF647. Analysis was performed by FACS. The expression of human PVRIG in Jurkat human cell line was observed by CPA.7.021 and CPA.7.050 only, as compared to isotype control expression. No binding for human PVRIG was observed in Jurkat cell line by using the other three mAbs.

[00400] Mouse cell line: to verify the cell-surface endogenous expression of mouse PVRIG, various mouse cell lines: J774A.1, NIH/3T3, SaI/N and Renca (detailed in Figure 55), were tested as described in Material & Methods above. The cell lines were stained with the custom polyclonal rat anti mouse PVRIG Ab (Aldevron), or with Isotype control (Aldevron) followed by a secondary goat anti rat PE Ab. Analysis was performed by FACS. No binding for mouse PVRIG protein was observed in either of the tested mouse cell lines by Aldevron polyclonal Ab (data not shown).

[00401] Knock down of human PVRIG in human cell lines: In order to further confirm endogenous expression of PVRIG protein in Jurkat cell line, human PVRIG siRNA pool was used for knock down as described in Material & Methods. 48 hours post siRNA transfection, cells were harvested for further analysis by qRT-PCR and by FACS.

[00402] Knock down of human PVRIG in human cell lines tested by qPCR: As shown in Figure 59, human PVRIG transcript level in Jurkat cells transfected with human PVRIG siRNA pool is significantly reduced (right histogram bar) as compared to cells transfected with scrambled siRNA (left histogram bar) analyzed by qRT-PCR as described in Material & Methods..

[00403] Knock down of human PVRIG in human cell lines tested by FACS: Further analysis of human PVRIG membrane expression in the same siRNA transfected cells was performed by FACS. As shown in Figure 60 membrane expressions of human PVRIG protein is reduced in cells transfected with PVRIG siRNA (green for CPA.7.021mAb or red for Sigma Ab) as compared to cells transfected with scrambled siRNA (orange). The fold change (anti PVRIG vs, Isotype control) in Jurkat cell line is decreased from 8 fold to 3.3 fold by using Sigma Ab, or from 15.3 fold to 2.8 fold by using CPA.7.021 mAb.

[00404] This report includes preliminary data on PVRIG endogenous expression in cell lines both at the RNA level and the protein level in human and mouse cell lines.

[00405] Various human cancer cell lines were tested by qRT-PCR, WB and FACS for endogenous expression of PVRIG.

[00406] Cell surface expression of human PVRIG was observed in Jurkat cell line by using the commercial polyclonal Abs (Sigma and Abnova) and the mouse monoclonal Abs (Inc), as shown in Figure 4A and 4B respectively. These observations are in correlation to RNA transcript levels as shown in Figure 1A & B, and to WB results as shown in Figure 3.

[00407] Additional confirmation of endogenous human PVRIG in Jurkat cell lines was done by knock down experiment confirming clear reduction in the RNA transcript following PVRIG siRNA transfection, as shown in Figure 5, and also reduction was observed in the protein cell surface expression in Jurkat cell lines as shown in Figure 6 by commercial Ab and by monoclonal Ab.

[00408] Various mouse cell lines were tested by qRT-PCR and FACS for endogenous expression of PVRIG. In the transcript level, presence of PVRIG was observed in J774A.1, NIH/3T3, SaI/N and Renca cell lines as shown in Figure 2A & B. Although no membrane expression of mouse PVRIG was observed in these tested cell lines detected by polyclonal Ab (Aldevron) (data not shown). Figure 61 and Figure 62 indicate the summary of the findings described in this report, highlighting the cell lines showing correlation between qPCR and FACS, confirmed by knock down.

[00409] Specifically incorporated by reference herein is USSN 62/141,168, filed 31 March 2015, and USSN 62/118,235, filed 19 February 2015, in their entirety, and in particular for the Examples therein, and for the associated Figures and Legends.

[00410] **Example 1H**

5 [00411] The aim of this experiments is to evaluate the expression of PVRIG protein on resting or activated human (Tumor infiltrating lymphocytes) TILs isolated from human melanoma samples and propagated in the presence of melanoma specific antigens and IL2. Human mAb were produced directed against the extracellular domain (ECD) of human PVRIG. These Abs were directly labeled with Alexa flour 647 in order to examine the
10 expression of PVRIG on cells by FACS analysis.

[00412] **Materials and Methods**

[00413] TILs: In this experiments series three different Tumor-infiltrating lymphocyte (TIL) from resected metastases of three melanoma patients were used: 1) TIL-412- HLA-A2-Mart1 specific; 2) TIL-F4- HLA-A2-gp100 specific, and 3) TIL-209- HLA-A2-gp100
15 specific. Human TILs (>90% CD8+), were thawed 24h prior to beginning of experiment. Cells were thawed in 12 ml of TIL medium (IMDM + 10% human serum + 1% Glutamax + 1% Na-Pyruvate + 1% non-essential amino acids + 1% Pen-Strep) supplemented with 300 U/ml of rhIL2 (Biolegend 509129). Cells were left to recover from freezing for 24 hours.

[00414] Assay conditions: After recovery, TILs were tested in four different
20 conditions: 1) Resting – with 300U/ml of IL2 (Biolegend cat-589106), 2) With polyclonal activation of T cells, using 1 µg/ml of plate bound anti CD3 antibody (eBioscience clone OKT3, cat-16-0037-85) + 2 µg/ml of anti CD28 ab (eBioscience clone CD28.2 cat-16-0289-85) + 300 U/ml of IL2. 3) Co-cultured (1:1) with Mel888 (LIMS ID: CL-216) melanoma cells (HLA-A2 negative) and 4) Co-cultured (1:1) with Mel624 (LIMS ID CL-218)
25 melanoma cells (HLA-A2 + Mart1/gp100 positive).

[00415] After 12 hours of resting / activation / co-culture, cells were tested by FACS for PVRIG expression as well as the expression of other members of PVRIG pathway and other surface markers.

[00416] Staining cells: Cells were harvested after 12 hours and washed twice with
30 PBS. Cells were stained in room temp for 20 minutes with PBS supplemented with 1/1000 of fixable viability stain efluor 450 (BD horizon cat-562247). After staining, cells were washed

twice with PBS and stained for 15 minutes on ice with FACS buffer (PBS + 0.5% BSA + 2 mM EDTA + 0.05% Azide) supplemented with 1/25 of human Truostain FC-Block (Biolegend, 422302). After FC-blocking, cells were stained on ice for 30 minutes with the Abs and concentrations that are listed in table 1.

Antibodies	Isotype	Conjugated to	Manufacturer	Catalog number	concentration (ug/ul)	Staining concentration
Anti-human PVRIG - CPA.7.021	Human IgG2	AF-647	Compugen - iNC	CPA.7.021	0.2	5 ug/ml
Human IgG2 isotype control	Human IgG2	AF-647	Compugen - iNC		0.2	5 ug/ml
CD96	mIgG1	APC	Biolegend	338410	0.2	4 ug/ml
PVR	mIgG1	APC	Biolegend	337618	0.05	1 ug/ml
PVRL2	mIgG1	APC	Biolegend	337412	0.1	2 ug/ml
TIGIT	mIgG1	APC	eBioscience	17-9500-42	0.025	0.5 ug/ml
DNAM1	mIgG1	APC	Biolegend	338312	0.1	2 ug/ml
PD1	mIgG1	AF647	Biolegend	329910	0.1	2 ug/ml
CD8	mIgG1	FITC	Biolegend	300906	0.15	3 ug/ml

5 [00417] After staining, cells were washed once and re-suspended in FACS buffer for analysis. Compensation calibration was done using compensation beads (BD, 552843). One drop of beads were stained for 30 minutes with above antibodies. Beads staining was done with same concentrations as cell staining. After beads staining, compensation was performed on MacsQuant FACS machine according to standard procedure. All samples were acquired
10 on a MACSQuant analyzer (Miltyeni) and data was analyzed using Tree Star FlowJo software (v10.0.8).

[00418] PVRIG is expressed on human resting TILs: Resting TILs, cultured for 12 hours with 300 U/ml of IL2 only, were stained for PVRIG expression and analyzed by FACS. Gating strategy for TILs: Lymphocytes were gated first according to size and granularity in
15 FCS:SSC graph, than single cells were gated according to FSC-H and FSC-A, than live cells were gated according to viability Dye staining in Vioblue:FSC graph, than CD8⁺ cells were gated according to CD8 staining in CD8:FSC graph. Expression levels of PVRIG was than plotted according to PVRIG staining in histograms.

[00419] PVRIG expression on human TILs is downregulated upon activation with anti
20 CD3 + anti CD28 abs: Human TILs, cultured for 12 hours with anti CD3 + anti CD28 abs + IL2 were stained for PVRIG expression and analyzed by FACS. PVRIG expression on

surface of all three TILs examined is downregulated upon activation, comparing to resting TILs (data not shown).

[00420] PVRIG expression on human TILs is slightly downregulated upon co-culture with Mel888: Human TILs, co-cultured for 12 hours with Mel888 cells were stained for PVRIG expression and analyzed by FACS. PVRIG expression on surface of all three TILs examined is slightly downregulated upon co-culture with Mel888 comparing to resting TILs.

[00421] PVRIG expression on human TILs is downregulated upon co-culture with Mel624: Human TILs, co-cultured for 12 hours with Mel624 cells were stained for PVRIG expression and analyzed by FACS. PVRIG expression on surface of all three TILs examined is slightly downregulated upon co-culture with Mel624 comparing to resting TILs.

[00422] Expression of other pathway members on resting TILs: Human TILs, co-cultured for 12 hours with IL2 only were stained for the expression of CD96, PVR, PVRL2, TIGIT and DNAM1 and analyzed by FACS. CD96, TIGIT and DNAM1 is expressed on all three examined TILs. PVR is expressed on the surface of all three TILs as well but to relatively low levels. PVRL2 is not detected on any of the TILs.

[00423] Expression of other pathway members on TILs activated with anti CD3 and anti CD28 abs: Human TILs, cultured for 12 hours with anti CD3 and anti CD28 abs were stained for the expression of CD96, PVR, PVRL2, TIGIT and DNAM1 and analyzed by FACS. Upon activation with anti CD3 + anti CD28 abs, CD96 is downregulated, PVR is slightly upregulated, TIGIT is slightly upregulated and DNAM1 is upregulated as well.

[00424] Expression of other pathway members on TILs Co-cultured with Mel888: Human TILs, co-cultured for 12 hours with Mel888 cells were stained for the expression of CD96, PVR, PVRL2, TIGIT and DNAM1 and analyzed by FACS. Upon co-culture with Mel888, CD96 is downregulated, PVR is highly upregulated, TIGIT and DNAM1 is downregulated, PVRL2 is slightly induced as well.

[00425] Expression of other pathway members on TILs Co-cultured with Mel624: Human TILs, co-cultured for 12 hours with Mel624 cells were stained for the expression of CD96, PVR, PVRL2, TIGIT and DNAM1 and analyzed by FACS. Gating strategy was done according to figure 1. Upon co-culture with Mel624, CD96 is downregulated, PVR is highly upregulated, TIGIT is stable or slightly upregulated, DNAM1 is downregulated and PVRL2 is slightly induced.

[00426] Expression of PD1 on TILs: Human TILs, cultured for 12 hours with IL2 only or activated with anti CD3 + anti CD28 abs or co-cultured with Mel888 or with Mel624 cells were stained for the expression of PD1 and analyzed by FACS. As can be seen in figure 16 and figures 17, PD1 is expressed on resting TIL412 only. No change in PD1 expression is noticed upon co-culture with Mel888, But, PD1 is upregulated in all three TILs upon co-culture with Mel624 or upon activation with anti CD3 + anti CD28 abs.

[00427] Summary and conclusions: For all TILs that were tested:

- Anti PVRIG - CPA.7.021 ab stains TILs (up to 2.6 fold)
- PVRIG expression is downregulated upon activation of 12 hours with anti CD3 + anti CD28 abs or upon co-culture with Mel624 (almost to background level).
- Resting TILs express CD96, TIGIT and DNAM1 (up to 35, 12 and 79 fold respectively)
- CD96 expression is downregulated upon activation (from up to 35 to ~11 fold) or co-culture with irrelevant (HLA-A2-) melanoma
- DNAM1 expression is upregulated upon activation with α CD3/CD28 abs (from up to 79 to 102 fold) but strongly downregulated upon co-culture of TILs with Mels (down to 8 fold).
- TIGIT expression is slightly downregulated upon co-culture of TILs with mel888 cell line, and was stable with a slight upregulation upon co-culture with Mel624 or activation with anti CD3 + anti CD28 abs.
- PD1 expression is upregulated upon activation (from 0 up to 18 fold)
- High levels of PVR were detected following TILs co-culture with melanomas (from <2 up to 18 fold).

[00428] Resting TIL-412 show positive staining for PD1. TIL-F4 is also slightly positive for PD1 whereas TIL-209 is negative. Summary of changes in expression levels of all parameters tested, in the different conditions can be seen in Table 2.

[00429] Table 2:

	+IL2	+αCD3+αCD28+IL2	+Mel888	+Mel624
PVRIG	1.4 – 2.6	0 – 12	1.3 – 1.7	0 – 1.2
CD96	23 – 35	12.7 – 16	11.7 – 17.6	11.1 – 16.6

TIGIT	5.7 – 12.6	7.8 – 12.5	4 – 7.3	6.1 – 12.5
DNAM1	43 – 79	56 – 100	14 – 20	17 – 25
PVR	1.6 – 1.8	2.6 – 3.2	13.6 – 18	11 – 17
PVRL2	0	0	1.4 – 2.3	1.2 – 1.8
PD1	0 – 4.5	2.3 – 18.4	0 – 4.6	2 – 9.3

[00430]

Specifically incorporated by reference herein is USSN 62/141,168, filed 31 March 2015, and USSN 62/118,235, filed 19 February 2015, in their entirety, and in particular for the Examples therein, and for the associated Figures and Legends.

5 **Example 1I: EXPRESSION OF PVRIG ON RESTING AND ACTIVATED HUMAN T CELLS AND TILS**

[00431] The aim of this example was to evaluate the expression of PVRIG protein on resting and activated human isolated primary CD4+ and CD8+ T cells, as well as TILs

10 (Tumor Infiltrating Lymphocytes) isolated from human melanoma samples and propagated in the presence of melanoma specific antigens and IL2. Human mAbs were produced against the extracellular domain (ECD) of human PVRIG. These Abs were directly labeled with Alexa flour 647 in order to examine the expression of PVRIG on cells by FACS analysis.

[00432] Materials and Methods

15 [00433] TILs: In this series of experiments, two different TILs, from resected metastases of three melanoma patients, were used:

[00434] TIL-Mart1- HLA-A2-Mart1 specific

[00435] TIL-209- HLA-A2-gp100 specific

[00436] Human TILs (>95% CD8+), were thawed 24h prior to beginning of
20 experiment. Cells were thawed in 12 ml of TIL medium (IMDM + 10% human serum + 1% Glutamax + 1% Na-Pyruvate + 1% non-essential amino acids + 1% Pen-Strep) supplemented with 300 U/ml of rhIL2 (Biolegend 509129). Cells were left to recover for 24 hours.

[00437] Primary T cell: In this series of experiments two different donors were used:

[00438] CD4+ and CD8+ from donor #147

[00439] CD4+ and CD8+ from donor #186

[00440] Human primary cells (>95% purity), were thawed 24h prior to beginning of experiment. Cells were thawed in RPMI complete medium (RPMI + 10% FBS + 1% Glutamax + 1% Na-Pyruvate + 1% Pen-Strep) supplemented with 300 U/ml of rhIL2 (Biolegend 509129). Cells were left to recover for 24 hours.

[00441] Assay conditions: After recovery, cells were activated using a polyclonal activation of T cells, with 1 µg/ml of plate bound anti CD3 antibody (BD-pharmingen clone Ucht-1, cat-555329), 2 µg/ml of anti CD28 ab (eBioscience clone CD28.2 cat-16-0289-85) and 300 U/ml of IL2.

[00442] Activation was carried out for 24h, 48h, 72h and 144h.

[00443] Staining cells: Cells were harvested and washed with PBS. Cells were stained at room temperature for 10 minutes with PBS supplemented with 1/1000 of fixable viability stain efluor 450 (BD horizon cat-562247). After staining, cells were washed twice with PBS and stained with the Abs at the concentrations listed in Figure 65 for 30 minutes on ice in FACS buffer (PBS + 0.5% BSA + 2 mM EDTA + 0.05% Azide) and concentrations that are listed in Figure 65. After staining, cells were washed once and re-suspended in FACS buffer for analysis.

[00444] Results: Human T cells from two different donors and TILs were left untreated (resting) or polyclonal stimulated for various timepoints as described in Materials and Methods. Cell activation state was evaluated by detection of surface expression of CD137 and PD-1 at each time point compared to isotype control (FMO), as shown for activated CD8+, CD4+ T cells and TILs (Figure 70A, B & C respectively). As expected, PD-1 and CD137 expression was detected and elevated upon activation (Figure 70A, B & C).

[00445] PVRIG expression was observed on both resting CD4+ and CD8+ T cells, with higher expression on CD8+ cells (6-8 fold) as compared to CD4+ cells (3 folds), and diminished upon activation (Figure 71A, B & C). On days 3-6 of activation, PVRIG expression was increased on CD8+ (4-5 fold) and CD4+ (2-3 fold) T cells, as can be seen in Figure 71.

[00446] In addition, PVRIG expression was also observed on Mart1 and 209 resting TILs, and expression was decreased upon activation (Figure 72A, B & C). On day 3-6 of

activation PVRIG expression was increased, as can be seen in Figure 72, compared to day 1-2 of activation.

Example 2: Generation and Characterization of PVRIG-Expressing Stable Transfectant

Cell Pools

- 5 [00447] Recombinant stable pools of cell lines overexpressing PVRIG human and mouse proteins were generated, for use in determining the effects of PVRIG on immunity, for PVRIG characterization and for identifying immunoregulatory PVRIG based therapeutic agents.
- [00448] Materials & Methods:
- 10 [00449] Reagents: DNA constructs:
- [00450] Human PVRIG flag pUC57
- [00451] Human PVRIG flag pCDNA3.1
- [00452] Human PVRIG flag pMSCV
- [00453] Recombinant cells:
- 15 [00454] HEK293 pCDNA3.1 Human PVRIG flag
- [00455] HEK293 pMSCV Human PVRIG flag
- [00456] Commercial antibodies:
- [00457] Anti PVRIG, Sigma cat. HPA047497 – Rabbit polyclonal
- [00458] Anti-PVRIG, Abnova cat. H00079037-B01 -Mouse polyclonal
- 20 [00459] Full length validation of mouse PVRIG was done using PCR reactions and sequencing of the PCR products.
- [00460] Three couples of primers were used (Table 3).
- [00461] Table 3: Sequence of primers used for mouse full length validation
- [00462] Primer name Sequence :
- 25 [00463] 200-554_mPVRIG_F CCACCAACCTCTCGTCTTTC
- [00464] 200-553_mPVRIG_R TCATGCCAGAGCATACAG
- [00465] 200-571_mPVRIG_F CAGTGCCTCTAACTGCTGAC

[00466] 200-572_mPVRIG_R TCACTGTTACCAGGGAGATGAG

[00467] 200-549_mPVRIG_F CACAGGCTGCCCCATGCAAC

[00468] 200-551_mPVRIG_R TGCCTGGGTGCTAGTGAGAG

[00469] 200-554_mPVRIG_F CCACCAACCTCTCGTCTTTC

5 [00470] 200-546_mPVRIG_R GACCCTGTTACCTGTCATTG

[00471] As a templet for the PCR reaction, cDNA of NIH 3T3 cell line or a mix of three commercial cDNA panels were used:

[00472] 1. cDNA panel I, Mouse, Biochain, Cat no. C8334501 (Heart, Brain, Kidney, Liver).

10 [00473] 2. cDNA panel II, Mouse, Biochain, Cat no. C8334502 (Lung, Pancreas, Spleen, Skeletal Muscle).

[00474] 3. cDNA, Clontech, Cat no. 637301, (Brain, Heart, day 7 Embryo, Testis, Spleen).

[00475] Expression constructs

15 [00476] Full length cloning of human and mouse PVRIG-flag was performed by gene synthesis (GenScript) using codon optimized sequence in pUC57 vector for human transcript and non optimized for mouse transcript and subcloned into a mammalian expression vector, pcDNA3.1 or to pMSCV, to create the expression plasmid.

20 [00477] Human PVRIG sequence that was subcloned into pcDNA3.1 initiate from the second methionine of human PVRIG protein, whereas the human PVRIG sequence that was subcloned into pMSCV initiate from the first methionine of human PVRIG protein.

[00478] -Construct encoding the Human PVRIG-flag.

[00479] Full length human PVRIG gene, synthesis by GenScript was subcloned into using pcDNA3.1 using BamI and NheI restriction enzymes.

25 [00480] Constructs encoding the mouse PVRIG proteins:

[00481] Four contracts encoding the mouse sequence were synthesize by GenScript as following:

[00482] 1. First Methionine no tag

- [00483] 2. First Methionine with Flag
- [00484] 3. Second Methionine no tag
- [00485] 4. Second Methionine with Flag
- [00486] The synthesize gene were subcloned into pCDNA3.1
- 5 [00487] Generation of stable transfectants over expressing PVRIG proteins
- [00488] The resulting expression construct was verified by sequence and subsequently used for transfections and stable pool generation as described below. The protein sequences encoded by the expression constructs are as set forth in Figure 103.
- [00489] Generation of stable transfectant pools expressing human PVRIG-flag protein
- 10 [00490] HEK293 (ATCC, catalog number: CRL-1573) cells were transfected with pCDNA3.1+ human PVRIG-flag plasmid or with empty vector (pCDNA3.1+ as negative control), using FUGENE 6 Reagent (Roch, catalog number 11-988-387). Geneticin, G418 (Gibco, catalog number: 11811-031) resistant colonies were selected for stable pool generation.
- 15 [00491] GP2-293 packaging cell line (Clontech cat#631458) was transfected with pMSCV-human PVRIG or with pMSCV empty vector using Lipofectamine 2000 transfection reagent (Invitrogen, catalog number 11668019). 48 hours post transfection supernatants containing virions were collected, and directly used for infection of the human cell line as follows:
- 20 [00492] HEK-293 (ATCC, CRL- CRL-1573) cells was infected with virions expressing human PVRIG or with pMSCV empty vector virions as negative control, Puromycin (Invivogen, catalog number: 58-58-2) resistant colonies were selected for stable pool generation.
- [00493] Expression validation
- 25 [00494] Expression validation by Western blot
- [00495] Whole cell extracts of cell pool (30ug of total protein) were analyzed by western blot. As negative control, whole cell extracts of stable cell pools transfected with the empty vector were used. For the human PVRIG-flag detection, anti-flag and anti PVRIG antibodies were used as follow:

[00496] • Mouse anti Flag M2-Peroxidase, Sigma, cat. A8592 diluted 1:1000 in TTBS/5% BSA;

[00497] • Anti PVRIG, Sigma cat. HPA047497– Rabbit polyclonal, diluted 1:200 in TTBS/5% BSA. Followed by Goat Anti Rabbit-HRP, Jackson, Cat: 111-035-003
5 diluted 1:20,000 in 5% milk/TTBS

[00498] solution.

[00499] Expression validation by Flow Cytometry (FACS)

[00500] In order to validate the cell surface expression of the human PVRIG protein in the recombinant stable pools, 1×10^5 cells were stained with Fixable viability stain 450 (BD, 562247) diluted 1:1000 in PBS, for 10 min at R.T. Mouse polyclonal anti PVRIG, (Abnova, 10 Cat.H00079037-B01) diluted 1:200 or with mouse IgG1 isotype control (Life Technologies), were then added to cells followed by staining with Goat Anti Mouse-PE (Jackson, cat.115-116-146).

[00501] Results Expression validation of HEK293 Stable pool cells over expressing
15 the Human PVRIG-Flag protein

[00502] To verify expression of the PVRIG protein in the stably transfected HEK293 cells pools, whole cell extracts were analyzed by western blot using anti-flag antibody or anti PVRIG antibodies (Abnova), as described in Material and Methods. The results, shown in Figure 24, demonstrate a band corresponding to the expected protein size of ~33kDa in the
20 extracts of HEK293 cell pools expressing human PVRIG, but not in the cells transfected with the empty vector.

[00503] In order to verify cell surface expression of the PVRIG protein, HEK293 stably transfected cells over-expressing the PVRIG- flag pCDNA3.1 vector were analyzed by FACS using mouse anti-PVRIG pAb (Abnova) as described in Material and Methods. The
25 results presented in Figure 25 show that the binding of mouse anti-PVRIG pAb to cells stably expressing the human PVRIG- flag (gray) is higher than that observed with cells transfected with the empty vector (light gray).

Example 3: PVRIG-ECD Ig fusion protein production

[00504] PVRIG mECD-mIg fusion protein (see Figure 103), composed of the ECD of
30 mouse PVRIG fused to the Fc of mouse IgG2a, was produced at ProBioGen (Germany) in CHO-DG44 cells by culturing stable cell pools for 12 days, followed by Protein A

purification of cell harvest and preparative SEC purification for aggregate removal. The final product was formulated in 5mM Na citrate, 5mM Na/K phosphate, 140mM NaCl, 0.01% Tween pH5.5.

[00505] Expression vector used was ProBioGen's PBG-GPEX6. PVRIG gene is driven
5 by CMV/EF1 hybrid promoter followed by polyadenylation signal pA-1. The vector contains puromycin N-acetyl-transferase gene that allows selection of transfected cells using puromycin, as well as dehydrofolate reductase gene that allows selection of transfected cells using methotrexate (MTX).

[00506] PVRIG hECD-hIg fusion protein (see Figure 103), composed of the ECD of
10 human PVRIG fused to the Fc of human IgG1 bearing C220, C226 and C229 to S mutations at the hinge, was produced at GenScript (China) by transient transfection in CHO-3E7 cells which were cultured for 6 days, followed by protein A purification of cell harvest. The final product was formulated in PBS pH 7.2.

[00507] Expression vector used was Mammalian Expression Vector pTT5, in which
15 PVRIG gene is driven by CMV promoter.

Example 4: Expression of PVRIG on human PBLs and binding of PVRIG-Fc to melanoma cell lines

[00508] PVRIG is a novel immune checkpoint protein, which without wishing to be
limited by a single theory functions as a CD28 like receptor on T cells. In this study, the
20 expression of PVRIG on human peripheral blood lymphocytes and the binding of PVRIG-ECD-Ig (composed of the extra-cellular domain of human PVRIG fused to human IgG1) to melanoma cell lines was evaluated.

[00509] Materials and Methods

[00510] Three human melanoma cell lines which present the MART-1 antigen in
25 HLA-A2 context (SK-MEL-23, Mel-624 and Mel-624.38) were used as targets for CTLs. Mel-888 which does not express HLA-A2, served as a negative control.

[00511] Buffy coats from human healthy donors were obtained from Tel Hashomer
Blood Bank. Peripheral blood mononuclear cells were stimulated with PHA and cultured for
3 days, and subsequently transduced with MSCV-based retroviral vector (pMSGV1).
30 Following transduction, cells were further grown in lymphocyte medium (Bio target medium,

fetal bovine serum (10%), L Glutamine Penicillin/ Streptomycin (100 units/ml), IL-2 300 IU) for additional 5 days.

[00512] To evaluate PVRIG expression on PBLs, cells were stained with a specific antibody for PVRIG (mouse poly clonal) at 5ug/ml for 30min at 4 degrees. Following washing, cells were stained with FITC conjugated Goat anti mouse mAb (1:250) (Invitrogen, Cat# A10667) in FACS buffer in the dark for 30 minutes at 4 degrees. Following two washes in FACS buffer, samples were read on a BD Bioscience FACS Calibur with a Cytex HTS.

[00513] To evaluate binding of PVRIG-Ig to the melanoma cell lines, SK-MEL-23, Mel-624, Mel-624.38 and mel-888, cells were co-cultured with F4 transduced or un-transduced (designated w/o) PBLs and subsequently stained with 20ug/ml of the fusion protein PVRIG-Ig HH batch #125. Following two washes in FACS buffer, samples were stained with a secondary goat anti-human PE (Jackson, cat# 109-116-098).

[00514] Results

[00515] To evaluate the endogenous expression of PVRIG on primary human leukocytes, PBLs were stimulated with PHA and subsequently transduced with an empty vector and stained with an anti-PVRIG specific antibody. As shown in Figure 11, in two different donors staining with anti-PVRIG is observed relative to an isotype matched control.

[00516] To evaluate the endogenous expression of PVRIG on melanoma cell lines and to determine whether the endogenous expression is affected by co-culture with antigen specific T cells, 4 different melanoma cell lines (SK-MEL-23, Mel-624, Mel-624.38 and mel-888) co-cultured with PBLs either expressing or not expressing the F4 (gp100 specific TCR). Cells were subsequently stained with the fusion protein composed of the extra-cellular domain of human PVRIG fused the Fc portion of human IgG1. As shown in Figure 12, all 4 tested human melanoma cell lines exhibit binding to PVRIG-Ig. Binding intensity is not affected by T cell dependent activation following co-culture with melanoma reactive engineered T cells.

[00517] Summary: The results presented herein suggest that PVRIG is expressed on PHA activated human primary peripheral blood leukocytes (PBLs). In addition, 4 melanoma cell lines that were tested in this study bind to the fusion protein composed of the extra-cellular domain of human PVRIG fused the Fc portion of human IgG1 suggesting that these cell lines express the counterpart for PVRIG.

Example 5: Receptor-Ligand identification and Validation

[00518] A first validation study was performed using a cell microarray technology was used to screen for interactions of PVRIG to 3559 full-length human plasma membrane proteins, which were individually expressed in human HEK293 cells.

5 [00519] Human HEK293 cells were grown over slides spotted with expression vectors encoding 3559 full-length human membrane proteins. An expression vector (pIRES-hEGFR-IRES-ZsGreen1) was spotted in quadruplicate on every slide, and was used to ensure that a minimal threshold of transfection efficiency had been achieved or exceeded on every slide. Human HEK293 cells were used for reverse transfection/expression. A fusion protein
10 composed of the ECD of PVRIG fused to a human IgG1 was added at 20ug/ml to each slide following cell fixation. Detection of binding was performed by using an appropriate fluorescent secondary antibody. Two replicate slide-sets were screened. Fluorescent images were analyzed and quantitated (for transfection efficiency) using ImageQuant software (GE).

[00520] A protein 'hit' was defined as a duplicate spot showing a raised signal
15 compared to background levels. This was achieved by visual inspection using the images gridded on the ImageQuant software. Hits were classified as 'strong, medium, weak or very weak', depending on the intensity of the duplicate spots. To confirm the hits, all vectors encoding the hits identified in the primary screen were arrayed on new slides. Confirmation/Specificity screen and analyses was carried out as for primary screening (n=2
20 replicate slides per sample), except that identical slides were also probed with appropriate negative controls. Additionally, all the vectors encoding the hits were sequenced. Vectors encoding every primary hit was sequenced confirming its identity.

[00521] Background screen showed negligible binding to untransfected HEK293 cells at 2, 5 and 20 ug/ml (Figure 13). Based upon the background data, 20 ug/ml was chosen for
25 full profiling. Primary screen resulted in multiple duplicate hits (clones), with the majority being weak or very weak intensity. All primary hits identified, and a control EGFR-ZsGreen1 vector, were spotted and re-expressed in duplicate and probed with PVRIG at 20ug/ml for the Confirmation/Specificity screen.

[00522] A single specific hit, PVRL2, with strong intensity, was identified (Figure 14).
30 Another weak hit, MAG, was later shown to bind also other fusion proteins tested (data not shown), thus suggesting that it is not specific. These results are consistent with the recently published abstract <https://www.yumpu.com/en/document/view/7263720/sunday-december-4->

late-abstracts-1-molecular-biology-of-the-/133 by G. Quinones in New Technologies & Frontiers. PVRL2 is known to play a role as a ligand for TIGIT and DNAM1, which are both modulators of T cell and NK cell activation. TIGIT has been recently reported to be a key player in the inhibition of the immune response directed against tumor cells (Noa Stanietzky, journal of immunology, vol. 106 no. 42, 17858–17863; Robert J Johnston, Cancer cell, Volume 26, Issue 6, p923–937, 8 December 2014). Results presented in Example 5, showing interaction of PVRIG with the same counterpart as TIGIT, suggests an involvement of PVRIG in an important regulatory pathway that regulates cancer immune surveillance and thus positions PVRIG as a potential target for cancer treatment.

10 [00523] Additional Validation Study 2

[00524] Materials and Methods

[00525] Materials

[00526] Fc fusion proteins, His-tagged proteins and control Ig: The Fc fusion protein PVRIG-Fc M:M was used for binding studies. Mouse IgG2a was used as isotype control.

15 Other commercial mouse proteins used in the study were PVRL2-his (R&D, 3869-N2), and PVRL2-his (Sino Biological, 50318-M08H).

[00527] Cells: HEK293 over-expressing (OX) mouse PVRIG and PVRIG-FLAG were generated (RC-287 and RC-286, respectively) and binding of PVRL2 to these cells was compared to HEK293 cells expressing empty vector (EV) (RC-83). HEK293 OX mouse

20 PVRL2 splice variants 1 and 2 (sv1 and sv2) were generated (RC-334 and RC-335, respectively) and binding of PVRIG to these cells was compared to HEK293 cells expressing EV. B16-F10 cells (CL-161, mouse skin melanoma cells endogenously expressing mPVRL2) were also used to study the interaction between PVRIG and PVRL2.

[00528] Antibodies: Anti-mouse PVRL2-PE Ab (R&D, FAB3869P, 25µg/ml, 1:100)

25 was used for detection of PVRL2. Rat IgG2A-PE (R&D, IC006P, 25µg/ml, 1:100) was used as isotype control. Anti-mouse-PE (Jackson ImmunoResearch, 115-115-206, 0.5mg/ml, 1:200) and anti-his Ab (Abcam, ab72467, 0.1mg/ml, 1:300) were used to detect binding of recombinant proteins. Anti-DYKDDDDK Tag (anti-FLAG) Ab (BioLegend, 637302, 0.5mg/ml, 1:300) was used for detection of PVRIG expression on HEK293 OX mouse
30 PVRIG-FLAG. For PVRIG labeling, Alexa Fluor® 647 Antibody Labeling Kit (Molecular Probes, A-20186) was used according to manufacturer's protocol. For biotinylation of PVRIG, DSB-X™ Biotin Protein Labeling Kit (Molecular Probes, D-20655) was used

according to manufacturer's protocol. Biotinylated PVRIG was detected by streptavidin-PE (SA-PE) (Jackson ImmunoResearch, 016-110-084, 0.5mg/ml, 1:300).

[00529] Methods

[00530] FACS analysis of mouse PVRIG-Fc binding to stable HEK293 cells over-

5 expressing (OX) mouse PVRL2 or to B16-F10 cells: HEK293 cells OX PVRL2 (sv1 or sv2) or B16-F10 cells were suspended to 10^6 cells/ml in PBS. For each 1ml of cells, 1 μ l of viability stain stock solution (BD Horizon Fixable Viability Stain 450, cat. 562247, BD Bioscience) was added. Cells were incubated for 10min protected from light at room temperature. The cells were then washed twice with PBS and suspended to 3×10^6 cells/ml in
10 the presence of 1:50 human TruStain FcXTM (BioLegend 422302) in FACS buffer (PBS supplemented with 2% FBS and 0.5mM EDTA) at room temperature for 15min for blocking of Fc γ -receptors. Without washing, 1×10^5 cells/well were then plated in 96-well V-shaped plates (Costar #3357). Expression of PVRL2 was examined by anti-PVRL2 antibody (see above). Binding of PVRIG-Fc to cells was examined with various batches (see above),
15 generally at 60 μ g/ml or with several concentrations. Cells were incubated with antibodies or PVRIG-Fc for 40min at room temperature, then washed once. Secondary antibody (anti-mouse-PE) was added for 15min at room temperature, cells were washed twice and were taken for analysis by MACSQuant® FACS analyzers (Miltenyi Biotec), followed by data analysis using Flow-Jo 10 software.

20 [00531] FACS analysis of mouse PVRL2-his binding to stable HEK293 cells OX mouse PVRIG: PVRIG levels were examined with anti-FLAG antibody. PVRL2-his binding was monitored by anti-his antibody. FACS analysis was performed as described above.

[00532] Biophysical SPR analysis of mouse PVRIG / PVRL2 interaction by Biacore:

The interaction between mouse PVRIG and PVRL2 was analyzed in a Biacore T100 SPR
25 biomolecular interaction analyzer at Bar-Ilan University. Proteins were diluted to 100nM in acetate buffer pH 4.0, and were covalently coupled to a unique flow cell of a CM5 Series S Biacore chip using standard amine coupling chemistry. Surfaces were activated with EDC-NHS, and later blocked by injection of 1M ethanolamine (pH 8.5). Running buffer was 10mM Hepes pH 7.3, 150mM NaCl, 3mM EDTA and 0.05% Tween-20 (HBS-EP+). Final
30 immobilization levels were ~1000RU. Proteins used as analytes were diluted to 2500nM, 500nM and 100nM. In each run one tube contained running buffer only for reference. After each run a regeneration step with 4M MgCl₂ for 30 sec at 20 μ l/sec was performed.

[00533] Results

[00534] Binding of mouse PVRL2 to HEK293 cells OX PVRL2 sv1: In order to validate the interaction between mouse PVRL2 and mouse PVRL2 we first tested the binding of PVRL2-Fc to cells over-expressing (OX) PVRL2. The level of PVRL2 expression on HEK293 OX PVRL2 sv1 was determined using specific anti-mouse PVRL2 antibodies. Mouse PVRL2 expression was 10-fold higher compared to HEK293 cells expressing empty vector (data not shown). Four batches of PVRL2-Fc were examined for binding to PVRL2 OX cells. All PVRL2-Fc batches showed 6-11-fold binding to cells OX PVRL2 compared to empty vector cells (data not shown). Binding of PVRL2-Fc to PVRL2 OX cells was also examined using biotinylated and fluorescently labelled (Alexa Fluor 647) PVRL2 proteins. While the biotinylated proteins displayed slightly stronger binding to PVRL2 OX cells compared to untagged PVRL2-Fc (data not shown), fluorescently labelled PVRL2 demonstrated much lower binding (data not shown). These results show that PVRL2 is detected on the membrane of HEK293 cells OX PVRL2; binding of mouse PVRL2-Fc to PVRL2 OX cells is detected by anti-mouse IgG2A antibodies; binding of biotinylated mouse PVRL2-Fc to PVRL2 OX cells is detected by streptavidin-PE, and binding of Alexa Fluor 647-labeled PVRL2-Fc to PVRL2 OX cells.

[00535] Binding of mouse PVRL2 to HEK293 cells OX PVRL2 : To further validate the interaction between mouse PVRL2 and mouse PVRL2 we tested the binding of PVRL2 to cells OX PVRL2 with or without a FLAG-tag. Membrane expression of mouse PVRL2 on HEK293 cells OX PVRL2 with a FLAG-tag was confirmed using an anti-FLAG antibody (data not shown). As expected, HEK293 cells OX PVRL2 without a FLAG-tag showed no expression using an anti-FLAG antibody. Using anti-PVRL2 supernatants (Aldevron), these cells demonstrated lower expression of PVRL2 compared to cells OX PVRL2 with a FLAG-tag. Commercial mouse PVRL2 recombinant protein was available only as a His-tagged protein. Therefore, extensive calibrations were required to obtain an appropriate anti-His antibody and conditions for detection. His-tagged PVRL2, from two different sources, were tested for binding to PVRL2 OX cells at 60µg/ml and demonstrated 2-fold (data not shown) and 3-4 fold (data not shown) binding compared to HEK293 cells expressing empty vector. That is, his-tagged mouse PVRL2 binds HEK293 OX mouse PVRL2, and mouse PVRL2 is expressed on membranes of HEK293 cells OX PVRL2.

[00536] Study of mouse PVRIG and mouse PVRL2 interaction using SPR-Biacore: In order to assess the interaction between mouse PVRIG-Fc and mouse His-tagged PVRL2, both proteins were immobilized to a Biacore chip. Following immobilization, both proteins, as well as PVRIG-Fc (data not shown) were run as analytes at three concentrations: 2500,
5 500 and 100nM (PVRIG batch #480 and PVRL2 were run twice as analytes). Interaction between the two proteins was detected in both directions and with both batches of PVRIG (data not shown). Due to complex kinetics, an exact KD could not be determined from the Biacore results.

[00537] Dose response binding of mouse PVRIG to HEK293 cells OX PVRL2 sv2 and
10 B16-F10 cells: As shown above, mouse PVRL2 binding to mouse PVRIG OX cells was relatively low. In order to establish a method for screening anti-mouse PVRIG antibodies capable of blocking the interaction between mouse PVRIG and mouse PVRL2, the binding of PVRIG-Fc to PVRL2 OX cells was selected. First, a dose response binding curve of mouse IgG2A and mouse PVRIG-Fc to cells OX mouse PVRL2 was generated and compared to
15 cells expressing empty vector (EV). The dose response was performed in two-fold serial dilutions (1:2) from 50µg/ml to 0.1µg/ml. While no difference in mouse IgG2A binding was observed (data not shown), PVRIG-Fc demonstrated saturation of binding at 12.5µg/ml and reduced binding in correlation with the decrease in protein concentration (data not shown). Similar results were obtained also with PVRIG-Fc (data not shown). These results suggest
20 that this binding assay can be considered for screening of blocking antibodies.

[00538] In order to consider also an endogenous system for screening of anti-mouse PVRIG antibodies, the expression of PVRL2 on B16-F10 cells was assessed using an anti-PVRL2 antibody. Results show that PVRL2 is highly expressed on B16-F10 cells (data not shown). Therefore, a similar dose response binding curve was produced also for binding of
25 mouse IgG2A and mouse PVRIG-Fc to B16-F10 cells. Similarly to the results obtained with HEK293 cells OX PVRL2, mouse PVRIG-Fc demonstrated dose response binding to B16-F10 cells reaching saturation at 12.5µg/ml, while no change in binding of mouse IgG2A was detected (data not shown).

[00539] Discussion and Conclusions: Human PVRIG interaction with human PVRL2
30 was identified using Cell Microarray Technology at Retrogenix. To validate this interaction also in mouse, several approaches were taken. Among them the use of PVRIG or PVRL2 OX cells, and biophysical measurements using SPR-Biacore. All approaches indicated that mouse

PVRIG interacts with mouse PVRL2. However, the binding of mouse PVRL2 to cells OX
PVRIG was relatively low compared to the binding of PVRIG to cells OX PVRL2. The
reason for this could be the fact that commercial PVRL2 is available only as a monomer His-
tagged protein and not as an Fc-fused protein (as for PVRIG). To this end, a custom Fc-fused
5 mouse PVRL2 was produced at GenScript. However, from preliminary data, only a minor
increase in binding was observed with this protein (~5-fold compared to 2-3 fold with the
PVRL2-his). Therefore, some other factors might influence this relatively low binding.

[00540] Due to the low PVRL2 binding to cells OX PVRIG, it was decided to establish
an anti-PVRIG antibody blocking assay using PVRIG-Fc binding to cells OX PVRL2.

10 According to the observed dose response curves we suggested three working concentrations:
0.1, 0.2 and 0.4 µg/ml. Following similar results obtained with binding of PVRIG to PVRL2
endogenously expressing B16-F10 cells, we suggested to perform the antibody blocking
assay also on these cells at the following concentrations: 0.2, 0.4, 0.8 µg/ml.

[00541] PVRIG is a presumed receptor, therefore, preferably the antibody blocking
15 assay should be performed with PVRL2 as a soluble protein and PVRIG expressed on the
cells. Thus, it should be considered to examine anti-mouse PVRIG antibodies that
demonstrate blocking activity in the current format also in this system.

[00542] Additional Validation Study 3

[00543] The objective of this study is to confirm the binding partners of PVRIG, a
20 novel immuno-oncology target. Preliminary studies indicate that one of these ligands is
PVRL2. In this study, binding of the recombinant PVRIG protein to several potential ligands
in the PVRIG axis has been investigated by ELISA.

[00544] Protocols

[00545] List of reagents: Current literature on the PVRIG proteins suggests that there
25 are three potential ligands: PVR (CD155), PVRL2 (CD112), and PVRL3 (CD113). To
investigate their ability to bind the PVRIG receptor, these three ligands were sourced
commercially, as follows: PVR and PVRL3 from Sino Biologicals Inc. and PVRL2 from
R&D Systems and Sino Biologicals Inc. The human PVRIG recombinant protein was
generated at Compugen as the PVRIG extra-cellular domain (ECD) fused to a human IgG1
30 Fc domain (PVRIGHH).

[00546] ELISA to determine receptor-ligand interaction: Commercially sourced His-tagged ligands, PVR, PVRL2, and PVRL3, were coated on the wells of a high binding EIA/RIA plate (Costar 9018) overnight at 4°C. An irrelevant His-tagged protein was included as a negative control. Coated plate wells were rinsed twice with PBS and incubated with 300
5 μ L blocking buffer (5% skim milk powder in PBS pH 7.4) at room temperature (RT) for 1 hr. Blocking buffer was removed and plates were rinsed twice more with PBS. Plate-bound ligands were incubated with varying concentrations of PVRIGHH in solution (linear range of 0.1 μ g/mL to 4 μ g/mL in a 50 μ L/well volume) at RT for 1 hr. Plates were washed three times with PBS-T (PBS 7.4, 0.05% Tween20), then three times with PBS and 50 μ L/well of a
10 HRP-conjugated secondary antibody was added (Human IgG Fc domain specific, Jackson ImmunoResearch). This was incubated at RT for 1hr and plates were washed again. ELISA signals were developed in all wells by adding 50 μ L of Sureblue TMB substrate (KPL Inc) and incubating for 5-20 mins. The HRP reaction was stopped by adding 50 μ L 2N H₂SO₄ (VWR) and absorbance signals at 450 nm were read on a SpectraMax (Molecular Devices) or
15 EnVision (PerkinElmer) spectrophotometer. The data were exported to Excel (Microsoft) and plotted in GraphPad Prism (GraphPad Software, Inc.).

[00547] Results: PVRIG preferably binds to PVRL2: The human PVRIG Fc-fusion protein was assayed for binding to PVR, PVRL2 and PVRL3, which were immobilized on an EIA/RIA plate. Varying concentrations of the receptor PVRIG in solution phase were
20 incubated with the immobilized ligand. The data clearly show dose-dependent binding of PVRIGHH to PVRL2, but no binding to ligands PVR, PVRL3 or the negative control protein (data not shown). The ELISA A₄₅₀ signal was plotted as a function of the receptor concentration using a one-site binding equation, revealing an equilibrium binding constant (K_D) of 13 ± 1 nM.

25 [00548] Summary and Conclusions: PVRIG is a novel immuno-oncology target for which the biology is not fully understood. In an effort to shed more light on this biology, we examined its binding to several potential ligands. PVRL2 was clearly identified as the binding partner of PVRIG. Quantitative analysis suggests that this interaction is very strong, with a K_D of 13 ± 1 nM. Our results also suggest that human PVRIG either does not bind the
30 human PVR and PVRL3, or the binding is too weak to detect by ELISA.

[00549] Additional Validation Study 4:

[00550] In this example, PVRIG expression on PBMC cell subsets was evaluated pre and post allo-activation. Following allo-activation the expression of PVRIG was upregulated on CD4⁺ T cells as well as on CD8⁺ T cells and double negative gamma delta T cells. This upregulation was observed in PBMCs of one out of two donors tested (see Figure 52).

5

Example 6 SURFACE PLASMON RESONANCE STUDIES OF PVR, PVRL2, AND PVRL3 BINDING TO PVRIG, DNAM, AND TIGIT

[00551] Materials and Methods

[00552] All experiments were performed using a ProteOn XPR 36 instrument at 22°C.

10 [00553] **Step 1:** A high density goat anti-human fc polyclonal antibody surface (Invitrogen H10500) was prepared over all six lanes of a GLC chip using a ProteOn XPR 36 biosensor. The activation step for the anti-human fc surface occurred in the horizontal flow direction while the immobilization step for the high density pAb occurred in the vertical flow direction. The blocking step occurred in both the vertical and horizontal positions so that the
15 horizontal “interspots” could be used as reference surfaces. An average of ~4400 RU of goat anti-human pAb was immobilized on each lane.

[00554] **Step 2:** For each cycle, three different lots of human PVRIG fusion protein (human fc, GenScript lots 451, 448, 125), human DNAM-1 fusion protein (human fc, R&D Systems), human TIGIT fusion protein (human fc, R&D Systems), and a control human IgG
20 (Synagis) were each captured over a different vertical lane for two minutes at a concentration of 2 µg/mL. PVR, two lots of PVRL2, and PVRL3 were each injected in the horizontal flow direction at six different concentrations over all six captured ligands at different ligand capture cycles. The injections were two minutes followed by 10 minutes of dissociation at a flow rate of 50µL/min. The PVR concentration range was 1.4nM-332nM in a 3-fold dilution series, both lots of PVRL2 were injected at a concentration range of 1.3nM-322nM in a 3-
25 fold dilution series, and PVRL3 was injected at a concentration range of 1.4nM-334nM in a 3-fold dilution series. All protein reagents were prepared in running buffer which was degassed PBS buffer with 0.05% Tween 20 and 0.01% BSA added. The anti-human fc capture surfaces were regenerated with two 30-second pulses of 146 mM phosphoric acid
30 after each cycle.

[00555] **Step 3:** Sensorgram data of the analytes binding to each captured ligand were processed and double-referenced using ProteOn Manager version 3.1.0.6 making use of interspot referencing and a pre-blank injection identical to the analyte injections.

[00556] Results

5 [00557] **a) PVR:** Binds weakly to captured DNAM-1 and TIGIT and shows no binding to all three lots of PVRIG and the control IgG. Not enough information was generated to estimate the K_D of the PVR interactions with DNAM-1 and TIGIT (data not shown).

10 [00558] **b) PVRL2:** Both lots of PVRL2 showed binding to all three lots of PVRIG and to DNAM-1 but minimal or no binding to TIGIT and no binding to the control IgG. Sensorgrams showed complex kinetics, therefore binding constants could not be estimated (data not shown).

[00559] **c) PVRL3:** Showed minimal binding to TIGIT and did not bind the other proteins (data not shown).

15 **Example 7: IN-VITRO IMMUNOMODULATORY ACTIVITIES OF PVRIG ECD-IG ON MOUSE T CELLS**

[00560] In these experiments the immunomodulatory activities of the recombinant fused protein PVRIG-ECD-Ig was investigated on mouse T cell activation. The effect of PVRIG-ECD-Ig on activation of mouse CD4 T cells was investigated using a number of in-
20 vitro T cell activation readouts: cell activation markers, cytokine secretion and proliferation.

[00561] In order to evaluate the activity of pvrig protein on t cell activation, recombinant protein was produced comprising the mouse extracellular domain (ecd) of the mouse pvrig fused to the fc of mouse igg2a (designated pvrig-ecd ig m:m) (seq id no:29). The effect of the fc fused protein co-immobilized with anti-cd3 on mouse cd4 t cell functions, as
25 manifested by activation markers and cytokines secretion was investigated.

[00562] Materials and Methods

[00563] Fc fusion protein and control Ig : Fc fusion protein, PVRIG-ECD-Ig (batch #198) was tested. Mouse IgG2a (clone MOPC-173; Biolegend or C1.18.4; BioXcell) was used as isotype control.

[00564] Mouse CD4 T cells isolation: Untouched CD4+CD25- T cells were isolated from pools of spleens of BALB/C mice using a T cell isolation Kit (Miltenyi Cat# 130-093-227) according to the manufacturer's instructions. The purity obtained was >90%.

[00565] Activation of mouse CD4 T cells: Anti-mouse CD3- ϵ mAb (clone 145-2C11; BD Biosciences) at 2 μ g/ml together with PVRIG-ECD-Ig protein or control Ig at various concentrations (1, 3 or 10 μ g/ml), were co-immobilized for 3hr at 37°C, on 96-well flat bottom tissue culture plates (Sigma, Cat. # Z707910). Control Ig was added to each well in order to complete a total protein concentration of 12 μ g/ml per well. Wells were washed 3 times with PBS and plated with 1x10⁵ purified CD4+CD25- T cells per well and kept in a humidified, 5% CO₂, 37°C incubator. In some experiments, soluble anti-CD28 (clone: 37.51; eBioscience; 1 μ g/ml) was added. Culture supernatants were collected at the indicated times post stimulation and analyzed for mouse IFN γ or IL-2 secretion by ELISA kits (R&D Systems). The effect of PVRIG-ECD-Ig protein (see Figure 103) on the expression of the activation marker CD69 on mouse CD4+ T cells was analyzed by flow cytometry. Cells were stained 48h post stimulation with a cocktail of antibodies including PerCP-anti-CD4 (clone G41.5; Biolegend), FITC or PE-anti-CD69 (clone H1.2F3; Biolegend), in the presence of anti-CD16/32 (clone 2.4g2; BD Biosciences) for blocking of Fc γ -receptors. Cells were evaluated using MACSQuant analyzer 9 (Miltenyi) and data analyzed using BD CellQuest or by MACSQuantify TM Software. Data was analyzed using Excel or Prism4 software.

[00566] Results and Summary

[00567] Effect of PVRIG-ECD Ig M:M (see Figure 103) on mouse CD4+ T cells function: Figure 15 shows in-vitro immunomodulatory activities of PVRIG-ECD-Ig (see Figure 103) on isolated mouse splenic T cells (CD4+, >95%purity) stimulated with microplates co-immobilized with anti-CD3 (2 μ g/ml) alone or co-immobilized with control Ig (mIgG2a) or PVRIG-ECD-Ig (see Figure 103)) (10 μ g/ml) in the presence of soluble anti-CD28 (1 μ g/ml). PVRIG-ECD-Ig (see Figure 103) suppressed mouse CD4 T cell activation in a dose dependent manner, as manifested by reduced CD69 up-regulation (Figure 15A, D), and reduction in TCR-induced cytokines (IL-2 and IFN γ) secretion (Figure 15B-C, E). The magnitude of the inhibitory effect of PVRIG-ECD-Ig (see Figure 103) was in the range of 30-100%. Inhibitory effect of PVRIG-ECD-Ig (see Figure 103) on IFN γ secretion was observed in concentrations as low as 3 μ g/ml (~60% inhibition vs. control Ig).

[00568] PVRIG-ECD-Ig (see Figure 103) inhibits T cell activation in a concentration-dependent manner when the Fc fusion protein is co-immobilized with anti-CD3 on plates. Maximal inhibitory effect was observed at 10ug/ml of PVRIG-ECD-Ig (see Figure 103).

[00569] The results demonstrate the inhibitory effect of PVRIG-ECD-Ig on mouse T cells activation, manifested by reduced cytokine secretion, and suppression of activation marker CD69 upregulation. This inhibition of T cell activation, supports the therapeutic potential of immunoinhibitory PVRIG proteins (PVRIG polypeptides and fusion proteins) according to the present invention in treating T cell-driven autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, psoriasis and inflammatory bowel disease, as well as for other immune related diseases and/or for reducing the undesirable immune activation that follows gene therapy. In addition, these results also support the therapeutic potential of immunostimulatory PVRIG proteins (PVRIG polypeptides and fusion proteins) that reduce the inhibitory activity of PVRIG for treating conditions which should benefit from enhanced immune responses, in particular enhanced CTL immunity and proinflammatory cytokines such as cancer, infectious diseases, particularly chronic infections and sepsis wherein T cell-mediated depletion of diseased cells is therapeutically advantageous.

Example 8: IN-VITRO IMMUNOMODULATORY ACTIVITIES OF PVRIG ON HUMAN CYTOTOXIC T CELLS (CTLs)

[00570] The experiments described in this example evaluated the effect of ectopic expression of human PVRIG on different melanoma cell lines on their ability to activate CTLs (cytotoxic T lymphocytes) and serve as targets for killing by these cells.

[00571] MATERIALS & METHODS:

[00572] Three human melanoma cell lines which present the MART-1 antigen in HLA-A2 context (SK-MEL-23, Mel-624 and Mel-624.38) were used as targets for CTLs. Mel-888 which does not express HLA-A2, served as a negative control.

[00573] Ectopic expression of human PVRIG on cytotoxic T lymphocytes (CTLs): In order to express human PVRIG in peripheral blood leukocyte (PBL) cultures, the cDNA encoding for PVRIG was amplified using specific primers and cloned into an MSCV-based retroviral vector (pMSGV1) or in tripartite vectors: the CD8-dependent F4 TCR α - and β - chains were linked with a P2A sequence and cloned into pMSGV1 vector, either followed by an internal ribosome entry site (IRES) and PVRIG. The retroviral vector encoding for NGFR1, as negative control or in tripartite vectors: the CD8-dependent F4 TCR α - and β -

chains were linked with a P2A sequence and cloned into pMSGV1 vector, either followed by an internal ribosome entry site (IRES) and NGFR. Verification of the cloning was done first using restriction enzyme digestion and subsequently by sequencing. Upon sequence confirmation, large amounts of the retroviral vector (Maxi-prep) were produced for subsequent use.

[00574] Peripheral blood leukocytes of healthy human donors were transduced with the retroviral constructs encoding PVRIG or with the retroviral vectors encoding for NGFR1 or an empty vector, as negative control. Transduction was carried out using a retronectin-based protocol; briefly, retroviral supernatant was produced in 293GP cells (a retroviral packaging cell line) following transfection with the retroviral vector and an amphotropic envelop gene (VSV-G). The retroviral supernatant was plated on retronectin-coated plates prior to the transduction to enable the binding of virions to the plate, and the PBLs were added to the plate for 6 hours. After that, the cells were replenished in a new culture vessel. Transduction efficiency and expression of the protein was determined by staining the transduced PBLs with commercial PVRIG specific rabbit polyclonal antibody or with commercial anti-NGFR (Cat.No 345108; BioLegend). Rabbit IgG (Sigma Cat. No. I5006) was used as isotype control, and as secondary antibody we used APC-conjugated anti-rabbit IgG (Jackson, Cat. No. 711-136-152).

[00575] Ectopic expression of the F4 T cell receptor on cytotoxic T lymphocytes

(CTLs): In order to obtain effector lymphocytes that express the MART-1-specific F4 TCR, specifically recognizing MART-126-35-/HLA-A2 peptide-MHC complex, freshly isolated human PBLs previously transduced to express either with PVRIG, NGFR or an empty vector were stimulated with PHA and cultured for 5-10 days, and subsequently transduced with in vitro-transcribed mRNA encoding both α and β chains from the MART-1-specific F4 TCR. The transduced lymphocytes were cultured in lymphocyte medium (Bio target medium, fetal bovine serum (10%), L Glutamine Penicillin/ Streptomycin (100 units/ml), IL-2 300 IU), replenished every 2-3 days. F4 TCR expression levels were verified by FACS staining using a specific monoclonal antibody that recognizes the extra-cellular domain of the beta-chain from the transduced specific TCR. (TCR-Vb12-PE, (Cat.No IM2291; Beckman Coulter).

[00576] Cytokine secretion from PVRIG, NGFR or an empty vector and F4-TCR transduced lymphocytes upon co-culture with melanoma cells: PBLs expressing PVRIG or NGFR along with F4-TCR were co-cultured with un-manipulated melanoma cells. 10^5

transduced PBLs were co-cultured with 10^5 melanoma target cells for 16 hours. In order to assess the response of the effector CD8 T cells to the different tumor cell lines, cytokine secretion (IFN γ , IL-2 and TNF- α) was measured by ELISA in culture supernatants (IFN γ (Cat.No DY285E), IL-2 (Cat.No DY202E), TNF- α (Cat.No DY210E) R&D SYSTEMS),
5 diluted to be in the linear range of the ELISA assay.

[00577] Cell mediated cytotoxicity assay: This assay was performed in order to assess target cell killing upon co-culture. PVRIG and F4 were expressed in PBLs using a bi-cystronic vector and co-cultured with CFSE labeled melanoma Target cells (labeled with 2 mM CFSE (eBioscience) for 6 min), at 37°C for 18hr, at E:T ratio of 3:1. Cells were
10 collected after 18hr and 1 mM propidium iodide (Sigma-Aldrich) was added for assigning the ratio of cell death. Samples were run on a CyAn-ADP flow cytometer (Beckman Coulter).

[00578] Results:

[00579] General design of the experimental system: In the experimental system
15 described herein, PVRIG is over expressed on human PBLs which are next manipulated to express the MART1-specific and HLA-A2 restricted F4 TCR. Over expressing cells are then co-cultured with HLA-A2 positive (name them) and HLA-A2 negative (names) melanoma cell lines (reference). The F4 TCR was recently used in clinical trials in terminally-ill melanoma patients to specifically confer tumor recognition by autologous lymphocytes from
20 peripheral blood by using a retrovirus encoding the TCR (Morgan et al, 2006 Science, 314:126-129). The effect of PVRIG expression on antigen-specific activation of CD8 T cells by co-culture with cognate melanoma cells was assessed by cytokine secretion.

[00580] Over expression of PVRIG on human PBLs – experiment 1: Human PBLs were transduced with a retroviral vector encoding the PVRIG or an empty vector as negative
25 control, as described in Materials & Methods. The levels of PVRIG were assessed by flow cytometry at 48hrs after transduction, and compared to cells transduced with an empty vector. The percentage of the transgene-expressing cells was 62.4% as shown in Figure 16.

[00581] Over expression of PVRIG on human PBLs – experiment 2: Human PBLs were transduced with a retroviral vector encoding the PVRIG or NGFR or an empty vector as
30 negative controls, as described in Materials & Methods. The levels of PVRIG were assessed by flow cytometry at 48hrs after transduction, and compared to cells transduced with an empty vector. The percentage of the PVRIG-expressing cells was in the range of 20%. The

expression of NGFR was of 63% as shown in Figure 17. A few additional attempts to over express PVRIG on PBLs were un-successful. One possibility is that the difficulty in expressing PVRIG in primary PBLs stems from a basal endogenous expression level in these cells.

5 **[00582]** Over expression of F4 TCR on human PBLs: To perform functional assays with human CTLs, we used PBLs engineered to express the F4 TCR, which recognizes HLA-A2+/MART1+ melanoma cells, as described in Materials & Methods. Figure 18A shows levels of F4 TCR expression obtained upon TCR transduction of leukocytes used in experiment 1, Figure 18B shows levels of F4 TCR expression obtained upon TCR
10 transduction of leukocytes used in experiment 2.

15 **[00583]** Effect of PVRIG expression on IFN γ secretion – experiment 1: PVRIG or Empty-vector and F4-transduced PBLs were co-cultured with melanoma cell lines. The levels of IFN γ secretion were measured at 16-hours of co-culture. As shown in Figure 19, the magnitude of inhibition of IFN γ secretion due to PVRIG over-expression was more than
15 90%. Co-culture with the HLA-A2 negative cell line Mel-888 which served as a negative control, caused only a minor activation dependent IFN γ secretion from F4-transduced lymphocytes. PBLs not expressing the F4 TCR (designated W/O) serve as an additional negative control.

20 **[00584]** Effect of PVRIG expression on IFN γ secretion – experiment 2: PVRIG, NGFR or Empty-vector and F4 were transduced into PBLs in co-transduction (Figure 20A) or using a bi-cystronic vector (Figure 20B). Transduced PBLs were co-cultured with melanoma cell lines. The levels of IFN γ secretion were measured at 16-hours of co-culture. As shown in Figure 20A, the magnitude of inhibition of cytokine secretion due to PVRIG over-expression was in the range of 30%. Co-culture with the HLA-A2 negative cell line
25 Mel-888 which served as a negative control, caused only a minor activation dependent IFN γ secretion from F4-transduced lymphocytes. PBLs not expressing the F4 TCR (designated W/O) serve as an additional negative control. As shown in Figure 20B, when PVRIG is co-transduced with the F4 TCR, no inhibition of IFN γ was observed.

30 **[00585]** Effect of PVRIG on CTL mediated killing activity – experiment 2: PVRIG or NGFR and F4 were transduced to PBLs using a bi-cystronic vector and co-cultured with CFSE labeled melanoma cell lines. As shown in Figure 21, the percentage of propidium Iodide positive events (reflecting intensity of killing activity) was decreased by ~50% by the

expression of PVRIG relative to negative control NGFR transduced cells. Killing activity of PVRIG expressing cells is similar to that of co-culture between melanoma and PBLs not expressing the F4 TCR (designated W/O).

[00586] Summary: Without wishing to be limited by a single hypothesis, the results presented herein indicate that overexpression on primary lymphocytes results in reduced cytokine secretion by CTLs, suggesting that PVRIG has an inhibitory effect on CTLs.

Example 9: HUMAN ANTI-PVRIG ANTIBODIES

[00587] The objective of this study was to isolate human antibodies that bind to the PVRIG immuno-oncology target with high affinity and specificity, and block the interaction of PVRIG with its binding partner, PVRL2. This was achieved by panning a human fab fragment phage display library against a recombinant protein comprising the human PVRIG extracellular domain (ECD) fused to the human IgG1 Fc region, and screening the resulting antibodies for their ability to block the PVRIG interaction with PVRL2.

[00588] Protocols

[00589] Functional QC of reagents: The purity of the panning reagent, PVRIG ECD fused to human IgG1 Fc domain (PVRIGHH), was determined by Microfluidics Capillary Electrophoresis using a LabChip System (PerkinElmer). Activity of the panning reagent was validated by its ability to bind its ligand PVRL2.

[00590] ELISA to detect protein-protein interaction: His-tagged PVRL2 recombinant protein was diluted to 2 µg/mL in phosphate buffered saline (PBS) and 50 µL aliquots were coated on the wells of a high binding EIA/RIA plate (Costar) overnight at 4°C. Coated plate wells were rinsed twice with PBS and incubated with 300 µL blocking buffer (5% skim milk powder in PBS pH 7.4) at room temperature (RT) for 1 hr. Blocking buffer was removed and plates were rinsed twice more with PBS. Plate-bound PVRL2 was incubated with varying concentrations of PVRIGHH in solution (linear range of 0.1 µg/mL to 4 µg/mL in a 50 µL/well volume) at RT for 1 hr. Plates were washed three times with PBS-T (PBS 7.4, 0.05% Tween20), then three times with PBS and 50µL/well of a HRP-conjugated secondary antibody was added (Human IgG Fc domain specific). This was incubated at RT for 1hr and plates were washed again. ELISA signals were developed in all wells by adding 50 µL of Sureblue TMB substrate (KPL Inc) and incubating for 5-20 mins. The HRP reaction was stopped by adding 50 µL 2N H2SO4 (VWR) and absorbance signals at 450 nm were read on a SpectraMax (Molecular Devices) or EnVision (PerkinElmer) spectrophotometer.

[00591] Preparation of biotinylated PVRIG: To facilitate phage panning in solution using streptavidin-coated magnetic beads, PVRIGHH and an irrelevant human IgG1 Fc isotype control were biotinylated using Lightning-Link® Biotin kit (Innova Biosciences). Biotinylation reactions were performed following the manufacturer's protocol and the biotinylated reagents were stored at 4°C for further QC and biopanning. The purity and activity of the biotin-labeled proteins was assessed by LabChip and functional ELISA, as described in Section 2.1. In addition, the degree of biotinylation was assessed by ELISA using two approaches: 1) the biotinylated reagents were adsorbed on a high binding EIA/RIA plate and the proteins were detected using HRP-conjugated streptavidin, and 2) the biotinylated proteins were incubated on EIA/RIA plate pre-coated with streptavidin and the binding was detected using a HRP-conjugated human IgG Fc domain specific secondary antibody.

[00592] Phage panning of human antibody library: Panning reactions were carried out in solution using streptavidin-coated magnetic beads to capture the biotinylated antigens.

Note that all washing and elution steps were conducted using a magnetic rack to capture the beads (Promega). All incubation steps were conducted at room temperature with gentle mixing on a tube rotator (BioExpress). Four panning sub-campaigns were conducted, each with a different combination of antigen concentrations, washes and Fc-binder depletion steps (Table 1).

[00593] All the panning sub-campaigns were carried out using the biotinylated PVRIGHH antigen. For each round of panning, the phage libraries were depleted against 100 pmol of an irrelevant human IgG1 Fc protein in two successive steps. Following depletion, sub-campaigns A and B involved panning against 50 nM of the antigen in each round, under low and high stringency wash conditions, respectively. Sub-campaigns C and D were identical to sub-campaign B, except that in campaign C the library was blocked with 10-fold excess of the irrelevant IgG1 Fc protein in panning rounds 2 and 3. Sub-campaign D differed in that 5 nM antigen was used in round 3.

[00594] Table 1: Antigen and washing conditions used for phage panning against PVRIGHH.

Sub-campaign	Round	Antigen Concentration	Washes	Fc Depletion
A	1	50 nM	3x PBS-T + 3x PBS	2X 100 pmol

[00596] A 1 50 nM 3x PBS-T + 3x PBS 2X 100 pmol

	[00597]	2	50 nM 3x PBS-T + 3x PBS	2X 100 pmol
	[00598]	3	50 nM 3x PBS-T + 3x PBS	2X 100 pmol
	[00599]	B 1	50 nM 3x PBS-T + 3x PBS	2X 100 pmol
	[00600]	2	50 nM 6x PBS-T + 6x PBS	2X 100 pmol
5	[00601]	3	50 nM 6x PBS-T + 6x PBS	2X 100 pmol
	[00602]	C 1	50 nM 3x PBS-T + 3x PBS	2X 100 pmol
	[00603]	2	50 nM 6x PBS-T + 6x PBS	2X 100 pmol + block with 1 nmol
	[00604]	3	50 nM 6x PBS-T + 6x PBS	2X 100 pmol
10	[00605]	block with 1 nmol		
	[00606]	D 1	50 nM 3x PBS-T + 3x PBS	2X 100 pmol
	[00607]	2	50 nM 6x PBS-T + 6x PBS	2X 100 pmol
	[00608]	3	5 nM 6x PBS-T + 6x PBS	2X 100 pmol
	[00609]			
15	[00610]	Preparation of phage library for panning: All phage panning experiments used the XOMA031 human fab antibody phage display library (XOMA Corporation, Berkeley, CA). Sufficient phage for a 50-fold over-representation of the library were blocked by mixing 1:1 with 10% skim milk powder in PBS (final skim milk concentration 5%) and incubating for 1hr.		
20	[00611]	Antigen coupling to streptavidin beads: For each sub-campaign, three 100 µL aliquots of Dynal streptavidin-coated magnetic beads (Life Technologies) were blocked by suspension in 1 mL of blocking buffer (5% skim milk powder in PBS) and incubated for 30 mins. One blocked bead aliquot was mixed with 100 pmols of biotinylated PVRIGHH. The other two aliquots were mixed with 100 pmols of the irrelevant antigen for depletion of Fc-		
25		only binders. Biotin-labeled antigens were coupled to the beads for 30 mins at RT. Bead suspensions were washed twice with PBS to remove free antigen and re-suspended in 100 µL blocking buffer.		
	[00612]	2.4.2. Depletion of human IgG1 Fc and streptavidin bead binders from the phage library: It was necessary to remove unwanted binders to streptavidin beads and the Fc		

region of PVRIGHH before phage panning could commence. To achieve this, blocked phage was mixed with one 100 μ L aliquot of uncoupled streptavidin beads and incubated for 45 mins. The beads (and presumably unwanted bead and human IgG1 Fc-binders) were discarded. This step was repeated once and depleted phage library supernatants were reserved for panning.

[00613] 2.5. Phage panning round 1: The blocked and depleted phage library was mixed with biotinylated PVRIGHH coupled to magnetic beads described above. This suspension was incubated for 1hr at RT with gentle rotation to allow binding of PVRIGHH specific phage. Non-specific binders were removed by washing according to the protocol in Table 1. After washing, bound phage were eluted by incubation with 500 μ L of 100 mM triethylamine (TEA) (EMD) for 15 mins at RT. The eluate was neutralized by adding 500 μ L of 1 M Tris-HCl pH 8.0 (Teknova).

[00614] 2.5.1. Determination of phage titer: 10 μ L of the initial phage library (input titer) or panning eluate (output titer) was serially diluted (10-fold) in PBS. A 90 μ L aliquot of each phage dilution was mixed with 500 μ L of TG1 E. coli cells grown to an optical density of $\sim$ 0.5 at 600 nm (OD 600nm). Phage were allowed to infect the cells by stationary incubation for 30 mins, then shaking incubation (250 rpm) for 30 mins, all at 37°C. A 10 μ L aliquot of each infected cell culture was spotted on a 2YT agar plate supplemented with 2% glucose and 100 μ g/mL carbenicillin (2YTTCG, Teknova). Plates were incubated overnight at 30°C. Colonies growing from each 10 μ L spot were counted and used to calculate input and output titers.

[00615] 2.5.2. Phage rescue: The remaining phage eluate ($\sim$ 1 mL) was mixed with 10 mL of TG1 E. coli cells grown to an OD 600 nm of 0.5. Phage were infected into cells as detailed in section 2.5.1. Infected cells were pelleted by centrifugation at 2500xG, re-suspended in 750 μ L 2YT medium (Teknova) and spread on 2YTTCG agar plates. These were incubated overnight at 37°C and the resulting E. coli lawns were scraped and re-suspended in $\sim$ 20 mL liquid 2YTTCG (Teknova). A small aliquot of re-suspended cells was inoculated into 50 mL 2YTTCG to achieve an OD 600nm of 0.05, and then grown at 37°C with 250 rpm shaking until the OD reached 0.5. The resulting culture was infected with M13K07 helper phage (New England Biolabs) and incubated overnight at 25°C with shaking to allow phage packaging. The culture supernatant containing rescued phage particles was cleared by

centrifugation at 2500xG and 1 mL was carried over for either a) a subsequent round of panning or b) fab binding screens.

[00616] Phage panning rounds 2-3: Second and third rounds of panning were conducted as per the steps above, except that the rescued phage supernatant from the previous
5 round was used in place of the phage library. The washing conditions, depletion and the antigen concentrations used are listed in Table 1.

[00617] Binding screens using fabs prepared from periplasmic extracts

[00618] Fab expression vectors: The XOMA031 library is based on phagemid constructs that also function as fab expression vectors. These vectors contain fab heavy chain
10 and light chain expression cassettes, a lac promoter to drive expression of the antibody genes, and an ampicillin resistance gene. The antibody chains are appended with N-terminal signal peptides to drive their secretion into the periplasmic space. The C-terminal of the heavy chain carries a truncated gene III protein sequence for incorporation into phage particles. The heavy chain also carries hexa-histidine, c-myc and V5 affinity tags. Transformation of these vectors
15 into E. coli and induction with isopropyl β -D-1-thiogalactopyranoside (IPTG) results in periplasmic expression of soluble fab molecules.

[00619] Fab PPE production: Eluted phage pools from panning round 3 were diluted and infected into TG1 E. coli cells (Lucigen) so that single colonies were generated when spread on a 2YTCG agar plate. This resulted in each colony carrying single fab clone.
20 Individual clones were inoculated into 1 mL 2YTCG starter cultures in 96-well deep well blocks (VWR) using a Qpix2 instrument (Molecular Devices). These starter cultures were grown overnight in a Multitron 3mm incubator (Infors) at 37°C with 700 rpm shaking. For fab expression, 20 μ L of 1 mL starter cultures were transferred into a second set of deep well plates containing 1 mL 2YT with 0.1% glucose and 100 μ g/mL ampicillin. Cultures were
25 grown until the average OD 600nm was 0.5-1.0 and protein expression was induced by adding IPTG (Teknova) to a final concentration of 1 mM. Expression cultures were incubated overnight in the Multitron instrument at 25°C with 700 rpm shaking.

[00620] Fab proteins secreted into the E. coli periplasm were extracted for analysis. Cells were harvested by centrifugation at 2500xG, the supernatants were discarded and
30 pellets were re-suspended in 75 μ L ice-cold PPB buffer (Teknova). Extracts were incubated for 10 mins at 4°C with 1000 rpm shaking, and 225 μ L ice-cold ddH₂O was added and incubated for a further 1hr. The resulting periplasmic extract (PPE) was cleared by

centrifugation at 2500xG and transferred to separate plates or tubes for ELISA and FACS analysis. All extraction buffers contained EDTA-free Complete Protease Inhibitors (Roche).

[00621] Each plate of samples also included duplicate “blank PPE” wells to serve as negative controls. These were created by intentionally leaving two 1 mL cultures un-
5 inoculated and then processing them in the same way as the fab PPEs, thereby creating a sample with no bacterial growth and therefore no fab expression.

[00622] Primary screen by ELISA: Two 96-well plates of PPE extracts per sub-campaign were tested for binding to PVRIGHH by ELISA. Note that a non-biotinylated version of the protein was used for the ELISA screen to avoid the selection of biotin or
10 streptavidin-binders. PVRIGHH recombinant protein was diluted to 2 µg/mL in phosphate buffered saline (PBS) and 50 µL aliquots were coated on the wells of a high binding EIA/RIA plate (Costar) overnight at 4°C. Coated plate wells were rinsed twice with PBS and incubated with 300 µL blocking buffer (5% skim milk powder in PBS pH 7.4) at room temperature (RT) for 1 hr. Blocking buffer was removed and plates were rinsed twice more
15 with PBS. Plate-bound PVRIG was incubated with the PPEs, pre-blocked with 3% skim milk, at RT for 1 hr. Plates were washed three times with PBS-T (PBS 7.4, 0.05% Tween20), then three times with PBS and 50µL/well HRP-conjugated, anti-human Fab secondary antibody (Jackson ImmunoResearch) was added at a 1:2000 dilution in 5% milk in PBS. This was incubated at RT for 1hr and plates were washed again. ELISA signals were developed in all
20 wells by adding 50 µL of Sureblue TMB substrate (KPL Inc) and incubating for 5-20 mins. The HRP reaction was stopped by adding 50 µL 2N H2SO4 (VWR) and absorbance signals at 450 nm were read on a SpectraMax (Molecular Devices) or EnVision (PerkinElmer) spectrophotometer. Wells that showed signal over background (blank PPE) ratio > 3 were selected as positive hits.

25 [00623] Sequence analysis of ELISA positive fabs: The positive hits from the ELISA screen were selected and re-arrayed into a new 96-well plate. The clones were grown overnight at 37°C and the plasmid DNA was sequenced using heavy chain and light chain-specific primers. The sequences were assembled and analyzed using Xabtracker (XOMA) software. The clones were deemed sequence-unique if there were more than one non-
30 conservative differences in the heavy chain CDR3. Clones with same or similar heavy chain but significantly different light chains were labeled as siblings of the original clone.

[00624] FACS screening of fabs as PPEs: The sequence-unique ELISA-positive fab clones were selected and analyzed for their ability to bind PVRIG over-expressing cells by fluorescence-activated cell sorting (FACS). Analyses were conducted using HEK293 cells over-expressing the human PVRIG antigen. In a parallel experiment, un-transfected HEK293
5 cells were used as a negative control for each fab sample.

[00625] The PPEs for the sequence-unique ELISA-positive fab clones were generated as described above. All the assays were conducted using FACS buffer (1% BSA and 0.1% sodium azide in PBS). The human PVRIG and un-transfected HEK293 cells were harvested, washed twice and re-suspended at a density of 2×10^6 cells/ml. A 25 μ l aliquot of cells was
10 mixed with 25 μ l of each PPE sample and incubated for 1 hr at 4°C with gentle shaking. Two blank PPE controls were also included in the analysis. Plates were washed one time in 200 μ l of FACS buffer and 50 μ L of a 2 μ g/mL dilution of a mouse anti-C-myc antibody (Roche) was added to each well. After incubation for 30 mins at 4°C, cells were washed again and 25 μ l of a 5 μ g/mL dilution of goat anti mouse fab-AF647 (Jackson ImmunoResearch) was added
15 to each PPE and negative control well. All secondary antibodies were incubated for 30 min at 4°C. After two washes, cells were re-suspended in a final volume of 50 μ l of fixation buffer (2% paraformaldehyde in FACS buffer). Samples were read on an Intellicyt HTFC screening system, recording approximately 5000 events per well in a designated live gate. Data was analyzed using FlowJo (De Novo Software, CA, USA) and exported to Excel. Ratio of Mean
20 Fluorescence Intensity (MFI) for the human PVRIG over-expressing HEK cells and the un-transfected 293 cells was calculated using Xabtracker software (XOMA). Positive hits on each plate were identified as those giving an MFI ratio 5-fold greater than the averaged blank PPE control signal.

[00626] Re-formatting of fab hits and production as human IgG molecules: Potential
25 PVRIG binding fabs were converted to full length human IgGs for further characterization. Protein expression constructs were derived by PCR-amplification of variable heavy, lambda and kappa domain genes, which were sub-cloned into pFUSE-CHlg-hG1 (human IgG1 heavy chain), pFUSE2-CLlg-hK (human kappa light chain) or pFUSE2-CLlg-hL2 (human lambda 2 light chain) vectors, respectively (all expression vectors were sourced from Invivogen).

30 [00627] Expi293 cells (Life Technologies) were seeded at 6×10^5 cells/ml in Expi293 medium (Life Technologies) and incubated for 72 hrs at 37°C in a humidified atmosphere of 8% CO₂ with shaking at 125 rpm. This cell stock was used to seed expression cultures at 2.0

$\times 10^6$ cells/ml in Expi293 medium. These cultures were incubated as above for 24 hrs with shaking at 135 rpm.

[00628] For transfection, cells were diluted again to 2.5×10^6 cells/ml in Expi293 medium. The protein expression constructs for antibody heavy chain and light chain were mixed at a ratio of 1:2. For every 30 mL of expression culture volume, 30 μ g of DNA and 81 μ L of Expifectamine (Life Technologies) were each diluted separately to 1.5 mL with Opti-MEM (Life Technologies) and incubated for five minutes. Diluted DNA and Expifectamine were then mixed and incubated at RT for 20 mins. This was then added to the expression culture in a shaker flask and incubated as described above, with shaking at 125 rpm.

[00629] Approximately 20 hrs post-transfection, 150 μ L of ExpiFectamine 293 transfection Enhancer 1 and 1.5mL of ExpiFectamine 293 Transfection Enhancer 2 was added to each flask. Cultures were incubated for a further five days (six days post-transfection in total) and supernatants were harvested by centrifugation. IgGs were purified from the supernatants using an AKTA Pure FPLC (GE Healthcare Bio-Sciences) and HiTrap MabSelect Sure affinity columns (GE Healthcare Bio-Sciences) according to manufacturer's instructions.

[00630] FACS screening of reformatted IgG1 antibodies: FACS screening of the reformatted antibodies was done similarly to the PPE based screen described herein, except that a dose-dependent titration of the purified antibodies was performed. The human PVRIG over-expressing HEK293 cells, or the un-transfected HEK293 cells, were incubated with varying concentrations (0 – 10 μ g/ml) of the anti PVRIG antibodies or isotype controls in FACS buffer at 4°C for 60 mins. Cells were washed once in FACS buffer, re-suspended in 50 μ L of Alexa Fluor 647 conjugated anti-human IgG (Fab fragment specific) diluted 1:200 and incubated for 30 mins at 4°C in the dark. Cells were washed twice and re-suspended in a final volume of 80 μ L of FACS buffer and Propidium Iodide (Biolegend cat# 421301) diluted 1:1000. Samples were analyzed using an Intellicyt HTFC screening system (Intellicyt). Data was analyzed using FlowJo (DeNovo), exported to Excel (Microsoft) and plotted in GraphPad Prism (GraphPad Software, Inc.).

[00631] Results

[00632] Functional QC of the PVRIGHH recombinant protein: The purity of the PVRIGHH protein was assessed by microfluidics capillary electrophoresis using a LabChip system. Under reducing conditions, the recombinant protein migrated at 80 kDa, consistent

with its calculated molecular weight of 80.4 kDa, and showed 99% purity (data not shown). Under non-reducing conditions, one additional peak was observed which likely resulted from the presence of a dimeric form of the protein due to Fc-Fc interaction.

[00633] The functional integrity of the recombinant protein was assessed by evaluating its binding to PVRL2 (a known ligand for PVRIG) in ELISA. A dose-dependent response was observed for the binding of PVRIGHH to PVRL2 (data not shown). In comparison, no binding was observed for an irrelevant human IgG1 Fc control. Taken together, this indicated that the PVRIGHH recombinant protein is of high purity and is functionally active, and thus is suitable for biopanning.

[00634] QC of the biotinylated PVRIGHH recombinant protein: The purity of the biotinylated PVRIGHH protein was assessed by microfluidics capillary electrophoresis using LabChip system. No significant differences were observed between the non-biotinylated and the biotinylated recombinant proteins (data not shown). Note that an additional 44.3 kDa peak observed in the biotinylated protein sample. This peak may result from the monomeric form of the PVRIGHH protein or maybe an artifact of the quenching reaction of the biotinylation kit.

[00635] Successful biotinylation was confirmed by incubating the biotinylated protein on a streptavidin-coated EIA plate and detecting the bound protein using a HRP-conjugated anti human IgG1 Fc secondary antibody. The binding of biotinylated PVRIGHH to the streptavidin-coated EIA plate was comparable to a commercially sourced irrelevant biotinylated protein (data not shown).

[00636] Phage panning: The biotinylated PVRIGHH protein was used for phage panning against the XOMA031 human fab antibody phage display library (XOMA Corporation, Berkeley, CA). Three rounds of biopannings were performed, under 4 different combinations of washing stringency, antigen concentration, and depletion of Fc binders (sub-campaigns A – D). The success of each round was estimated using the phage output titers. Qualitative guidelines were used to define the success of the panning sub-campaigns, such as significant reduction in phage titers after round 1, increase or maintenance of phage titers after rounds 2 and 3, and decrease in phage titers upon increasing wash stringency or decreasing antigen concentration. All 4 sub-campaigns resulted in phage titers in the expected range that were consistent among the sub-campaigns (data not shown).

[00637] Screening of phage output as fab PPEs: Two 96-well plates of fab clones (as PPEs) for each of the four sub-campaigns were screened to evaluate the success of biopanning. The results are summarized in table 3 and are discussed in further detail below. Overall, all 4 sub-campaigns yielded significant numbers of PVRIGHH specific fabs. A total of 49 target-specific unique fabs were identified. The sub-campaigns B and D showed the highest ELISA hit rates and FACS correlation and were selected for an extended screen.

[00638] Table 3: Summary of pilot screen of fab PPEs. For each sub-campaign, the total number of clones screened, ELISA hits, FACS hits and sequence uniqueness are listed. Open reading frames (ORFs) represent the clones that were successfully sequenced as a full-length fab. Specificity is based on the lack of non-specific binding to irrelevant proteins in ELISA. FACS correlation represents the percent of ELISA hits that were also FACS positive (specifically bound to PVRIG over-expressing HEK293 cells).

[00639]	Sub A	Sub B	Sub C	Sub D	Overall	
[00640]	Clones screened	182	182	182	182	728
[00641]	ELISA positive (>3 S/N)	48	51	44	68	211
[00642]	ELISA Hit rate	26%	28%	24%	37%	29%
[00643]	ORFs 36 (75%)	45 (88%)	35 (80%)	63 (93%)	179 (85%)	
[00644]	Unique sequences	25	21	17	31	73
[00645]	Diversity	69%	47%	49%	49%	41%
[00646]	Specificity by ELISA*	100%	100%	100%	100%	100%
[00647]	FACS Binders (>5 S/N)	14	17	14	24	49**
[00648]	FACS correlation	56%	81%	82%	77%	67%

[00649] *No non-specific binding to irrelevant Fc conjugates or PVRL2; **35 unique HCs, 14 siblings

[00650] Primary fab screen (ELISA): Two 96-well plates (182 fab clones) of PPEs for each sub-campaign were screened by ELISA against the PVRIGHH recombinant protein. Note that although biotinylated protein was used for panning, the non-biotinylated version was used for the ELISA screen, which avoided detection of biotin or streptavidin-specific binders. The 4 sub-campaigns resulted in ELISA hit rates ranging from 24 – 37% when the

threshold for a 'positive' signal was set at a 3-fold ratio of target-specific binding: blank PPE control signal.

[00651] Secondary screen (DNA sequence analysis, ELISA and FACS) fabs: The ELISA positive clones were sequenced to select non-redundant fabs. Seventy-three sequence-unique fab clones were identified. 19 clones were unique to sub-campaign A, 13 clones were unique to sub-campaign B, 10 clones were unique to sub-campaign C, 18 clones were unique to sub-campaign D, while the remaining 23 clones were shared between the campaigns. Sequence-unique, ELISA-positive fab clones were re-expressed as PPEs and screened for specific binding by FACS. A total of 49 out of 73 unique clones were identified as PVRIG specific ELISA and FACS binders (following the criteria established in 2.6.5). The 49 FACS binders corresponded to 35 antibodies with unique heavy chains and 14 siblings that have unique light chains but share the heavy chain with one of the unique clones. A summary of FACS binding data is presented in Table 4.

[00652] The sequence unique fabs were also tested for non-specific binding. All the fab PPEs analyzed bound to the PVRIGHH recombinant protein with an assay signal greater than 3-fold over the blank PPE control. In a parallel assay, fab PPEs were tested for binding to two irrelevant proteins with the same IgG1 Fc region, as well as the PVRL2 recombinant protein. None of the clones showed significant non-specific binding to the controls, suggesting that the selected fabs are specific for PVRIG.

[00653] Table 4: FACS binding summary for PVRIG fabs. All unique ELISA positive fabs were analyzed by FACS. The mean fluorescence intensity (MFI) was measured for the PVRIG over-expressing HEK293 cells as well as the un-transfected HEK293 cells. The MFI ratio for the target-specific vs off-target binding was calculated. Clones with MFI ratio > 5 were selected as hits and are listed below.

[00654]	fab clone	MFI ratio	fab clone	MFI ratio
[00655]	CPA.7.001	11	CPA.7.026	5.3
[00656]	CPA.7.002	8.9	CPA.7.027	9.2
[00657]	CPA.7.003	9.5	CPA.7.028	17
[00658]	CPA.7.004	9.3	CPA.7.029	6.7
[00659]	CPA.7.005	6.5	CPA.7.030	15

	[00660]	CPA.7.006	9.6	CPA.7.031	8.5
	[00661]	CPA.7.007	14	CPA.7.032	7.6
	[00662]	CPA.7.008	14	CPA.7.033	22
	[00663]	CPA.7.009	10	CPA.7.034	7.7
5	[00664]	CPA.7.010	7.6	CPA.7.035	14
	[00665]	CPA.7.011	10	CPA.7.036	5
	[00666]	CPA.7.012	19	CPA.7.037	5.3
	[00667]	CPA.7.013	12	CPA.7.038	6.3
	[00668]	CPA.7.014	14	CPA.7.039	12
10	[00669]	CPA.7.015	15	CPA.7.040	12
	[00670]	CPA.7.016	7.6	CPA.7.041	7.6
	[00671]	CPA.7.017	13	CPA.7.042	5.4
	[00672]	CPA.7.018	7.8	CPA.7.043	13
	[00673]	CPA.7.019	16	CPA.7.044	7.9
15	[00674]	CPA.7.020	6.9	CPA.7.045	7.8
	[00675]	CPA.7.021	15	CPA.7.046	10
	[00676]	CPA.7.022	7.5	CPA.7.047	8.4
	[00677]	CPA.7.023	12	CPA.7.049	10
	[00678]	CPA.7.024	9.8	CPA.7.050	22
20	[00679]	CPA.7.025	6		

[00680]

[00681] Reformatting of the ELISA and FACS positive fabs into hIgG1: All unique ELISA and FACS binders were reformatted for expression as human IgG1 molecules in Expi293 cells. Out of the original 49 antibodies, 44 were successfully expressed as full-length antibodies. These reformatted antibodies were tested for retained binding to PVRIG over-expressing HEK293 cells alongside an irrelevant human IgG1 isotype control. All antibodies were also tested against un-transfected HEK293 cells. The resulting binding results were used

to demonstrate the specificity of the antibodies and also plotted to calculate the equilibrium binding constant (KD). Nine out of the remaining 44 antibodies showed weak binding or significant non-specific binding. The remaining 35 antibodies were selected for further analysis in cell-based functional assays. The FACS-based KD of these antibodies are listed in Table 6. The KD values range from 0.30 nM to 96 nM, with a median of 9.4 nM, suggesting that most antibodies obtained from the panning campaign are very specific and bind to PVRIg with high affinity.

[00682] Table 5: Expression and binding summary of reformatted antibodies. All unique ELISA and FACS positive fabs were reformatted into the human IgG1 backbone.

FACS KD values were determined by dose titration against the PVRIg over-expressing HEK293 cells. Off-target binding was determined by dose titration against the un-transfected HEK293 cells.

[00683]	Antibody	FACS KD (nM)	Antibody	FACS KD (nM)
[00684]	CPA.7.001	No-expression	CPA.7.026	Non-binder
[00685]	CPA.7.002	44.35	CPA.7.027	Non-binder
[00686]	CPA.7.003	Non-specific binding	CPA.7.028	7.14
[00687]	CPA.7.004	21.71	CPA.7.029	Weak binding
[00688]	CPA.7.005	95.56	CPA.7.030	No-expression
[00689]	CPA.7.006	No-expression	CPA.7.031	Non-binder
[00690]	CPA.7.007	0.73	CPA.7.032	8.78
[00691]	CPA.7.008	No-expression	CPA.7.033	12.8
[00692]	CPA.7.009	33.00	CPA.7.034	14.2
[00693]	CPA.7.010	21.89	CPA.7.035	Non-binder
[00694]	CPA.7.011	66.02	CPA.7.036	6.0
[00695]	CPA.7.012	0.30	CPA.7.037	Non-specific binding
[00696]	CPA.7.013	No-expression	CPA.7.038	20.26
[00697]	CPA.7.014	2.04	CPA.7.039	3.76
[00698]	CPA.7.015	1.34	CPA.7.040	0.79

	[00699]	CPA.7.016	22.02	CPA.7.041	52.2
	[00700]	CPA.7.017	1.82	CPA.7.042	24.26
	[00701]	CPA.7.018	9.29	CPA.7.043	13.2
	[00702]	CPA.7.019	0.45	CPA.7.044	9.4
5	[00703]	CPA.7.020	86.97	CPA.7.045	3.73
	[00704]	CPA.7.021	11.22	CPA.7.046	Non-specific binding
	[00705]	CPA.7.022	4.17	CPA.7.047	5.36
	[00706]	CPA.7.023	4.08	CPA.7.049	19.9
	[00707]	CPA.7.024	9.08	CPA.7.050	68.3
10	[00708]	CPA.7.025	Non-binder		
	[00709]	Summary and Conclusions			
	[00710]	A phage display antibody discovery campaign was conducted to isolate binders against the immuno-oncology target PVRIG using a recombinant Fc-tagged version of the antigen. Quality control analysis showed that the panning antigen was pure and			
15		functionally active. The panning effort yielded 49 unique fab clones that specifically bound to the PVRIG target, both as a recombinant protein and on the cell surface. Of these, 35 were successfully produced as human IgG1 antibodies and were shown to retain specific binding to the PVRIG. This pool of antibodies displayed high affinities in a FACS assays, with 18 out of 35 antibodies binding with a KD < 10 nM.			
20		Example 10 Demonstration of the ability of the anti-human PVRIG fabs to block the interaction between PVRIG and PVRL2 by ELISA.			
	[00711]	Method: The human PVRL2-His (Catalog #2229-N2-050/CF, R&D Systems), was coated on the ELISA plate. Fab periplasmic extracts (PPEs), diluted 1:1 in 5% skim milk, were preincubated with 1 ug/ml (final concentration) of the human PVRIG-Fc, for 15			
25		min at RT. The fab-receptor mixture was allowed to bind the PVRL2-His coated on the ELISA plate. The PVRIG-Fc/ PVRL2-His interaction was probed using anti-human Fc antibody, conjugated to HRP (Jackson Immuno Research catalog #709-035-098). In the absence of PPE (negative wells), a strong positive signal was expected. For blocking fabs, the signal would be significantly reduced. The fab clones with >5-fold lower signal than the			
30		negative wells (>80% blocking) could be selected as blocking fabs.			

[00712] Protocol:

[00713] ELISA plates (Costar 9018) were coated with 50 μ l of 2 μ g/ml antigen and were stored at 4 C overnight. The antigen-coated plates were washed 3 times with 1X PBS. The plate was blocked with 200 μ l of 5% skim milk in PBS and incubated 1 hr at RT (room
5 temperature). Next the plate was washed with 1X PB.

[00714] After adding 50 μ l/well of Fab PPEs (diluted in 5% skim milk), the plate was preincubated with 1 μ g/ml of the human PVRIG-Fc that was added to the respective wells. The “no fab” control was performed with 2 wells.

[00715] The plate was incubated 1 hr at RT.

10 [00716] The plates were washed 3 times with 1X PBST and 3 times with 1X PBS.

[00717] After adding 50 μ l/well of the HRP-conjugated secondary antibody (Jackson Immuno Research, 709-035-098), diluted in 5% milk in PBS, the plate was incubated 1 hr at RT.

[00718] The plates were washed 3 times with 1X PBST and 3 times with 1X PBS.

15 [00719] After adding 50 μ l/well of the TMB substrate and waiting until the color develops, the reaction was stopped by adding 50 μ l/well of 2N H₂SO₄. Absorbance was measured at 450 nm.

[00720] Results

[00721] Figure 52 shows the results of testing anti-PVRIG antibodies for their ability
20 to block at least 80% of PVRL2 binding to PVRIG. As shown, a large number of such antibodies were able to successfully block at least 80% of the binding. Specifically the antibodies which blocked successfully are designated as follows:

[00722] CPA.7.001, CPA.7.003, CPA.7.004, CPA.7.006, CPA.7.008, CPA.7.009,
CPA.7.010, CPA.7.011, CPA.7.012, CPA.7.013, CPA.7.014, CPA.7.015, CPA.7.017,
25 CPA.7.018, CPA.7.019, CPA.7.021, CPA.7.022, CPA.7.023, CPA.7.024, CPA.7.033,
CPA.7.034, CPA.7.036, CPA.7.040, CPA.7.046, CPA.7.047, CPA.7.049, CPA.7.050,

**Example 11: Surface Plasmon Resonance Study of Epitope Binning of 37 Anti PVRIG
IgG Antibodies binding to human PVRIG fusion protein**

[00723] Materials and Methods

[00724] Experiments were performed using a ProteOn XPR 36 instrument at 22°C with all samples kept at 4°C during the experiment.

[00725] Step 1: The following anti-PVRIG mAbs were each diluted to ~10 µg/mL in 10mM sodium acetate, pH 5.0 and covalently immobilized on independent spots on a
5 ProteOn GLC biosensor chip using standard amine coupling:

	[00726]	CPA.7.002	CPA.7.017	CPA.7.033
	[00727]	CPA.7.003	CPA.7.018	CPA.7.034
	[00728]	CPA.7.004	CPA.7.019	CPA.7.036
	[00729]	CPA.7.005	CPA.7.020	CPA.7.037
10	[00730]	CPA.7.007	CPA.7.021	CPA.7.038
	[00731]	CPA.7.009	CPA.7.022	CPA.7.039
	[00732]	CPA.7.010	CPA.7.023	CPA.7.040
	[00733]	CPA.7.011	CPA.7.024	CPA.7.043
	[00734]	CPA.7.012	CPA.7.026	CPA.7.045
15	[00735]	CPA.7.014	CPA.7.028	CPA.7.046
	[00736]	CPA.7.015	CPA.7.029	CPA.7.047
	[00737]	CPA.7.016	CPA.7.032	CPA.7.050

[00738] The activation step occurred in the horizontal flow direction for five minutes while the immobilization step occurred in the vertical flow direction. MAb were injected for
20 four minutes after surface activation. The blocking step occurred in both the vertical and horizontal positions at five minutes each so that the horizontal “interspots” could be used as reference surfaces. MAb were immobilized at a range of ~450RU-5000RU. An additional mAb CPA.7.041 was also binned in this study, but only as an analyte in solution. See below.

[00739] Step 2: Preliminary experiments involved several cycles of injecting ~20 nM
25 PVRIG antigen (PVRIGHH-2-1-1 #448, GenScript) over all immobilized mAbs for three minutes at a flow rate of 25 µL/min followed by regeneration with a 30-second pulse of 10 mM glycine-HCl, at either pH 2.0 or pH 2.5, depending on the horizontal row of mAbs in the GLC chip array. Antigen samples were prepared in degassed PBST (PBS with 0.05% Tween 20) running buffer with 100 µg/mL BSA. These preliminary experiments showed that clones

CPA.7.026 and CPA.7.029 did not bind to the antigen and were therefore not binned. The remaining mAbs on the ProteOn array showed reproducible binding to the antigen.

[00740] Step 3: A “pre-mix” epitope binning protocol was performed because of the bivalency of the fc-fusion PVRIG antigen. In this protocol each mAb listed in Step 1, plus
5 mAb CPA.7.041, was pre-mixed with PVRIG antigen and then injected for three minutes over all immobilized mAbs. The molar binding site concentration of each mAb was in excess of the molar antigen binding site concentration. The final binding site concentration of each mAb was ~400nM and the final binding site concentration of the antigen was ~20nM. An antigen-only control cycle was performed after every eight mAb injection cycles to monitor
10 the activity of the immobilized mAbs throughout the experiment. Buffer blank injections were also performed after about every eight mAb injection cycles for double-referencing. Additional controls included each mAb injected alone over all immobilized mAbs at concentrations identical to the pre-mix injection cycles. All surfaces were regenerated with a 30 second pulse of 10 mM glycine-HCl at either pH 2.0 or pH 2.5 depending on which row of
15 mAbs in the array was being regenerated, and all cycles were run at a flow rate of 25 μ L/min. MAb and antigen samples were prepared in degassed PBST running buffer with 100 μ g/mL BSA.

[00741] Step 4: Sensorgram data were processed and referenced using ProteOn Manager Version 3.1.0.6 using interspots and buffer blanks for double-referencing. The
20 mAb-only control injections were used as the injection references where significant binding with the mAb-only injections was observed. An antibody pair was classified as having a shared antigen binding epitope (designated as a red “0” in the matrix in Figure 43) if no binding was observed from the injection of mixed mAb and antigen over the immobilized mAb, or if binding was significantly reduced as compared to the antigen-only control
25 injection over the same immobilized mAb. An antibody pair was classified as binding to different antigen epitopes, or “sandwiching” the antigen (designated as a green “1” in the matrix in Figure 43) if the injection of mixed mAb and antigen showed binding to the immobilized mAb similar to or greater than the antigen-only control over the same immobilized mAb.

[00742] Step 5: The blocking pattern for mAb CPA.7.041 (#37) was studied only as an analyte because the GLC chip array has only 36 spots. Therefore for consistency, hierarchical clustering of the binding patterns in the binary matrix for each mAb pre-mixed

with antigen (vertical patterns in Figure 42) was performed using JMP software version 11.0.0. The blocking patterns of the immobilized mAbs (horizontal patterns in Figure 42) were also clustered as a comparison to the blocking patterns of the mAbs pre-mixed in solution (data not shown, see Results for discussion).

5 **[00743]** Results: Figure 42 shows the binary matrix of the blocking (“0”) or sandwiching (“1”) between each mAb pair where the mAbs are listed in identical order both vertically (mAbs on the surface – “ligands”) and horizontally (mAbs in solution – “analytes”). Identical “bins” of blocking patterns for all mAbs as analytes are highlighted in Figure 42 with a black box around each group of similar vertical patterns. Figure 43 shows
10 the dendrogram of the vertical (analyte) blocking patterns in the matrix in Figure 42. For the strictest definition of an epitope “bin” where only those mAbs which show identical blocking patterns technically bin together, there are a total of 4 discrete bins. Specifically, 33 of the 35 mAbs that were binned comprise two bins where the only difference between these two bins is whether a mAb sandwiches (Bin 2, see Figure 42 and 43) with or blocks (Bin 1, see Figure
15 42 and Figure 43) binding to CPA.7.039. This means that CPA.7.039 is in its own separate bin. The fourth bin consists only of mAb CPA.7.050 which is unable to block antigen binding to any of the other 34 mAbs. Hierarchical clustering of the blocking patterns of the mAbs as ligands (horizontal patterns in Figure 42) showed mAb CPA.7.016 sandwiching antigen with mAb CPA.7.039 whereas as an analyte it blocks antigen binding to immobilized
20 CPA.7.039. Hence clone CPA.7.016 would be placed in bin 2 rather than in bin 1. The mAbs in each bin are listed in Figure 43. Processed sensorgram data representative of each bin are shown in Figure 44 to Figure 47.

[00744] Summary: 35 anti-PVRIG IgG mAbs were binned using SPR according to their pair-wise blocking patterns with fc fusion human PVRIG. By the strictest definition of
25 an epitope bin, there are a total of four discrete bins. 33 of the 35 mAbs comprise two bins which differ only by whether their respective component mAbs block or sandwich antigen with clone CPA.7.039.

Example 12 SURFACE PLASMON RESONANCE KINETIC SCREEN OF 50 ANTI-PVRIG HUMAN FABS PREPARED IN PERIPLASMIC EXTRACTS

30 **[00745]** Materials and Methods

[00746] All experiments were performed using a Biacore 3000 instrument and a ProteOn XPR 36 instrument at 22°C.

[00747] **Step 1:** The molar concentration of all 52 fabs in periplasmic extract supernatant were quantitated using a Biacore 3000 instrument at 22°C. Each fab was diluted 20-fold and then injected for 2 minutes at 5µL/min over high density anti-human fab (GE Healthcare 28-9583-25) surfaces prepared using standard amine coupling with a CM5 Biacore chip (GE Healthcare). A standard human fab at a known concentration (Bethyl P80-115) was then injected over the anti-fab surface with the same conditions as the fab supernatants. Samples were prepared in the running buffer which was degassed HBSP (0.01 M HEPES, 0.15 M NaCl, 0.005% P20, pH 7.4) with 0.01% BSA added. The association slopes of each SPR sensorgram from each fab supernatant was fit against the SPR association slope of the standard human fab of known concentration using CLAMP 3.40 software to estimate the molar concentrations of each fab in supernatant.

[00748] **Step 2:** A high density goat anti-human fc polyclonal antibody surface (Invitrogen H10500) was prepared using standard amine coupling over two lanes of a GLC chip using a ProteOn XPR 36 biosensor. A high density anti-mouse fc polyclonal antibody surface (GE Healthcare BR-1008-38) was prepared using standard amine coupling over two different lanes of the same GLC chip. The activation and blocking steps for all four capture surfaces occurred in the vertical flow direction. Each fab in supernatant was then injected at three concentrations over fc-fusion human PVRIG (PVRIG-HH-2-1-1 #448, GenScript) and fc-fusion mouse PVRIG (PVRIG-MM-2-1-1 #198, GenScript) which were captured to one high density anti-human fc surface and one anti-mouse fc surface (respectively) at an average of ~200RU and ~290RU per cycle, respectively. Each fab concentration series was injected for two minutes followed by 10 minutes of dissociation at a flow rate of 50 µL/min. The starting concentration range (as determined in Step 1) was ~20nM - ~400nM with two three-fold dilutions of the highest concentration for each fab. Fabs were diluted into the running buffer which was degassed PBS with 0.05% Tween 20 and 0.01% BSA added. The anti-human fc capture surfaces were regenerated with two 30-second pulses of 146 mM phosphoric acid after each cycle and the anti-mouse fc surfaces were regenerated with two 30-second pulses of 10mM glycine, pH 1.7 after each cycle.

[00749] **Step 3:** Sensorgram data of fabs in supernatant binding to captured PVRIG were processed and double-referenced using ProteOn Manager version 3.1.0.6. The sensorgrams were double-referenced using the corresponding anti-species capture surfaces with no captured PVRIG as reference surfaces and a blank injection over the captured PVRIG under identical conditions as the injections of the fabs. Where possible, the sensorgrams for

the three different concentrations of each fab were then globally fit to a 1:1 kinetic model (with a term for mass transport) to estimate the association and dissociation rate constants. Sensorgrams which did not show simple 1:1 binding were not fit with the kinetic model and therefore were not assigned estimates for k_a and k_d .

5 **[00750]** Results

[00751] None of the fabs included in this study showed binding activity to mouse PVRIG (data not shown). Sensorgrams for 17 of the 50 fabs screened against the human PVRIG could be fit for reliable estimates of their rate constants. Twenty eight clones showed complex kinetics, five of the fabs did not show any binding to the captured human PVRIG fusion protein (CPA.7.025, CPA.7.026, CPA.7.027, CPA.7.029, CPA.7.035) and one clone (CPA.7.035) showed no titer when performing the concentration determination in Step 1. The rate constants and their corresponding sensorgrams are shown below in Figure 49 and Figure 50. The clones listed below showed complex kinetics. Figure 51 shows some examples of these data.

15	[00752]	CPA.7.001	CPA.7.006	CPA.7.013	CPA.7.045
	[00753]	CPA.7.030	CPA.7.036	CPA.7.014	CPA.7.046
	[00754]	CPA.7.031	CPA.7.037	CPA.7.041	CPA.7.017
	[00755]	CPA.7.032	CPA.7.009	CPA.7.042	CPA.7.018
	[00756]	CPA.7.033	CPA.7.038	CPA.7.043	CPA.7.047
20	[00757]	CPA.7.034	CPA.7.039	CPA.7.016	CPA.7.023
	[00758]	CPA.7.003	CPA.7.011	CPA.7.044	CPA.7.024

**EXAMPLE 13 MEASURING THE BINDING AFFINITY OF IGG CLONE
CPA.7.021 TO PVRIG EXPRESSED ON HEK CELLS USING FLOW
CYTOMETRY**

25

[00759] Materials and Methods

[00760] Flow cytometry was used to measure the affinity of CPA.7.021 IgG binding to human PVRIG expressed on HEK 293 cells. CPA.7.021 conjugated with Alexa 647 was added in duplicate at a binding site concentration range of 3 pM – 101 nM in a 2-fold serial dilution to a constant number of cells (100,000 cells/well) over 17 wells in a 96-well plate.

30

One well contained cells without any added IgG to serve as a blank well. The cells were equilibrated for 4 hours with IgG at 4°C. Cells were washed twice and then the Mean Fluorescence Intensity (MFI) was recorded over approximately 10,000 “events” using an Intellicyte flow cytometer. The resulting MFI values as a function of the CPA.7.021 IgG binding site concentration are shown below. The KD of CPA.7.021 binding to HEK 293 cells expressing human PVRIG was estimated by fitting the MFI vs. the IgG binding site concentration curve with a 1:1 equilibrium model as detailed in Drake and Klakamp, Journal of Immunol Methods, 318 (2007) 147–152.

[00761] Results: Alexa647 labelled CPA.7.021 IgG was titrated with HEK 293 cells expressing human PVRIG and the binding signal was measured using flow cytometry. The resulting binding isotherm, showing MFI in duplicate vs. the binding site concentration of CPA.7.021, is presented below. The red line is a 1:1 equilibrium fit of the curve that allows for a KD estimate of $2.5 \text{ nM} \pm 0.5 \text{ nM}$ (95% confidence interval of the fit, N=1).

[00762]

Example 14 Effect of PVRIG Knock down (KD) and anti-PVRIG antibody on human Melanoma specific TILs function

[00763] The aim of these assays is to evaluate the functional capacity of PVRIG in human derived TILs, as measured by activation markers and cytokine secretion, upon co-culture with melanoma target cells. PD1 was used as a benchmark immune-checkpoint for the knock down (siRNA) studies. The effect of anti-PVRIG antibody (CPA.7.21), which has been shown to block the interaction of PVRIG and PVRL2, alone or in combination with other antibodies (e.g aTIGIT, DNAM1) was evaluated.

[00764] Materials and Methods

[00765] TILs

[00766] Tumor-infiltrating lymphocytes (TILs) from three melanoma patients were used:

[00767] ☐ TIL-412- HLA-A2-Mart1 specific

[00768] ☐ TIL-F4- HLA-A2-gp100 specific

[00769] ☐ TIL-209- HLA-A2-gp100 specific

[00770] TILs were thawed in IMDM (BI, 01-058-1A) full medium supplemented with 10% human serum (Sigma, H3667) + 1% Glutamax (Life technologies, 35050-038) + 1% Na-Pyruvate (Biological Industries, 03-042-1B) + 1% non-essential amino acids (Biological Industries, 01-340-1B) + 1% Pen-Strep (Biological Industries, 03-031-1B) + 300 U/ml of
5 rhIL2 (Biolegend, 509129).

[00771] Tumor cell lines: Human melanoma cells Mel-624 express MART-1 and gp-100 antigens in the context of MHC-I haplotype HLA-A2. Cells were cultured in complete DMEM medium (Biological Industries, 01-055-1A) supplemented with 10% FBS (BI, 04-127-1A), 25 mM HEPES buffer (BI, 03-025-1B), 1% Glutamax (Life technologies, 35050-038), and 1% Pen-Strep (Biological Industries, 03-031-1B).
10

[00772] Knock down in TILs: Knock-down (KD) of human PVRIG and human PD1 in TILs was done using 100pmol of Dharmacon ON-TARGETplus human PVRIG siRNA - SMARTpool (L-032703-02) or Human PD1 siRNA - SMARTpool (L-004435) or non-targeting siRNA (D-001810-01-05). siRNA were electroporated to TILs (AMAXA, program
15 X-005). Electroporation was done on resting TILs cultured in full IMDM supplemented with IL-2 24hr post thawing. After the electroporation TILs were seeded in 96 well TC plate to recover for 24hr. After 24 hr, cells were harvested and stained with viability dye (BD Horizon; Cat# 562247, BD biosciences), washed with PBS and stained with anti-human PVRIG – CPA.7.021 (CPA.7.021 IgG2 A647, 7.5ug/ml) or with anti-human PD-1
20 (Biolegend, #329910 AF647, 5ug/ml) in room temperature for 30min. isotype control used are synagis (IgG2 A647, 7.5ug/ml) and mouse IgG1 (Biolegend #400130 A647, 5ug/ml) respectively. All samples were run on a MACSQuant analyzer (Miltenyi) and data was analyzed using FlowJo software (v10.0.8).

[00773] Co-culture of TILs with 624 melanoma cells: siRNA electroporated TILs were
25 harvested and seeded in 96 TC plate 5x10⁴/well. Mel-624 cells were harvested as well and seeded in 1:1 / 1:3 E:T ratios in co-culture. The plate was incubated overnight (18hr) in 37°C, 5% CO₂.

[00774] To assess the effect of anti-PVRIG antibody (CPA.7.021), anti-TIGIT (Clone 10A7) and anti-DNAM1 (clone DX11) on melanoma specific TIL activity, TILs (1x10⁵
30 cells/well) were pre-incubated with tested antibodies or relevant isotype controls in mono-treatment (10µg/mL) or in combination-treatment (final 10µg/mL for each) prior to the

addition of 624 Melanoma target cells at a 1:1 Effector:target ratio. The plate was incubated overnight (18hr) in 37°C, 5% CO₂.

[00775] Assessment of TILs activation: 16 hours post co-culture, cells were stained with viability dye (BD Horizon; Cat# 562247, BD biosciences), washed with PBS and
5 exposed to Fc blocking solution (cat# 309804, Biolegend), followed by surface staining with anti-CD8a (Cat #301048, Biolegend) and anti-CD137 (Cat #309804, Biolegend) in 4°C for 30min. All samples were run on a MACSQuant analyzer (Miltenyi) and data was analyzed using FlowJo software (v10.0.8). Culture supernatants were collected and analyzed for cytokine secretion by CBA kit (Cat #560484, BD).

10 [00776] Results

[00777] PVRIG Knock-Down in TILs: TIL MART-1 and TIL F4 were cultured 24 hr with IL-2. 100 pmol of ON-TARGETplus human PVRIG siRNA - SMART pool (L-032703-02) or Human PD1 siRNA - SMARTpool (L-004435) or non-targeting siRNA (D-001810-01-05) were electroporated to TILs (AMAXA, program X-005). Detection of PVRIG or PD-1
15 was performed 24 hr post electroporation (and prior to co-culture). Cells were stained for viability dye followed by 30min RT incubation with anti PVRIG or anti PD-1. The percentage of KD population is indicated in Figure 82.

[00778] Functional assay using knocked down TILs: Human TILs, cultured for 24 hours with IL2 were electroporated with siRNA encoding for human PVRIG or PD-1 or
20 scrambled sequence as control. TILs were tested for PVRIG and PD-1 expression 24 hr post electroporation. ~80% knock down of PVRIG and ~50% knock down of PD-1 compared to scrambled-electroporated TILs was observed (Figure 82).

[00779] KD TILs were cultured with Mel-624 cells in 1:1 or 1:3 E:T for 18hr and were stained for the expression of CD137. Elevated levels of activation marker CD137 were shown
25 in TIL MART-1 electroporated with PVRIG siRNA, similarly to TILs that were electroporated with PD-1 siRNA, compared to control scrambled siRNA (Error! Reference source not found.A). Co-culture supernatant was collected and tested for the presence of secreted cytokines. TILs that were electroporated with PVRIG siRNA show a significant increase in IFN γ and TNF levels compared to control SCR siRNA. A similar effect was
30 shown in TILs that were electroporated with PD-1 siRNA (Figure 83B-C).

[00780] The same trend of increase in activation levels was observed in TIL F4. Co-culture of PVRIG siRNA electroporated TIL F4 with Mel-624 in 1:3 E:T led to increased

levels of CD137 surface expression (Figure 84A) as well as increased secretion of IFN γ in co-culture supernatant (Figure 84B). Similar trends were observed in TILs that were electroporated with PD-1 siRNA.

[00781] Functional assay using blocking Abs:

5 [00782] In vitro monotherapy and combo therapy of anti-PVRIG and anti-TIGIT: 209
TILs were cultured with Mel-624 cells in 1:1 E:T for 18hr. Co-culture supernatant was collected and tested for the presence of secreted cytokines. Treatment with anti TIGIT did not affect IFN γ or TNF secretion levels. However, an increase in IFN γ and TNF levels was observed when anti TIGIT and anti PVRIG were added to co-culture in combination (Figure
10 85A-B).

[00783] In vitro monotherapy and combo therapy of anti-PVRIG and anti-TIGIT: 209
TILs were cultured with Mel-624 cells in 1:1 E:T for 18hr. TILs were stained for surface expression of activation marker CD137 and showed reduced level of expression upon treatment with anti DNAM-1. Co-culture supernatant was collected and tested for presence of
15 secreted cytokines. Treatment of anti DNAM-1 mediated a trend to increase secreted cytokines IFN γ and TNF. Treatment with anti DNAM-1 and anti PVRIG in combination partially reversed the effect on CD137 expression (Figure 86C) and enhanced the effect on cytokine secretion IFN γ and TNF (Fig. 5A-B). MART-1 TILs were cultured with Mel-624 cells in 1:1 E:T for 18hr. Co-culture supernatant was collected and tested for the presence of
20 secreted cytokines. Treatment with anti DNAM-1 reduced CD137 surface expression on TILs and also the secreted cytokines IFN γ and TNF. Treatment with anti DNAM-1 and anti PVRIG in combination partially reversed these effects (Figure 86D-F).

[00784] Summary and conclusions

[00785] PD1 KD improved TIL activity, as measured by IFN γ and secretion in F4 and
25 MART-1 TILs. An increase (~20%) of IFN γ and TNF secretion was observed upon PVRIG KD in MART-1 TILs compared to control siRNA. The same trend was observed in CD137 expression upon co-culture with 624 Melanoma cells on F4 TILs.

[00786] Treatment of anti-TIGIT did not affect IFN γ or TNF secretion levels from TILs co-cultured with 624 Mels, however, an increase in IFN γ and TNF levels was observed
30 when anti TIGIT and anti PVRIG (CPA.7.021) were added to co-culture in combination.

[00787] Anti DNAM-1 treatment reduced TIL-MART-1 activation manifested by reduced CD137 and cytokine secretion and anti-PVRIG (CPA.7.21) could partially reverse this effect in combo treatment with DNAM-1 Ab. In TIL 209, IFN γ and TNF secretion levels were slightly elevated (~10%) with anti DNAM-1, and an increase in IFN γ and TNF levels (~40% and 30%, respectively) was observed when anti DNAM1 and anti PVRIG (CPA.7.021) were added to co-culture in combination. Collectively, our results suggest that PVRIG is a new co-inhibitory receptor for PVRL2.

EXAMPLE 15 EFFECT OF ANTI-PVRIG ANTIBODY ON HUMAN MELANOMA SPECIFIC TILS FUNCTION IN COMBINATION WITH ANTI-TIGIT AND ANTI-PD1 ANTIBODIES

[00788] Materials and Methods

[00789] TILs: Tumor-infiltrating lymphocytes (TILs) from three melanoma patients were used:

15 [00790] ☐ TIL-412- HLA-A2-Mart1 specific

[00791] ☐ TIL-F4- HLA-A2-gp100 specific

[00792] ☐ TIL-209- HLA-A2-gp100 specific

[00793] TILs were thawed in IMDM (BI, 01-058-1A) full medium supplemented with 10% human serum (Sigma, H3667) + 1% Glutamax (Life technologies, 35050-038) + 1% Na-Pyruvate (Biological Industries, 03-042-1B) + 1% non-essential amino acids (Biological Industries, 01-340-1B) + 1% Pen-Strep (Biological Industries, 03-031-1B) + 300 U/ml of rhIL2 (Biolegend, 509129).

[00794] Tumor cell lines: Human melanoma cells Mel-624 express MART-1 and gp-100 antigens in the context of MHC-I haplotype HLA-A2. Cells were cultured in complete DMEM medium (Biological Industries, 01-055-1A) supplemented with 10% FBS (BI, 04-127-1A), 25 mM HEPES buffer (BI, 03-025-1B), 1% Glutamax (Life technologies, 35050-038), and 1% Pen-Strep (Biological Industries, 03-031-1B).

[00795] Co-culture of TILs with 624 melanoma cells in the presense of anti-PVRIG, anti-TIGIT and PD1 blocking antibodies: To assess the effect of anti-PVRIG antibody (CPA.7.021), anti-TIGIT (Clone 10A7) and anti-PD1 (mAb 1B8, Merck) on melanoma

specific TIL activity, TILs (3x10⁴ cells/well) were pre-incubated with tested antibodies or relevant isotype controls in mono-treatment (10 µg/mL) or in combination-treatment (final 10 µg/mL for each) prior to addition of 624 Melanoma target cells at 1:3 Effector:target ratio. Plate was incubated overnight (18hr) in 37°C, 5% CO₂.

5 [00796] Assessment of TILs activation: Culture supernatants were collected and analyzed for cytokine secretion by CBA kit (Cat #560484, BD).

[00797] In vitro monotherapy anti-PVRIG and combo-therapy of with anti-TIGIT and PD1 blocking antibodies: F4 TILs (gp100 sepecific) were cultured with Mel-624 cells in 1:3 E:T for 18hr. Co-culture supernatant was collected and tested for presence of secreted
10 cytokines. Treatment of anti-TIGIT or anti-PD1 did not affect IFN γ or TNF secretion levels. However, an increase in IFN γ and TNF levels was observed when anti TIGIT or anti-PD1 in combination with anti PVRIG were added to co-culture in combination (Figure 87A-B).

[00798] Treatment of anti-PVRIG, anti-TIGIT and PD1 alone did not affect IFN γ or TNF secretion levels from TILs co-culture with 624 Mels, however, an increase in IFN γ and
15 TNF levels was observed when anti-TIGIT or anti-PD1 antibodies were added in combination with anti PVRIG (CPA.7.021). The presented data suggest that there is synergistic effect for combinatory therapy with anti-TIGIT or anti-PD1 antibodies.

EXAMPLE 16: EFFECT OF ANTI-PVRIG ANTIBODIES ON TCR SIGNALING USING REPORTER GENE ASSAY

20 [00799] A reporter assay system for TCR signaling, such as the Jurkat-NFAT-Luc cell line, is used to test the effect of anti-PVRIG antibodies on TCR mediated signaling. This Jurkat cell line derivative expresses the luciferase reporter gene under the control of the NFAT response element. These cells are transfected with a vector encoding full length human PVRIG. As negative control, cells transfected with empty vector are used. Transfectants with
25 vectors encoding for costimulatory or coinhibitory reference molecules, such as CD28 and PD-1, serve as positive control. Transfectants are stimulated by the addition of anti-human CD3 (e.g. OKT3) in the absence or presence of anti-PVRIG antibodies. Isotype control serves as negative control. Known functional antibodies against the reference molecules serve as positive controls. A functional agonistic crosslinking antibody is expected to show an
30 inhibitory effect on the luciferase activity.

EXAMPLE 17 EFFECT OF ANTI-PVRIG ANTIBODIES ON T CELL ACTIVATION USING PVRL2-FC

[00800] A plate bound assay is used to test the effect of anti-PVRIG antibodies on T cell activation, proliferation and cytokine secretion. Purified human bulk T cells are stimulated using 1 ug/ml plate bound anti-human CD3 (e.g. OKT3) and 5 ug/ml PVRL2-Fc (recombinant fused protein composed of the ECD of PVRL2, the counterpart of PVRIG) or negative control. T cell activation is evaluated by expression of activation markers, e.g. CD137, or by cell division as evaluated by dilution of CFSE dye (T cells are labeled with CFSE prior to their stimulation). Cytokine production (e.g. IFN γ , IL-2) is also assessed as additional readout of T cell activation. T cell subtype markers are used to distinguish specific effects on CD4 or CD8 T cells. The co-immobilized PVRL2-Fc could have a basal stimulatory effect on T cell activation, mediated through endogenous DNAM1 - a known costimulatory counterpart receptor of PVRL2 on T cells. In the presence of antagonistic anti-PVRIG Abs, this stimulatory basal effect of PVRL2-Fc is expected to be further enhanced, due to their blocking of the inhibitory influence of endogenous PVRIG on T cell activation. Accordingly, agonistic anti-PVRIG Abs are expected to show inhibition of T cell activation.

EXAMPLE 18: Effect of anti-PVRIG antibodies on T cell activation using PVRL2 ectopic expressing cells

[00801] A cell based assay is used to test the effect of anti-PVRIG antibodies on T cell activation, proliferation and cytokine secretion. Purified human bulk or CD4 or CD8 T cells are stimulated upon co-culture with CHO stimulator cells (CHO cells expressing membrane-bound anti-CD3) ectopically expressing PVRL2 or empty vector. T cell activation is evaluated by expression of activation markers, e.g. CD137, or by cell division as evaluated by dilution of CFSE dye (T cells are labeled with CFSE prior to their stimulation). Cytokine production (e.g. IFN γ , IL-2) is also assessed as additional readout of T cell activation. T cell subtype markers are used to distinguish specific effects on CD4 or CD8 T cells. The PVRL2-expressing CHO stimulators are expected to have a basal stimulatory effect on T cell activation, mediated through endogenous DNAM1 - a known costimulatory counterpart receptor of PVRL2 on T cells. In the presence of antagonistic anti-PVRIG Abs, this stimulatory basal effect of surface expressed PVRL2 is expected to be further enhanced, due to their blocking of the inhibitory influence of endogenous PVRIG on T cell activation. Accordingly, agonistic anti-PVRIG Abs are expected to show inhibition of T cell activation.

EXAMPLE 19 EFFECT OF ANTI-PVRIG ANTIBODIES ON T CELL ACTIVATION USING THE SEB ASSAY

[00802] Anti-PVRIG antibodies are tested for their effect on T cell activity using blood cells from healthy volunteers and SEB (Staphylococcus enterotoxin B) superantigen to engage and activate all T cells expressing the V β 3 and V β 8 T cell receptor chain. Human PBMCs are cultured in 96-well round-bottom plates and pre-incubated for 30-60 min with the tested antibodies. SEB is then added at various concentrations ranging from 10 ng/mL to 10 μ g/mL. Supernatants are collected after 2 to 4 days of culture and the amount of cytokine (e.g. IL-2, IFN γ) produced is quantified by ELISA or using standard CBA kit. SEB stimulates cytokine production by whole-blood cells in a dose dependent manner. The effect of anti-PVRIG mAbs on cytokine production is tested at several Ab doses. Blocking anti-PVRIG mAbs are expected to enhance IL-2 production over control IgG. In addition to IL-2, the effect of the Abs on the levels of additional cytokines such as TNF α , IL-17, IL-7, IL-6 and IFN γ can be tested in this assay using a CBA kit.

EXAMPLE 20 Effect of anti-PVRIG antibodies in Ag-specific assays

[00803] An assay that is used to profile the functional effect of anti-human PVRIG antibodies on Ag specific stimulation of pre-existing memory T cells in healthy donor blood is the tetanus toxoid (TT) assay. To this end, freshly prepared PBMC (2×10^5 cells) are plated in 96 well round-bottom plates in complete RPMI 1640 medium (containing 5% heat inactivated human serum), pre-incubated with tested antibodies at varying concentration and stimulated with TT (Astarte Biologics) at a concentration of 100 ng/mL. The cells are incubated for 3-7 days at 37°C, after which supernatants are harvested. Cytokine concentrations (e.g. IL-2, IFN- γ) are determined by ELISA and/or CBA kit. Blocking anti-PVRIG Abs are expected to enhance T cell proliferation and cytokine production compared to that obtained with TT antigen alone.

[00804] Similarly to the method described above, which uses TT to stimulate human memory T cells, we can test the effect of anti-PVRIG Abs on T cell activation upon recall responses to additional antigens such as CMV, EBV, influenza HIV, mumps, and TB, using a similar experimental setup as described above. This can also be used to test the effect of anti-PVRIG antibodies on stimulation of naïve cells using neo-antigens such as KLH.

[00805] In addition, the effect of anti-PVRIG Abs is tested on the antigen specific responses of tetramer-sorted Ag-specific CD8 T cells from peripheral blood of patients suffering from viral infections such as HCV and HIV. Tetramer sorted CD8 T cells are co-cultured with peptide-loaded autologous PBMCs for 5 days. Proliferation of CD8 Ag-specific

T cells and secretion of cytokines (e.g. IFN γ , IL2, TNF- α) are evaluated. We expect anti-PVRIG antibodies to enhance proliferation and cytokine production, compared to antigen alone.

EXAMPLE 21 BINDING AND FUNCTIONAL ANALYSIS OF HYBRIDOMA-DERIVED ANTIBODIES AGAINST PVRIG

[00806] This example shows the characterization of binding of hybridoma-derived antibodies (the CHA antibodies) to human and cynomolgus PVRIG protein in cell lines and primary leukocytes, as well as the characterization of the capacity of hybridoma-derived antibodies to block the interaction between PVRIG and PVRL2.

[00807] Protocols

[00808] FACS analysis of hPVRIG over-expressing cells: The following cell lines were used to assess the specificity of anti-human PVRIG antibodies: HEK parental and HEK hPVRIG over-expressing cells. These cells were cultured in DMEM (Gibco) + 10% fetal calf serum (Gibco) + glutamax (Gibco). For the HEK hPVRIG over-expressing cells, 0.5ug/ml puromycin (Gibco) was also added to the media for positive selection. For FACS analysis, all cell lines were harvested in log phase growth and 50,000-100,000 cells per well were seeded in 96 well plates. Anti- human PVRIG antibodies (mIgG1 or mIgG2a) and their respective controls were added in single point dilutions (5ug/ml), or as an 8 point titration series starting at 10ug/ml on ice for 30 mins-1 hr. The titration series were conducted as either 1:3 or 1:3.3 fold serial dilutions. Data was acquired using a FACS Canto II (BD Biosciences) or IntelliCyt (IntelliCyt Corporation) and analyzed using FlowJo (Treestar) and Prism (Graphpad) software.

[00809] FACS analysis of human cell lines for hPVRIG: The following cell lines were used to assess the expression and specificity of anti-human PVRIG antibodies: Jurkat and HepG2. Jurkat cells were cultured in RPMI media + 10% fetal calf serum, glutamax, non-essential amino acids (Gibco), sodium pyruvate (Gibco), and penicillin/streptomycin (Gibco). HepG2 cells were cultured in DMEM + 10% fetal calf serum + glutamax. For FACS analysis, all cell lines were harvested in log phase growth and 50,000-100,000 cells per well were seeded in 96 well plates. Anti- human PVRIG antibodies (mIgG1 or mIgG2a) and their respective controls were added in single point dilutions (5ug/ml), or as an 8 point titration series starting at 10ug/ml on ice for 30 mins-1 hr. The titration series were conducted as either 1:3 or 1:3.3 fold serial dilutions. Data was acquired using a FACS Canto II or IntelliCyt and analyzed using FlowJo and Prism software.

[00810] FACS analysis of naïve human primary leukocytes for hPVRIG: Primary

leukocytes were obtained by Ficoll (GE Healthcare) gradient isolation of peripheral blood (Stanford Blood Bank). Leukocytes as isolated peripheral blood mononuclear cells (PBMC) were frozen down in liquid nitrogen at a density between 1×10^7 and 5×10^7 cells/ml in a 10%

5 DMSO (Sigma), 90% fetal calf serum mixture. To assess protein expression of PVRIG on PBMC, antibody cocktails towards major immune subsets were designed that included human anti-PVRIG antibodies. Anti- human PVRIG antibodies (mIgG1 or mIgG2a) and their respective controls were added in single point dilutions (5ug/ml), or in some cases, as a 4 point titration series starting at 10ug/ml on ice for 30 mins-1 hr.

10 **[00811]** Briefly, antibody cocktail mixtures were added to resuscitated PBMC that

were seeded at 5×10^5 – 1×10^6 cells/well upon prior Fc receptor blockade and live/dead staining (Aqua Live/Dead, Life Technologies). Antibody cocktails were incubated with PBMC for 30mins – 1hr on ice. PBMC were then washed and data was acquired by FACS using a FACS Canto II. Data was analysed using FlowJo and Prism software. Immune

15 subsets that were analysed include CD56 dim NK cells, CD56 bright NK cells, CD4+ T cells, CD8+ T cells, non-conventional T cells (e.g. NKT cells and $\gamma\delta$ T cells), B cells, and monocytes.

[00812] FACS analysis of cynomolgus PVRIG engineered over-expressing cells: The

following cell lines were used to assess the cross-reactivity of anti-human PVRIG antibodies with cynomolgus PVRIG (cPVRIG): expi parental and expi cPVRIG over-expressing cells.

20 These cells were cultured in DMEM + 10% fetal calf serum + glutamax. expi cPVRIG transient over-expressing cells were generated by electroporating cPVRIG DNA into parental expi cells using the Neon transfection system. For FACS analysis, expi cPVRIG cells were used between 1-3 days post transfection. Parental expi cells were harvested from log growth

25 phase. 50,000-100,000 cells of per well of each type were seeded in 96 well plates. Anti-human PVRIG antibodies (mIgG1 or mIgG2a) and their respective controls were added in single point dilutions (5ug/ml), or as an 8 point titration series starting at 10ug/ml on ice for 30 mins-1 hr. The titration series were conducted as either 1:3 or 1:3.3 fold serial dilutions.

30 Data was acquired using a FACS Canto II or IntelliCyte and analyzed using FlowJo and Prism software.

[00813] FACS analysis of naïve primary cynomolgus monkey leukocytes: Primary

cynomolgus monkey (cyno) leukocytes were obtained from fresh blood which was drawn no

longer than 24 hours prior to expression analysis. Blood was sourced from Bioreclamation. To assess protein expression of PVRIG on cyno PBMC, antibody cocktails towards major immune subsets were designed that included human anti-PVRIG antibodies. Anti-human PVRIG antibodies (mIgG1 or mIgG2a) and their respective controls were added in single point dilutions (5ug/ml), or as an 8 point titration series starting at 10ug/ml on ice for 30 mins-1 hr.

[00814] Briefly, antibody cocktail mixtures were added to PBMC that were seeded at $5 \times 10^5 - 1 \times 10^6$ cells/well upon prior Fc receptor blockade and live/dead staining. Antibody cocktails were incubated with PBMC for 30mins – 1hr on ice. PBMC were then washed and data was acquired by FACS using a FACS Canto II. Data was analysed using Prism software. Immune subsets that were analysed include CD16+ lymphocytes, CD14+/CD56+ monocytes/myeloid cells, and CD3+ T cells.

[00815] Cellular-based competition assays: The ability of PVRIG antibodies to inhibit the interaction of PVRIG with its ligand PVRL2 was assessed in a cellular competition assay. In this assay, the ligand PVRL2 is endogenously expressed on un-manipulated HEK cells and soluble Fc-tagged PVRIG (manufactured on demand by Genscript) is added. In this case, the ability of PVRIG antibodies to block soluble PVRIG binding to HEK cells were assessed through the concomitant addition of 33nM of soluble PVRIG protein and PVRIG antibodies (0.066-66 nM) to 100,000 HEK cells and incubated for 1 hour on ice. The extent of PVRIG Fc binding was detected by addition of anti-human Fc Alexa 647 (Jackson Laboratories) for 20-30 minutes on ice. Cells were washed twice in PBS for acquisition using a FACS Canto II. Data was analyzed using FlowJo (Treestar), Excel (Microsoft) and Prism (GraphPad).

[00816] Results

[00817] Hybridoma PVRIG antibodies recognize PVRIG on overexpressing cells: To screen for antibodies that were specific for PVRIG, we assessed the ability of antibodies that were generated from two hybridoma campaigns to bind HEK cell lines that were engineered to overexpress human PVRIG. The majority of antibodies from these campaigns bound to the HEK hPVRIG cells, albeit with varying affinity. Furthermore, the majority of these antibodies also showed low background binding to HEK parental cell lines indicating high specificity towards PVRIG. Figure 77 shows one example of the specificity of the PVRIG antibodies. A summary of all binding characteristics of the antibodies towards HEK hPVRIG

cells relative to control that were generated in the hybridoma campaigns are displayed in Figure 79.

[00818] PVRIG antibodies recognize PVRIG protein on naïve NK and T cells: The populations which displayed the highest level of PVRIG on naïve PBMC subsets were NK and CD8 T cells, and the absolute level of expression between these two cell subsets was similar (gMFI). CD4 T cells showed lower levels of PVRIG, while B cells and monocytes had very low/no detectable expression. A summary of expression on naïve NK cells and CD8 T cells as detected by the antibodies is shown in Figure 91. Other minor subsets also displayed PVRIG expression and included non-conventional T cells such as NKT cells and $\gamma\delta$ T cells. The expression pattern on PBMC subsets was very similar across all donors sourced and analyzed.

[00819] PVRIG is detected on Jurkat cell lines by hybridoma-derived PVRIG antibodies: In addition to screening PBMC for PVRIG protein expression, we wanted to understand whether it was also expressed on cancer cell lines. We chose to screen our antibodies on Jurkat cells given their high expression of PVRIG RNA. We also chose HepG2 as a negative control cell line to further validate the specificity of our antibodies. Most of the hybridoma-derived antibodies did detect PVRIG protein expression on Jurkat cells (Figure 79), but not the HepG2 cells (data not shown). An example of PVRIG detection on Jurkat is shown in Figure 78 with a representative antibody, CHA.7.518.

[00820] Cellular-based biochemical assays: Upon screening our 29 hybridoma antibodies in the cellular biochemical assays, we found that there were 20 clear blockers and 9 non-blockers of the PVRIG-PVRL2 interaction. All of the blocking antibodies were able to inhibit the interaction of PVRIG Fc with HEK cells by at least 50%, with most of these antibodies completely abolishing PVRIG Fc binding. The IC_{50} values associated with those antibodies that did show blocking capacity are reported in Figure 92. The majority of IC_{50} values were between 20-60nM.

[00821] Summary and Conclusions

[00822] Using a hybridoma platform, we have been able to successfully generate monoclonal antibodies towards the human PVRIG antigen. Using engineered over-expressing cells as well as a suite of cancer cell lines, we showed that our antibodies are highly specific to the PVRIG antigen, and are able to detect protein expression which correlated with RNA expression. Upon analysis of human PBMC subsets, we showed that the PVRIG protein is

most highly expressed on NK and T cells, with low/negative expression on B cells and myeloid cells. We also showed that a proportion of these antibodies are cross-reactive with the cynomolgus monkey (cyno) PVRIG antigen through assessing their binding to over-expressing cells. Furthermore, the expression pattern on cyno PBMC is similar to human PBMC. Lastly, we were able to show through a FACS-based competition assay, that a proportion of our hybridoma antibodies are able to inhibit the interaction of PVRIG with its ligand, PVRL2. The antibodies which showed the best characteristics regarding all the aforementioned data were CHA-7-518, CHA-7-524, CHA-7-530, and CHA-7-538.

[00823]

10 **EXAMPLE 22. Effect of CHA anti-PVRIG antibodies in the MLR assay**

[00824] An assay used to profile the functional effect of anti-human PVRIG antibodies on allo-antigen responses is proliferation of Human CD8⁺ T Cells in a Mixed Lymphocyte Reaction (MLR) assay. As is known in the art, MLR is an ex vivo cellular immune assay that provides an in vitro correlation of T cell function.

15 [00825] Anti-PVRIG antibodies are expected to enhance proliferation of human CD4 and CD8 T cells in response to cells from an MHC-mismatched donor. Human T cells are enriched from whole blood of one donor (e.g. donor A) by using Human T cell RosetteSep RTM (StemCell Technologies) as per manufacturer's instructions. After separation, cells are fluorescently labeled with CFSE dye (Molecular Probes). To serve as allogeneic antigen presenting cells (APCs), mononuclear cells are first isolated from whole blood from a MHC-mismatched donor (e.g. donor B) and then depleted of CD3⁺ T cells. APCs are then irradiated with 2500 rads in a cesium irradiator.

25 [00826] In general, an MLR assay is done as follows. Human T cells and allogeneic 150,000 APCs are co-cultured in a 96-well flat-bottom plate with 150,000 CD8⁺ T cells and APCs for 5 days with anti-PVRIG antibodies at different concentrations. On day 5, cells are harvested, washed and stained with anti-CD8-biotin followed by streptavidin-PerCp. Samples are run by FACS to assess the degree of proliferation as depicted by CFSE dilution. Functional blocking anti-PVRIG antibodies are expected to enhance T cells proliferation and cytokine secretion in response to cells from a MHC-mismatched donor.

30 [00827] An MLR assay was used to characterize the biochemical effect of the CHA antibodies of the invention on resting and activated human T cells, and to characterize the capacity of hybridoma-derived antibodies to modulate T cell proliferation in an MLR setting

[00828] Protocols

[00829] Mixed Lymphocyte Reaction (MLR): A mixed lymphocyte reaction was established by co-culturing dendritic cells (DCs) and T cells derived from distinct donors in an allogeneic setting. DCs were generated by culturing purified monocytes with 100 ng/ml GM-CSF (R&D systems) and 100ng/ml IL-4 (R&D systems) for 7 days. After 7 days, purified CFSE-labelled CD3 T cells were combined with DCs at a 10:1 ratio and were cultured in X vivo-20 serum free media (Lonza) for 5 days. In some conditions, unconjugated anti-PVRIG antibodies or isotype control antibodies were added to the plates at 10ug/ml. Three MLR assay permutations were set up, where DCs from one donor were co-cultured with CD3 T cells from 3 separate allogeneic donors. All blood products were sourced from Stanford Blood Bank.

[00830] Expression and functional analysis: After the 5 day MLR culture, the level and extent of T cell activation and proliferation was assessed by CFSE dilution and expression of activation markers such as CD25 and PD-1. In-house anti-PVRIG antibodies from both phage and hybridoma campaigns were used to assess the expression of PVRIG. Expression of the PVRIG ligand, PVRL2, was also assessed in a kinetic fashion on DC. All data was acquired using flow cytometry and data analysis was performed using FlowJo (Treestar) and Prism (Graphpad) software.

[00831] FACS-based epitope analysis: As we tested an array of antibodies in the MLR, we were interested in determining whether these antibodies could be epitope ‘binned’ based on FACS-based binding, and whether this ‘binning’ would correlate to changes in T cell activation and proliferation in the assay. To do this, T cells harvested from the assay were pre-incubated with unconjugated PVRIG antibodies, and then counter-stained with a conjugated PVRIG antibody of a different clone. The extent to which the conjugated PVRIG antibody gave a signal on T cells indicated the extent to which this antibody had to compete for PVRIG binding on T cells with the unconjugated antibody. A negative or low signal would indicate that there is high competition, indicating the two antibodies are in the same epitope ‘bin’. A high signal would indicate low or no competition and thus the antibodies would be considered to be in different ‘bins’.

[00832] Results

[00833] Expression of PVRL2 on monocyte-derived DC: To determine whether PVRL2 would be expressed on DC for the MLR assay, DC were generated from monocytes,

and PVRL2 expression was assessed in a kinetic fashion at daily intervals after addition of GM-CSF and IL-4. As indicated in Figure 72, PVRL2 expression increased from Day 0 until Day 5 where expression peaked. At Day 6, expression decreased slightly compared to Day 5. At Day 7, expression was similar to Day 6 indicating stabilization of PVRL2 expression at these time points. Thus, DC expressed PVRL2 at the appropriate time point for use in the MLR assay.

[00834] Expression of PVRIG on T cells after MLR culture: Many T cell receptors than modulate function in the MLR are expressed on proliferating T cells. Thus, we wanted to determine whether PVRIG is also expressed. We analysed proliferating T cells at Day 5 post MLR co-culture initiation and were characterized by their dilution of CFSE (i.e. CFSE low). As shown in Figure 73 and Figure 74, relative to isotype control (mIgG1), PVRIG was expressed on CFSE low cells as determined by multiple PVRIG antibodies on both CD4 and CD8 T cells across three donors analysed. FACS plots are shown in Figure 73 to indicate PVRIG on CFSE low cells, and bar graphs in Figure 74 indicate the level of expression of PVRIG relative to mIgG1.

[00835] PVRIG antibodies enhance T cell proliferation: Having shown that PVRIG expression is expressed on proliferating T cells in the MLR, we wanted to determine whether treatment with PVRIG antibodies could affect levels of T cell proliferation. As shown in Figure 4, addition of PVRIG antibodies into the MLR assay was able to increase the percentage of CFSE low cells across all the hybridoma antibodies tested compared to control. This was observed across all donors analysed.

[00836] PVRIG antibodies bind to multiple epitopes on PVRIG: To compare the PVRIG antibodies for their ability to bind different epitopes on PVRIG, we performed a competition experiment where T cells from the MLR were cultured with unlabeled anti-PVRIG antibodies derived from our hybridoma campaigns for 5 days. T cells were then harvested at day 5 and counter-stained with a conjugated anti-PVRIG antibody that was derived from our phage campaign (CPA.7.021). As shown in Figure 76, complete or near complete reduction of CPA.7.021 binding was observed in conditions that contained CHA.7.516-M1, CHA.7.518-M1, CHA.7.524-M1, CHA.7.530-M1, and CHA.7.538-M1 when compared to background fluorescence levels, suggesting that these antibodies may overlap in epitope recognition. Partial reduction in CPA.7.021 binding was observed with CHA.7.537-M1, CHA.7.528-M1, and CHA.7.548-M1, suggesting partial overlap in epitope

recognition. No reduction in CPA.7.021 binding was observed in cells pre-cultured with CHA.7.543-M1 suggesting an absence of epitope recognition. Collectively, this data indicates that the PVRIG antibodies from our campaigns, when assessed relative to CPA.7.021, could recognize at least 3 different epitopes on PVRIG.

5 [00837] Conclusions We characterized our PVRIG antibodies for their ability to bind to proliferating and resting T cells, as well as their functional activity in a MLR. Binding of multiple PVRIG antibodies was detected on proliferating T cells and was higher on proliferating T cells as compared to resting, especially the CD8+ subset. This data demonstrates that PVRIG expression is increased upon T cell activation. Furthermore, 10 several PVRIG antibodies increased T cell proliferation as compared to mIgG1 isotype indicating that they can also modulate T cell function. As above, these antibodies all have ability to block PVRIG with its ligand, PVRL2. Based on this, we conclude that by blocking the PVRIG-PVRL2 interaction, these antibodies lead to an increase in T cell activation and proliferation, which is a hallmark indication of a desired effect for an immune checkpoint 15 inhibitor that would be used to treat cancer. Lastly, we performed competition experiments comparing the binding of multiple hybridoma-derived PVRIG antibodies to activated T cells, relative to a phage-derived antibody. From this series of experiments, we provide evidence for epitope diversity of our phage and hybridoma-derived antibodies.

20 **EXAMPLE 23. EFFECT OF ANTI-PVRIG ANTIBODIES ON T CELL ACTIVATION UPON COMBINATION WITH IMMUNE CHECKPOINT BLOCKADE**

[00838] The combination of PVRIG blockade with blocking Abs of a known immune checkpoint (e.g. PD1, PDL-1 or TIGIT), is expected to further enhance the stimulatory effect on T cell activation in the assays depicted above.

EXAMPLE 24. FUNCTIONAL ANALYSIS OF PVRIG ANTIBODIES

25 [00839] The human PVRIG antibodies of the invention were characterized for the ability to inhibit the interaction of PVRIG with its ligand PVRL2, and their ability to modulate effector lymphocyte function in primary cell-based assays.

[00840] Protocols

[00841] Cellular-based biochemical assays

30 [00842] The ability of PVRIG antibodies to inhibit the interaction of PVRIG with its ligand PVRL2 was assessed in a cellular biochemical assay format in two orientations.

[00843] In the first orientation, the ligand PVRL2 is endogenously expressed on un-manipulated HEK cells and soluble biotinylated Fc-tagged PVRIG (manufactured on demand by Genscript) is added. In this case, the ability of PVRIG antibodies to block soluble PVRIG binding to HEK cells were assessed through two permutations. In the first permutation, various concentrations of PVRIG antibodies (range 0.066-66nM) were pre-incubated with 33nM of soluble PVRIG in phosphate buffered saline (PBS, Gibco) for 30 minutes on ice. This complex was subsequently added to 100,000 HEK cells in and incubated for a further 1 hour on ice. After 1 hour, HEK cells were washed twice in PBS and the extent of soluble PVRIG bound to HEK cells was detected by addition of streptavidin conjugated to Alexa 647 (Jackson Laboratories) for 30 minutes on ice. HEK cells were washed twice in PBS, and resuspended in 100ul of PBS for acquisition on the FACS Canto II (BD Biosciences). Data was analysed using FlowJo (Treestar) and Prism (Graphpad) software. In the second permutation, 33nM of soluble PVRIG protein and PVRIG antibodies (0.066-66 nM) were added concomitantly to 100,000 HEK cells and incubated for 1 hour on ice. Subsequent steps to analysis for this permutation are equivalent to the first permutation.

[00844] In the second orientation, HEK cells were engineered to over-express PVRIG and soluble biotinylated Fc-tagged PVRL2 (CD Biosciences) was added. In this case, various concentrations of PVRIG antibodies (range 0-200nM) with 160nM soluble PVRL2 were added concomitantly to 100,000 HEK hPVRIG or parental HEK cells, and incubated in PBS + 1% BSA + 0.1% sodium azide (FACS buffer) for 1hr on ice. Soluble PVRL2 binding was detected by addition of streptavidin Alexa 647 in FACS buffer for 30 minutes on ice. Cells were washed twice in FACS buffer, and re-suspended in 50ul of PBS for acquisition on the Intellicyt HTFC (Intellicyt). Data was analyzed using FlowJo (Treestar), Excel (Microsoft) and Prism (GraphPad).

[00845] Primary NK cell assay

[00846] The PBMC subset with the most robust expression profile for PVRIG was on NK cells. As such, we designed an NK cell-based co-culture assay with PVRL2-expressing tumor cells to determine whether our antibodies could modulate NK cell-mediated cytotoxicity towards these targets. The targets we chose were the acute B cell lymphocytic leukemia cell line, Reh (ATCC cell bank), and the acute myeloid leukemia cell line, MOLM-13 (DSMZ cell bank). Reh and MOLM-13 cells were grown in RPMI media (Gibco) + 20% fetal calf serum (Gibco), glutamax (Gibco), penicillin/streptomycin (Gibco), non-essential

amino acids (Gibco), sodium pyruvate (Gibco), HEPES (Gibco), and beta-mercaptoethanol (Gibco).

[00847] Two days prior to the co-culture assay, primary NK cells were isolated using the human NK cell isolation kit (Miltenyi Biotec) and cultured in RPMI media + 20% fetal calf serum, glutamax, penicillin/streptomycin, non-essential amino acids, sodium pyruvate, HEPES, beta-mercaptoethanol, and 250U/ml IL-2 (R&D systems). On the day of the assay NK cells were harvested, enumerated and pre-incubated with PVRIG antibodies for 15-30 minutes at room temperature. During this incubation, target cells were harvested from culture, labelled with Calcein AM (Life Technologies) for 30 minutes at 37°C, washed in media, and enumerated for the assay. NK cell-mediated cytotoxicity assays were set up where a constant number of target cells (50,000) were co-cultured with increasing concentrations of NK cells pre-incubated with 5 ug/ml of PVRIG antibodies (thus altering the NK cell to target ratio). Alternatively, a fixed NK cell to target ratio was used in the assay, but NK cells were pre-incubated with altering concentrations of PVRIG antibody (range 3.9 ng/ml - 5 ug/ml) in a dose titration. Upon addition of the NK cells and targets, plates were pulse spun at 1,400 rpm for 1 minute and placed at 37°C in a 5% CO₂ atmosphere for 4 hours. After 4 hours, plates were spun at 1,400 rpm for 4 minutes, and 80ul of supernatant was harvested to quantitate the release of Calcein AM from the target cells. The quantity of Calcein AM released from targets was assessed by a Spectramax Gemini XS fluorometer (Molecular Devices). As controls for Calcein AM release, total and spontaneous release was assessed by exposing target cells to 70% ethanol or media only for the duration of the assay. Levels of killing (as a percentage) by NK cells were calculated using the following formula:

$$[\text{00848}] \quad (\text{Sample release} - \text{spontaneous release}) / (\text{total release} - \text{spontaneous release}) * 100$$

[00849] In addition to PVRIG antibodies, in some cases, other antibodies towards NK cell receptors such as TIGIT (Genentech, clone 10A7, Patent number: WO2009126688 A2) and DNAM-1 (Biolegend, clone 11A8) were also added as comparators.

[00850] Results

[00851] Cellular-based biochemical assays: Upon screening a panel of our PVRIG antibodies in the cellular biochemical assays, we found that there was variable levels of inhibition across the antibodies tested, and the level of inhibition was dependent on the permutation and orientation of the assay (Figure 98). Four antibodies are specifically shown

in Figure 93 to illustrate these points. The orientation and permutation of the assay which gave the most robust inhibitory effect relative to control, was when soluble PVRIG pre-incubated with PVRIG antibodies was added to HEK cells (Figure 93a). In this permutation, CPA.7.021 showed the best absolute blocking capacity compared to the other three antibodies (CPA.7.002, CPA.7.005, and CPA.7.050). Despite the differences in level of blocking, all antibodies in this permutation showed similar IC_{50} values which were in the low nanomolar range, and the blocking capacity plateaued at higher concentrations.

[00852] When the absolute level of inhibition invoked by the four PVRIG antibodies was then measured when soluble PVRIG and PVRIG antibodies were concomitantly added to HEK cells, more variability of blocking in the assay was observed (Figure 93b). CPA.7.021 remained the best blocking antibody. However, CPA.7.002 and CPA.7.005 showed markedly less ability to inhibit soluble PVRIG binding to HEK cells relative to the control antibody. CPA.7.050 showed an intermediate level of blocking as compared to CPA.7.021, CPA.7.002, and CPA.7.005. This difference in absolute level of inhibition also corresponded to differences in the IC_{50} values of each antibody. CPA.7.021 and CPA.7.050 again showed low nanomolar IC_{50} values, although they were both higher than in the first permutation of the assay. In contrast, the IC_{50} values of CPA.7.002 and CPA.7.005 increased substantially, CPA.7.002 by approximately 20-fold, and CPA.7.005 by approximately 30-fold. This data indicates that how the antibody has to compete for PVRIG binding with its cognate ligand, will indicate the potency with which the antibody can block this interaction.

[00853] When the orientation of the biochemical assay was reversed (i.e. PVRL2Fc was assessed to bind to HEK hPVRIG cells), the ability of the four PVRIG antibodies to block PVRL2 Fc interaction was variable (Figure 93c). Consistent with the biochemical assays which used HEK cells as targets (Figure 93a-b), CPA.7.021 and CPA.7.050 inhibited PVRL2 Fc binding to HEK hPVRIG cells, and their ability to block the binding was similar. Surprisingly however, we saw enhancement of PVRL2 Fc binding in the presence of CPA.7.002 and CPA.7.005 antibodies which we did not observe when HEK cells were used as targets.

[00854] NK cell cytotoxicity assay with Reh cells: The first target we investigated in the NK cell cytotoxicity assay was the Reh line. Reh was initially selected as it showed robust levels of PVRL2 by flow cytometry, but a low frequency of other activating ligands such as NKG2D ligands, and low expression of PVR (Figure 94). Traditional NK cell targets

were not used, such as K562, due to their expression of a high frequency of NKG2D ligands, and high expression of PVR, which may mask a functional effect of the PVRIG antibodies. Importantly, Reh cells did not express any NK cell receptors known to interact with PVRL2 and PVR such as TIGIT, DNAM-1, and PVRIG.

5 [00855] Upon screening our panel of PVRIG antibodies in this assay, we found four antibodies that were able to modulate NK cell-mediated cytotoxicity (Figure 99). These four antibodies were those that were discussed in the biochemical assay results section- CPA.7.002, CPA.7.005, CPA.7.021, and CPA.7.050. In all cases, addition of these antibodies enhanced NK cell-mediated cytotoxicity against Reh cells (Figure 95a-c). Addition of
10 CPA.7.002 and CPA.7.005 enhanced cytotoxicity most robustly (Figure 95a-b), followed by CPA.7.021 and CPA.7.050 which showed similar levels of enhancement (Figure 95c). Figure 95d shows a concentration-dependent analysis of enhancement of NK cell-mediated cytotoxicity by CPA.7.002 and CPA.7.021. Blocking antibodies towards receptors that have been reported to also bind PVRL2 such as TIGIT and DNAM-1 were added to the assay with
15 Reh cells as comparators. As shown in Figure 95e-f, the addition of TIGIT and DNAM-1 antibodies did not show functional effects in this assay.

[00856] NK cell assay with MOLM-13 cells: To assess whether PVRIG antibodies were able to modulate NK cell-mediated cytotoxicity against a second target, MOLM-13 cells were utilized. MOLM-13 also express PVRL2 analogous to Reh cells, but also have
20 robust expression of PVR (Figure 94). Like the Reh cells, MOLM-13 did not express any NK cell receptors. Utilization of this cell line, in addition to Reh cells, would indicate whether PVRIG antibodies can modulate NK cell-mediated cytotoxicity in the context of different receptor-ligand interactions, particularly when PVR is expressed.

[00857] Upon screening our PVRIG antibodies in this assay, we found that the
25 functional effect of CPA.7.021 was diminished and did not show significant enhancement of NK cell-mediated cytotoxicity above control levels (Figure 97a). In contrast, CPA.7.002 and CPA.7.005 were able to enhance NK cell-mediated cytotoxicity in this assay (Figure 97a). Using a comparator antibody, blockade of TIGIT did not show functional effects in this assay when compared to control (Figure 97b).

30 [00858] Summary and Conclusions

[00859] Using our antibody phage platform, we generated a panel of antibodies against the human PVRIG antigen that showed an ability to block the interaction of PVRIG with its

ligand PVRL2, and enhance NK cell-mediated cytotoxicity against two hematological cell lines. The ability of the PVRIG antibodies to inhibit PVRIG and PVRL2 interaction was influenced by the orientation of the assay as well as pre-incubation steps, representative of potential antibody dynamics with PVRIG in physiological settings such as cancer. Four
5 antibodies showed an ability to enhance NK cell-mediated cytotoxicity against the Reh cell line, but only two antibodies showed an ability to enhance cytotoxicity against MOLM-13 cells. This difference may be attributed to the alternate receptor-ligand interactions involved in NK cell-mediated recognition of each cell line, and/or differential properties of the antibodies and their potency in modulating the function of PVRIG.

10 **EXAMPLE 25. EFFECT OF ANTI-PVRIG ANTIBODIES ON GD T CELL
ACTIVATION USING PVRL2 ECTOPIC OR NATURALLY EXPRESSING CELLS**

[00860] A cell based assay is used to test the effect of anti-PVRIG antibodies on gamma delta T cell activation, proliferation and cytokine secretion. Purified human gamma delta T cells are activated with HMBPP or IPP and co-cultured with target cells (e.g. REH,
15 MOLM-13)_that naturally express PVRL2 or with target cells ectopically expressing PVRL2 or empty vector (e.g. CHO, Raji, 721.221). Gamma delta T cell function is assessed by examining cytokine production (e.g. IFN- γ , IL-17)_in cultured supernatants or cytotoxic activity on the target cells. PVLR2 expression is expected to have a basal stimulatory effect on gamma delta T cell activation, mediated through endogenous DNAM1 - a known
20 costimulatory counterpart receptor of PVRL2 on gamma delta T cells. In the presence of antagonistic anti-PVRIG Abs, cytokine production or cytotoxic activity is expected to be further enhanced, due to their blocking of the inhibitory function of endogenous PVRIG on gamma delta T cell activation. Accordingly, agonistic anti-PVRIG Abs are expected to show inhibition of gamma delta T cell activation.

25 **Example 26: Effect of Proteins On Human T Cells Activated Using Anti-CD3 and Anti-
CD28 in the Presence of Autologous PBMCs**

[00861] MATERIALS AND METHODS

[00862] In these experiments the effects of PVRIG on human T cells which were activated using anti-CD3 and anti-CD28 in the presence of autologous PBMCs is evaluated.
30 Conversely, this assay can also be used to assay the effects of anti-PVRIG antibodies on T cell activation.

[00863] PVRIG hECD-hIg fusion protein (Figure 92BA), composed of the ECD of human PVRIG fused to the Fc of human IgG1 bearing C220, C226 and C229 to S mutations at the hinge, was produced at GenScript (China) by transient transfection in CHO-3E7 cells which were cultured for 6 days, followed by protein A purification of cell harvest.

5 The final product was formulated in PBS pH 7.2. Expression vector used was Mammalian Expression Vector pTT5, in which PVRIG gene is driven by CMV promoter.

[00864] CD4⁺ Human T cell Isolation Kit II is purchased from Miltenyi (Cat. #130-094-131). hIgG1 control (Synagis®) is obtained from Medimmune Inc. Anti-human CD3 Ab (OKT3, Cat# 16-0037) and anti-human CD28 Ab (clone CD28. 2; Cat# 16-0289) are

10 purchased from eBioscience. Dynabeads M-450 Epoxy (Cat. # 140. 11) are purchased from Invitrogen. Buffy coats of human blood are obtained from LifeSource. Ficoll-Paque Plus (Cat. #17-1440-02), is purchased from GE HealthCare.

[00865] Isolation of PBMCs from buffy coats using Ficoll separation: Total PBMCs are suspended in Ex-Vivo 20 medium, and irradiated at 3000rad. Naïve CD4⁺ T cells are

15 isolated from buffy coats of three healthy human donors' blood using CD4⁺ Human T cell Isolation Kit II (Miltenyi) according to manufacturer's instructions and co-cultured with irradiated autologous PBMCs at a ratio of 1:1 (1. 5x10⁵ T cells with 1. 5x10⁵ irradiated PBMCs per well). The cultures are activated with anti-CD3 (0. 5ug/ml) and anti-CD28 (0. 5 ug/ml) antibodies. Either an anti-PVRIG antibody or a PVRIG ECD protein are added to the

20 culture at the indicated concentrations. After 24 hr in culture, cells are pulsed with H3-thymidine. Cells are harvested after 72 hours in culture.

[00866] For the ECD experiment, the results are expected to cause a dose dependent inhibition of T cell proliferation and/or activation, supporting the therapeutic potential of immunoinhibitory PVRIG based therapeutic agents (e.g. PVRIG polypeptides or PVRIG

25 fusion proteins according to at least some embodiments of the invention) for treating T cell-driven autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, psoriasis and inflammatory bowel disease, as well as for treating other immune related diseases and/or for reducing the undesirable immune activation that follows gene or cell therapy. Essentially, immunoinhibitory PVRIG proteins that agonize PVRIG should prevent or reduce the

30 activation of T cells and the production of proinflammatory cytokines involved in the disease pathology of such conditions.

[00867] In addition, these results are also expected to support a therapeutic potential of immunostimulatory anti-PVRIG antibodies that reduce the inhibitory activity of PVRIG for treating conditions which will benefit from enhanced immune responses such as immunotherapy of cancer, infectious diseases, particularly chronic infections and sepsis.

- 5 Essentially, immunostimulatory anti-PVRIG antibodies will promote the activation of T cells and elicit the production of proinflammatory cytokines thereby promoting the depletion of cancerous or infected cells or infectious agents.

Example 27: Inhibition of T cell Activation Assay.

- [00868] In these experiments the effects of PVRIG ECDs or anti-PVRIG antibodies on
10 T cell activation in a bead assay.

[00869] MATERIALS & METHODS

- [00870] Isolation of human T Cells: Buffy coats are obtained from Stanford Blood Bank from healthy human donors. CD3⁺ T cells are isolated from buffy coats using RosetteSep kit (StemCell Technologies) following manufacturer's instructions. Cells are
15 analyzed with anti-CD45 and anti-CD3 by flow cytometry to evaluate the % of CD3⁺ cells obtained. Viability is evaluated after thawing prior to the assay.

- [00871] Bead Coating and QC: Tosyl activated beads (Invitrogen, Cat# 14013) at $500 \times 10^6/\text{ml}$ are coated with anti-CD3 mAb and either PVRIG ECD proteins or anti-PVRIG antibodies in a two-step protocol: with 50ug/ml human anti-CD3 clone UTCH1 (R&D
20 systems, Cat# mab 100) in sodium phosphate buffer at 37 ° C. overnight, followed with 0-320ug/ml of either PVRIG ECD proteins or anti-PVRIG antibodies for another overnight incubation at 37° C.

- [00872] The amount of PVRIG protein (either ECD or antibody) bound to the beads is analyzed.

- 25 [00873] Bead assay setup: 100k human CD3⁺ T cells are cultured with 100k or 200k beads coated with various concentrations of the PVRIG protein for 5 days in complete IMDM (Gibco, Cat #12440-053) supplemented with 2% AB human serum (Gibco, Cat# 34005-100), Glutmax (Gibco, Cat #35050-061), sodium pyruvate (Gibco, Cat #11360-070), MEM Non-Essential Amino Acids Solution (Gibco, Cat #11140-050), and 2-mercaptoethanol (Gibco,
30 Cat #21985). At the end of 5 day culture, cells are stained with anti-CD25, anti-CD4, anti-CD8, and fixable live dead dye to determine CD25 expression levels on each subset of cells.

Supernatants are collected and assayed for IFN γ secretion by ELISA (Human IFN γ duoset, R&D systems, DY285).

[00874] In these experiments human CD3 T cells co-cultured with beads coated with various concentration of PVRIG-protein are analyzed for their level of expression of CD25.

- 5 Both CD4⁺ and CD8⁺ cells are anticipated to show dose dependent inhibition by the PVRIG-ECD- fusion protein, or, conversely, both CD4⁺ and CD8⁺ cells are anticipated to show dose dependent activation by the PVRIG-antibody.

Example 29: Epitope mapping of anti-human PVRIG antibodies based on cynomolgus cross-reactivity

- 10 [00875] Rationale and Objectives

[00876] The objective of this study is to identify the epitopes on the PVRIG protein that determine cross-reactivity of anti-human PVRIG antibodies against the cynomolgus monkey (cyno) orthologue. Many of the lead antibodies against human PVRIG target show varied degrees of cyno cross-reactivity despite the fact that many of these antibodies belong
15 to the same epitope bin. To shed light on the molecular basis of human/cyno cross-reactivity (or lack thereof), several cyno-to-human mutations of the PVRIG recombinant proteins were designed, expressed and purified, and tested for binding to a panel of anti-human PVRIG antibodies in ELISA.

[00877] Methods

- 20 [00878] Design of cyno-to-human PVRIG variants: Sequence alignment of human and PVRIG extracellular domains (ECDs) shows 90% sequence identity and 93% sequence homology between human and cyno orthologs (Figure 100). Based on the nature of the mutations (conserved vs non-conserved) and the secondary structure prediction (coil vs extended) of the mutation region, three site-directed mutants of the cyno PVRIG were
25 designed to probe the cyno-cross reactivity focused epitope mapping. These mutants include H61R, P67S, and L95R/T97I cyno PVRIG. Wild type cyno and human PVRIG were also generated.

- [00879] Expression and purification of cyno, human, and hybrid PVRIG variants: All the PVRIG variants were expressed as ECD fusions with a C-terminal 6XHis tag in
30 mammalian cells. The proteins were purified by affinity purification, ion-exchange chromatography, and size-exclusion chromatography. The purified proteins were buffer-exchanged into PBS buffer (pH 7.4) and stored at 4°C.

[00880] ELISA to determine PVRIG-antibody interaction: The functional ELISA was performed as follows: cyno, human, and cyno/human hybrid PVRIG (His-tagged) recombinant proteins were adsorbed on an IA plate overnight at 4°C. Coated plate wells were rinsed twice with PBS and incubated with 300 µL blocking buffer (5% skim milk powder in PBS pH 7.4) at room temperature (RT) for 1 hr. Blocking buffer was removed and plates were rinsed twice more with PBS. Plate-bound PVRIG variants were incubated with anti-human PVRIG mAbs (human IgG1 isotype) in solution (linear range of 0.1 µg/mL to 8 µg/mL in a 50 µL/well volume) at RT for 1 hr. Plates were washed three times with PBS-T (PBS 7.4, 0.05% Tween20), then three times with PBS and 50µL/well of a HRP-conjugated secondary antibody was added (Human IgG Fc domain specific, Jackson ImmunoResearch). This was incubated at RT for 1hr and plates were washed again. ELISA signals were developed in all wells by adding 50 µL of Sureblue TMB substrate (KPL Inc) and incubating for 5-20 mins. The HRP reaction was stopped by adding 50 µL 2N H2SO4 (VWR) and absorbance signals at 450 nm were read on a SpectraMax (Molecular Devices) or EnVision (PerkinElmer) spectrophotometer. The data were exported to Excel (Microsoft) and plotted in GraphPad Prism (GraphPad Software, Inc.).

[00881] Results

[00882] S67, R95, and I97 residues as determinants of cyno cross-reactivity: The binding data shown in Figure 101 clearly shows that the S67, R95, and I97 residues affect the cyno cross-reactivity of various antibodies. While the P67S cyno-to-human mutation negatively impacts the binding of CPA.7.002 and CPA.7.041, the L95R/T97I cyno-to-human mutation significantly improves the binding of CPA.7.002, CPA.7.021, CPA.7.028, and CPA.7.041. On the other hand, H61R cyno-to-human mutation does not affect the binding of any of the antibodies tested.

[00883] Relative binding to cyno-to-human variants suggests three epitope groups: The relative binding of the antibodies to cyno, human and hybrid PVRIG variants suggests 3 distinct epitope groups: Group 1 binds to R95/I97 residues (CPA.7.021 and CPA.7.028). Group 2 binds to S67 and R95/I97 residues (CPA.7.002 and CPA.7.041). Group 3 does not bind to S67 or R95/I97 residues (CPA.7.024 and CPA.7.050). The epitope groups show strong correlation to the degree of cyno cross-reactivity of these antibodies (Figure 102).

[00884] Summary and Conclusions

[00885] The restricted epitope mapping based on cyno-to-human variations in the PVRIG ECD identified S67, R95, and I97 residues as determinants of cyno cross-reactivity of anti-human PVRIG antibodies. The complete restoration of binding to L95R/T97I cyno PVRIG for CPA.7.021 and CPA.7.028 antibodies and improved binding of CPA.7.002 to this mutant strongly suggests that R95 and I97 residues are critical human PVRIG epitopes for these antibodies. These findings also suggest a possible way to predict cross-reactivity to non-human primate PVRIG orthologs based on their primary amino acid sequence.

CLAIMS

1. A method of screening for inhibitors of the binding association of PVRIG polypeptide with PVLR2, said method comprising:
 - a) providing a surface comprising a first ligand protein comprising one of
5 PVRIG polypeptide or PVLR2 polypeptide;
 - b) contacting said surface with a candidate agent under physiological conditions, wherein if said candidate agent binds to said first ligand protein it forms a first binding complex,
 - c) contacting said surface with a second ligand protein comprising the other of
10 PVRIG polypeptide or PVLR2; and
 - d) determining whether said PVRIG polypeptide and said PVLR2 are bound as an indication of whether said candidate agent inhibits said binding association.
2. A method of screening for inhibitors of the binding association of PVRIG polypeptide
15 with PVLR2, said method comprising:
 - a) providing a cell comprising an exogenous recombinant nucleic acid encoding a human PVRIG polypeptide, wherein said cell expresses said human PVRIG polypeptide;
 - b) contacting said cell with a candidate agent and a labeled PVLR2 polypeptide;
 - 20 c) determining whether said PVRIG polypeptide binds to PVLR2 as an indication of whether said candidate agent inhibits the binding of PVRIG polypeptide with PVLR2.
3. A method of screening for inhibitors of the binding association of PVRIG polypeptide with PVLR2, said method comprising:
 - 25 a) providing a cell comprising an exogenous recombinant nucleic acid encoding a human PVLR2 polypeptide, wherein said cell expresses said human PVLR2 polypeptide;
 - b) contacting said cell with a candidate agent and a labeled PVRIG polypeptide;
 - c) determining whether said PVLR2 polypeptide binds to PVRIG as an indication of
30 whether said candidate agent inhibits the binding of PVRIG polypeptide with PVLR2.
4. A method of screening for inhibitors of the binding association of PVRIG polypeptide with PVLR2, said method comprising:

- a) providing a test solution comprising:
 - i) a PVRIG polypeptide comprising a first FRET label;
 - ii) a PVLR2 polypeptide comprising a second FRET label;
 - c) providing a candidate agent;
 - 5 d) detecting a FRET signal between said first and second label, wherein a difference in said FRET signal in the presence or absence of said candidate agent indicates that the candidate agent inhibits said binding association.
5. A method according to any of claims wherein a plurality of candidate agents are tested.
- 10 6. A method according to any of claims 1 to 5 wherein said candidate agent is a protein.
7. A method according to claim 6 wherein said protein is an anti-PVRIG antibody.
8. A method according to claim 6 wherein said protein comprises an extracellular domain (ECD) of PVRIG.
9. A method according to claim 8 wherein said protein is a fusion protein comprising said
- 15 ECD and a fusion partner.
10. A method according to claim 9 wherein said fusion partner is selected from the group consisting of a human IgG Fc domain and a human serum albumin (HSA).
11. A method according to any of claims 1 to 10 wherein said method further comprises:
- 20 a) contacting said candidate agent with a population of cytotoxic T cells (CTLs) under conditions wherein said CTLs would normally be activated; and
- b) determining the effect of said agent on said activation.
12. A method according to any of claims 1 to 10 wherein said method further comprises:
- 25 a) contacting said candidate agent with a population of cytotoxic T cells (CTLs); and b) determining the effect of said agent on IFN γ production.
13. A method according to any of claims 1 to 10 wherein said method further comprises:
- a) contacting said candidate agent with a population of $\gamma\delta$ T cells under conditions wherein said $\gamma\delta$ T cells would normally be activated; and
- b) determining the effect of said agent on said activation.
- 30 14. A method according to any of claims 1 to 10 wherein said method further comprises:
- a) contacting said candidate agent with a population of Th1 cells under conditions wherein said Th1 cells would normally be activated; and
- b) determining the effect of said agent on said activation.

15. A method according to any of claims 1 to 10 wherein said method further comprises:
a) contacting said candidate agent with a population of regulatory T cells
(Tregs) under conditions and determining the effect of said agent on Treg cell number
or activity.
16. A method according to claim 11 to 15 wherein said determination is done by
measuring the presence or absence of increased expression of a protein selected from
the group consisting of IFN γ , TNF α , GM-CSF, CD25, CD137, CD69, PD1, CD107A,
HLA-DR, IL-2, IL-6, IL-4, IL-5, IL-10 and IL-13, wherein increased expression is an
indication of activation.
17. A method of treating a disorder associated with the interaction of PVRIG and PVLR2
comprising administering to a patient a composition comprising a stimulator of PVRIG
to effect treatment.
18. A method according to claim 17 wherein said treatment is a decrease in immune
response.
19. A method according to claim 17 wherein said treatment is a decrease in activation of
 $\alpha\beta$ and/or $\gamma\delta$ T cells.
20. A method according to claim 17 wherein said treatment is a decrease in cytotoxic T
cell activity
21. A method according to claim 17 wherein said treatment is a decrease in NK and/or
NKT cell activity.
22. A method according to claim 17 wherein said treatment is a decrease of $\alpha\beta$ and/or $\gamma\delta$
T-cell activity.
23. A method according to claim 17 wherein said treatment is a decrease in pro-
inflammatory cytokine secretion
24. A method according to claim 17 wherein said treatment is a decrease in IL-2 secretion.
25. A method according to claim 17 wherein said treatment is a decrease in interferon- γ
production.
26. A method according to claim 17 wherein said treatment is a decrease in Th1 response.
27. A method according to claim 17 wherein said treatment is a increase in Th2 response.

28. A method according to claim 17 wherein said treatment is an increase in inhibition of T cell activity.
29. A method according to claim 17 wherein said treatment is an increase in inhibition of CTL activity.
- 5 30. A method according to claim 17 wherein said treatment is an increase in inhibition of NK cell activity.
31. A method according to claim 17 wherein said treatment is an increase in $\alpha\beta$ and/or $\gamma\delta$ T cell exhaustion.
32. A method according to claim 17 wherein said treatment is a decrease in $\alpha\beta$ and/or $\gamma\delta$ T
10 cell response.
33. A method according to claim 17 wherein said treatment is a decrease in activity of cytotoxic cells.
34. A method according to claim 17 wherein said treatment is a reduction in antigen-specific memory responses.
- 15 35. A method according to claim 17 wherein said treatment is an inhibition of apoptosis or lysis of cells.
36. A method according to claim 17 wherein said treatment is a decrease in cytotoxic or cytostatic effect on cells.
37. A method according to claim 17 wherein said treatment is a reduction in direct killing
20 of cells.
38. A method according to claim 17 wherein said treatment is a decreases in Th17 activity.
39. A method according to claim 17 wherein said treatment is an reduction of complement dependent cytotoxicity and/or antibody dependent cell-mediated cytotoxicity.
40. A method according to any of claims 17 to 38 wherein said stimulator is selected from
25 the group consisting of a protein and a nucleic acid.
41. A method according to claim 40 wherein said protein comprises an extracellular domain (ECD) of PVRIG.
42. A method according to claim 40 wherein said protein is a fusion protein comprising said ECD and a fusion partner.
- 30 43. A method according to claim 42 wherein said fusion partner is selected from the group consisting of a human IgG Fc domain and a human serum albumin (HSA).
44. A method according to any of claims 17 to 43 wherein said patient has an immune disorder.

45. A method according to 44 wherein said immune disorder is selected from the group consisting of an autoimmune disease, organ transplant rejection and inflammation.
46. A method according to claim 45 wherein said autoimmune disease is selected from the group consisting of rheumatoid arthritis, lupus, Inflammatory bowel disease, psoriasis, multiple sclerosis and diabetes type I.
47. A composition comprising an isolated PVRIG polypeptide consisting of a PVRIG polypeptide ECD domain having at least 95% identity to the ECD domain of an amino acid sequence selected from the group consisting of the sequences depicted in Figure 67, Figure 91 and Figures 92A to 92AT.
48. A composition according to claim 47 wherein said isolated PVRIG polypeptide has at least 99% identity to an amino acid sequence selected from the group consisting of the sequences depicted in Figure 67, Figure 91 and Figures 92A to 92AT.
49. A composition according to claim 47 wherein said isolated PVRIG polypeptide is selected from the group consisting of the sequences depicted in Figure 67, Figure 91 and Figures 92A to 92AT.
50. A composition comprising a PVRIG fusion polypeptide comprising:
a) an ECD from a PVRIG polypeptide; and
b) a covalently attached fusion partner moiety.
51. A composition according to claim 50 wherein said fusion partner moiety is selected from the group consisting of a human IgG Fc domain, a human serum albumin (HSA) and a polyethylene glycol (PEG).
52. A composition according to claim 50 wherein said ECD has an amino acid sequence selected from the group consisting of the sequences depicted in Figure 67, Figure 91 and Figures 92A to 92AT.
53. A composition according to claim 50 to 52 wherein said PVRIG polypeptide and said fusion partner moiety are directly covalently attached.
54. A composition according to claim 53 wherein said fusion partner moiety is a polyethylene glycol (PEG) moiety.
55. A composition according to claim 50 to 52 wherein said PVRIG polypeptide and said fusion partner moiety are covalently attached using an exogeneous linker.

56. A composition according to claim 55 wherein said exogeneous linker is selected from the group consisting of those depicted in Figure 93.

57. A composition according to claim 55 or 56 wherein said exogenous linker has the formula (GGGS)_n, wherein n is from 1 to 5.

5 58. A composition according to any of claims 50 to 53 and 55 to 57 wherein said fusion partner moiety is a human serum albumin (HSA).

59. A composition according to any of claims 50 to 53 and 55 to 57 wherein said fusion partner moiety is an Fc domain.

10 60. A composition according to claim 59 wherein said Fc domain is a human IgG Fc domain.

61. A composition according to claim 60 wherein said human IgG Fc domain is selected from the group consisting of the Fc domain of human IgG1, the Fc domain of human IgG2, the Fc domain of human IgG3 and the Fc domain of human IgG4.

15 62. A composition according to claim 59 wherein said Fc domain is a variant human Fc domain from IgG1 or IgG2.

63. A composition according to any of claims 50 to 62 further comprising a pharmaceutically acceptable carrier.

20 64. A method of suppressing T cell activation of a patient comprising administering a composition according to any of claims 50 to 63 to said patient such that said patient's immune response is suppressed as a result of treatment.

65. A method according to claim 64 wherein said patient has an immune disorder.

66. A method according to claim 65 wherein said immune disorder is selected from the group consisting of an autoimmune disease, and organ transplant rejection.

25 67. A method according to claim 66 wherein said autoimmune disease is selected from the group consisting of rheumatoid arthritis, lupus, Inflammatory bowel disease, psoriasis, multiple sclerosis, Diabetes type I.

30

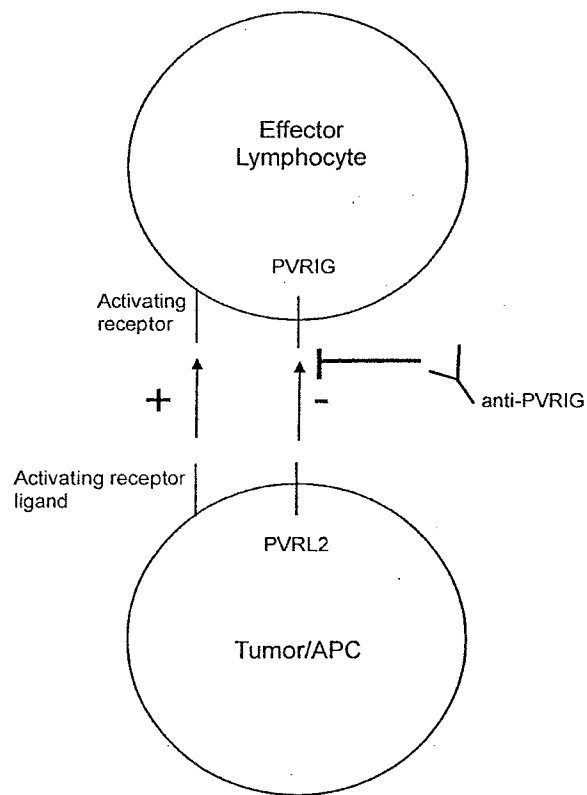
Figure 1

Figure 2.

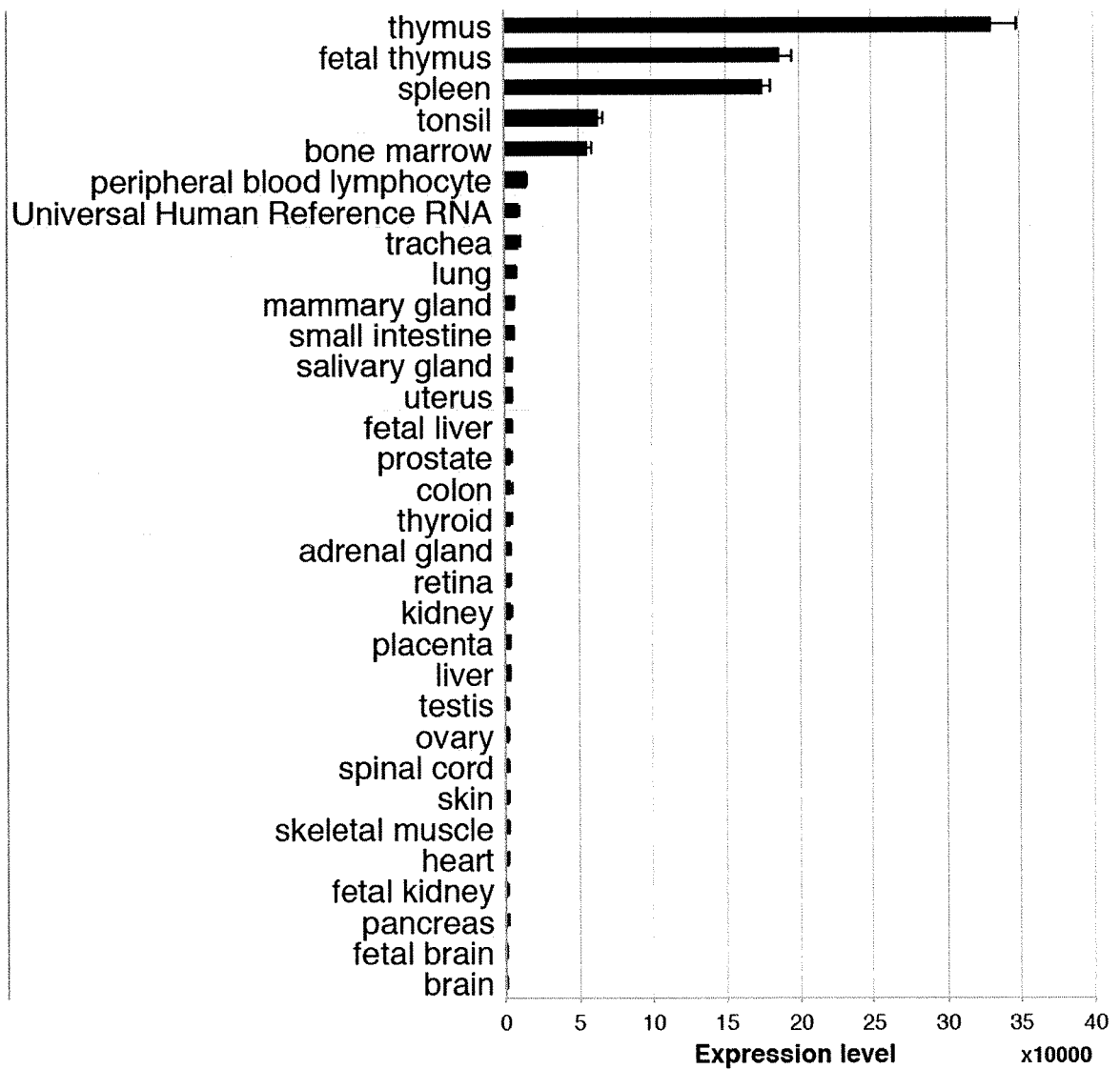


Figure 3:

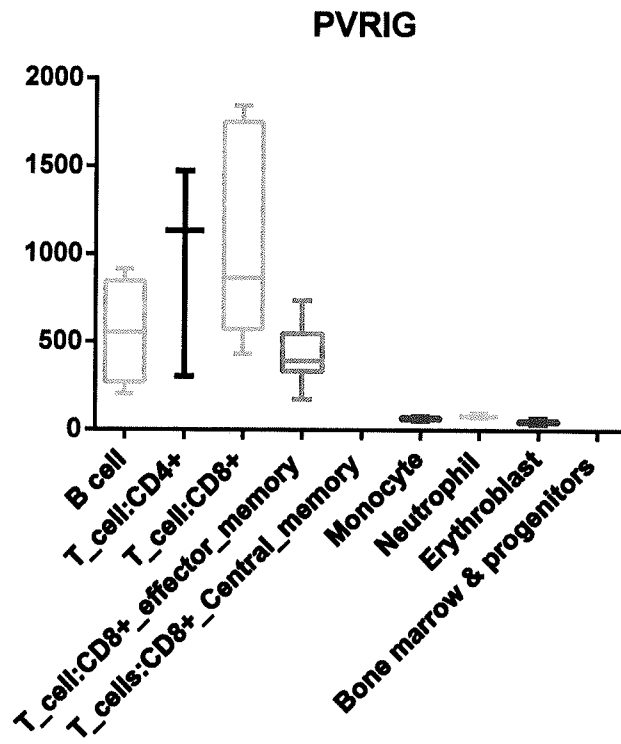


Figure 4

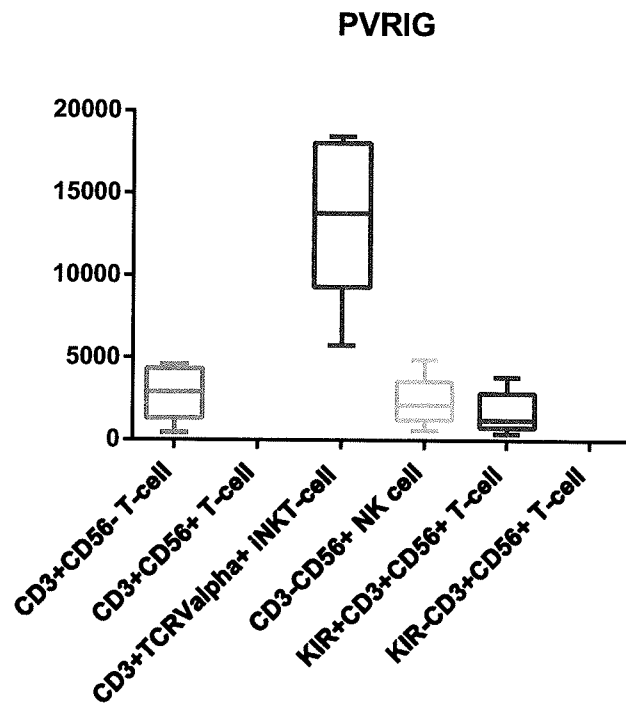


Figure 5a:

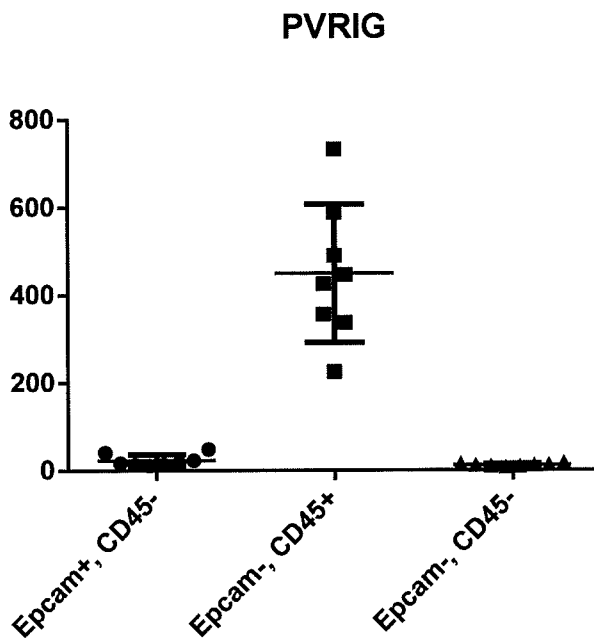


Figure 5b:

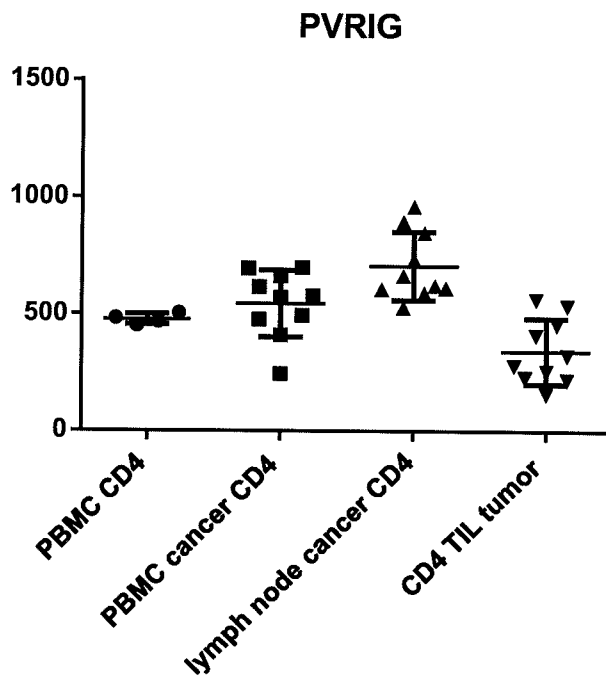


Figure 5c:

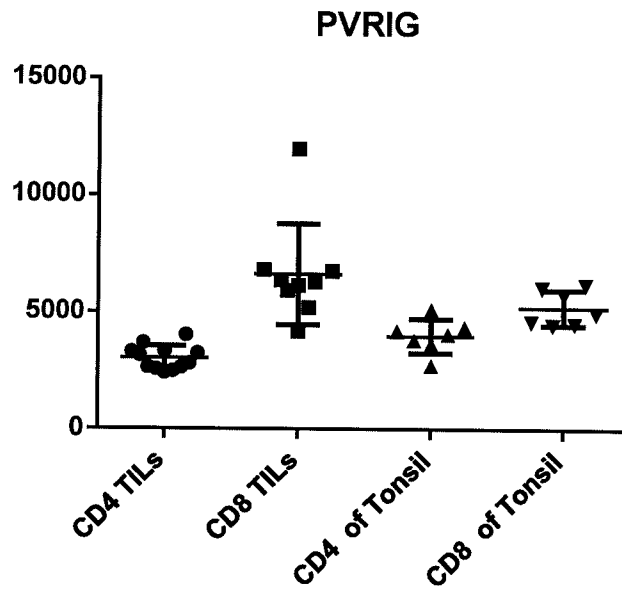


Figure 6

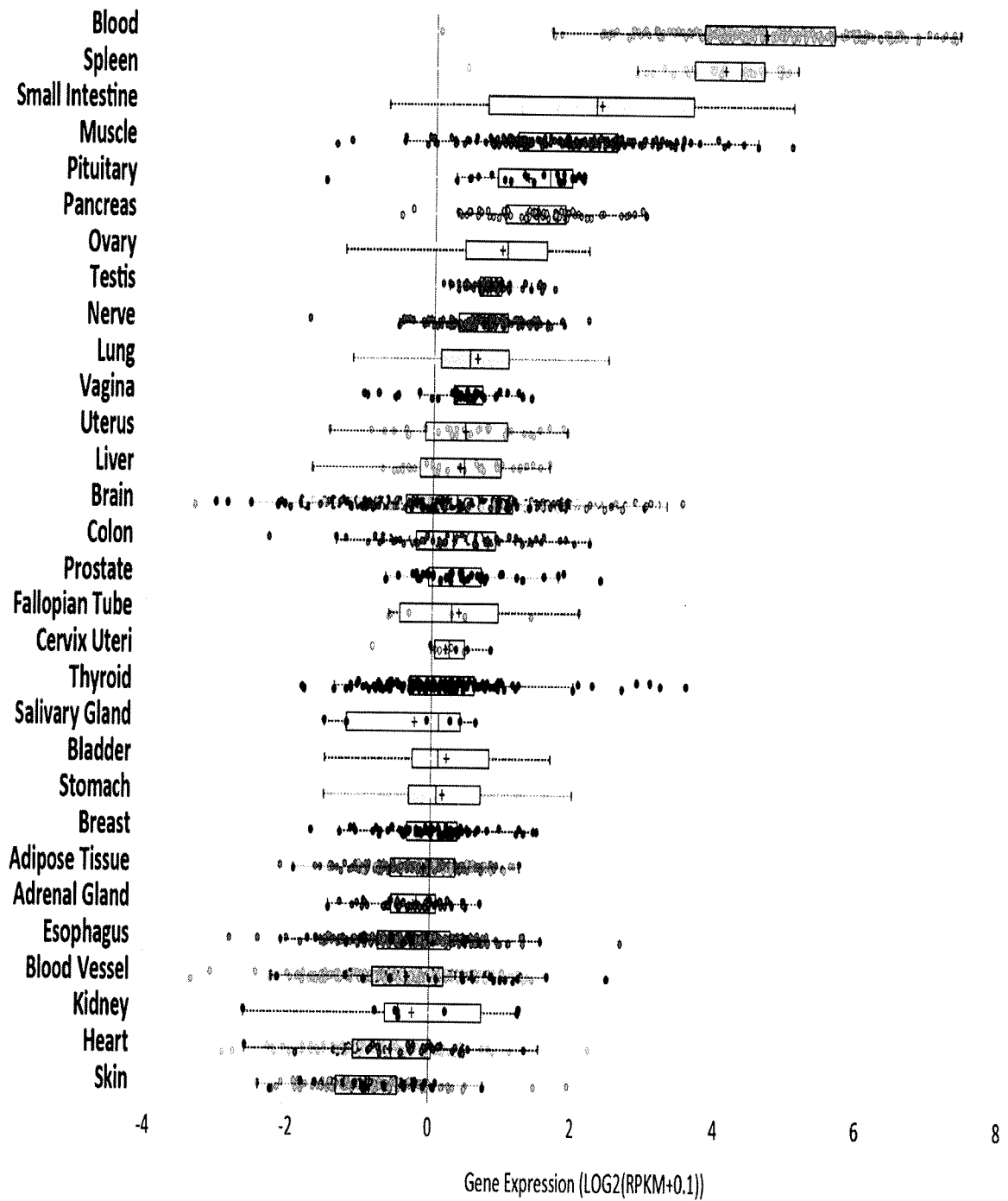


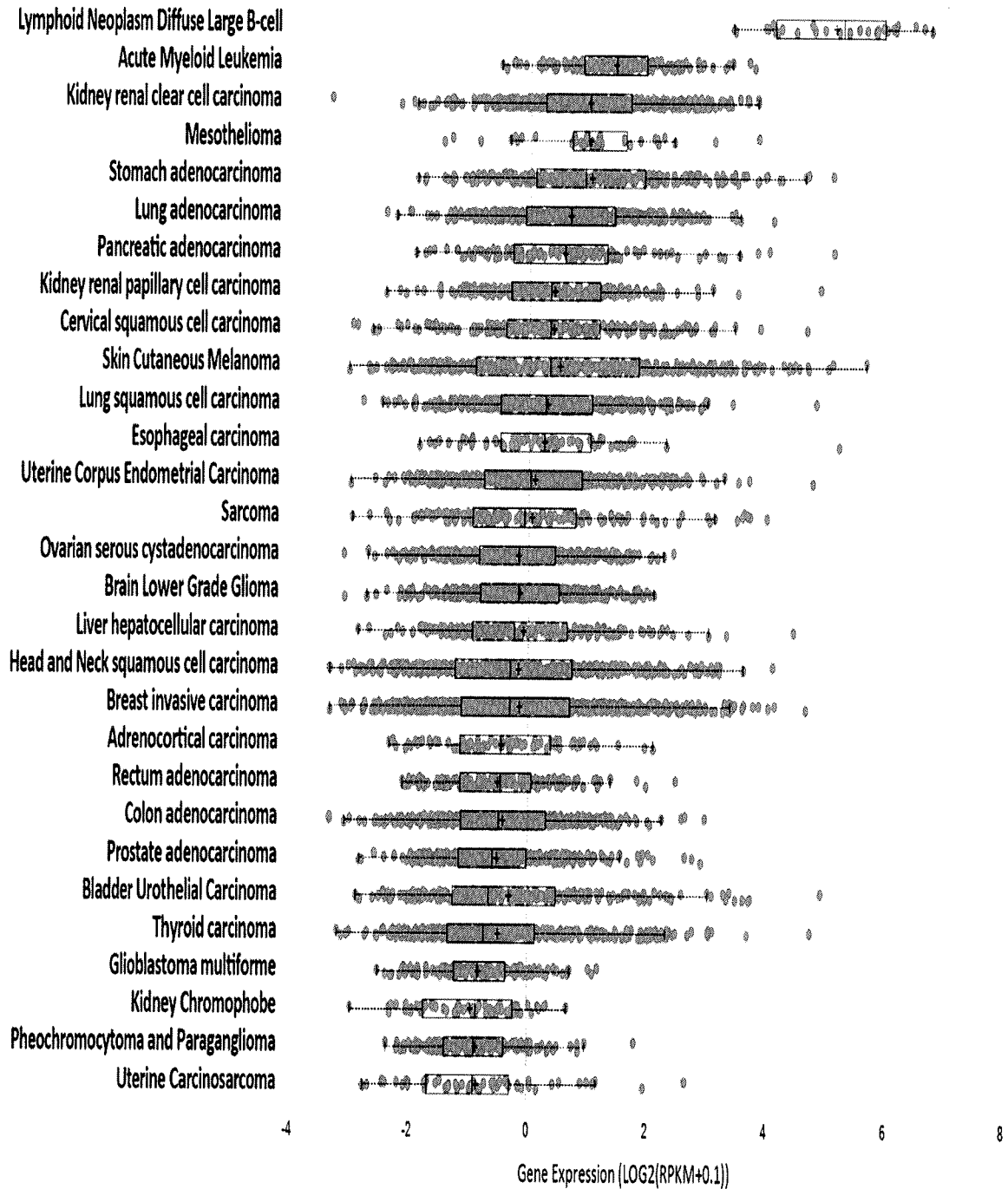
Figure 7

Figure 8

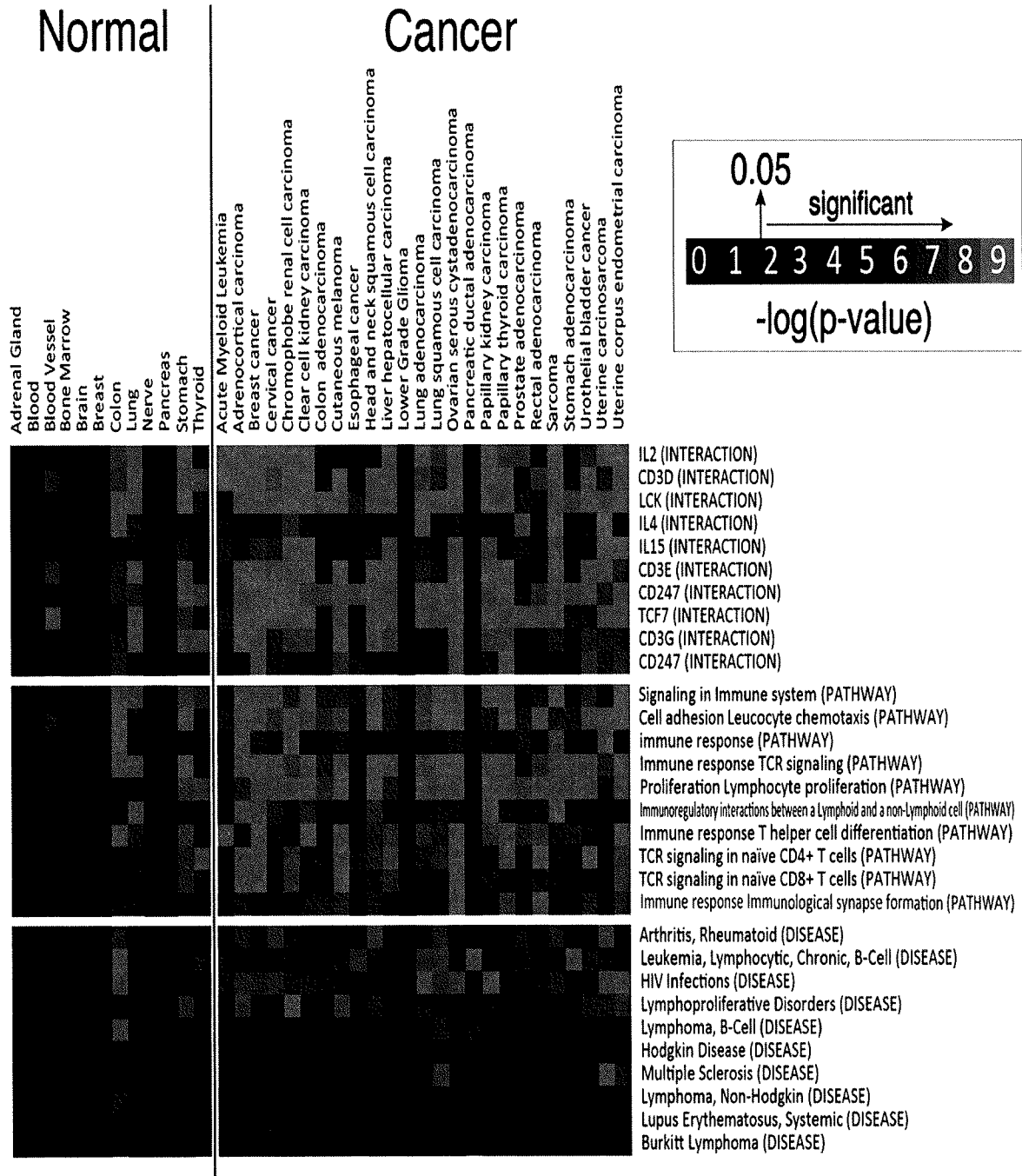


Figure 9

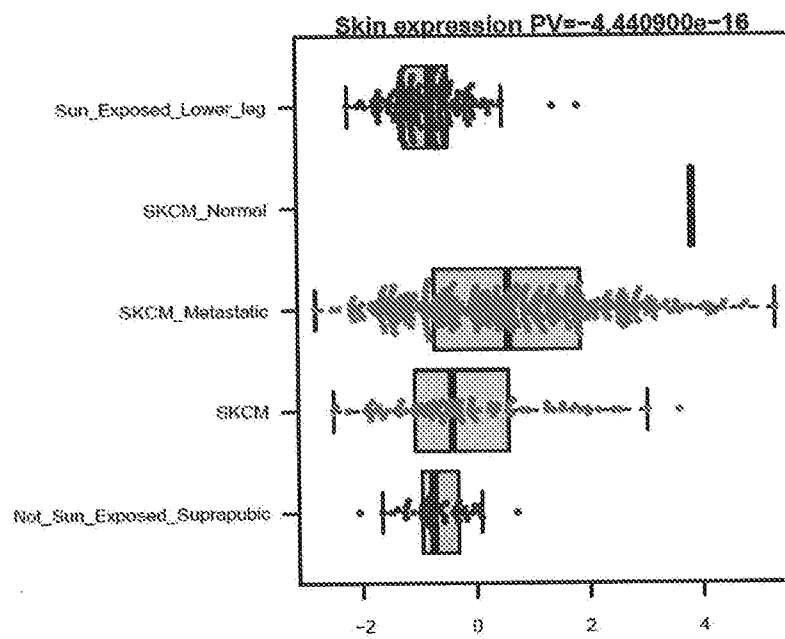


Figure 10

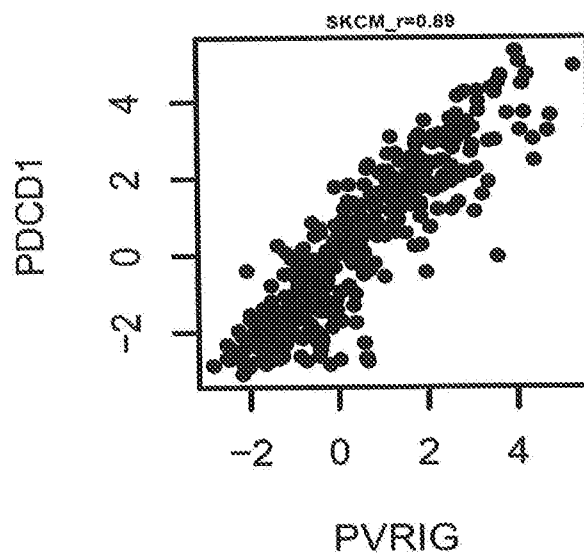


Figure 11

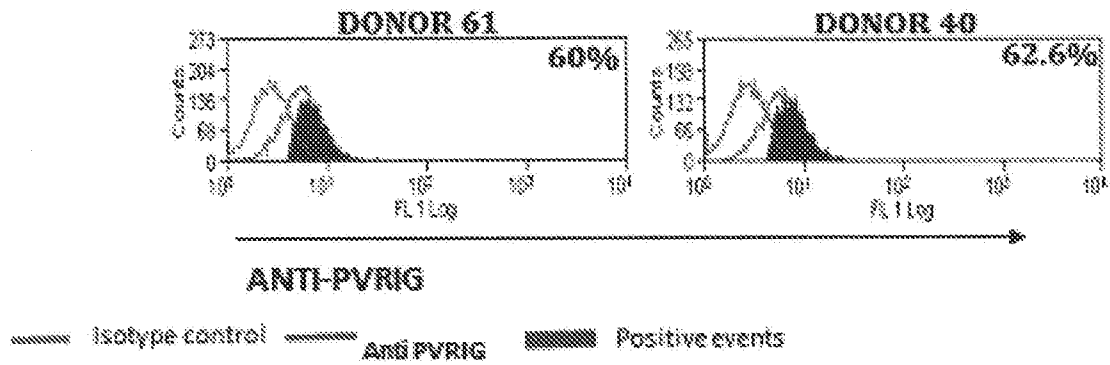


Figure 12

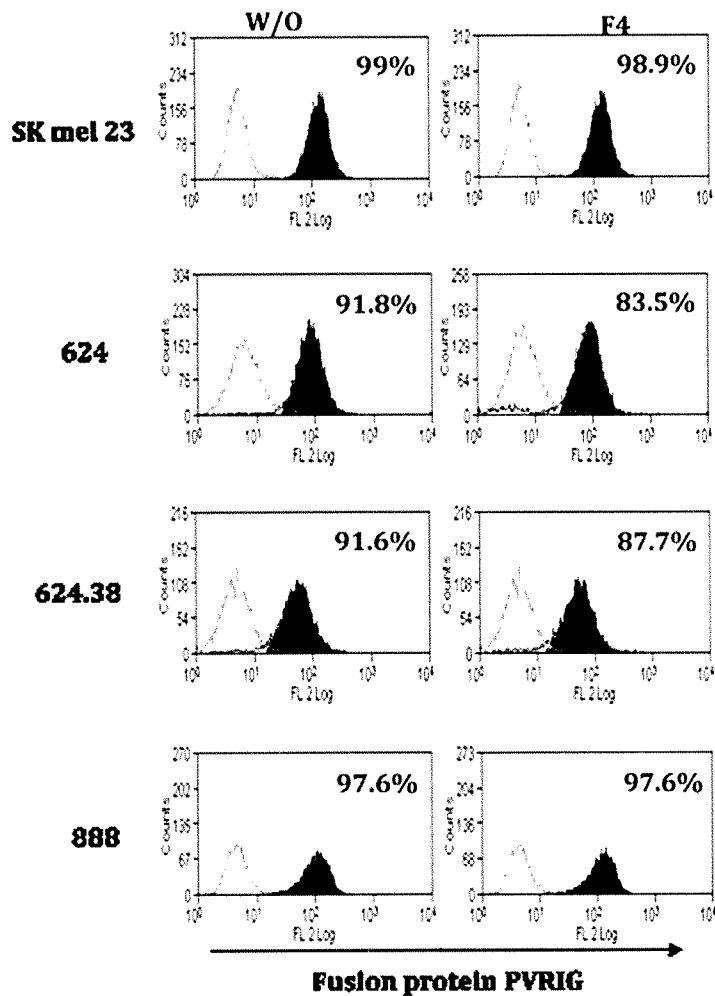


Figure 13

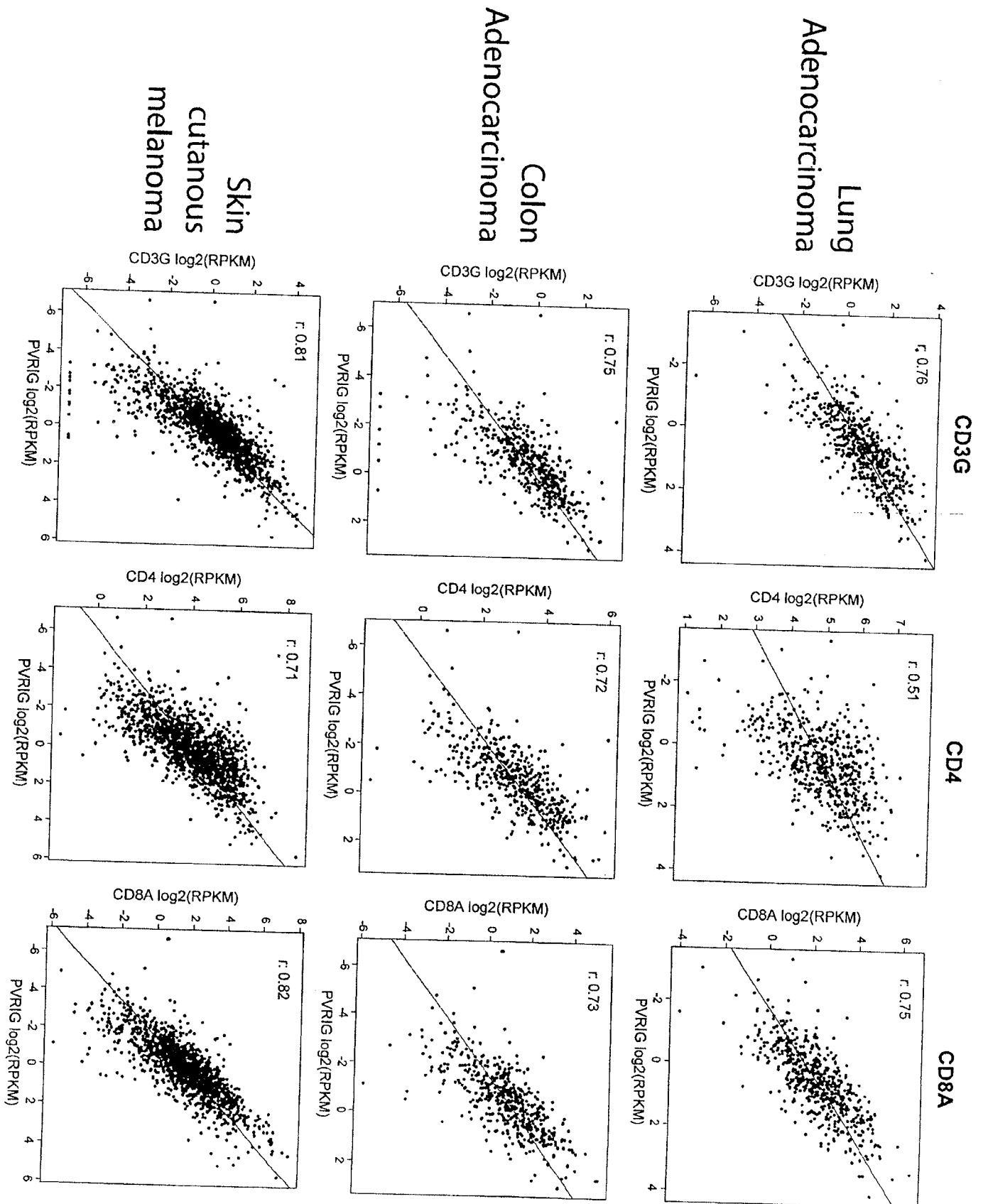


Figure 14:

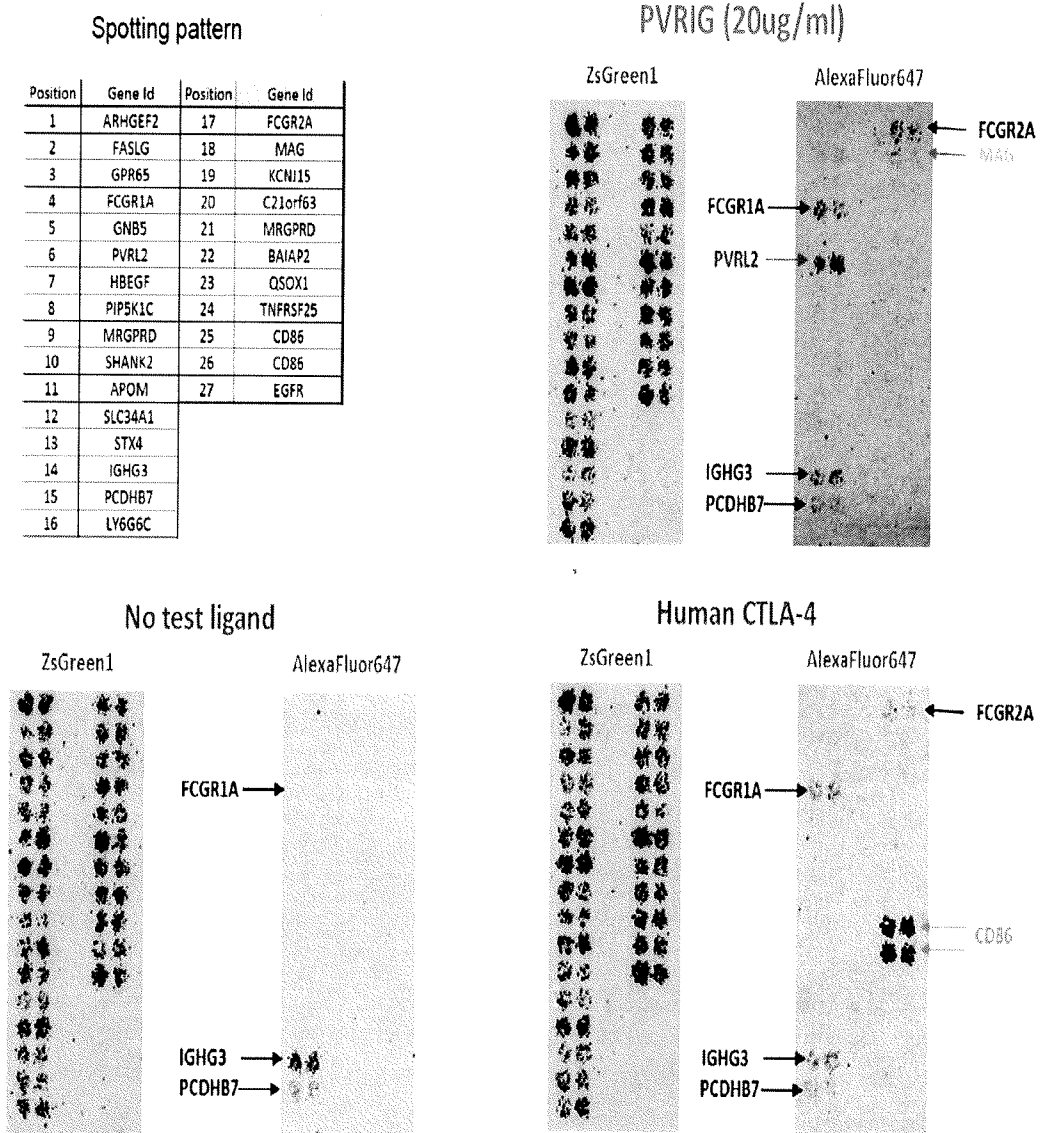


FIG. 15A

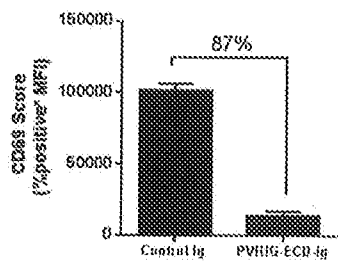


FIG. 15B

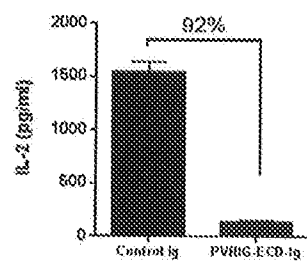


FIG. 15C

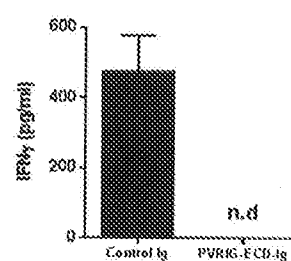


FIG. 15D

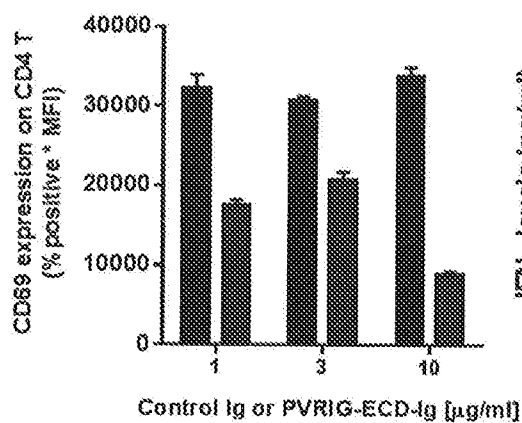


FIG. 15E

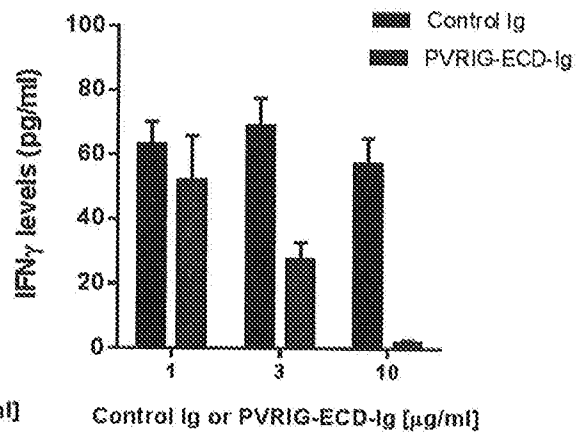


Figure 16

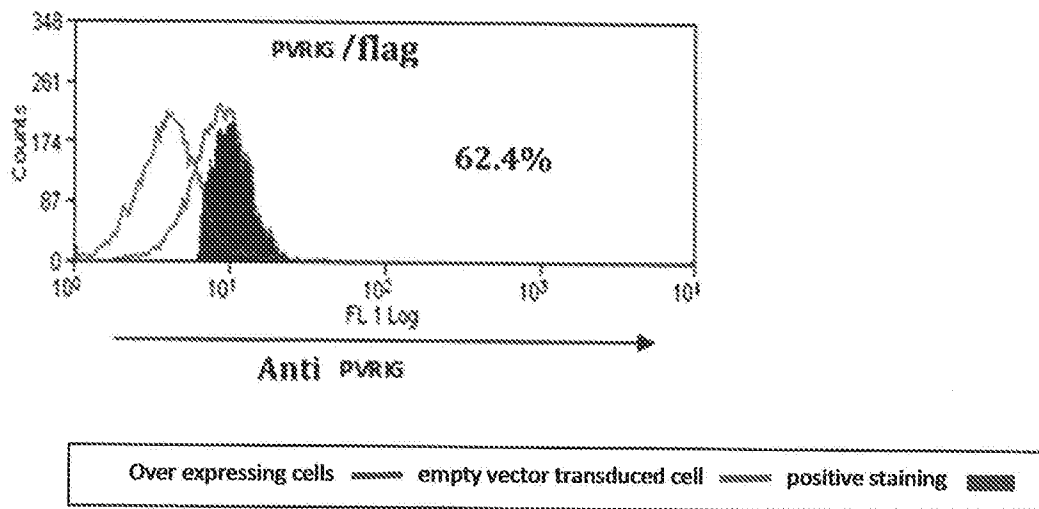


Figure 17

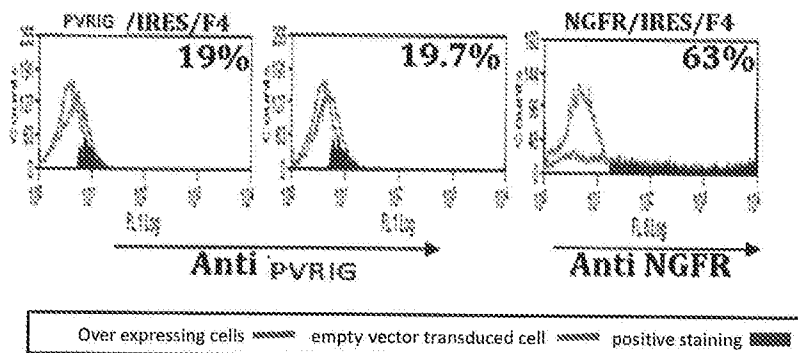


Figure 18A

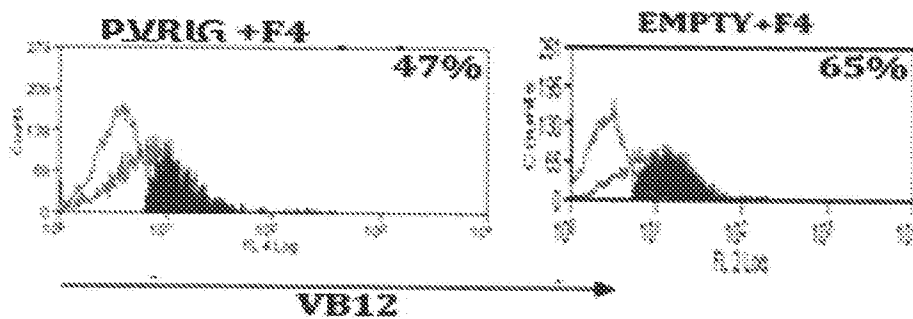


Figure 18B

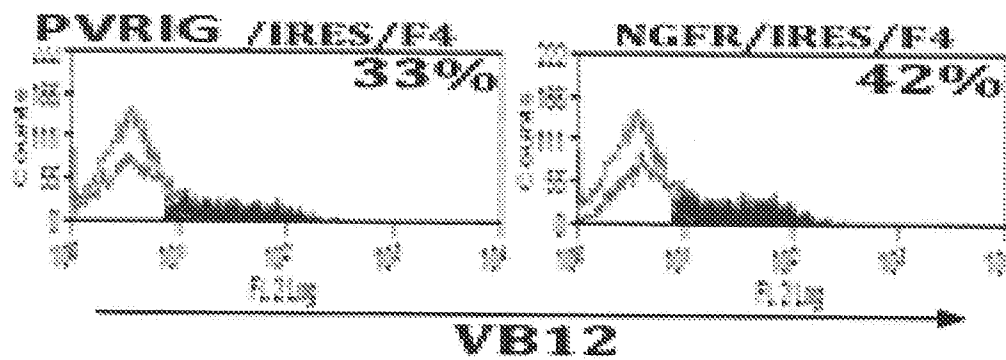


Figure 19

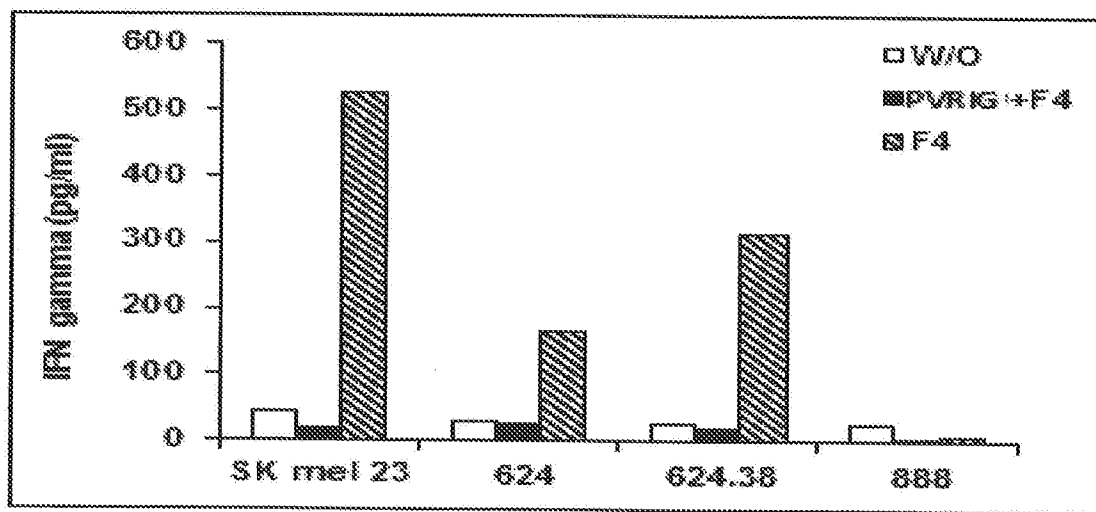


Figure 20A

A

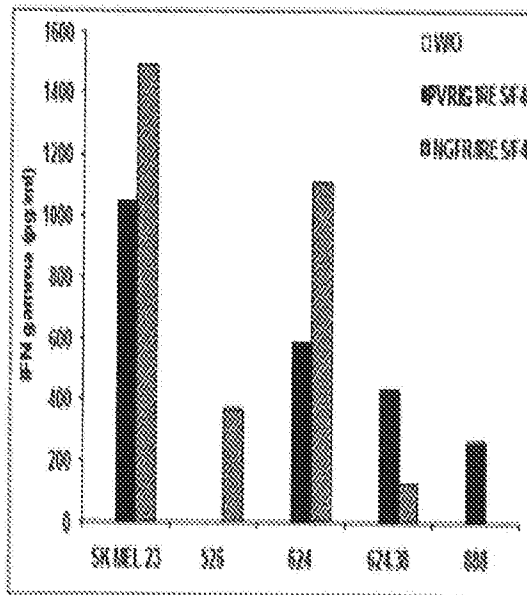


Figure 20B

B

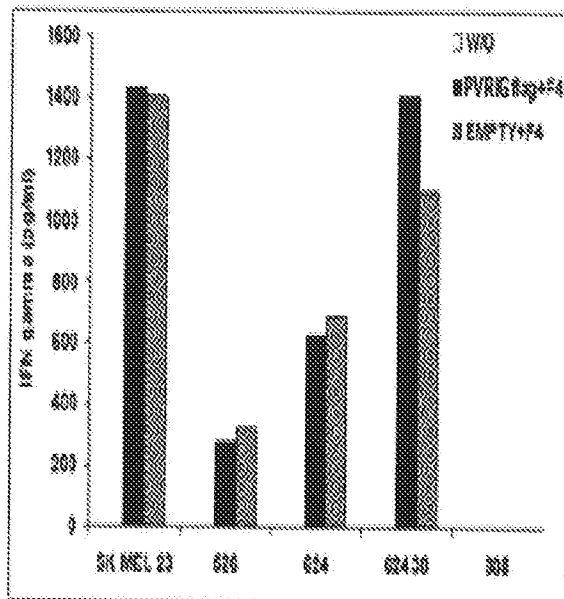
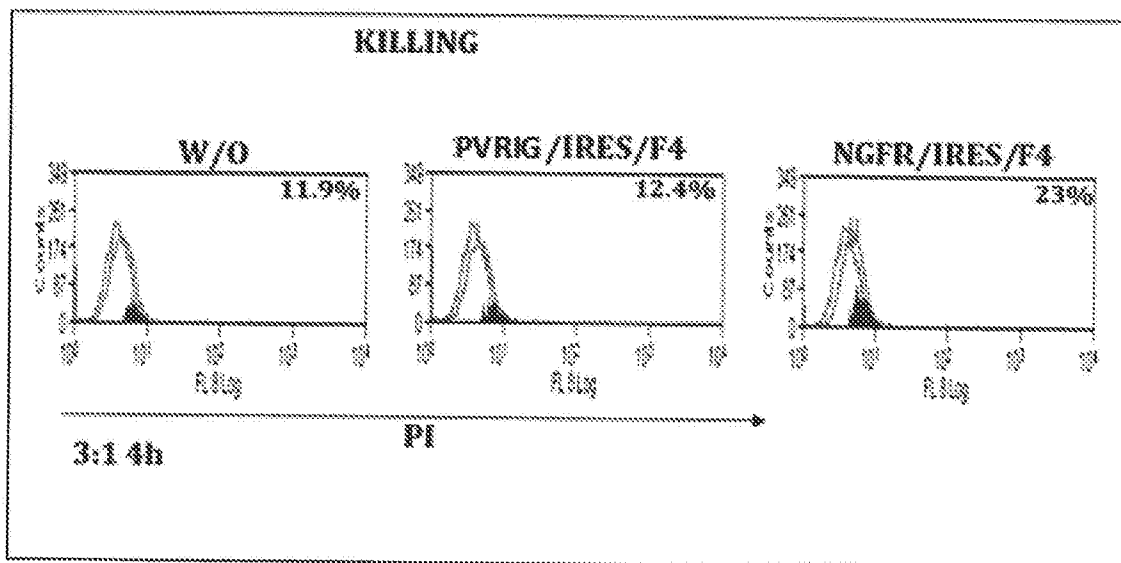


Figure 21



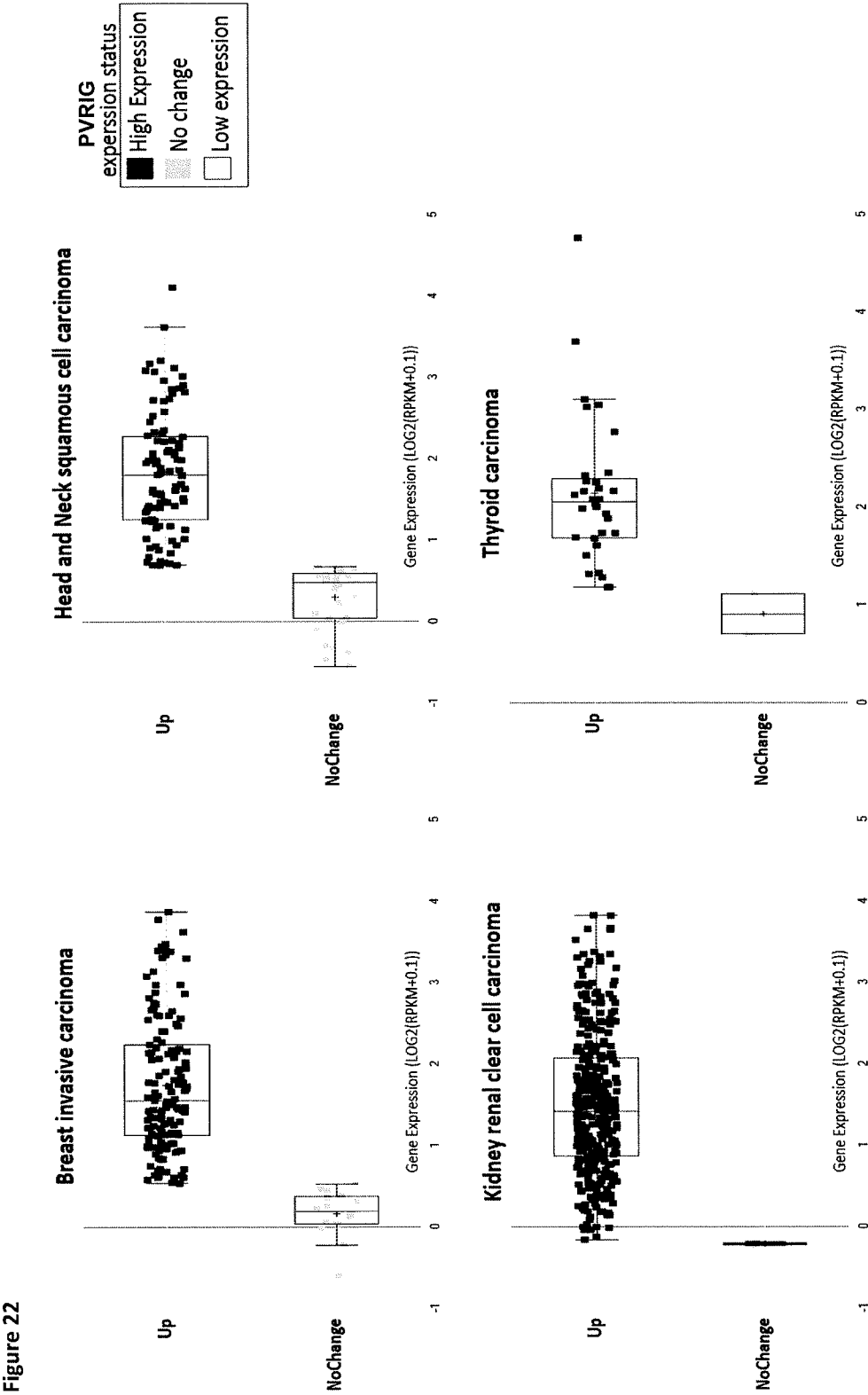


Figure 23A

Figure 23B

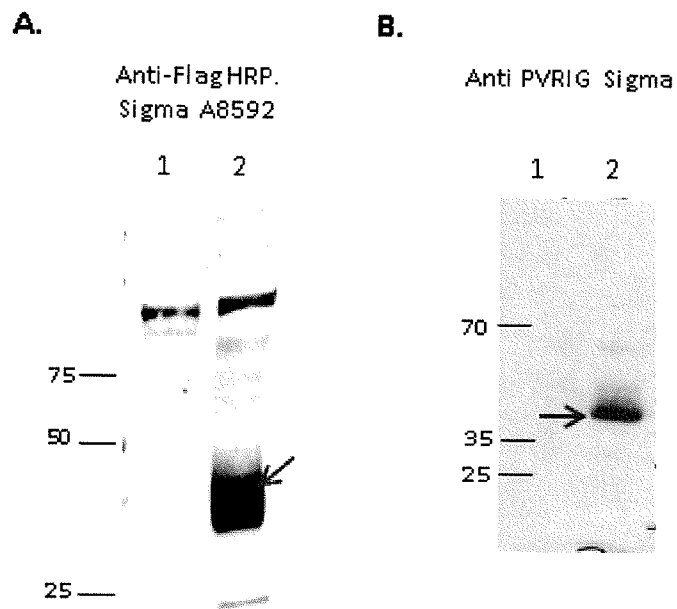


Figure 24

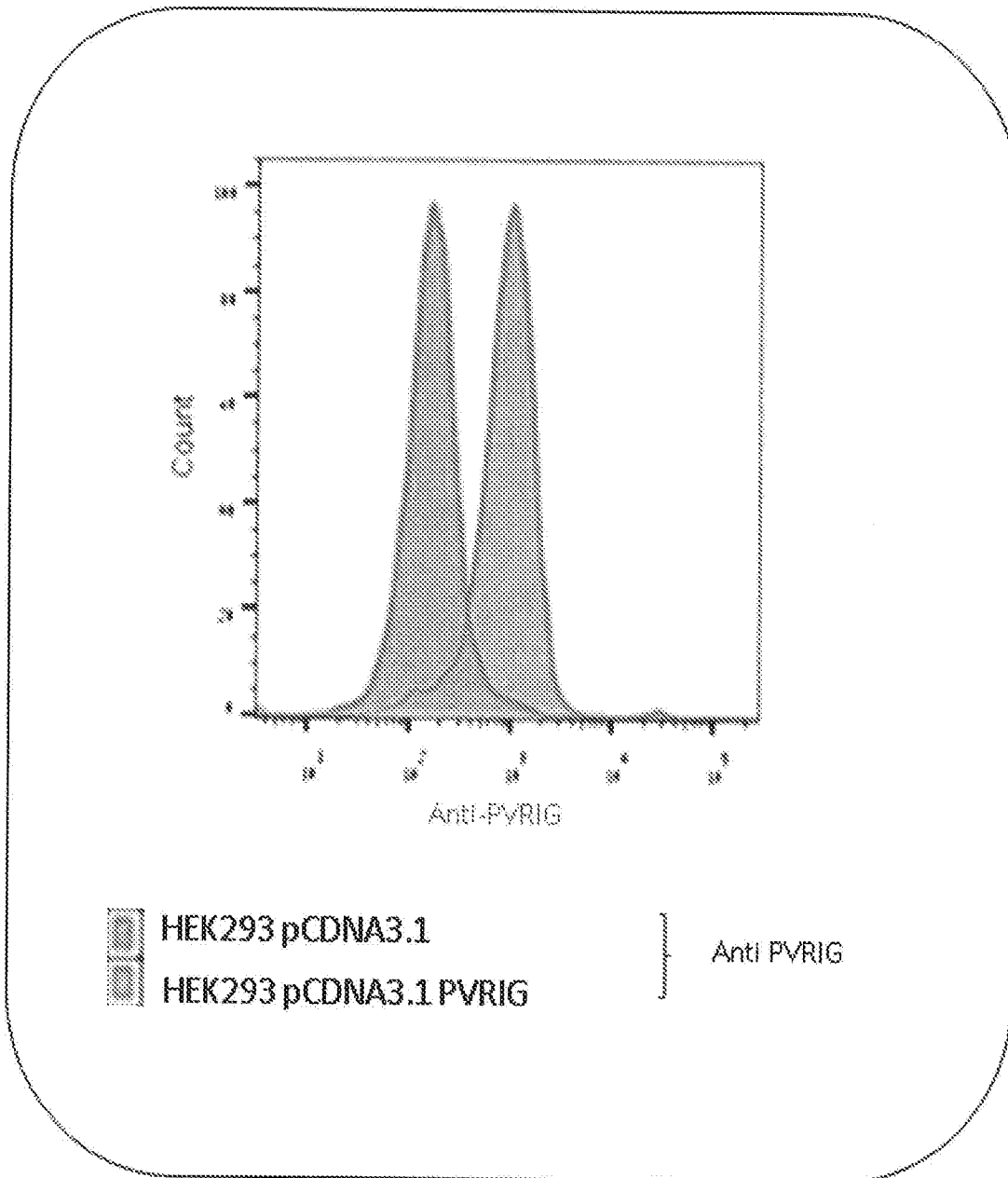


Figure 25

Human PVRIG WT Full length

Human PVRIG sequence starting from position 21 - alternative methionine

MGHRTLVLPPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIR
QWAPARQARWETOSSISLILEGSGASSPCANTTECCKEASFEGSWEACGSLPPSSDPGLSAPPTPAPILRADLAGILGV
SGVLLFGCVYLLHLLRRHKHRPAPRLQPSRTSPQAPRARAWAPSQA (SEQ ID NO: 8)

Human PVRIG sequence starting from position 1 methionine

MRTEAQVPALQPPEPGLEGAMGHRTLVLPPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWG
GPNGAGGTTLAVLHPERGIQWAPARQARWETOSSISLILEGSGASSPCANTTECCKEASFEGSWEACGSLPPSSDPG
LSAPPTPAPILRADLAGILGVSGVLLFGCVYLLHLLRRHKHRPAPRLQPSRTSPQAPRARAWAPSQA (SEQ ID NO: 9)

Alternative signal P with three C to S Mutations at the Fc domain

CGEN-PVRIGHH-2 Alternative SP (MGWSCILFLVATATGVHS (SEQ ID NO: 10)) + CGEN-PVRIG (41-171 of PVRIG_HUMAN) + Human IgG1 Fc mutated at C220S, C226S, C229S of hinge

MGWSCILFLVATATGVHSTPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGGPNGAGGTTLAVLHPERGIQW
APARQARWETOSSISLILEGSGASSPCANTTECCKEASFEGSWEACGSLPPSSDPGLSAPPTPAPILRADEPKSSDKTHI
SPSPAPELLGGPSVELEPPKPKDTLMISRTPEVTCVYVDYSHEDPEYKENWYVDGVEVHNAKTKPREEQYNSTYRVYS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGEYPSDIAVEWESN
GQPENNYKTIPTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSISLSPGK (SEQ ID NO: 11)

Figure 26

Human PVLR2 alpha isoform

```
>gi|5360210|ref|NP_002847.1| nectin-2 isoform alpha precursor [Homo sapiens]  
MARAAALLPSRSPTPLLWPLLLLLLLETGAQDVRVQVLPEVRGQLGGTVLPCHELLPPVPGLYISLVTW  
QRPDAPANHQNVAAAFHPKMGPSFSPKPGSERLSFVSAKQSTGQDTEAELQDATLALHGLTVEDEGNYTC  
EFATFPKGSVRGMTWLRVIAKPKNQAEAQKVTFSDPTTVALCISKEGRPPARISWLSSLDWEAKETQVS  
GTLAGTVTVTSRFTLVPSGRADGVTVTCKVEHESFEPPALIPVTLVRYPPPEVSISGYDDNWYLGRTDAT  
LSCDVRNPEPTGYDWSTTSPTSAVAQGSQSLVIHAVDSLNTTFVCTVTNAVGMGRAEQVIFVRETP  
RASPRDVGPVLVWGAVGGTLLVLLLLAGGSLAFILLRVRRRRKSPGGAGGGASGDGGFYDPKAQVLGNGDP  
VFWTPVVPMPMEPDGKDEEEEEEEKAEGKMLMLPPPPALEDDMESQLDGSLSRRAVYV (SEQ ID NO: 12)
```

Human PVLR2 delta isoform

```
>gi|112789532|ref|NP_001036189.1| nectin-2 isoform delta precursor [Homo sapiens]  
MARAAALLPSRSPTPLLWPLLLLLLLETGAQDVRVQVLPEVRGQLGGTVLPCHELLPPVPGLYISLVTW  
QRPDAPANHQNVAAAFHPKMGPSFSPKPGSERLSFVSAKQSTGQDTEAELQDATLALHGLTVEDEGNYTC  
EFATFPKGSVRGMTWLRVIAKPKNQAEAQKVTFSDPTTVALCISKEGRPPARISWLSSLDWEAKETQVS  
GTLAGTVTVTSRFTLVPSGRADGVTVTCKVEHESFEPPALIPVTLVRYPPPEVSISGYDDNWYLGRTDAT  
LSCDVRNPEPTGYDWSTTSPTSAVAQGSQSLVIHAVDSLNTTFVCTVTNAVGMGRAEQVIFVRETP  
NTAGAGATGGIIGGIIAIIATAVAATGILICRQQRKEQTLQGAEEDEDELEGPSPYKPPTPKAKLEAQEM  
PSQLFTLGASEHSPLKTPYFDAGASCTEQEMPRYHELPTLEERSGPLHPGATSLGSPIPVPPGPPAVEDV  
SLDLEDEEGEEEEYLDKINPIYDALSY (SEQ ID NO: 13)
```

Figure 27

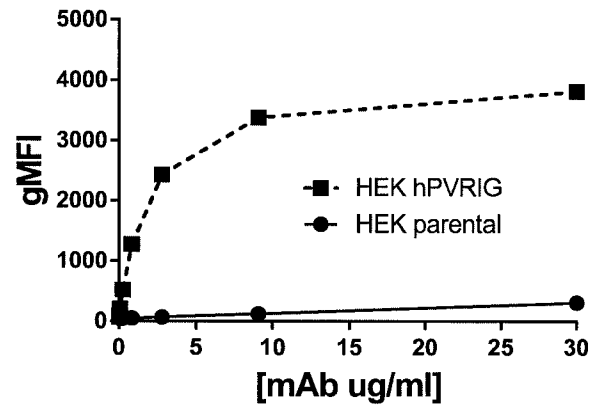


Figure 28

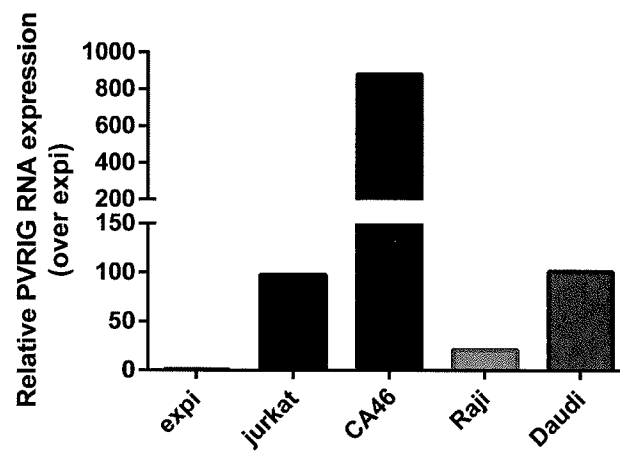


Figure 29

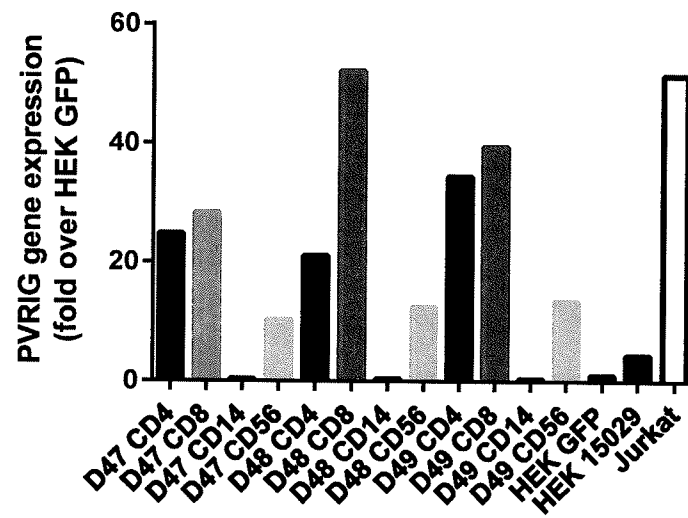


Figure 30A

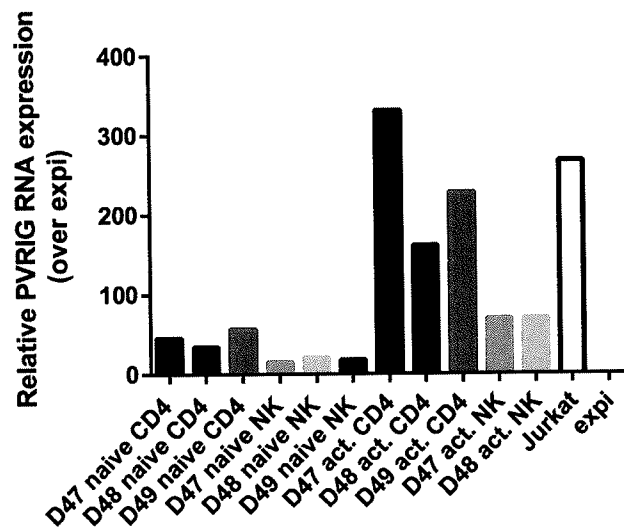


Figure 30B

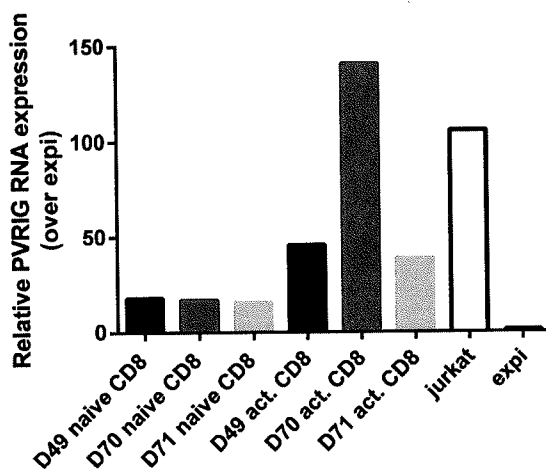


Figure 31A

Antibody (hlgG1)	EC ₅₀ (HEK OE, nM)	HEK OE/par (5ug/ml, gMFlr)	Jurkat (5ug/ml, gMFlr)	CA46 (5ug/ml, gMFlr)
CPA.7.002	53.47	11.97	1.25	1.16
CPA.7.003	7.12	1.28	3.85	6.71
CPA.7.004	43.74	25.18	1.03	1.16
CPA.7.005	125.50	15.77	0.93	1.02
CPA.7.007	0.99	2.90	4.42	9.82
CPA.7.008	Not tested	Not tested	Not tested	Not tested
CPA.7.009	67.30	8.35	0.93	0.99
CPA.7.010	30.00	18.76	1.08	1.01
CPA.7.011	128.80	10.86	0.93	0.96
CPA.7.012	0.40	2.01	4.89	8.23
CPA.7.014	19.66	5.90	1.14	0.92
CPA.7.015	1.74	6.64	4.3	7.33
CPA.7.016	28.38	2.20	1.11	1.04
CPA.7.017	2.62	4.56	0.99	1.49
CPA.7.018	20.38	2.06	1.05	0.96
CPA.7.019	2.11	1.94	4.66	9.23
CPA.7.020	108.30	1.37	1.64	1.45
CPA.7.021	12.41	30.59	4.47	7.96
CPA.7.022	7.73	4.60	1.01	1.24
CPA.7.023	10.90	20.31	0.9	1.01
CPA.7.024	22.91	9.92	1.14	1.25
CPA.7.025	No binding	No binding	Not tested	Not tested
CPA.7.026	8.169	1.16	1.00	0.99
CPA.7.027	No binding	No binding	Not tested	Not tested
CPA.7.028	21.5	2.34	9.57	3.16
CPA.7.029	3.87	1.24	1.17	0.85
CPA.7.031	No binding	No binding	Not tested	Not tested
CPA.7.032	49.62	4.50	1.81	1.64
CPA.7.033	221	1.8221999	1.94	1.17
CPA.7.034	55.69	4.12	1.25	1.01
CPA.7.035	No binding	No binding	Not tested	Not tested
CPA.7.036	14.19	5.68	1.69	1.46
CPA.7.037	Not reliable fit	4.56	1.78	1.28

Figure 31B

Antibody (hlgG1)	EC ₅₀ (HEK OE, nM)	HEK OE/par (5ug/ml, gMFir)	Jurkat (5ug/ml, gMFir)	CA46 (5ug/ml, gMFir)
CPA.7.038	2436	4.36	Not tested	Not tested
CPA.7.039	2.99	6.52	1.55	1.40
CPA.7.040	0.84	7.91	1.78	1.59
CPA.7.041	14.35	3.25	Not tested	Not tested
CPA.7.042	5.42	4.20	1.57	1.28
CPA.7.043	7.63	5.47	1.20	1.08
CPA.7.044	43.64	3.24	1.03	0.97
CPA.7.045	5.17	4.04	1.68	1.05
CPA.7.046	Not reliable fit	Not tested	Not tested	Not tested
CPA.7.047	4.61	5.89	0.81	0.92
CPA.7.049	1.94	2.56	Not tested	Not tested
CPA.7.050	121.5	4.29	2.72	2.02

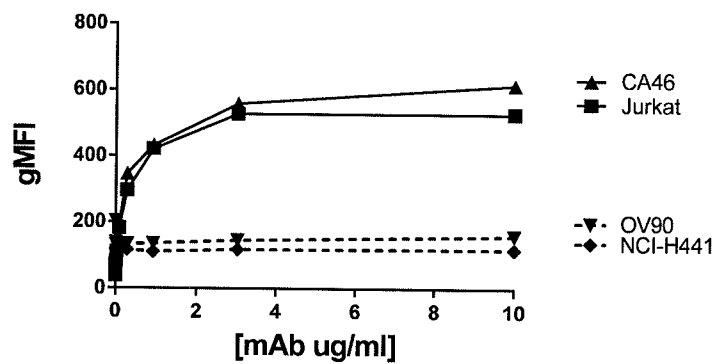
Figure 32A

Antibody (hIgG1)	Human CD56 int. NK (gMFlr, 5ug/ml)	Human CD8+ T cells (gMFlr, 5ug/ml)	Expi cyno OE/par (gMFlr, 5ug/ml)	Cyno NK cells (gMFlr, 5ug/ml)	Cyno CD3+ T cells (gMFlr, 5ug/ml)
CPA.7.002	3.21	1.01	1.16	2.24	1.01
CPA.7.003	16.21	1.41	1.36	Not tested	Not tested
CPA.7.004	1.30	0.98	1.1	Not tested	Not tested
CPA.7.005	2.35	1.07	1.21	1.12	0.99
CPA.7.007	5.31	1.32	1.11	Not tested	Not tested
CPA.7.008	Not tested	Not tested	Not tested	Not tested	Not tested
CPA.7.009	1.34	0.98	1.08	Not tested	Not tested
CPA.7.010	2.09	1.06	1.41	Not tested	Not tested
CPA.7.011	1.19	1.02	1.06	Not tested	Not tested
CPA.7.012	1.62	1.18	1.98	Not tested	Not tested
CPA.7.014	1.57	0.98	1.06	Not tested	Not tested
CPA.7.015	2.82	1.14	1.11	Not tested	Not tested
CPA.7.016	2.39	1.05	1.43	Not tested	Not tested
CPA.7.017	1.73	0.86	1.30	Not tested	Not tested
CPA.7.018	1.62	1.04	1.06	Not tested	Not tested
CPA.7.019	4.23	1.36	1.23	Not tested	Not tested
CPA.7.020	3.12	1.07	3.10	Not tested	Not tested
CPA.7.021	3.74	1.23	1.08	1.91	1.01
CPA.7.022	1.27	0.87	Not tested	Not tested	Not tested
CPA.7.023	1.11	0.99	1.08	Not tested	Not tested
CPA.7.024	1.20	1.02	5.67	0.30	1.06
CPA.7.025	Not tested	Not tested	1.41	Not tested	Not tested
CPA.7.026	1.72	0.92	1.31	Not tested	Not tested
CPA.7.027	Not tested	Not tested	1.45	Not tested	Not tested
CPA.7.028	21.42	1.40	1.34	Not tested	Not tested
CPA.7.029	1.24	0.90	1.29	Not tested	Not tested
CPA.7.031	Not tested	Not tested	1.47	Not tested	Not tested
CPA.7.032	10.43	0.93	1.46	Not tested	Not tested
CPA.7.033	2.46	0.93	1.39	Not tested	Not tested
CPA.7.034	1.05	0.89	1.33	Not tested	Not tested
CPA.7.035	Not tested	Not tested	1.24	Not tested	Not tested
CPA.7.036	4.17	0.87	1.24	Not tested	Not tested
CPA.7.037	1.64	1.01	1.40	Not tested	Not tested
CPA.7.038	18.08	1.03	7.88	3.35	1.01
CPA.7.039	1.43	0.92	7.53	0.31	1.01

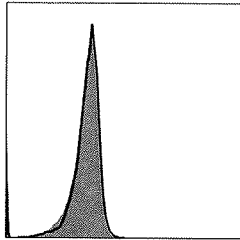
Figure 32B

Antibody (hIgG1)	Human CD56 int. NK (gMFIr, 5ug/ml)	Human CD8+ T cells (gMFIr, 5ug/ml)	Expi cyno OE/par (gMFIr, 5ug/ml)	Cyno NK cells (gMFIr, 5ug/ml)	Cyno CD3+ T cells (gMFIr, 5ug/ml)
CPA.7.040	1.73	0.88	1.32	Not tested	Not tested
CPA.7.041	6.15	1.01	3.31	1.26	0.98
CPA.7.042	6.10	0.92	3.71	1.38	0.99
CPA.7.043	1.10	0.83	1.50	Not tested	Not tested
CPA.7.044	8.79	0.88	1.31	Not tested	Not tested
CPA.7.045	1.28	0.84	1.43	Not tested	Not tested
CPA.7.046	Not tested	Not tested	4.42	Not tested	Not tested
CPA.7.047	0.99	0.90	1.29	Not tested	Not tested
CPA.7.049	Not tested	Not tested	1.37	Not tested	Not tested
CPA.7.050	4.98	1.23	1.47	0.64	1.03

Figure 33



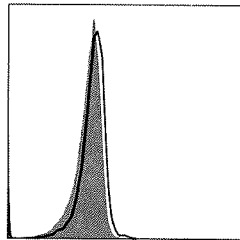
a



expi parental

Figure 34A

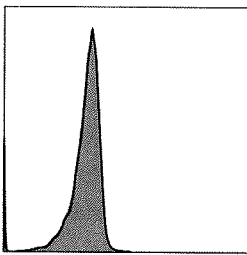
b



cPVRIG transient

Figure 34B

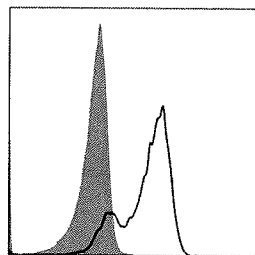
c



expi parental

Figure 34C

d



cPVRIG transient

Figure 34D

Figure 35

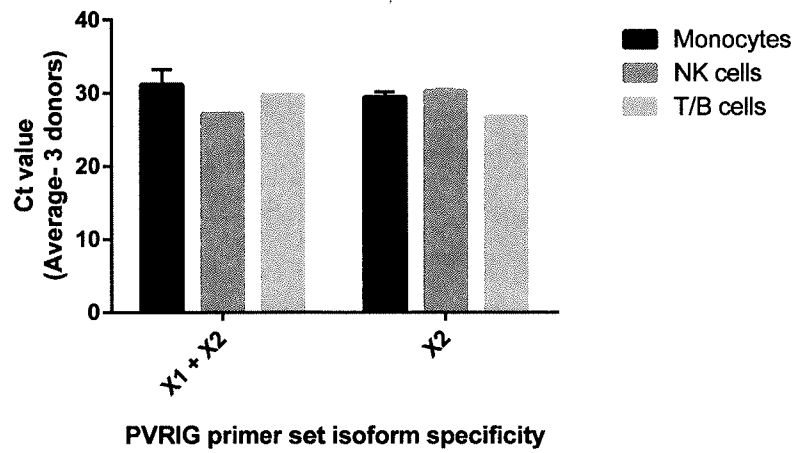


Figure 36A

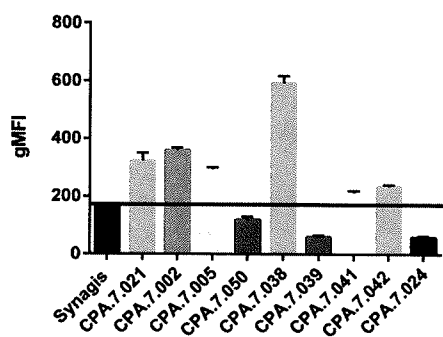


Figure 36B

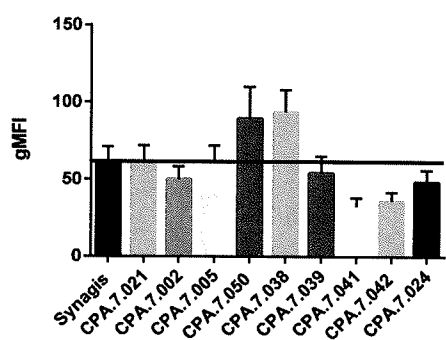


Figure 36C

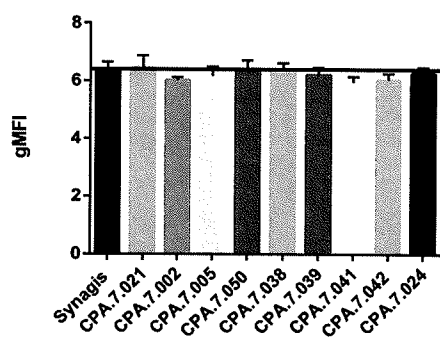


Figure 37A

Name	Hcd1r1	SEQ ID NO:			Hcd3	SEQ ID NO:			Hcd1r2	SEQ ID NO:			Hcd3	SEQ ID NO:			Hcd1r3	SEQ ID NO:		
		ID	NO:	SEQ		ID	NO:	SEQ		ID	NO:	SEQ		ID	NO:	SEQ		ID	NO:	SEQ
CPA.7.001	GTFSSYA	14		IPIFGTA	15	AREEVSPYGM DV	16	TGAVTSGHY	17	DTG		18	LLSYSGASWV	19						
CPA.7.003	GFSLSHFS	20		FDPEEGGT	21	ATGIWYSSGW PVDY	22	QSLDSSGYNV	23	LGS		24	MQALQTPIT	25						
CPA.7.004	GTLTELS	26		DPEDGET	27	ATVSRVRGVINYYYMDV	28	QSLLYRNGNNY	29	LGS		30	MQALQTPPT	31						
CPA.7.006	GGTFGTYA	32		ITPISATI	33	ARGFEYSDGLLDD	34	QSLFYSDDGNTY	35	RLS		36	MQHMEFPLT	37						
CPA.7.008	SGSISSTNW	38		IYHSGST	39	ARVGPAAIY	40	SNNVGEV	41	RNN		42	SAWDSSLNNAV	43						
CPA.7.009	GTLTELS	44		DPEDGET	45	ATAKPGIAVAGQNYYYMDV	46	QSLLYRNGNNY	47	LGS		48	MQALQTPPT	49						
CPA.7.010	GTFSSYA	50		ISYDGSNK	51	ASSPIGYSYWGGM DV	52	SGIDVRTNK	53	FQSDSDK		54	LIWHTSGWV	55						
CPA.7.011	GTLTELS	56		DPEDGET	57	ATGPAAAGVGYYYMDV	58	QSLLYRNGNNY	59	LGS		60	MQALQTPPT	61						
CPA.7.012	GTFSSYA	62		ISYDGSNK	63	ARDVMVYCSSTCYFGMDV	64	QDIRDY	65	DAS		66	QQFENLPIT	67						
CPA.7.013	GTLTELS	68		DPEDGET	69	ATGGYSSGFNYYYMDV	70	QSLLYRNGNNY	71	LGS		72	MQALQTPPT	73						
CPA.7.014	GTLTELS	74		DPEDGET	75	ATGVTTYYYGMDV	76	QSLLYSNGNMF	77	LGS		78	MQALQTPPT	79						
CPA.7.015	GTFSSYG	80		IRYDGSNK	81	ARDLDFFWWDGMDV	82	QSVSSMY	83	GAS		84	QQYVSSPMYT	85						
CPA.7.017	GGTFNNYG	86		IPLFGTT	87	ARDMAADGMVAFDY	88	SSNIGRHF	89	KND		90	SSWDAALNGV	91						
CPA.7.018	GTLTELS	92		DPEDGET	93	ATEVPMVRGARRYYYMDV	94	QTLLYNENNY	95	LGS		96	MQGLQTPPT	97						

DB2/28144952.4

Figure 37B

Name	Hcd1	SEQ ID NO:	Hcd2	SEQ ID NO:	Hcd3	SEQ ID NO:	Lcd1	SEQ ID NO:	Lcd2	SEQ ID NO:	Lcd3	SEQ ID NO:
CPA.7.019	GGISNSNY	98	IYSGST	99	ARGAWELSLGDWFD	100	SSNIGAGYD	101	GNN	102	QSYDSSLSVYV	103
CPA.7.021	GFTFGTSS	104	ISFDGTEI	105	AKGSGNIYFVSGMDV	106	QSIGSW	107	ETS	108	QQWYSYPLT	109
CPA.7.022	GTYLTELS	110	FDPEDGET	111	ATGVPAAIGVYYYYYMDV	112	QSLLYSNGYNY	113	LGS	114	MQALQSPVT	115
CPA.7.023	GTYLTELS	116	FDPEDGET	117	ATDSRDGPAARGGYYYMDV	118	QSLLYINGYNY	119	LGS	120	MQALQTPPT	121
CPA.7.024	GGTFSSYA	122	IIPIFGTA	123	ARDAVYDSSGGYNPDAFDI	124	QSLHSHNGYNY	125	LGS	126	MQGLQTPRT	127
CPA.7.033	GGTFSSA	128	IIPIYGIT	129	ARDDTARRVRGVYYYYYAMDV	130	QDIDDD	131	EAS	132	LQHDNLPLT	133
CPA.7.034	GTYLTELS	134	FDPEDGET	135	ATEDPGPVAGPYYYGMDV	136	QSLLYINGYHY	137	LGS	138	MQALQTPPT	139
CPA.7.036	GGTFSSA	140	IIPIYGIT	141	ARDDTARRVRGVYYYYYAMDV	142	QSLDSDDGNTY	143	TLS	144	MQRQLQFPLT	145
CPA.7.040	GTYLTELS	146	FDPEDGET	147	ATGVPAAIGVYYYYYMDV	148	QSLLYRNGYNY	149	WGS	150	MQAVQNPPT	151
CPA.7.046	GGTFSSA	152	IIPIYGIT	153	ARDDTARRVRGVYYYYYAMDV	154	QTMNNY	155	DAS	156	QQYGDWLPIT	157
CPA.7.047	GTYLTELS	158	FDPEDGET	159	ATAFPEATISYYYMDV	160	QSLLYRNGYNY	161	WGS	162	MQAVQNPPT	163
CPA.7.049	GGTFSSA	164	IIPIYGIT	165	ARDDTARRVRGVYYYYYAMDV	166	RSLDSDDGNTY	167	SLS	168	MQRKEFPLT	169
CPA.7.050	GGTFSSYA	170	IIPIFGTA	171	ARGPWYDSSGGYSSYAYYMDV	172	QSLHSDGYNY	173	LGS	174	MQALHTPGVT	175

Figure 38A

CPA.7.001

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QMLVQSGAEVKKPGSSVKVCKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTAYME LSSLRSEDTAVVYCAR ~~EEVSSPYGMDV~~ WGQGT ~~TVVSS~~	176
vhCDR1	GGTFSSYA	177
vhCDR2	IPIFGTA	178
vhCDR3	AREEVSSPYGMDV	179
Full length HC	QMLVQSGAEVKKPGSSVKVCKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTAYME LSSLRSEDTAVVYCAR ~~EEVSSPYGMDV~~ WGQGT ~~TVVSS~~ ASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSWTVPSSSLGTQYICNVNHPKSNITKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLT ~~VLHQDWLNGKEY~~ CKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSLKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	180
Variable light (vl) domain	QAVVTQEP ~~SLTVSPGGTVTL~~ TCGTS ~~TGAVTSGHY~~ PYWFQQKPGQAPK ~~TLIYDTGNKHSWTPARFSGSLGGKAA~~ LTLSGAQPE DEADYY ~~CLLSYSGASWV~~ FGGGTKLTVLG	181
vlCDR1	TGAVTSGHY	182
vlCDR2	DTG	183
vlCDR3	LLSYSGASWV	184
Full length light chain	QAVVTQEP ~~SLTVSPGGTVTL~~ TCGTS ~~TGAVTSGHY~~ PYWFQQKPGQAPK ~~TLIYDTGNKHSWTPARFSGSLGGKAA~~ LTLSGAQPE DEADYY ~~CLLSYSGASWV~~ FGGGTKLTVLGQPKAAPSV ~~TLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTT~~ PSKQSNINKYAASSYLSTPEQWKSHRYSQCQVTHEGSTVEKTVAPTECS	185

Figure 38B
CPA.7.003

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGASVKVCKVSGFSLSHFSMHVVRQVPGKGLEWLGGFDP EEGGTIPAKQFQGRLTMTEDTST ETAYMELSLRSED TAVVYCATGIWYSSGWPVDYWGPGLVTVSS	186
vhCDR1	<u>GFSLSHFS</u>	187
vhCDR2	<u>FDPEEGGT</u>	188
vhCDR3	<u>ATGIWYSSGWPVDY</u>	189
Full length HC	EVQLVQSGAEVKKPGSSVKVCKKASGGTFSSYAISWVROAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTST AYMELSLRSED TAVVYCARGPWYDSSGYSSAYYMDVWVGQGT TVVSSASTKGPSVFP LAPSSKSTSGGTAALGCL VKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHPKPSNTKVDKKVEPKSCDKTH TCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVAVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	190
Variable light (vl) domain	DVVMTQSPSLPVT PGGPASISCRSSQSLDSSGYNVVDWYLQKPGQSPQLLISLGS DRASGVPDRFSGSGSGTDFTLKI SRVEAEDVG IYVCMQALQTPITFGGQTRLEIKR	191
viCDR1	<u>QSLDSSGYNV</u>	192
viCDR2	<u>LGS</u>	193
viCDR3	<u>MQALQTPIT</u>	194
Full length light chain	DVVMTQSPSLPVT PGGPASISCRSSQSLDSSGYNVVDWYLQKPGQSPQLLISLGS DRASGVPDRFSGSGSGTDFTLKI SRVEAEDVG IYVCMQALQTPITFGGQTRLEIKR TVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYFPREAKVQWKVDNAL QSGNSQESVTEQDSKDSYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	195

Figure 38C
CPA.7.004

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVCKVSGYTLTSLMHWVRQAPGKGLWVGDFDPEDGETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYCATVSRVGVINYYMDV WGKGTTVTVSS	196
vhCDR1	<u>GYTLIELS</u>	197
vhCDR2	<u>FDPEDGET</u>	198
vhCDR3	<u>ATVSRVGVINYYMDV</u>	199
Full length HC	EVQLVQSGAEVKKPGASVKVCKVSGYTLTSLMHWVRQAPGKGLWVGDFDPEDGETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYCATVSRVGVINYYMDVWGKGTTVTVSSASTKGPSVFPPLAPSSKSTSGGTAALGCLV KDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSVTVPSSSLGTQTYICNVNHPKPSNTKVDKKVEPKSCDKHTC PPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTTPVLDSDGSFELYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK	200
Variable light (vl) domain	DVWMTQSPSLSPVTPGEPASISCRSSQSLLYRNGNNYLDWYLQKPGQSPQLLIYLGSNRASGVDPDRFSGSGGTDFTLKI SRVEAEDVGVYYCMQALQTPPTFGGGTKLEIKR	201
vICDR1	<u>QSLLYRNGNNY</u>	202
vICDR2	<u>LGS</u>	203
vICDR3	<u>MQALQTPPT</u>	204
Full length light chain	DVWMTQSPSLSPVTPGEPASISCRSSQSLLYRNGNNYLDWYLQKPGQSPQLLIYLGSNRASGVDPDRFSGSGGTDFTLKI SRVEAEDVGVYYCMQALQTPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	205

Figure 38D
CPA.7.006

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVRRPGSSRVSCPKPSGGTFTGYAFTWVRQAPGQGLEWMGGITPISATINRAQNLQDRLTITADESTT TVHMDLTSLRSED ~~TAVYYCARGFEYSDGLLDDWGQGTLVTVSS~~	206
vhCDR1	<u>GGTFTGYA</u>	207
vhCDR2	<u>ITPISATI</u>	208
vhCDR3	<u>ARGFEYSDGLLDD</u>	209
Full length HC	QVQLVQSGAEVRRPGSSRVSCPKPSGGTFTGYAFTWVRQAPGQGLEWMGGITPISATINRAQNLQDRLTITADESTT TVHMDLTSLRSED ~~TAVYYCARGFEYSDGLLDDWGQGTLVTVSS~~ ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVAVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPGK	210
Variable light (vl) domain	DIVMTQTPLSLPVIPGEPASISCRSSQSLFYSDDGNTYLDWYLQKPGQSPQLLIYRLSHRASGVPDRFSGSGSGTDFTLKI SRVEAEDGVVYCMQHMEFPLTFGGGKVEIKR	211
viCDR1	<u>QSLFYSDDGNTY</u>	212
viCDR2	<u>RLS</u>	213
viCDR3	<u>MQHMEFPLT</u>	214
Full length light chain	DIVMTQTPLSLPVIPGEPASISCRSSQSLFYSDDGNTYLDWYLQKPGQSPQLLIYRLSHRASGVPDRFSGSGSGTDFTLKI SRVEAEDGVVYCMQHMEFPLTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSSTYSLSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	215

Figure 38E
CPA.7.008

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQESGPGLVKPSGTLSTLCVSSGSISSNTNWTVWRQPPGKLEWIGEYHSGSTSYNSSLKSRVTISEDKSKNQIS LRLSSVTAADTAVVYCARVGPAAIYWGQGGLVTVSS	216
vhCDR1	<u>SGSISSNTNW</u>	217
vhCDR2	<u>IYHSGST</u>	218
vhCDR3	<u>ARVGPAAIY</u>	219
Full length HC	QVQLQESGPGLVKPSGTLSTLCVSSGSISSNTNWTVWRQPPGKLEWIGEYHSGSTSYNSSLKSRVTISEDKSKNQIS LRLSSVTAADTAVVYCARVGPAAIYWGQGLTVTVSSASTKGPSVFPPLAPSSKSTSGGTAALGCLVKDYFPEPVT/SWN SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQYICNVNHNKPSNTKV/DKKVEPKSCDKTHTCPPCPAPPELLGGPS VFLPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWVVDGVEVHNAKTRPEEQYNSTYRVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFPYSDIAVEWESNGQPENNYKTTPP VLDSGSEFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	220
Variable light (vl) domain	QAGLTQPPSVSKGLRQTATLTCTGNSNNVGEAAWLQHQHGHAPKLLLYRNNRPPSGISKRFASRSNGNTASLTITG LQPEDEADYYCSAWDSSLNAVVFGGGTQLTVLG	221
viCDR1	<u>SNNVGYEG</u>	222
viCDR2	<u>RNN</u>	223
viCDR3	<u>SAWDSSLNAV</u>	224
Full length light chain	QAGLTQPPSVSKGLRQTATLTCTGNSNNVGEAAWLQHQHGHAPKLLLYRNNRPPSGISKRFASRSNGNTASLTITG LQPEDEADYYCSAWDSSLNAVVFGGGTQLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADS SPVKAGVETTTPSKQSNKNKYAASSYLSTPEQWKSRSYSCQVTHEGSTVEKTVAPTECS	225

Figure 38F
CPA.7.009

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVCKVSGYTLTSLMHWVRQAPGKGLEWMGGFDPEDEGETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYCATAPGIAVAGQNYYYMDVWVGKTTVTYSS	226
vhCDR1	<u>GYTLTSL</u>	227
vhCDR2	<u>FDPEDEGET</u>	228
vhCDR3	<u>ATAKPGIAVAGQNYYYMDV</u>	229
Full length HC	EVQLVQSGAEVKKPGASVKVCKVSGYTLTSLMHWVRQAPGKGLEWMGGFDPEDEGETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYCATAPGIAVAGQNYYYMDVWVGKTTVTYSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHITCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWVVDGVEVHNAKTKPREEQYNST YRVSVLTVLIHQDWLNGKEYCKVSNKALPAPIEKTIKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPGK	230
Variable light (vl) domain	DVWMTQSPPLSPVTPGEPASISCRSSQSLLYRNGNNYLDWYLQKPGQSPQLLIYLGSNRASGVDPDRFSGSGGTDFTLKI SRVEAEDVGVYYCMQALQTPPTFGGGTKLEIKR	231
viCDR1	<u>QSLLYRNGNNY</u>	232
viCDR2	<u>LGS</u>	233
viCDR3	<u>MQALQTPPT</u>	234
Full length light chain	DVWMTQSPPLSPVTPGEPASISCRSSQSLLYRNGNNYLDWYLQKPGQSPQLLIYLGSNRASGVDPDRFSGSGGTDFTLKI SRVEAEDVGVYYCMQALQTPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSSTYSLSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	235

Figure 38G
CPA.7.010

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVESGGGLVQPGRSRLRSCAASGFTFSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYVCASSPIGYSYGYWGGMDVWGQGTTVTVSS	236
vhCDR1	<u>GTFSSYA</u>	237
vhCDR2	<u>ISYDGSNK</u>	238
vhCDR3	<u>ASSPIGYSYGYWGGMDV</u>	239
Full length HC	EVQLVESGGGLVQPGRSRLRSCAASGFTFSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYVCASSPIGYSYGYWGGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSWVTPSSSLGTQYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQWYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSEFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK	240
Variable light (vl) domain	QAVLTQPASLSASPGASASLTCTLRSGIDVRTNKIFWYQVKPGSPQHLLTFQSDSDKQQGGSGVPSRFSGSKDASANA GILISGLQSEDEADYYCLIWHTSGWVFGGGTQLTVLG	241
vlCDR1	<u>SGIDVRTNK</u>	242
vlCDR2	<u>FQSDSDK</u>	243
vlCDR3	<u>LIWHTSGWV</u>	244
Full length light chain	QAVLTQPASLSASPGASASLTCTLRSGIDVRTNKIFWYQVKPGSPQHLLTFQSDSDKQQGGSGVPSRFSGSKDASANA GILISGLQSEDEADYYCLIWHTSGWVFGGGTQLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK ADSSPVKAGVETTTPTPSKQSNKYYAASSLTLPTEQWKSRSRSCQVTHEGSTVEKTVAPTECS	245

Figure 38H
CPA.7.011

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVCKVSGYTLT <u>ELSMHWVRQAPGKGLEWMGGFDPE</u> <u>GETIYAQKFQGRVTMTEDTS</u> TDTAYMELSSLRSEDTAVYCATGPAAGVGYYYMDV WGKGTTVTVSS	246
vhCDR1	<u>GYTLTELS</u>	247
vhCDR2	<u>FDPE</u> <u>GET</u>	248
vhCDR3	<u>ATGPAAGVGYYYMDV</u>	249
Full length HC	EVQLVQSGAEVKKPGASVKVCKVSGYTLT <u>ELSMHWVRQAPGKGLEWMGGFDPE</u> <u>GETIYAQKFQGRVTMTEDTS</u> TDTAYMELSSLRSEDTAVYCATGPAAGVGYYYMDVWGKGTTVTVSSASTKGPSVFPPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQYICNVNHPKPSNTKVDKKVEPKSCDKTHTC PPCPAPPELLGGPVFLFPPPKDTLMISRTPEVTCVAVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYVTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSPGK	250
Variable light (vl) domain	DVWMTQSPPLSPVTPGEPASISCRSSQSLYRNGYNYLDWYLQKPGQSPQLLIY <u>LGSN</u> RASGVDRFSGSGSGTDFTLKI SRVEAEDVGVVYCMQALQTPPTFGQGTKEIKR	251
viCDR1	<u>QSLYRNGYNY</u>	252
viCDR2	<u>LGS</u>	253
viCDR3	<u>MQALQTPPT</u>	254
Full length light chain	DVWMTQSPPLSPVTPGEPASISCRSSQSLYRNGYNYLDWYLQKPGQSPQLLIY <u>LGSN</u> RASGVDRFSGSGSGTDFTLKI SRVEAEDVGVVYCMQALQTPPTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSYLSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	255

Figure 38I
CPA7.012

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVETGGGLIQGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLVAVAVISYDGSNKKYYADSVKGRFTISRDNKNT LYLQMNSLRAEDTAVYCCARDVMVYCSSTSCYFYGMVDVWGQGTITVSS	256
vhCDR1	<u>GFTFSSYA</u>	257
vhCDR2	<u>ISYDGSNK</u>	258
vhCDR3	<u>ARDVMVYCSSTSCYFYGMIDV</u>	259
Full length HC	EVQLVETGGGLIQGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLVAVAVISYDGSNKKYYADSVKGRFTISRDNKNT LYLQMNSLRAEDTAVYCCARDVMVYCSSTSCYFYGMVDVWGQGTITVSSASTKGPSVFLAPSSKSTSGGTAALGCL VKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHPKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVAVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPPVLDSGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	260
Variable light (vl) domain	DIQMTQSPSSLSASVGDRVTTCQASQDIRDYLNWYQKKPKGKAPKLLIYDASNLEAGVPSRFGSGGTDFTTISGLQ PEDVATYYCQQFENLPITFGQGTRLEIKR	261
viCDR1	<u>QDIRDY</u>	262
viCDR2	<u>DAS</u>	263
viCDR3	<u>QQFENLPIT</u>	264
Full length light chain	DIQMTQSPSSLSASVGDRVTTCQASQDIRDYLNWYQKKPKGKAPKLLIYDASNLEAGVPSRFGSGGTDFTTISGLQ PEDVATYYCQQFENLPITFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSYSLSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC	265

Figure 38J
CPA.7.013

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGASVKVCKVSGYTLIELSMHWVRQAPGKGLWMMGGFD <u>PEDGETIYAQKFQGRVTMTEDTS</u> TDTAYMELSLRSED ²⁶⁶ TA ²⁶⁷ VVYCATGGYSSGFN ²⁶⁸ YYYMDV ²⁶⁹ <u>WGKGT</u> TVTVSS	266
vhCDR1	<u>GYTLIELS</u>	267
vhCDR2	<u>FDPEDGET</u>	268
vhCDR3	<u>ATGGYSSGFNYYYMDV</u>	269
Full length HC	QVQLVQSGAEVKKPGASVKVCKVSGYTLIELSMHWVRQAPGKGLWMMGGFD <u>PEDGETIYAQKFQGRVTMTEDTS</u> TDTAYMELSLRSED ²⁷⁰ TA ²⁷¹ VVYCATGGYSSGFN ²⁷² YYYMDV ²⁷³ WGKGT ²⁷⁴ TVTVSS ²⁷⁵ KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC PPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVWS VLT ²⁷⁶ VLHQDWLNGKEYCKVSNKALPAPIEKTKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC ²⁷⁷ SCVMHEALHNHYTQKSLSLSPGK	270
Variable light (vl) domain	DVVMTQSP ²⁷⁸ LSLPVTPGEPASISCRSSQSLLYRNGNNYLDWYLQKPGQSPQLLYLGSNRASGVDPDRFSGSGGTDFTLKI SRVEAEDVG ²⁷⁹ VVYCMQALQTPPTFGGGTKLEIKR	271
vlCDR1	<u>QSLLYRNGNNY</u>	272
vlCDR2	<u>LGS</u>	273
vlCDR3	<u>MQALQTPPT</u>	274
Full length light chain	DVVMTQSP ²⁷⁵ LSLPVTPGEPASISCRSSQSLLYRNGNNYLDWYLQKPGQSPQLLYLGSNRASGVDPDRFSGSGGTDFTLKI SRVEAEDVG ²⁷⁶ VVYCMQALQTPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDS ²⁷⁷ STYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC	275

Figure 38K
CPA.7.014

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVCKVSGYTLT <u>ELSMHWVRQAPGKGLEWMGGFD</u> <u>PEDGETIYAQKFQGRVT</u> MTEDTS TDTAYMELSSLRSEDTAVYCATGVTTYYGMDVWGGGTTTVSS	276
vhCDR1	<u>GYTLIELS</u>	277
vhCDR2	<u>FPEDGET</u>	278
vhCDR3	<u>ATGVTTYYGMDV</u>	279
Full length HC	EVQLVQSGAEVKKPGASVKVCKVSGYTLT <u>ELSMHWVRQAPGKGLEWMGGFD</u> <u>PEDGETIYAQKFQGRVT</u> MTEDTS TDTAYMELSSLRSEDTAVYCATGVTTYYGMDVWGGGTTTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSWTPSSSLGTQYICNVNHPKSTKVDKKVEPKSCDKTHTCTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWVVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYCKVSNKALPAPIEKTIKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	280
Variable light (vl) domain	DWMTQSPSLSPVTPGEPASISCRSSQSLLYSNGNFDWYIQKPGQSPRLISLGSNRASGVDPDRFSGSGGTDFTLKI SRVEAEDVGIYYCMQALQTPPTFGGQGTKVEIKR	281
vlCDR1	<u>QSLLYSNGNFD</u>	282
vlCDR2	<u>LGS</u>	283
vlCDR3	<u>MQALQTPPT</u>	284
Full length light chain	DVVMQTSPSLSPVTPGEPASISCRSSQSLLYSNGNFDWYIQKPGQSPRLISLGSNRASGVDPDRFSGSGGTDFTLKI SRVEAEDVGIYYCMQALQTPPTFGGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNAL QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	285

Figure 38L
CPA.7.015

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QITLKESGGGVVQPGGSLRLSCAASGFTFSYGMHWRQAPGKGLEWVAFIRYDGSNKYYADSVKGRFTISRDN SKN TLYLQMNSLRAEDTAVVYCARDLDFWWDGMDVWVGQGTTVTVSS	286
vhCDR1	<u>GFTFSYG</u>	287
vhCDR2	<u>IRYDGSNK</u>	288
vhCDR3	<u>ARDLDFWWDGMDV</u>	289
Full length HC	QITLKESGGGVVQPGGSLRLSCAASGFTFSYGMHWRQAPGKGLEWVAFIRYDGSNKYYADSVKGRFTISRDN SKN TLYLQMNSLRAEDTAVVYCARDLDFWWDGMDVWVGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSWTPVSSSLGTQYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVWSVLT LHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQWYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGK	290
Variable light (vl) domain	EIVLTQSPGTLSLSPGEKATLSCRVSQSVSSMYLAWYQQKPGQAPRLIYGASYRATGIPDRFSGSGSGTDFNLTISRLEP EDFAVYVCQQVWSSPMYTFGLGTLKLEIKR	291
viCDR1	<u>QSVSSMY</u>	292
viCDR2	<u>GAS</u>	293
viCDR3	<u>QQWSSPMYT</u>	294
Full length light chain	EIVLTQSPGTLSLSPGEKATLSCRVSQSVSSMYLAWYQQKPGQAPRLIYGASYRATGIPDRFSGSGSGTDFNLTISRLEP EDFAVYVCQQVWSSPMYTFGLGTLKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLINFPREAKVQWKVDNALQSGN SQESVTEQDSKDSYSTSLSTLTSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	295

Figure 38M
CPA.7.017

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVCKASGGTFNNGISWVRQAPGKGLEWMGQIPLFGTTKYAQKFQGRVTIAADEPTS TAYMELGSLRSEDTAIYYCARDMAADGMAVFDYWGQGTLVTVSS	296
vhCDR1	<u>GGTFNNG</u>	297
vhCDR2	<u>IPLFGTT</u>	298
vhCDR3	<u>ARDMAADGMAVFDY</u>	299
Full length HC	QVQLVQSGAEVKKPGSSVKVCKASGGTFNNGISWVRQAPGKGLEWMGQIPLFGTTKYAQKFQGRVTIAADEPTS TAYMELGSLRSEDTAIYYCARDMAADGMAVFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF PEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWVVDGVEVHNAKTKPREEQYNSTYRVWSVLTVL HQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYVTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSPGK	300
Variable light (vl) domain	QSVLTQPPSVSGTPGQKVIISCSGSSNIGRHFVFWYQQLPGTAPKLLIYKNDERPSGVPDRFSGSKGTSASLAVSGLRS EDEADYYCSSWDAALNGVWFGGGTKLTVLG	301
viCDR1	<u>SSNIGRHF</u>	302
viCDR2	<u>KND</u>	303
viCDR3	<u>SSWDAALNGVW</u>	304
Full length light chain	QSVLTQPPSVSGTPGQKVIISCSGSSNIGRHFVFWYQQLPGTAPKLLIYKNDERPSGVPDRFSGSKGTSASLAVSGLRS EDEADYYCSSWDAALNGVWFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPV KAGVETTTPSKQSNINKYAASSYLSLTPEQWKSHRYSYSCQVTHEGSTVEKTVAPTECS	305

Figure 38N
CPA.7.018

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVCKVSGYTLTSLMHWVRQAPGKGLWVGDFDPEDGETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYCATTEVPMVRGARRYYMDVWGKGTITVSS	306
vhCDR1	<u>GYTLTSL</u>	307
vhCDR2	<u>FDPEDGET</u>	308
vhCDR3	<u>ATEVPMVRGARRYYMDV</u>	309
Full length HC	EVQLVQSGAEVKKPGASVKVCKVSGYTLTSLMHWVRQAPGKGLWVGDFDPEDGETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYCATTEVPMVRGARRYYMDVWGKGTITVSSASTKGPSVFPPLAPSSKTSGGTAALGC LVKDYFPEPVTVSWSGALTSGVHTFPAVLQSSGLYSLSVWTVPSLSLTQTYICNVNHPKSNKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVAVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTIKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	310
Variable light (vl) domain	DVVMTQSPFLAVTPGEPASISCRSSQTLLYINENNYLDWVYQKPGQSPQLLIYLGSTRASGVDPDRFSGGGSGTDFTLTI SRVEAEDVGLYYCMQGLQTPPTFGQGTREIKR	311
viCDR1	<u>QTLLYINENNY</u>	312
viCDR2	<u>LGS</u>	313
viCDR3	<u>MQGLQTPPT</u>	314
Full length light chain	DVVMTQSPFLAVTPGEPASISCRSSQTLLYINENNYLDWVYQKPGQSPQLLIYLGSTRASGVDPDRFSGGGSGTDFTLTI SRVEAEDVGLYYCMQGLQTPPTFGQGTREIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSSTLSKADYEKHKVACEVTHQGLSSPVTKSFNRGEC	315

Figure 380
CPA.7.019

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQESGPGLVKSETLSLTCSVSGGISNSNYWGIRPPGKGLEWIGGIYSGSTYYNPSLESRTISEDTSKNQIS LKLSSVTAADTAVVYCARGAWELSLGDWFDPPWGPGLTVTVSS	316
vhCDR1	<u>GGISNSNY</u>	317
vhCDR2	<u>IYSGST</u>	318
vhCDR3	<u>ARGAWELSLGDWFDPP</u>	319
Full length HC	QVQLQESGPGLVKSETLSLTCSVSGGISNSNYWGIRPPGKGLEWIGGIYSGSTYYNPSLESRTISEDTSKNQIS LKLSSVTAADTAVVYCARGAWELSLGDWFDPPWGPGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWVVDGVEVHNAKTPREEQYNSTYRVVSVLTVLH QDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	320
Variable light (vl) domain	QSVLTQPPSVSGAPGQRVTISCTGSSNIGAGYDIQWYQLPGTAPKLLIYGNNNRPSPGVDRFSGSRGTSASLAITGL QAEDEADYYCQSYDSSLSVYVVFGGGTQLTVLG	321
vlCDR1	<u>SSNIGAGYD</u>	322
vlCDR2	<u>GNN</u>	323
vlCDR3	<u>QSYDSSLSVYV</u>	324
Full length light chain	QSVLTQPPSVSGAPGQRVTISCTGSSNIGAGYDIQWYQLPGTAPKLLIYGNNNRPSPGVDRFSGSRGTSASLAITGL QAEDEADYYCQSYDSSLSVYVVFGGGTQLTVLGPQKAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSS PVKAGVETTTTPSKQSNKNKYAASSYLSTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS	325

Figure 38P
CPA.7.021

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVESGGGVVKPGGSLRLSCAASGFTGTSSMNWVRQAPGKGLEWVAVISFDGTEIHVADSVKGRFTISRDNKST VFLQMNSLRPDDTALYYCAKSGNIYFSGMDVVGQGTITVTVSS	326
vhCDR1	<u>GFTFGTSS</u>	327
vhCDR2	<u>ISFDGTEI</u>	328
vhCDR3	<u>AKSGNIYFSGMDV</u>	329
Full length HC	EVQLVESGGGVVKPGGSLRLSCAASGFTGTSSMNWVRQAPGKGLEWVAVISFDGTEIHVADSVKGRFTISRDNKST VFLQMNSLRPDDTALYYCAKSGNIYFSGMDVVGQGTITVTVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSWTVPSSSLGTQTYICNVNHPKSNKVDKKVEPKSCDKTHTCTPCP APELLGGPSVFLFPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWVDGVEVHNKTKPREEQYNSTYRVWSVLTVL HQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPGK	330
Variable light (vl) domain	DIQMTQSPSTLSASVGDRVTITCRAGQSIGWLAWFQQKPGKAPNLLIYETISTLESQVPSRFGSGSGTEYTLTISSLQP DDFATYYCQQYYSYPLTFGQGTKEIKR	331
vlCDR1	<u>QSIGW</u>	332
vlCDR2	<u>ETS</u>	333
vlCDR3	<u>QQYYSYPLT</u>	334
Full length light chain	DIQMTQSPSTLSASVGDRVTITCRAGQSIGWLAWFQQKPGKAPNLLIYETISTLESQVPSRFGSGSGTEYTLTISSLQP DDFATYYCQQYYSYPLTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSYSTLSLSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC	335

Figure 38Q
CAP.7.022

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVCKVSGYTLT <u>ELSMHWVRQAPGKGL</u> EWMGGFD <u>PEDGETIYAQKFQGRVTMTEDTS</u> TDTAYMELSSLRSEDTAVYYCATGVPAAIGVYYYYMDVWVGKGTTVTVSS	336
vhCDR1	<u>GYTLIELS</u>	337
vhCDR2	<u>FDPEDGETI</u>	338
vhCDR3	<u>ATGVPAAIGVYYYYMDV</u>	339
Full length HC	EVQLVQSGAEVKKPGASVKVCKVSGYTLT <u>ELSMHWVRQAPGKGL</u> EWMGGFD <u>PEDGETIYAQKFQGRVTMTEDTS</u> TDTAYMELSSLRSEDTAVYYCATGVPAAIGVYYYYMDVWVGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTPSSSLGTQYICNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPPVLDSDGSEFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	340
Variable light (vl) domain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYSNGYNYLDWYLQPKPGQSPQLLISLGSNRSAGVDPDRFSGSGSGTDFTLKI SRVEAEDVGYYCYCMQALQSPVTFGGGTKEIKR	341
vlCDR1	<u>QSLLYSNGYNY</u>	342
vlCDR2	<u>LGS</u>	343
vlCDR3	<u>MQALQSPVT</u>	344
Full length light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLLYSNGYNYLDWYLQPKPGQSPQLLISLGSNRSAGVDPDRFSGSGSGTDFTLKI SRVEAEDVGYYCYCMQALQSPVTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSSTLSLSTLTSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC	345

Figure 38R
CPA.7.023

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVCKVSGYTLT <u>ELSMHWVRQAPGKGLEWMGGFDPE</u> <u>DG</u> ETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYYCATDSRDGPAARGGYYYMDVWGGQTTTVSS	346
vhCDR1	<u>GYTLIELS</u>	347
vhCDR2	<u>FDPE</u> <u>DGET</u>	348
vhCDR3	<u>ATDSRDGPAARGGYYYMDV</u>	349
Full length HC	EVQLVQSGAEVKKPGASVKVCKVSGYTLT <u>ELSMHWVRQAPGKGLEWMGGFDPE</u> <u>DGET</u> IYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYYCATDSRDGPAARGGYYYMDVWGGQTTTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVAVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR WVSFLTLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	350
Variable light (vl) domain	DVWMTQSPSLSPVTLGQPASISCRSSQSLLYINGNYLDWYLQKPGQSPQLLYL <u>LGS</u> NRASGVPDRFSGSGSGTDFTLKI SRVEAEDVGVVYCMQALQTPPTFGQGTKEIKR	351
viCDR1	<u>QSLLYINGNY</u>	352
viCDR2	<u>LGS</u>	353
viCDR3	<u>MQALQTPPT</u>	354
Full length light chain	DVWMTQSPSLSPVTLGQPASISCRSSQSLLYINGNYLDWYLQKPGQSPQLLYL <u>LGS</u> NRASGVPDRFSGSGSGTDFTLKI SRVEAEDVGVVYCMQALQTPPTFGQGTKEIKRTVAAPSVFIPPSDEQLKSGTASVVCLLNNFYFPREAKVQWKVDNA LQSGNSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	355

Figure 38S
CPA.7.024

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGSSVKVCKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDAYYYDSSGYNPD ~~AFDI~~ WGQGTMTVSS	356
vhCDR1	<u>GGTFSSYA</u>	357
vhCDR2	<u>IPIFGTA</u>	358
vhCDR3	<u>ARDAYYYDSSGYNPD~~AFDI~~</u>	359
Full length HC	EVQLVQSGAEVKKPGSSVKVCKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDAYYYDSSGYNPD ~~AFDI~~ WGQGTMTVSSASTKGPSVFLAPSSKTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHPKPSNTKVDKKVEPKSCDKHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLIHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	360
Variable light (vl) domain	DVVMTQSP ~~LS~~ LPVTPGEPASISCRSSQSL ~~L~~ HSNGYNYLDWYLQKPGQSPQLLIYLGSSRASGVPDRFSGSVSGTDFTLKISRVEAEDVGYYCMQGLQTPRTFGRGTKLEIKR	361
vlCDR1	<u>QSL~~L~~HSNGYNY</u>	362
vlCDR2	<u>LGS</u>	363
vlCDR3	<u>MQGLQTPRT</u>	364
Full length light chain	DVVMTQSP ~~LS~~ LPVTPGEPASISCRSSQSL ~~L~~ HSNGYNYLDWYLQKPGQSPQLLIYLGSSRASGVPDRFSGSVSGTDFTLKISRVEAEDVGYYCMQGLQTPRTFGRGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWVKVDNALQSGNSQESVTEQDSKDSYSTLSLSTLTLSKADYEKHKVYACEVTHQGLSSLSPVTKSFNRGEC	365

Figure 38T

CPA.7.033

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVCKASGGTFSSAISWVRQAPGQGFEWMGGIPIYGITDYAQKFQGRVTITDESTST AYMELSSLTSED ~~TA~~ VVYCARDDTARRVRGVPPYY ~~Y~~ AMDVWVGQGT ~~TV~~ TVSS	366
vhCDR1	<u>GGTFSSA</u>	367
vhCDR2	<u>IPIYGIT</u>	368
vhCDR3	<u>ARDDTARRVRGVPPYY~~Y~~AMDV</u>	369
Full length HC	QVQLVQSGAEVKKPGSSVKVCKASGGTFSSAISWVRQAPGQGFEWMGGIPIYGITDYAQKFQGRVTITDESTST AYMELSSLTSED ~~TA~~ VVYCARDDTARRVRGVPPYY ~~Y~~ AMDVWVGQGT ~~TV~~ TVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYPEPVT ~~VS~~ WN ~~SG~~ ALTS ~~GV~~ HTFPAVLQSSGLYSLSSV ~~TV~~ PSSSLGTQTYICNVN ~~HK~~ PSNTKVDKKVEPKSCDK ~~TH~~ TCPPCPAPELLGGPSVFLPPKPKD ~~TL~~ MISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN ~~AK~~ TKPREEQYNSTYRV VSVLT ~~VL~~ HQDWLNGKEYCKVSNKALPAPIEK ~~TS~~ KAKGQPREPQVY ~~TL~~ PPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYK ~~TT~~ PPVLDSDGSFFLYSKLTVDKSRWQQGNV ~~F~~ SCSV ~~M~~ HEALHNHYTQKSLSLSPGK	370
Variable light (vl) domain	ETTLTQSPAFMSATPGDEVNISKASQDIDDDVSWYQQKPGGAPIFLIQEASTLVP ~~GI~~ PPRFGSGFGTDFTLTKNMES EDAA ~~Y~~ YFCLQH ~~DN~~ LN ~~PL~~ TFGGG ~~TK~~ V ~~DI~~ KR	371
vlCDR1	<u>QDIDDD</u>	372
vlCDR2	<u>EAS</u>	373
vlCDR3	<u>LQHDN~~LN~~PLT</u>	374
Full length light chain	ETTLTQSPAFMSATPGDEVNISKASQDIDDDVSWYQQKPGGAPIFLIQEASTLVP ~~GI~~ PPRFGSGFGTDFTLTKNMES EDAA ~~Y~~ YFCLQH ~~DN~~ LN ~~PL~~ TFGGG ~~TK~~ V ~~DI~~ KRTVAAPSVFIFPPSDEQLKSGTASV ~~V~~ CL ~~LN~~ FN ~~FP~~ REAKVQWKVDNALQSGN SQESVTEQDSKDS ~~TS~~ LS ~~SL~~ STL ~~SK~~ ADY ~~E~~ KHKVYACEVTHQGLSPV ~~TK~~ SFNRGEC	375

Figure 38U

CPA.7.034

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVCKVSGYTLTSLMHWVVRQAPGKGLWVGDFPEDGETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYCATDPGPVAGPYYYGMDVWVGQGTITVTVSS	376
vhCDR1	<u>GYTLTSL</u>	377
vhCDR2	<u>FDPEDGET</u>	378
vhCDR3	<u>ATEDPGPVAGPYYYGMDV</u>	379
Full length HC	EVQLVQSGAEVKKPGASVKVCKVSGYTLTSLMHWVVRQAPGKGLWVGDFPEDGETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYCATDPGPVAGPYYYGMDVWVGQGTITVTVSSASTKGPSVFLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGLVHTFPAVLQSSGLYSLSVTVPSSSLGTQTYICNVNHPKSNKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQWYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPGK	380
Variable light (vl) domain	DVVMTQSPLVLPVTPGEPASISCRSSQSLLYINGHYLDWVYVQRPQGSPQLLIFLGSTRASGVPDFRSGSGSGTDFLTLEIS KVEAEDVGIFYFCMQALQTPPTFGGGTKVEIKR	381
vlCDR1	<u>QSLLYINGHY</u>	382
vlCDR2	<u>LGS</u>	383
vlCDR3	<u>MQALQTPPT</u>	384
Full length light chain	DVVMTQSPLVLPVTPGEPASISCRSSQSLLYINGHYLDWVYVQRPQGSPQLLIFLGSTRASGVPDFRSGSGSGTDFLTLEIS KVEAEDVGIFYFCMQALQTPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC	385

Figure 38V

CPA.7.036

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPYIGITDYAQKFQGRVTITTDESTST AYMELSLTSED ~~AVVYCARD~~ DTARRVRGV ~~PYYYYAMD~~ VWGQGT ~~TVVSS~~	386
vhCDR1	<u>GGTFSSA</u>	387
vhCDR2	<u>IPIYGIT</u>	388
vhCDR3	<u>ARDDTARRVRGV~~PYYYYAMD~~V</u>	389
Full length HC	QVQLVQSGAEVKKPGSSVKVCKASGGTFSSSAISWVRQAPGQGFEWMGGIIPYIGITDYAQKFQGRVTITTDESTST AYMELSLTSED ~~AVVYCARD~~ DTARRVRGV ~~PYYYYAMD~~ VWGQGT ~~TVVSS~~ ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV ~~PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH~~ TCPPCPAPELLGGPSVFLPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLT ~~VLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW~~ ESNGQPENNYKTT ~~PPVLDSDGSFFLYSKLTVDKSRWQQGNV~~ FSCSV ~~MHEALHNHYTQKSLSLSPGK~~	390
Variable light (vl) domain	DIVMTQTPLSLPVTPGEPASISCRPSQSLDSDDDGNTYLDWYLQKPGQSPQLLIHTLSYRASGV ~~PDRFSGSGSGTDFTLK~~ I SRVEAEDVG ~~VVYCMQRLQFPLTFGGG~~ TKVEIKR	391
vlCDR1	<u>QSLDSDDGNTY</u>	392
vlCDR2	<u>ILS</u>	393
vlCDR3	<u>MQRLQFPLT</u>	394
Full length light chain	DIVMTQTPLSLPVTPGEPASISCRPSQSLDSDDGNTYLDWYLQKPGQSPQLLIHTLSYRASGV ~~PDRFSGSGSGTDFTLK~~ I SRVEAEDVG ~~VVYCMQRLQFPLTFGGG~~ TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDS ~~TYSL~~ STLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC	395

Figure 38W

CPA.7.040

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVCKVSGYTLTELSMHWVVRQAPGKGLWVGDFPEDGETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYCATGVPAALGVVYVYMDVWGKGTITVTVSS	396
vhCDR1	<u>GYTLTELS</u>	397
vhCDR2	<u>FDPEDGET</u>	398
vhCDR3	<u>ATGVPAALGVVYVYMDV</u>	399
Full length HC	EVQLVQSGAEVKKPGASVKVCKVSGYTLTELSMHWVVRQAPGKGLWVGDFPEDGETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYCATGVPAALGVVYVYMDVWGKGTITVTVSSASTKGPSVFPPLAPSSKSTSGGTAALGCL VKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHPKSNKVDKKVEPKSCDKTH TCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	400
Variable light (vl) domain	DVVMQTQSPSLPVTGPGEPAISCRSSQSLLYRNGYNYLDWYLQKPGQSPQLLIYWGSYRASGVPDRFSGSGGTDFLK ISRVEAEDGVVYVYCMQAVQNPPITFGQGTGVKDIKR	401
vlCDR1	<u>QSLLYRNGYNY</u>	402
vlCDR2	<u>WGS</u>	403
vlCDR3	<u>MQAVQNPPIT</u>	404
Full length light chain	DVVMQTQSPSLPVTGPGEPAISCRSSQSLLYRNGYNYLDWYLQKPGQSPQLLIYWGSYRASGVPDRFSGSGGTDFLK ISRVEAEDGVVYVYCMQAVQNPPITFGQGTGVKDIKRVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	405

Figure 38X

CPA.7.046

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKPGSSVKVCKASGGTFSSSAISWVRQAPGQGFEWMGGIPIYGITDYAQKFQGRVTITTDESTST AYMELSLTSED ~~TA~~ VVYCARDDTARRVRGVPPYYVAMDVWGQGT ~~TV~~ TVSS	406
vhCDR1	<u>GGTFSSA</u>	407
vhCDR2	<u>IPIYGIT</u>	408
vhCDR3	<u>ARDDTARRVRGVPPYYVAMDV</u>	409
Full length HC	QVQLVQSGAEVKPGSSVKVCKASGGTFSSSAISWVRQAPGQGFEWMGGIPIYGITDYAQKFQGRVTITTDESTST AYMELSLTSED ~~TA~~ VVYCARDDTARRVRGVPPYYVAMDVWGQGT ~~TV~~ TVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYPEPVT ~~VS~~ WNSGALTSGVHTFPAVLQSSGLYSLSSVTV ~~PS~~ SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLT ~~VL~~ HQDWLNGKEYCKVSNKALPAPIEKTIKAKGQPREPQVY ~~TV~~ TPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYK ~~TT~~ PPVLDSDGFFLYSKLTVDKSRWQQGNV ~~FC~~ SCVMHEALHNHYTQKSLSLSPGK	410
Variable light (vl) domain	DWMTQSPAF ~~LS~~ VPGERVTL ~~SC~~ KASQ ~~TM~~ MNNY ~~LA~~ WYQQKPGQAPRL ~~LI~~ YDAST ~~RA~~ TDTTPRFSGSGSGTEFTLTISV QSEDFALY ~~YC~~ Q ~~Q~~ YGDWLPITFGQGRLEIKR	411
vlCDR1	<u>QTMNNY</u>	412
vlCDR2	<u>DAS</u>	413
vlCDR3	<u>QQYGDWLPIT</u>	414
Full length light chain	DVMTQSPAF ~~LS~~ VPGERVTL ~~SC~~ KASQ ~~TM~~ MNNY ~~LA~~ WYQQKPGQAPRL ~~LI~~ YDAST ~~RA~~ TDTTPRFSGSGSGTEFTLTISV QSEDFALY ~~YC~~ Q ~~Q~~ YGDWLPITFGQGRLEIKR ~~TV~~ AAAPSVFIPPSDEQLKSGTASV ~~W~~ CLLN ~~NF~~ YPREAKVQWKVDNALQS GNSQESVTEQDSKDSYSLSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC	415

Figure 38Y

CPA.7.047

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVSGYTLTSLMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYYCATAFPEATISYYYMDVWVGKGTITVSS	416
vhCDR1	<u>GYTLTSL</u>	417
vhCDR2	<u>FDPEDGET</u>	418
vhCDR3	<u>ATAFPEATISYYYMDV</u>	419
Full length HC	EVQLVQSGAEVKKPGASVKVSGYTLTSLMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYYCATAFPEATISYYYMDVWVGKGTITVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKD YFPEPTVSWNSGALTSVHTFPAVLQSSGLYSLSVVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLT VLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGK	420
Variable light (vl) domain	DVVMTQSPSLPVTGPGEPAISCRSSQSLLYRNGYNYLDWYLQKPGQSPQLLIYWGSYRASGVDPDRFSGSGGTDFTLK ISRVEAEDVGYYCMQAVQNPPPTFGQGTKVEIKR	421
vlCDR1	<u>QSLLYRNGYNY</u>	422
vlCDR2	<u>WGS</u>	423
vlCDR3	<u>MQAVQNPPPT</u>	424
Full length light chain	DVVMTQSPSLPVTGPGEPAISCRSSQSLLYRNGYNYLDWYLQKPGQSPQLLIYWGSYRASGVDPDRFSGSGGTDFTLK ISRVEAEDVGYYCMQAVQNPPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC	425

Figure 38Z

CPA.7.049

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QMLVQSGAEVKKPGSSVKVCKASGGTFSSAISWVRQAPGQGFEWMGGIPIYGITDYAQKFQGRVTITTDESTST AYMELSSLTSED ~~TAVVYCARDDTARRVRGVPY~~ YYYAMDVWGGGTTVTVSS	426
vhCDR1	<u>GGTFSSA</u>	427
vhCDR2	<u>IPIYGIT</u>	428
vhCDR3	<u>ARDDTARRVRGVPYYYYAMDV</u>	429
Full length HC	QMLVQSGAEVKKPGSSVKVCKASGGTFSSAISWVRQAPGQGFEWMGGIPIYGITDYAQKFQGRVTITTDESTST AYMELSSLTSED ~~TAVVYCARDDTARRVRGVPY~~ YYYAMDVWGGGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVWTVPSSSLGTQTYICNVNHPKSNKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKD ~~TL~~ MISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTSKAKGQPREPQVWTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFC ~~SV~~ MHEALHNHYTQKSLSLSPGK	430
Variable light (vl) domain	DIVMTQTPLSLPVTPGEPASMSCSRSLDSDDGNT ~~HL~~ DWYLQKPGQSPQLLIQSLSYRASGV ~~PD~~ RFSGSGSGTDFTL EISRVEAEDVGIYYC <u>MQRKEFPLTF</u> GGGKVEIKR	431
vlCDR1	<u>RSLLDSDDGNTH</u>	432
vlCDR2	<u>SLS</u>	433
vlCDR3	<u>MQRKEFPLT</u>	434
Full length light chain	DIVMTQTPLSLPVTPGEPASMSCSRSLDSDDGNT ~~HL~~ DWYLQKPGQSPQLLIQSLSYRASGV ~~PD~~ RFSGSGSGTDFTL EISRVEAEDVGIYYC <u>MQRKEFPLTF</u> GGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPR ~~EAKVQWKVDN~~ ALQSGNSQESVTEQDSKSDSTYSLSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	435

Figure 38AA

CPA.7.050

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGGGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTST AYMELSSLRSEDTAVYYCARGPWYDSSGYSSAYYMDVWGQGTITVTVSS	436
vhCDR1	<u>GGTFSSYA</u>	437
vhCDR2	<u>IIPIFGTA</u>	438
vhCDR3	<u>ARGPWYDSSGYSSAYYMDV</u>	439
Full length HC	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGGGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTST AYMELSSLRSEDTAVYYCARGPWYDSSGYSSAYYMDVWGQGTITVTVSSASTKGPSVFPPLAPSSKSTSGGTAALGCL VKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHHKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTSKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPGK	440
Variable light (vl) domain	DVVMTQSPSLPVTGPGEPAISCRSSQSLHSDGYNLYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKI SRVEAEDVGYYCMQALHTPGVTFGGGTKVEIKR	441
vlCDR1	<u>QSLHSDGYNLY</u>	442
vlCDR2	<u>LGS</u>	443
vlCDR3	<u>MQALHTPGVT</u>	444
Full length light chain	DVVMTQSPSLPVTGPGEPAISCRSSQSLHSDGYNLYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKI SRVEAEDVGYYCMQALHTPGVTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSSTYSLSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	445

Figure 39A
CPA.7.028

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QLQLQESGPGLVKPSSETLSLTCTVTGGSISSSYYWAWIRQPPGKGLEWIGGIYSGSTYNNVLSLRVTISQDTSKNQFS LKLTSTVAADTAVVYCARGAWELRLGDWFDPPWGQGTILTVSS	446
vhCDR1	GGSISSSSYY	447
vhCDR2	IYSGST	448
vhCDR3	ARGAWELRLGDWFDPP	449
Full length HC	QLQLQESGPGLVKPSSETLSLTCTVTGGSISSSYYWAWIRQPPGKGLEWIGGIYSGSTYNNVLSLRVTISQDTSKNQFS LKLTSTVAADTAVVYCARGAWELRLGDWFDPPWGQGTILTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGPSVFLFPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVWVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	450
Variable light (vl) domain	QSVLTQPPSVSGAPGQRVTISCTGSSNIGAGYDIQWYQQLPGTAPKLLIYGYSNRPSGVPPDRFSGSKSGTSASIAITGL QAEDEADYYCQSYDSSLSVWVFGGGTQLTVLGQPKAA	451
vlCDR1	SSNIGAGYD	452
vlCDR2	GYS	453
vlCDR3	QSYDSSLSVWV	454
Full length light chain	QSVLTQPPSVSGAPGQRVTISCTGSSNIGAGYDIQWYQQLPGTAPKLLIYGYSNRPSGVPPDRFSGSKSGTSASIAITGL QAEDEADYYCQSYDSSLSVWVFGGGTQLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSS PVKAGVETITTPSKQSNKNKYAASSYLSTLTPQWKSHRSYSCQVTHEGSTVEKTVAPTECS	455

Figure 39B
CPA.7.030

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQESGGGVVQPGGSLRLSCAASRFTFEDYAMHWVRQPPGKGLVWVSGISWKS GGIN YADSVKGRFTISRDN AQ NSLYLQMNSLRAEDTALYVCVKDPTLVATDRAFNWGGQTMVTYSS	456
vhCDR1	RFTFEDYA	457
vhCDR2	ISWKSGGI	458
vhCDR3	VKDPTLVATDRAFNI	459
Full length HC	QVQLQESGGGVVQPGGSLRLSCAASRFTFEDYAMHWVRQPPGKGLVWVSGISWKS GGIN YADSVKGRFTISRDN AQ NSLYLQMNSLRAEDTALYVCVKDPTLVATDRAFNWGGQTMVTYSSASTKGPSVFPLAPSSKTSGGTAALGCLVKDY FPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVWTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCTPPC PAPELLGGPSVFLFPPKPKDITLMISRTPETVCWVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTQKSLSPGK	460
Variable light (vl) domain	DVVMTQSPLSLPVTPGEPASISCRSSQSLHSGNYLDWYLVKPGQSPQLLIYLGSNRASGVDPDRFSGSGGTDFTLKI SRVEAEDVGYYVYCMQYLQTPDTFGQGTKEIKRAAPS	461
vlCDR1	QSLHSGNYNY	462
vlCDR2	LGS	463
vlCDR3	MQYLQTPDT	464
Full length light chain	DVVMTQSPLSLPVTPGEPASISCRSSQSLHSGNYLDWYLVKPGQSPQLLIYLGSNRASGVDPDRFSGSGGTDFTLKI SRVEAEDVGYYVYCMQYLQTPDTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVCLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC	465

Figure 39C
CPA.7.041

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVCKASGGTFSSAISWVRQAPGQGFEWMGGIPIYGITDYAQKFQGRVTITTDESTST AYMELSLTSEDYAVVYCARDDETARRVRGVPIYYAMDVWGQGTITVTVSS	466
vhCDR1	GGTFSSA	467
vhCDR2	IPIYGIT	468
vhCDR3	ARDDTARRVRGVPIYYAMDV	469
Full length HC	QVQLVQSGAEVKKPGSSVKVCKASGGTFSSAISWVRQAPGQGFEWMGGIPIYGITDYAQKFQGRVTITTDESTST AYMELSLTSEDYAVVYCARDDETARRVRGVPIYYAMDVWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSEFLYSLKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	470
Variable light (vl) domain	EIVLTQSPDFQSVTPKEKVTITCRASQNISSSLHWYQQKPGQSPKLLINYSQSFSGVPSRFGSGGTDFTLTIDSLPE DAATYFCHQSSSLPTFGGGTKVEIRRTVAAPS	471
vlCDR1	QNIDSS	472
vlCDR2	YAS	473
vlCDR3	HQSSSLPT	474
Full length light chain	QSVLTQPPSVSGAPGQRVTISCTGSSNIGAGYDIQWYQQLPGTAPKLLIYGYSNRPSGVDPDRFGSGSGTSASLAITGL QAEDEADYYCQSYDSSLVYVFGGGTQLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAMKADSS PVKAGVETTTTPSKQSNKNKYAASSYLSTLPEQWKSRSYSCQVTHEGSTVEKTVAPTECS	475

Figure 39D
CPA.7.016

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGSSVKVCKTSGGTSSAISWVRQAPGQGFEWMGGIPIYGITDYAQKFQGRVTITTDSTSTAYMELSSLTSEDYAVYCARDDTARRVRGVPYPPYYAMDVWGQGTITVTVSS	476
vhCDR1	GGTFSSA	477
vhCDR2	IPIYGIT	478
vhCDR3	ARDDTARRVRGVPYPPYYAMDV	479
Full length HC	EVQLVQSGAEVKKPGSSVKVCKTSGGTSSAISWVRQAPGQGFEWMGGIPIYGITDYAQKFQGRVTITTDSTSTAYMELSSLTSEDYAVYCARDDTARRVRGVPYPPYYAMDVWGQGTITVTVSSASTKGPSVFPPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHPKPSNTKVDKKEPKSCDKHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPGK	480
Variable light (vl) domain	EIVLTQSPGTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLIYDASNRRATGIPARFSGSGSGTDFTLTISLQSEDFAVYYCQYDDWPQTFGGQGRLEIKRTVAAPS	481
vlCDR1	QSVSSY	482
vlCDR2	DAS	483
vlCDR3	QQYDDWPQT	484
Full length light chain	EIVLTQSPGTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLIYDASNRRATGIPARFSGSGSGTDFTLTISLQSEDFAVYYCQYDDWPQTFGGQGRLEIKRTVAAPSVFIIPPSDEQLKSGTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTSLSTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	485

Figure 39E
CPA.7.020

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QMLVQSGAEVKPGSSVKVSCKASGGTFSSAISWVRQAPGQGFWMGGIPIYGITDYAQKFQGRVTITDES TSTAYMELSLTSEDYAVYCARDDTARRVRGVPYVYVYAMDVWGGQTTTVSS	486
vhCDR1	GGTFSSA	487
vhCDR2	IPIYGIT	488
vhCDR3	ARDDTARRVRGVPYVYVYAMDV	489
Full length HC	QMLVQSGAEVKPGSSVKVSCKASGGTFSSAISWVRQAPGQGFWMGGIPIYGITDYAQKFQGRVTITDES TSTAYMELSLTSEDYAVYCARDDTARRVRGVPYVYVYAMDVWGGQTTTVSSASTKGPSVFLPAPSKSTSGG TAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHNKPSNTKVDKKV EPKCDKHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTSKAKGQPREPQVYTLPPSREEMTKNQVSLTLC LVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSL SLSPGK	490
Variable light (vl) domain	EIVMTQSPATLSLTGERATLFCRTSQNVYGEVAWYQQKPGQAPRLLIYDTFERAAGIPAKFSGSGGTDFTLTISR VEPEDFAVYCCQRRDWPITFGQGRLEIKRTVAAPS	491
viCDR1	QNVYGE	492
viCDR2	DTF	493
viCDR3	QQRDWPIT	494
Full length light chain	EIVMTQSPATLSLTGERATLFCRTSQNVYGEVAWYQQKPGQAPRLLIYDTFERAAGIPAKFSGSGGTDFTLTISR VEPEDFAVYCCQRRDWPITFGQGRLEIKRTVAAPSFIFFPSDEQLKSGTASVCLLNINFYPRKAVQWKVDN ALQSGNSQESVTEQDSKDSYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	495

Figure 39F
CPA.7.038

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLVQSGAEVKKPGSSVKVCKASGGTFSSAISWVRQAPGQGFWMGGIPIYGITDYAQKFQGRVTITTTDESTST AYMELSLTSEDYAVYCARDDTARRVRGVPIYYYAMDVWVGQGTITVSS	496
vhCDR1	GGTFSSA	497
vhCDR2	IPIYGIT	498
vhCDR3	ARDDTARRVRGVPIYYYAMDV	499
Full length HC	QVQLVQSGAEVKKPGSSVKVCKASGGTFSSAISWVRQAPGQGFWMGGIPIYGITDYAQKFQGRVTITTTDESTST AYMELSLTSEDYAVYCARDDTARRVRGVPIYYYAMDVWVGQGTITVSSASTKGPSVFLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCWVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSVMHREALHNYHTQKSLSPGK	500
Variable light (vl) domain	DIQMTQSPSSLSASVGDRVTITCQASRDISDSLSWYQQKPKAPKLLIFDASNLKGTGVSSRFGSGSGTDFTFTISSLQPE DIATYYCHQYDNLPLTFGGGKVEIKRTVAAPS	501
vlCDR1	RDISDS	502
vlCDR2	DAS	503
vlCDR3	HQYDNLPLT	504
Full length light chain	DIQMTQSPSSLSASVGDRVTITCQASRDISDSLSWYQQKPKAPKLLIFDASNLKGTGVSSRFGSGSGTDFTFTISSLQPE DIATYYCHQYDNLPLTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFVPREAKVQWVKVDNALQSGNSQ ESVTEQDSKDSYSTSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	505

Figure 39G
CPA.7.044

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QMLVQSGAEVKPGSSVKVCKASGGTFSSSAISWVRQAPGQGFEWMGGIPIYGITDYAQKFQGRVTITTDESTST AYMELSSLTSEDYAVVYCARDDETARRVRGVPYVYYAMDVWGQGTITVTVSS	506
vhCDR1	GGTFSSA	507
vhCDR2	IPIYGIT	508
vhCDR3	ARDDTARRVRGVPYVYYAMDV	509
Full length HC	QMLVQSGAEVKPGSSVKVCKASGGTFSSSAISWVRQAPGQGFEWMGGIPIYGITDYAQKFQGRVTITTDESTST AYMELSSLTSEDYAVVYCARDDETARRVRGVPYVYYAMDVWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHNKPSNTKVDDKKVEPKSCDKTH TCPPCPAPELLGSPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTPREEQYNSTYRV VSVLT/LHQDWLNGKEYCKVSNKALPAPIEKTIKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTPPVLDSDGSGFFLYSKLTVDKSRWQQGNVFSCVMEALHNHYTQKSLSLSPGK	510
Variable light (vl) domain	EIVMTQSPATLSLSPGERATLSCRASESVTTFLAWYQQKPGQAPRLITDASNRTATGIPGRFGSGSGTDFTLTISSELEPE DFAVYYCHQHTNWPLTFGGGKLEIKRTVAAPS	511
vlCDR1	ESVTTF	512
vlCDR2	DAS	513
vlCDR3	HQHTNWPLT	514
Full length light chain	EIVMTQSPATLSLSPGERATLSCRASESVTTFLAWYQQKPGQAPRLITDASNRTATGIPGRFGSGSGTDFTLTISSELEPE DFAVYYCHQHTNWPLTFGGGKLEIKRTVAAPSVFIPSPDEQLKSGTASVCLLNFPYFPAKVKQWVDNALQSGNS QESVTEQDSKDYSTLSLTLSKADYEEKHKVYACEVTHQGLSPVTKSFNRGEC	515

Figure 39H
CPA.7.045

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLVQSGAEVKKPGASVKVCKVSGYTLTSLMHWVRQAPGKGLEWMGGFDPEDGETIYAKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYCATTEVGYCSGGSCYISYYGMDVWVGQGTITVSS	516
vhCDR1	GYTLTSL	517
vhCDR2	FDPEDGET	518
vhCDR3	ATEVGYCSGGSCYISYYGMDV	519
Full length HC	EVQLVQSGAEVKKPGASVKVCKVSGYTLTSLMHWVRQAPGKGLEWMGGFDPEDGETIYAKFQGRVTMTEDTS TDTAYMELSSLRSEDTAVYCATTEVGYCSGGSCYISYYGMDVWVGQGTITVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK THTCPPCPAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR WVSLT/LHQQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	520
Variable light (vl) domain	DVVMTQSPSLPVTGPGEPAISCRSSQSLLYRNGHNFLDWVYQKPGQSPQLLIYLGSNRASGVDPDRFSGSGGTDFTLKI SRVEAEDVGVYYCMQALQTPPTFGQGTKEIKRTVAAPS	521
vlCDR1	QSLLYRNGHNF	522
vlCDR2	LGS	523
vlCDR3	MQALQTPPT	524
Full length light chain	DVVMTQSPSLPVTGPGEPAISCRSSQSLLYRNGHNFLDWVYQKPGQSPQLLIYLGSNRASGVDPDRFSGSGGTDFTLKI SRVEAEDVGVYYCMQALQTPPTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC	525

Figure 40A

CDRs

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.001	GGTFSSYA	526	IPIFGTA	527	AREEVSSPYGMDV	528	TGAVTSGHY	529	DTG	530	LLSYSGASWV	531
CPA.7.002	GGTFSSSA	532	IPIYGIT	533	ARDDTARRVRGVPYPPYYAMDV	534	QGIGNY	535	SAS	536	QQLKDYPT	537
CPA.7.003	GFSLSHFS	538	FDPEEGGT	539	ATGIWYSSGWVPVDY	540	QSLDSSGYNV	541	LGS	542	MQALQTPIT	543
CPA.7.004	GTYLTELS	544	FDPEDGET	545	ATVSRVRGVINYYYMDV	546	QSLLYRNGNNY	547	LGS	548	MQALQTPPT	549
CPA.7.005	GGTFSSSA	550	IPIYGIT	551	ARDDTARRVRGVPYPPYYAMDV	552	QSVDS	553	DAS	554	QYKDWPF	555
CPA.7.006	GGTFGTYA	556	ITPISATI	557	ARGFEYSDGLLDD	558	QSLFYSDDGNTY	559	RLS	560	MQHMEFPLT	561
CPA.7.007	GGSISSSY	562	IYSGST	563	ARGAWELRLGDWFDP	564	SSNIGAGYD	565	GNN	566	QSYDSSLSIYV	567
CPA.7.008	SGSISSTNW	568	IYHSGST	569	ARVGPAAIY	570	SNNVGYEG	571	RNN	572	SAWDSSLNNAV	573
CPA.7.009	GTYLTELS	574	FDPEDGET	575	ATAKPGIAGVAGQNYPPYYMDV	576	QSLLYRNGNNY	577	LGS	578	MQALQTPPT	579
CPA.7.010	GTFSSYA	580	ISYDGSNK	581	ASSPIGYSYGYWGGMDV	582	SGIDVRTNK	583	FQSDSDK	584	LIWHTSGWV	585
CPA.7.011	GTYLTELS	586	FDPEDGET	587	ATGPAAAGVGYPPYYMDV	588	QSLLYRNGNNY	589	LGS	590	MQALQTPPT	591
CPA.7.012	GTFSSYA	592	ISYDGSNK	593	ARDVMVYCSSTSCYFYGMDV	594	QDIRDY	595	DAS	596	QQFENLPIT	597
CPA.7.013	GTYLTELS	598	FDPEDGET	599	ATGGYSSGFNYYYMDV	600	QSLLYRNGNNY	601	LGS	602	MQALQTPPT	603

Figure 40B

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.014	GYLTELS	604	FDPEDGET	605	ATGVTTYYYYGMDV	606	QSLYSNGNMF	607	LGS	608	MQALQTPPT	609
CPA.7.015	GFTFSSYG	610	IRYDGSNK	611	ARDLDFWWDGMDV	612	QSVSSMY	613	GAS	614	QQYVSSPMYT	615
CPA.7.016	GGTFSSSA	616	IPIYGIT	617	ARDDTARRVRGVPPYYYYAMDV	618	QSVSSY	619	DAS	620	QQYDDWPQT	621
CPA.7.017	GGTFNNYG	622	IIPLFGTT	623	ARDRMAADGMAVFDY	624	SSNIGRHF	625	KND	626	SSWDAALNGVV	627
CPA.7.018	GYLTELS	628	FDPEDGET	629	ATEVPMVARGARRYYYYMDV	630	QTLLYINENNY	631	LGS	632	MQGLQTPPT	633
CPA.7.019	GGISNSNY	634	IYSGST	635	ARGAWELSLGDWFDP	636	SSNIGAGYD	637	GNN	638	QSYDSSLVWV	639
CPA.7.020	GGTFSSSA	640	IPIYGIT	641	ARDDTARRVRGVPPYYYYAMDV	642	QNVYGE	643	DTF	644	QQRDWPIT	645
CPA.7.021	GFTFGTSS	646	ISFDGTEI	647	AKGSGNIYFYSMDV	648	QSIGW	649	ETS	650	QQYYSYPLT	651
CPA.7.022	GYLTELS	652	FDPEDGET	653	ATGVPAAGVYYYYYMDV	654	QSLYSNGYNY	655	LGS	656	MQALQSPVT	657
CPA.7.023	GYLTELS	658	FDPEDGET	659	ATDSRDGPAARGGYYYYYMDV	660	QSLLYINGYNY	661	LGS	662	MQALQTPPT	663
CPA.7.024	GGTFSSYA	664	IPIFGTA	665	ARDAYYYDSSGYNPDADF	666	QSLHNSNGYNY	667	LGS	668	MQGLQTPRT	669
CPA.7.025	GFSLTGGMS	670	IDWNDDK	671	ARIRGMTWGFDS	672	QSVSSY	673	GAS	674	QQYGTTPFA	675
CPA.7.026	GYTFTAY	676	INPNSGGT	677	ARDGAFYGYSENYNAGWFD	678	QSLSGNGYNY	679	LGS	680	MQALKSPLT	681
CPA.7.027	GYFTINY	682	INPSSGGT	683	ARAGLGYNWVAPSGMDV	684	SSDVGGYNY	685	EVS	686	SSYAGSNNLV	687
CPA.7.028	GGSISSSY	688	IYSGST	689	ARGAWELRLGDWFD	690	SSNIGAGYD	691	GYS	692	QSYDSSLVWV	693

Figure 40C

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.029	GFTFDDYA	694	ISWNSGSI	695	AKDVNRILVAGMVDY		QSLHSHNGYNY	696	LGS	697	MQGLQTPIT	699
CPA.7.030	RFTFEDYA	700	ISWKSGGI	701	VKDPTLVATDRAFI		QSLHSHNGYNY	702	LGS	703	MQYLQTPDT	705
CPA.7.031	GGTFSSA	706	IPIYGIT	707	ARDDTARRVRGVPYNYAMDV		QSLDSDDGNIH	708	TLS	709	MQRKEFLT	711
CPA.7.032	GFTFSSYG	712	ISYDGSNK	713	AKEDRLRFLEWLFYGMVDV		HDIYTY	714	DAS	715	QQYDNHPPEVT	717
CPA.7.033	GGTFSSA	718	IPIYGIT	719	ARDDTARRVRGVPYNYAMDV		QDIDDD	720	EAS	721	LQHDNLPLT	723
CPA.7.034	GVTLELS	724	FDPEDGET	725	ATEDPGPVAGPYNYGMVDV		QSLLYNGYHY	726	LGS	727	MQALQTPPT	729
CPA.7.035	GYMFTNYP	730	INAGTGNT	731	AREGMFYGLESYKGGWFDP		QSLHRNGYNY	732	LAS	733	MQALQTPIT	735
CPA.7.036	GGTFSSA	736	IPIYGIT	737	ARDDTARRVRGVPYNYAMDV		QSLDSDDGNTY	738	TLS	739	MQRLQFPLT	741
CPA.7.037	TYFTTTY	742	IYPSGGNT	743	VRDQNYYSAMDV		QDIRNY	744	DAS	745	QQFENLPIT	747
CPA.7.038	GGTFSSA	748	IPIYGIT	749	ARDDTARRVRGVPYNYAMDV		RDISDS	750	DAS	751	HQYDNLPLT	753
CPA.7.039	GFNFRGYA	754	ISGSGGTT	755	AQSYAQIGYGGHIDH		SGIDVATYM	756	YKSDSDK	757	LIWHGSHYV	759
CPA.7.040	GVTLELS	760	FDPEDGET	761	ATGVPAALGVYNYNYMDV		QSLLYRNGYNY	762	WGS	763	MQAVQNPPT	765
CPA.7.041	GGTFSSA	766	IPIYGIT	767	ARDDTARRVRGVPYNYAMDV		QNIDSS	768	YAS	769	HQSSSLPLT	771
CPA.7.042	GGTFSSA	772	IPIYGIT	773	ARDDTARRVRGVPYNYAMDV		QSVYNNY	774	DAS	775	QQYNSWPPYT	777
CPA.7.043	GGTFSSYA	778	IPIFGTA	779	ARDAYYDSSGYNPDADF		QSLLYSNGYNY	780	LGS	781	MQARQTPYT	783

Figure 40D

Name	Hcdr1	SEQ ID		SEQ ID		SEQ ID		SEQ ID		SEQ ID		SEQ ID
		NO:	Hcdr2	NO:	Hcdr3	NO:	Lcdr1	NO:	Lcdr2	NO:	Lcdr3	
CPA.7.044	GGTFSSA	784	IPIYGIT	785	ARDDTARRVRGVPYYYYAMDV	786	ESVTTF	787	DAS	788	HQHTNWPLT	789
CPA.7.045	GYTLTELS	790	FPEDGET	791	ATEVGYCSGGSCYISYYGMDV	792	QSLLYRNGHNF	793	LGS	794	MQALQTPPT	795
CPA.7.046	GGTFSSA	796	IPIYGIT	797	ARDDTARRVRGVPYYYYAMDV	798	QTMNNY	799	DAS	800	QQYGDWLPIT	801
CPA.7.047	GYTLTELS	802	FPEDGET	803	ATAFPEATISYYYMDV	804	QSLLYRNGYNY	805	WGS	806	MQAVQNPPT	807
CPA.7.049	GGTFSSA	808	IPIYGIT	809	ARDDTARRVRGVPYYYYAMDV	810	RSLLDSDDGNTNTH	811	SLS	812	MQRKEFPLT	813
CPA.7.050	GGTFSSYA	814	IPIFGTA	815	ARGPWYDSSGSYSSAYYMDV	816	QSLHSDGYNY	817	LGS	818	MQALHTPGVT	819

Figure 41A

CHA.7.502

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	DVQLVESGGDLVQPGGSRKLSCTASGFTFSNFGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRFTISRDNPEPTLFLQMTSLRSEDAMYYCTRLDYYTNSYSMDHWGQGTSTVTVSS	820
vhCDR1	GFTFSNFG	821
vhCDR2	ISSGSSTI	822
vhCDR3	TRLDYYTNSYSMDH	823
Variable light (vl) domain	QIVLTQSPALMSASPGEKVTLTCSASSSLPYIYWYQQKPGSSPKPWIYLTSLNLSGVPARFSGSRSGTSYSLTISSVEAEDAATYYCQQWSSNPFTFGSGTKLEIK	824
vlCDR1	SSLPY	825
vlCDR2	LTS	826
vlCDR3	QQWSSNPFT	827

Figure 41B

CHA.7.503

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGAELAKPGASVKMSCKASGYTFTSNWMHWVKQRPQGQLEWIGYINP SNGYTECNQKFRDKATLSADKSSSTAYMQLNSLTADSAVVYCALMISAWLPYWG QGTLVTVSA	828
vhCDR1	GYTFTSNW	829
vhCDR2	INPSNGYT	830
vhCDR3	ALMISAWLPY	831
Variable light (vl) domain	DIVLTQSPASLAISLGQRATISCRASQSVSASSYSYVHWYQQKPGQPPKLLIKYASSLE SGVPARFSGSGSGTDFTLNHPVEEEDTATYYCLHTWEIPYTFGGGTKLEIK	832
vlCDR1	QSVSASSYSY	833
vlCDR2	YAS	834
vlCDR3	LHTWEIPYT	835

Figure 41C

CHA.7.506

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGAELTRPGASVNLSCKASGYTFTSYWMQWVKQRPGQGLEWIGAIYPG DGDTRFNQKFKGKATLTADESSSTAYMQLSSLASEDSAVYYCATYYRYDDYWGQGT TLTVSS	836
vhCDR1	GYTFTSYW	837
vhCDR2	IYPGDGDT	838
vhCDR3	ATYYRYDDY	839
Variable light (vl) domain	QIVLTQSPAIMASAPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDTSKLASG VPTRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPYTFGGGTKLEIK	840
vlCDR1	SSVS	841
vlCDR2	DTS	842
vlCDR3	QQWSSNPYT	843

Figure 41D

CHA.7.508

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLQQSGPDLVKPGASMKISCKAS <u>GYTFIDYN</u> IHVVKQSQGKSLDWIGYIYPYNG <u>GTGYNQKFKNKATLTVDSSSSTAYMEVRS</u> LT FEDSAVYFC <u>AREADYYGNRGQFDYW</u> GQGTLVTVSA	844
vhCDR1	GYTFIDYN	845
vhCDR2	IYPYNGGT	846
vhCDR3	AREADYYGNRGQFDY	847
Variable light (vl) domain	DIQMTQSPASLSVSVGETVTITCRASENIFSNLAWYQQKQKGKSPQLLVYGEANLAD GVPSRFGSGSGTQYSLKINSLQSEDFGNYC <u>QHFWGTPYT</u> FGGGTTLEIK	848
vlCDR1	ENIFSN	849
vlCDR2	GEA	850
vlCDR3	QHFWGTPYT	851

Figure 41E

CHA.7.510

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVTLKESGPGILQPSQTL ~~SLT~~ CSFSG <u>FS</u> LNTSGTGVGWIRQPSGKGLEWLTHI <u>WWN</u> <u>DNK</u> FYNTFLKSRLTISKETSNNQVFLKIASVDTADAATYYCARMAYGNLWFVNWGQ GTLVAVST	852
vhCDR1	GFSLNTSGTG	853
vhCDR2	IWWNDNK	854
vhCDR3	ARMAYGNLWFVN	855
Variable light (vl) domain	DIVLTQSPASLAVSLGQRASISCRASRSVTISGYSYMYWYQQKPGQPPRLLFY <u>L</u> ASNL ASGVPARFSGSGSGTDFTLNHPVEEEDAAIYYC <u>QHS</u> RELPTYFGGGTKLEIK	856
vlCDR1	RSVTISGYSY	857
vlCDR2	LAS	858
vlCDR3	QHSRELPTY	859

Figure 41F

CHA.7.512

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QIQLVQSGPELKKPRETVKISCKASGYTFTDYSMHVVKQAPGKGLKWMGWINTET <u>GEPTYADDFKGRFAFSLEASASSAYLQINILKDEDTATYFCARSRGGYEDYYALDYW</u> GQGTSVTVSS	860
vhCDR1	GYTFTDYS	861
vhCDR2	INTETGEP	862
vhCDR3	ARSRGGYEDYYALDY	863
Variable light (vl) domain	DIQMTQSPASLSASVSGESVTITCRASGNIHYYLAWYQQKQKGKSPQLLVYNAK ~~N~~ LAD GVPSRFSGSGGTQFSLKINSLQPEDFGSYYCQHF ~~W~~ ISPPTFGGGTKLEIK	864
vlCDR1	GNIHYY	865
vlCDR2	NAK	866
vlCDR3	QHF ~~W~~ ISPPT	867

Figure 41G

CHA.7.514

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLRQSGADLVKPGASVKLSCTASGFNIKDTYIDWVKQRPEQGLDWIGRIDPANG NTKYDPKFQGKATIITDTSSNTAYLQLSNLTSEDTAVYYC <u>ARYGSYPYFDYWGRG</u> TTL AVSS	868
vhCDR1	GFNIKDTY	869
vhCDR2	IDPANGNT	870
vhCDR3	ARYGSYPYFDY	871
Variable light (vl) domain	SIVMTQTPKFLLISAGDRVITITCKASQSVRNDVAWYQQKPGQSPKLLMY <u>YAS</u> NRYT GVPDRFTGSGYGTDFTFTISTVQAEDLAVYFC <u>QQDYSSPPT</u> FGGGTKLEIK	872
vlCDR1	QSVRND	873
vlCDR2	YAS	874
vlCDR3	QQDYSSPPT	875

Figure 41H

CHA.7.516

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGPELVKPGVSVKISCKVSGYTFTDYVMHWVKQSHAKSLEWIGIISPYS GNTNYNQNFKGKATMTVDKSSSTAYMALARLTSEDSAIYYCAREGDLPMFAYWGQG TLVTVSA	876
vhCDR1	GYTFTDYV	877
vhCDR2	ISPYSGNT	878
vhCDR3	AREGDLPMFAY	879
Variable light (vl) domain	QIVLTQSPTIMSASPGEKVTMTCSASSSVSYIYWYQQNPGSSPRLLIYDTSILASGV PFRFSGSGSGTSYSLTISRMEAEDAATYYCQQWTSYPLTFGSGTKLELK	880
vlCDR1	SSVSY	881
vlCDR2	DTS	882
vlCDR3	QQWTSYPLT	883

Figure 41l

CHA.7.518

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLQQSGPELVKPGASVKISCKASGYTFTDYNINWVKQSHGKSLEWIGYIYPYIGGS GYNQKF ~~SKATLSADNP~~ SSTAYMELRSLTSEDSAVYYCAREDKTARNAMDYWGQG TPVTVSS	884
vhCDR1	GYTFTDYN	885
vhCDR2	IYPYIGGS	886
vhCDR3	AREDKTARNAMDY	887
Variable light (vl) domain	DIQMTQSPASLSVSVGETVTIICRVSENIYSNLAWYQQKQKGKSPQLLVYEATNLAEG VPSRFSGSGSGTQYSLKINSLQSEDFGSYYCQHFWGTPYTFGGGTKLEIK	888
vlCDR1	ENIYSN	889
vlCDR2	EAT	890
vlCDR3	QHFWGTPYT	891

Figure 41J

CHA.7.520_1

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	DVQLQESGPGLVKPSQSLTCTVT <u>GYSITSDYA</u> WNWIRQFPGNKLEWMGYISYSGSTSYNPSLKSRISTRDTSKNQFFLQLNSVTTEDTATYYC <u>ARHYRPPYAMDY</u> WGQGTSVTVSS	892
vhCDR1	GYSITSDYA	893
vhCDR2	ISYSGST	894
vhCDR3	ARHYRPPYAMDY	895
Variable light (vl) domain	DIVMTQSPSSLAMSVGQKVTMSCKSSQSLNSSNQKNYLAWYQQKPGQSPKLLVYFAS <u>TRESGVPDRFIGSGSGTDFTLTITSVQAEDLADYFCQQHYSTPFT</u> FGSGTKLEIK	896
vlCDR1	QSLNSSNQKNY	897
vlCDR2	FAS	898
vlCDR3	QQHYSTPFT	899

Figure 41K

CHA.7.520_2

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLKQSGPGLVQPSSLSITCTVSGFSLTSFGVHWVRQSPGKGLEWLGVIWSSGGT TVYDAAFISRLSISKDNSKSQVFFKMNSLQTNDTAIYYCARKRGNFYVMDYWGQGT SVTVSS	900
vhCDR1	GFSLTSG	901
vhCDR2	IWSGGT	902
vhCDR3	ARKRGNFYVMDY	903
Variable light (vl) domain	DIVMTQSPSSLAMSVGQKVTMSCKSSQSLNSSNQKNYLAWYQQKPGQSPKLLVY FASTRESGVPDRFIGSGSGTDFTLTITSVQAEDLADYFCQQHYSTPFTFGSGTKLEIK	904
vlCDR1	QSLNSSNQKNY	905
vlCDR2	FAS	906
vlCDR3	QQHYSTPFT	907

Figure 41L

CHA.7.522

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQPGSELVRPGTSVKLSCKASGYTFTSYWVHWVRQRHGQGLEWIGNVYPG <u>SGST</u> NYDEKFKSKGTLTVDTSSTAYMHLSSLTSEDSAVYYCTRGVLRFPDLYWGQG TTLTVSS	908
vhCDR1	GYTFTSYW	909
vhCDR2	VYPGSGST	910
vhCDR3	TRGVLRFPDLY	911
Variable light (vl) domain	DIVMTQAAPSPVPTPGESVSISCRSSKSLLSHNGNTYLYWFLQRPQGSPHLLIYRMS NLASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPLTFGAGTKLELK	912
vlCDR1	KSLLSHNGNTY	913
vlCDR2	RMS	914
vlCDR3	MQHLEYPLT	915

Figure 41M

CHA.7.524

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGPEVVRPGVSVKISCKGSGYKFPDYVMHWVKQSHAKSLEWIGII <u>SIYSG</u> <u>NTN</u> YNQKFKGKATMTVDKSSSTAYMELARLTSEDSAIYYCAREGDLPMFAYWGQG TLVTVSA	916
vhCDR1	GYKFPDYV	917
vhCDR2	ISIYSGNT	918
vhCDR3	AREGDLPMFAY	919
Variable light (vl) domain	QIVLTQSPAIMASASPGEKVTMTCNASSSVSYMYWYQQKPISSPRLLIYDTSNLASGV PVRFSGSGSGTSYSLTIGRMEAEDAATYYC <u>QQWSSYPLT</u> FGAGTKVEVK	920
vlCDR1	SSVSY	921
vlCDR2	DTS	922
vlCDR3	QQWSSYPLT	923

Figure 41N

CHA.7.526

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLKESGPGLVAPSQSL SITCTVSGFSLTAYNINWVRQPPGKGLEWLGMIWGDG NTDYN SPLKSR LTISKDNSKSQVFLKMDSLQTD DTARYYCARDLKVRRDSPYTMDY WGQGTSTVTVSS	924
vhCDR1	GFSLTAYN	925
vhCDR2	IWGDGNT	926
vhCDR3	ARDLKVRRDSPYTMDY	927
Variable light (vl) domain	NIMMTQSPSSLAVSAGEKVTMSCKSSQSVLYSSNQKNYLAWYQQKPGQSPKLLIY WASNRESGVPDRFTGSGSGTDFTLTIS SVQAEDLAVYYCHQYLSSYTFGGGTKLEIK	928
vlCDR1	QSVLYSSNQKNY	929
vlCDR2	WAS	930
vlCDR3	HQYLSSYT	931

Figure 410

CHA.7.527

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	DVQLQESGPGLVKPSQSLTCTVT <u>GYSLTSDYA</u> WNWIRQFPGNKLEWMGY <u>ITYSG</u> <u>G</u> TTYNP SLKSRISITRDT SKNQFFLQLTSVTTEDTATYYC <u>ARRGSGTTVVDWYFDV</u> WGAGTTTVSS	932
vhCDR1	GYSLTSDYA	933
vhCDR2	ITYSGGT	934
vhCDR3	ARRGSGTTVVDWYFDV	935
Variable light (vl) domain	DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSFNQKYYLAWYQQKPGQSPKLLIYW <u>A</u> STRESGVPDRFTGSGSGTDFTPTISSVTAEDLAVYYC <u>QQFYTPYT</u> FGGGTKLEMK	936
vlCDR1	QSLLYSFNQKYY	937
vlCDR2	WAS	938
vlCDR3	QQFYTPYT	939

Figure 41P

CHA.7.528

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQVQQSGPELVKPGASVKISCKASGYTFTKSNMHWVKQSHGKSLEWIGYIYPYNG GTGYNQNFKSKATLTVDISSSTAYMELRSLTLEDSAVYLCAREADYYGNRGQFDYW GQGTLVTVSA	940
vhCDR1	GYTFTKSN	941
vhCDR2	IYPYNGGT	942
vhCDR3	AREADYYGNRGQFDY	943
Variable light (vl) domain	DIQMTQSPASLSVSVGETVTITCRASDNIFSNLAWYHQKQGKSPHLLVYGATNLAD GVPSRFGSGSGTQYSLKINSLSQSEDFGDYYCQHFWGTPYTFGGGKLEIK	944
vlCDR1	DNIFSN	945
vlCDR2	GAT	946
vlCDR3	QHFWGTPYT	947

Figure 41Q

CHA.7.530

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGAELMRPGTSVKVSCKASGYAFTNHLIEWIKQRPQGQLEWIGVINPGSDSTDYNEKFKDKATLTADKSSSTAYMQLSSLTSDDSAVYFCARSLYYNSWVFWGQGTLLTVSA	948
vhCDR1	GYAFTNHL	949
vhCDR2	INPGSDST	950
vhCDR3	ARSLYYNSWVFWY	951
Variable light (vl) domain	DIQMTQSPASLSASVGETVTITCRASENIYSYLAWYQQKRGKSPQLLVYNNAKTLVEGVPSRFGSGSGTQFSLKINSLQPEDFGSYQCQHHYGTPYTFGGGKLEIK	952
vlCDR1	ENIYSY	953
vlCDR2	NAK	954
vlCDR3	QHHYGTPYT	955

Figure 41R

CHA.7.534

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLQQSGAELVKGASVRLSCTAS <u>GFSIRDTY</u> IHWVKQRPEQGLDWIGKIDPANG <u>KSEYDPKFQGRATMTTDTSSNTAYLQLSSLTSED</u> AVYYCTRYGYYPYFDVWGAGTT VTVFS	956
vhCDR1	GFSIRDTY	957
vhCDR2	IDPANGKS	958
vhCDR3	TRYGYYPYFDV	959
Variable light (vl) domain	SIVMTQTPKFLLVSAGDRVAITCKASQSVRHDVVWYQQKPGQSPKLLIYY <u>ASSRYTG</u> VPDRFTGSGYGTDFTFTISTVQAEDLALYFCLQDFSSPWTFGGGTKLEIK	960
vlCDR1	QSVRHD	961
vlCDR2	YAS	962
vlCDR3	LQDFSSPWT	963

Figure 41S

CHA.7.535

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLQQSGPELVKPGASVKISCKTSGYTFTKNTMHWVRQSHGKSLEWIGGINPNSG GASFNQKFMGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARDGYDGDWFFDVWG AGTTVTVSS	964
vhCDR1	GYTFTKNT	965
vhCDR2	INPNSGGA	966
vhCDR3	ARDGYDGDWFFDV	967
Variable light (vl) domain	DIQMNQSPFSLASLGDTVTITCHASQNIYVWLSWYQQKPGNIPKLLIYKASDLHTG VPSRFSGSGSGTDFLTNISSLQPEDIATYYCQQGQSYPRTFGGGTKLEIK	968
vlCDR1	QNIYVW	969
vlCDR2	KAS	970
vlCDR3	QQGQSYPRT	971

Figure 41T

CHA.7.537

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLKESGPGLVAPSQSL SITCTVSG <u>FSLIGHG</u> VNWI RQPPGKGLEWLGVIWGDGN IDYNSALKSRLSISKDNSKSQVFLKMNSLQTDDTARYFC <u>AVNSAMDY</u> WGQGTAVT VSS	972
vhCDR1	GFSLIGHG	973
vhCDR2	IWGDGNT	974
vhCDR3	AVNSAMDY	975
Variable light (vl) domain	NIVMTQSPKSMMSVGERVTLNCTASENVASFVSWYQQKPEQSPKLLIY <u>GTS</u> NRYT GVPDRFTGSGSATDFTLTISVQAEDLGDYHCGQSYNYPFTFGSGTKLEIE	976
vlCDR1	ENVASF	977
vlCDR2	GTS	978
vlCDR3	GQSYNYPFT	979

Figure 41U

CHA.7.538_1

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGAELVRPGASVKVSCCKTSGYAFTNYLIEWVKQRPQGQGLEWIGVINPGSG GIYYNDKFKVKTTLTADKSSSTAYMQLSSLTSDDSAVYFCARSETHDTWFAYWGQG TLVTVSA	980
vhCDR1	GYAFTNYL	981
vhCDR2	INPGSGGI	982
vhCDR3	ARSETHDTWFAY	983
Variable light (vl) domain	DIVMTQSQKFISTSVGDRVSITCKASQSVRIAIAVWFQQKPGQSPKALIYLASTRHTG VPDRFTGSGSGTDFLTISNVQSEDLADYFCLQHWNPYPYTFGGGKLEIKR	984
vlCDR1	QSVRIA	985
vlCDR2	LAS	986
vlCDR3	LQHWNPYT	987

Figure 41V

CHA.7.538_2

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQSGAELVRPGTSVKMSCKAAGYTFTNYWIGWVKQRPGHGLEWIGDIYPG GGYTNYNEKFKGKATLTADTSSSTAYMQLSSLTSEDSAIYYCASPYYGSSYGFAFWG QGTLVTVSA	988
vhCDR1	GYTFTNYW	989
vhCDR2	IYPGGGYT	990
vhCDR3	ASPYYGSSYGFAF	991
Variable light (vl) domain	DIVMTQSQKFISTSVGDRVSITCKASQSVRIAVAWFQQKPGQSPKALIYLASTRHTG VPDRFTGSGSGTDFTLTISNVQSEDLADYFCLQHWNPYPYTFGGGTKLEIKR	992
vlCDR1	QSVRIA	993
vlCDR2	LAS	994
vlCDR3	LQHWNPYPY	995

Figure 41W

CHA.7.543

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLKESGPGLVAPSQSL SITCTVSGFSLSGYG IKWVRQPPGKGLEWLGT IWGDGS IDYNSALKSRLSISKDNSKSQVFLKMTSLQTDDTARYYC ASDSLGITFGYWGQGTLV TVSA	996
vhCDR1	GFSLSGYG	997
vhCDR2	IWGDGST	998
vhCDR3	ASDSLGITFGY	999
Variable light (vl) domain	DIQMTQTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVKLLIYYTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPLTFGAGTKLELK	1000
vlCDR1	QDISNY	1001
vlCDR2	YTS	1002
vlCDR3	QQGNTLPLT	1003

Figure 41X

CHA.7.544

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLKESGPGLVAPSQSL SITCTVSG <u>FS</u> LSYAITWVRQPPGKGLEWLGVIWPGGGT NYNSALKSRLSISKDNSKSHLFLKMNSLQTDDTARYYC <u>VRSYDGYLDWYFDV</u> WG TTVTSS	1004
vhCDR1	GFSLSYA	1005
vhCDR2	IWPGGGT	1006
vhCDR3	VRSYDGYLDWYFDV	1007
Variable light (vl) domain	NIVMTQSPKSMMSVGERVTLCKASENVGTYVSWYQQKPDQSPKLLIYGASNRYT GVPDRFTGSGSATDFTLISSVQAEDLSDYHCGQSYSPYTFGGGTKLEII	1008
vlCDR1	ENVGTY	1009
vlCDR2	GAS	1010
vlCDR3	GQSYSPYT	1011

Figure 41Y

CHA.7.545

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QIQLVQSGPDLKKPGETVKISCKASGYTFTTYGMSWVKQAPGRGLKWMGWINTYS <u>GVSTFPDDFKGRFAFSLETSASTAYLQINNKNEDSATYFCARLGMGSTTGAGYFDV</u> WGTGTTVTVSS	1012
vhCDR1	GYTFTTYG	1013
vhCDR2	INTYSGVS	1014
vhCDR3	ARLGMGSTTGAGYFDV	1015
Variable light (vl) domain	DIVLTQSPAIMSASPGEKVTMTCSASSSVSSWYLHWYQQKSGASPKLWIYGTSNLA SGVPARFSGSGSGTSYSLTISSVEAEDAATYYCQQYRSDPYTFGSGTKLEIK	1016
vlCDR1	SSVSSWY	1017
vlCDR2	GTS	1018
vlCDR3	QQYRSDPYT	1019

Figure 41Z

CHA.7.546

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QIQLVQSGPELKKPGETVKISCKASGYTFTTYGMSWVKQAPGKGLKWMGWINTYS GVSTYADDFEGRFAFSLETSVSTAYLQINNKNEDTATYFCARLGRGSTTGAGYLDV WGTGTTVTVSS	1020
vhCDR1	GYTFTTYG	1021
vhCDR2	INTYSGVS	1022
vhCDR3	ARLGRGSTTGAGYLDV	1023
Variable light (vl) domain	DIVLTQSPAIMASAPGEKVSMTCSASSVSSWYLHWYQQKSGASPKLWIYGTSNLA SGVPARFSGSGSGTSYSLTISSVEAEDAATYYCQQYHSDPYTFGSGTKLEIK	1024
vlCDR1	SSVSSWY	1025
vlCDR2	GTS	1026
vlCDR3	QQYHSDPYT	1027

Figure 41AA

CHA.7.547

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QIQLVQSGPDLKKPGETVKISCKASGYTFTTYGMSWVKQAPGRGLKWMGWINTYS GVSTFPDDFKGRFAFSLETSASTAYLQINNLIKNEFSATYFCARLGMGSTTGAGYFDV WGTGTTVTVSS	1028
vhCDR1	GYTFTTYG	1029
vhCDR2	INTYSGVS	1030
vhCDR3	ARLGMGSTTGAGYFDV	1031
Variable light (vl) domain	ENVLTQSPAISASLGEKVTLSRASSSVNYMYWYQQKSDASPKLWIYYTSNLAPG VPARFSGSGSGNSYSLTISVVEGEDAATYYCQQFTSSPWTFGGGTKLEIK	1032
vlCDR1	SSVNY	1033
vlCDR2	YTS	1034
vlCDR3	QQFTSSPWT	1035

Figure 41BB

CHA.7.548

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	EVQLQQSGPELVKPGASVKISCKAS <u>GYTFTDYS</u> MNWWVKQSHGKSLEWIGDITPNNG <u>SPN</u> YNQKFKDKATLTVDKSSSTVYME <u>LRSLT</u> SEDSAVYYC <u>ASLFFDY</u> WGHGTTTVSS	1036
vhCDR1	GYTFTDYS	1037
vhCDR2	ITPNNGSP	1038
vhCDR3	ASLFFDY	1039
Variable light (vl) domain	DIVMTQSPSSLSVSAGEKVTMSCKSSQ <u>SLLNSGNQKNY</u> LAWYQQKPGQPPKLLIYG <u>A</u> STRDSGVPDRFTGSGSGTDFTLTITSVQAEDLAVYYC <u>QNDHTYPYT</u> FGGGTKLEIK	1040
vlCDR1	QSLNSGNQKNY	1041
vlCDR2	GAS	1042
vlCDR3	QNDHTYPYT	1043

Figure 41CC

CHA.7.549

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQQPGTELVKPGASVKLSCKAS <u>GFTFTTHW</u> MHWVRQRPQGQGLEWIGNI <u>YPS</u> <u>NGGS</u> NYNEKF ~~TK~~ KATLTVD ~~RS~~ STAYMHLSSLTSEDSAVYYC <u>ARRVNWDGYYFDY</u> W GQGTTLVSS	1044
vhCDR1	GFTFTTHW	1045
vhCDR2	IYPSNGGS	1046
vhCDR3	ARRVNWDGYYFDY	1047
Variable light (vl) domain	DIVMTQSQKFMSTSVGDRVSVTCKAS <u>QNVGTN</u> VAWYQQKPGQSPKLLIY <u>SAS</u> RY SGVPDRFTGSGSGTDFTLTISNVQSEDLADYFC <u>QQYNSYPLT</u> FGGGTKLEIK	1048
vlCDR1	QNVGTN	1049
vlCDR2	SAS	1050
vlCDR3	QQYNSYPLT	1051

Figure 41DD

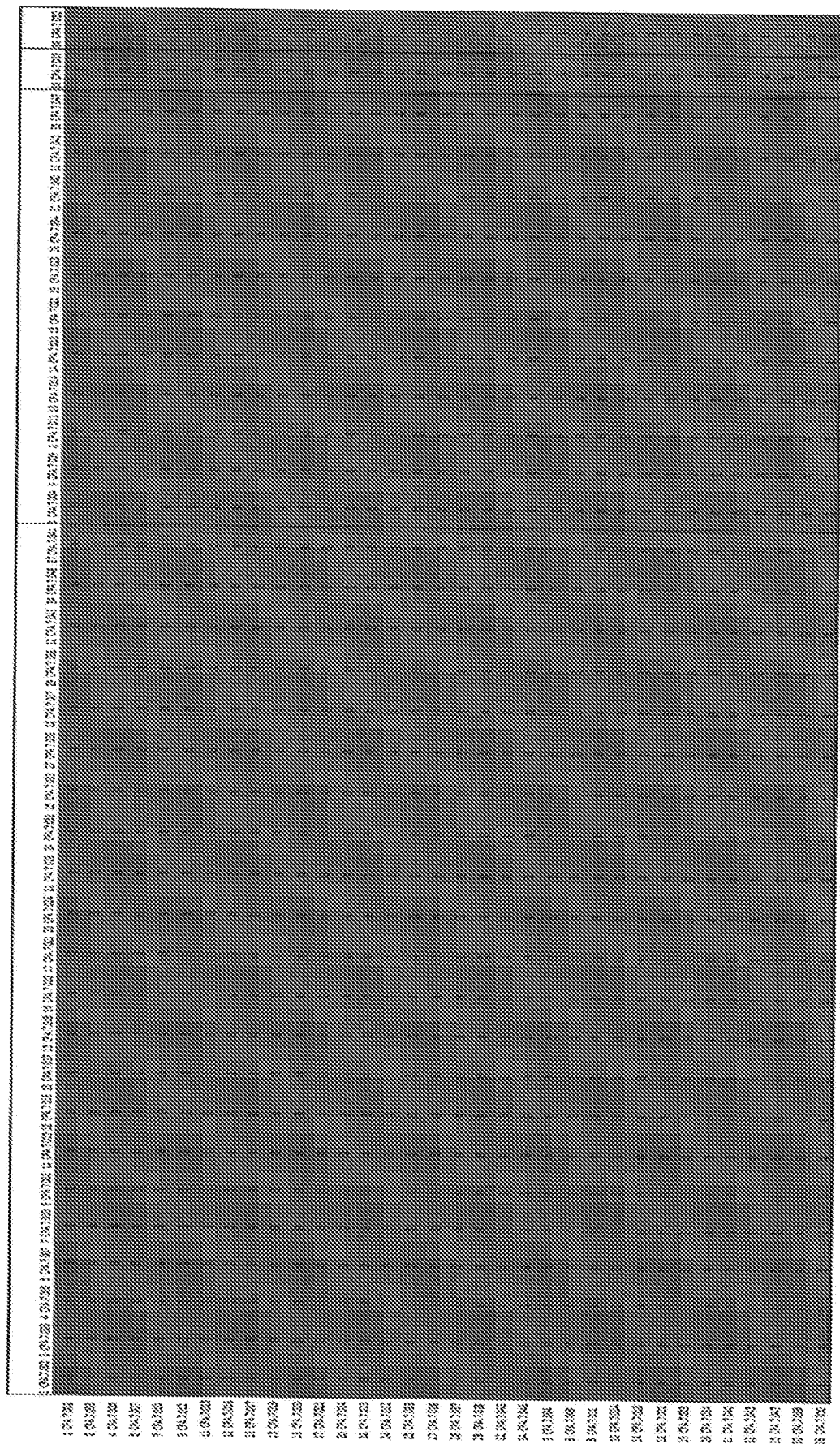
CHA.7.550

What	sequence	SEQ ID NO:
Variable heavy (vh) domain	QVQLQESGTELVKPGASVKLSCKASGYTFTSYWIHWVKQRPGQGLEWIGNINPSN GGTNYNEKFKSKAALTVDKSSSTAYMQLSSLTSEDSAVYYCARRGLPYFFDYWGQG TTLTVSS	1052
vhCDR1	GYTFTSYW	1053
vhCDR2	INPSNGGT	1054
vhCDR3	ARRGLPYFFDY	1055
Variable light (vl) domain	DIVMTQSQKFMSTSVGDRVSVTCKGSQNVGYNVAWYQQKPGQSPKALVYSASDR HSGVPDRFAGSGSGTDFLTISNVQSEDLAEYFCQQYNSYPLTFGAGTKLELK	1056
vlCDR1	QNVGYN	1057
vlCDR2	SAS	1058
vlCDR3	QQYNSYPLT	1059

Figure 42

1	2	3	4
CPA.7.002	CPA.7.004	CPA.7.039	CPA.7.050
CPA.7.003	CPA.7.009		
CPA.7.005	CPA.7.011		
CPA.7.007	CPA.7.014		
CPA.7.010	CPA.7.018		
CPA.7.012	CPA.7.022		
CPA.7.015	CPA.7.023		
CPA.7.016	CPA.7.034		
CPA.7.017	CPA.7.040		
CPA.7.019	CPA.7.045		
CPA.7.020	CPA.7.047		
CPA.7.021			
CPA.7.024			
CPA.7.028			
CPA.7.032			
CPA.7.033			
CPA.7.036			
CPA.7.037			
CPA.7.038			
CPA.7.043			
CPA.7.046			
CPA.7.041			

Figure 43



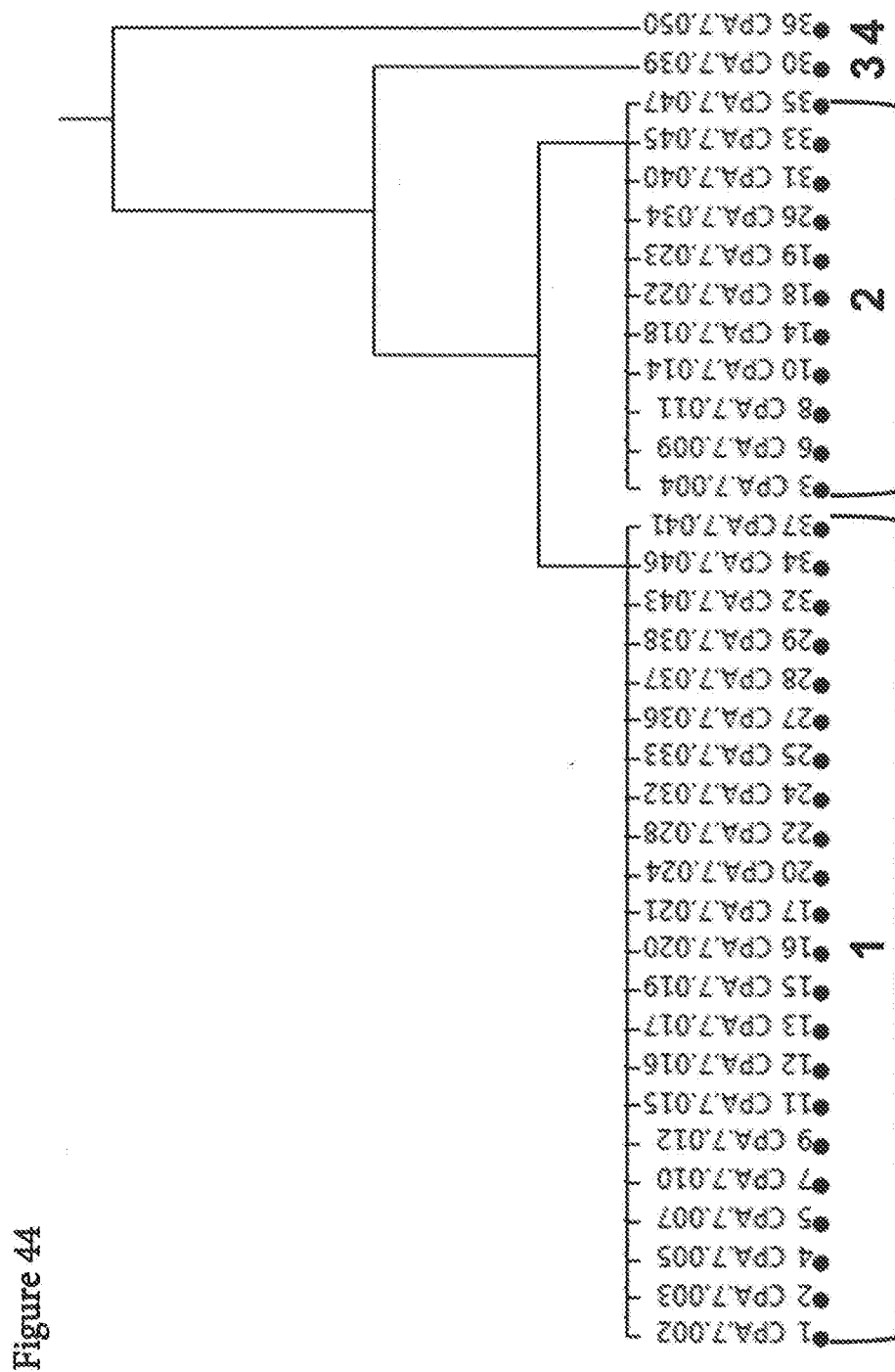
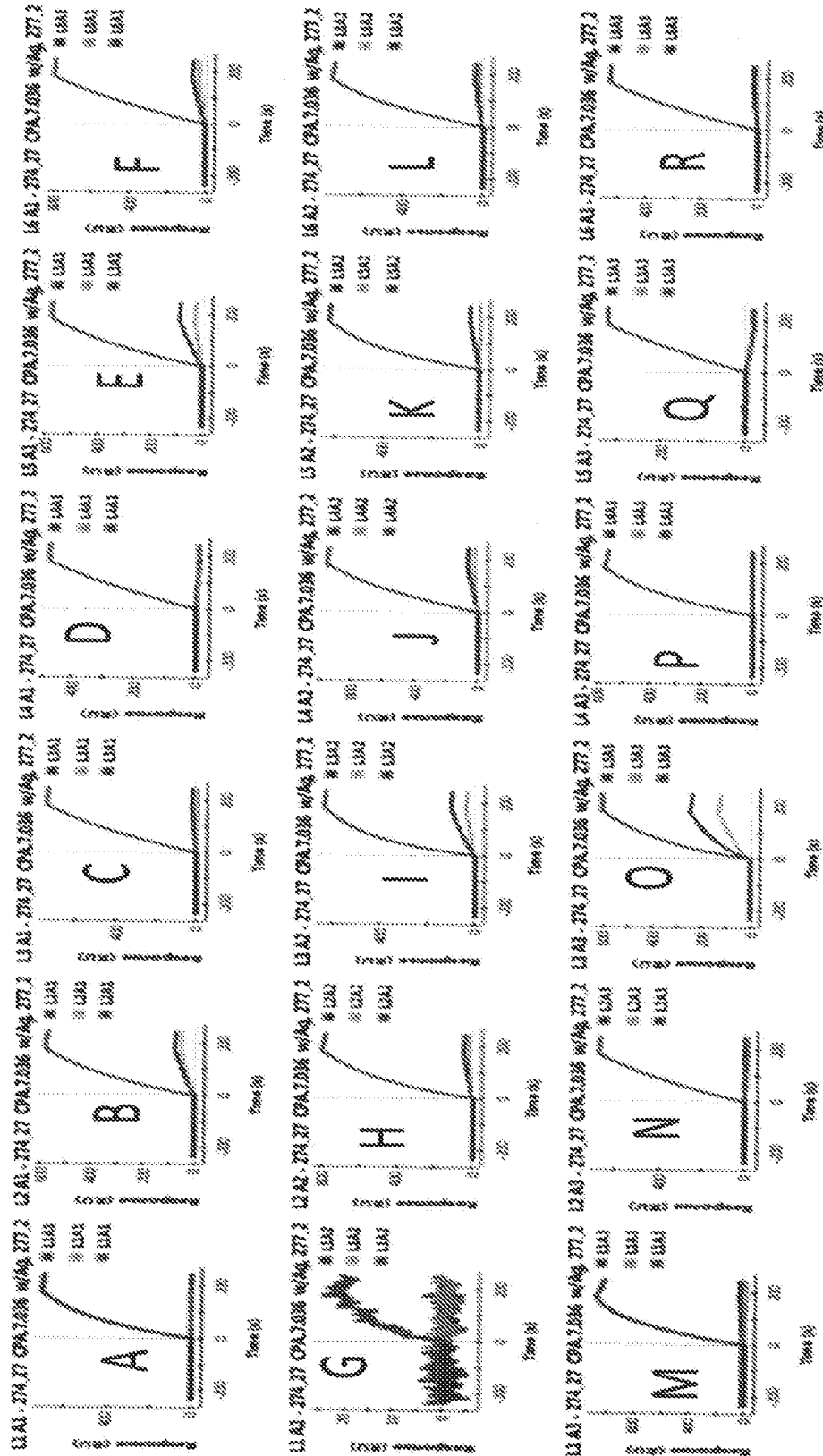


Figure 44

Figures 45A - 45R



Figures 45S - 45JJ

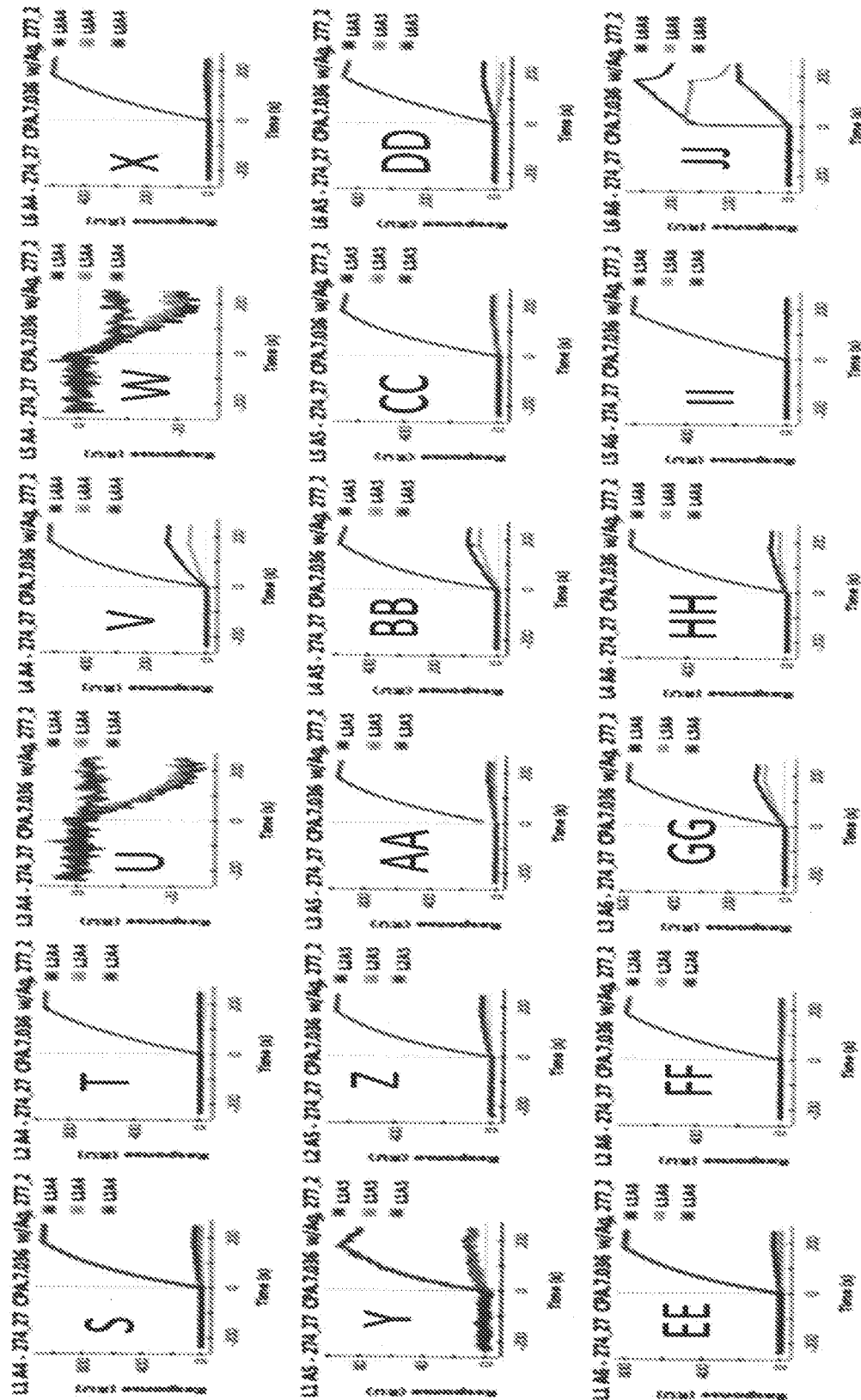
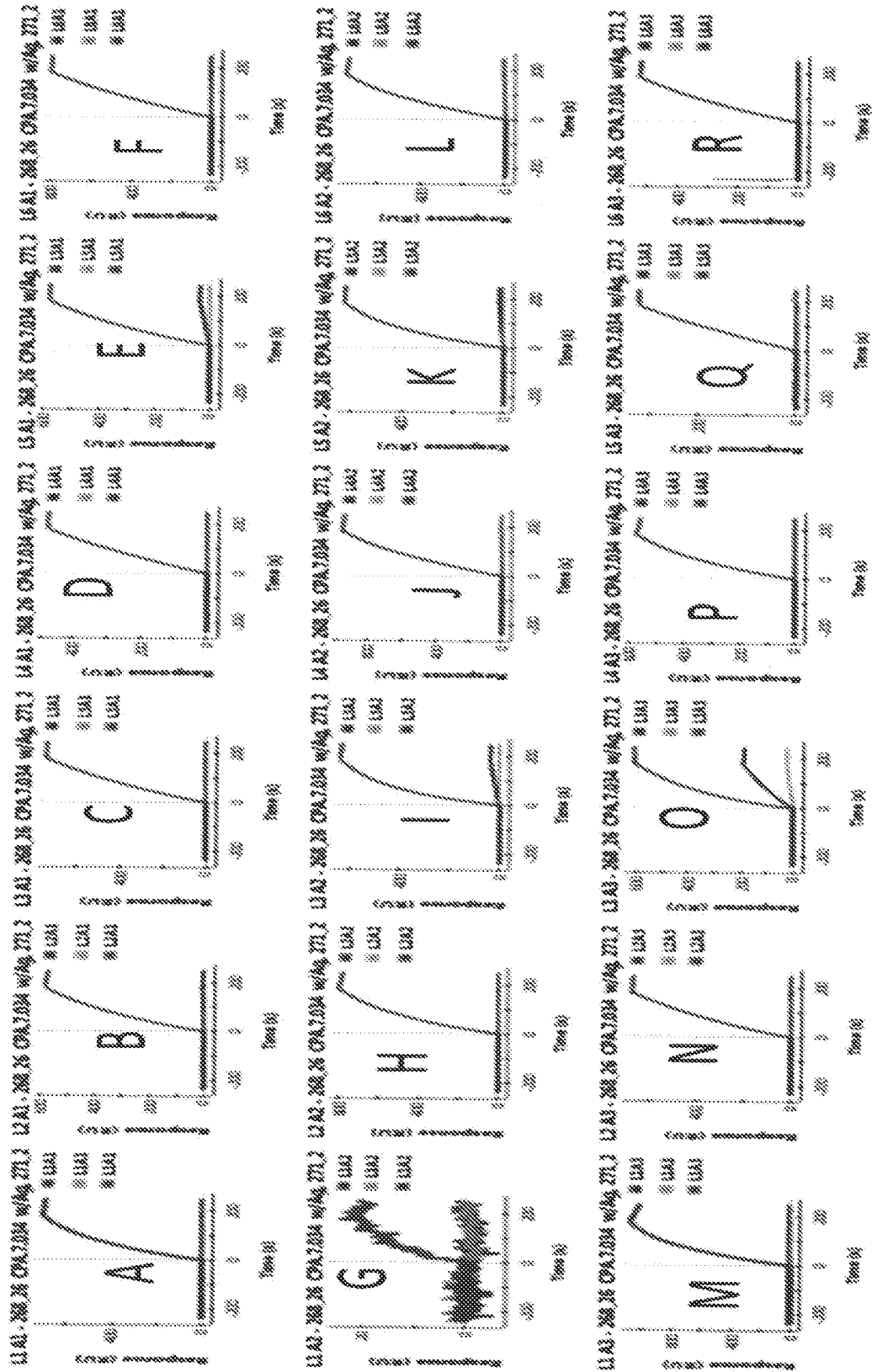
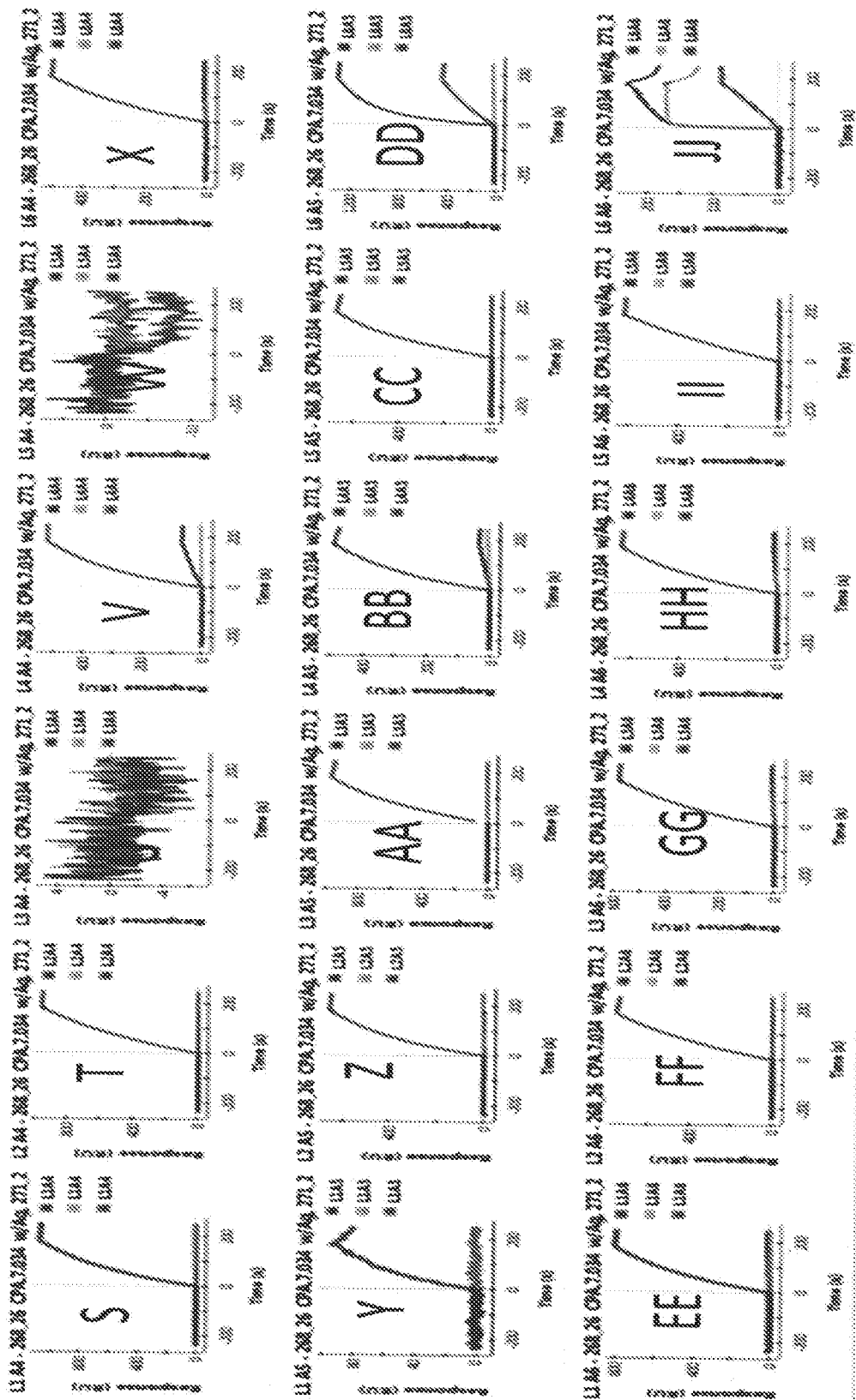


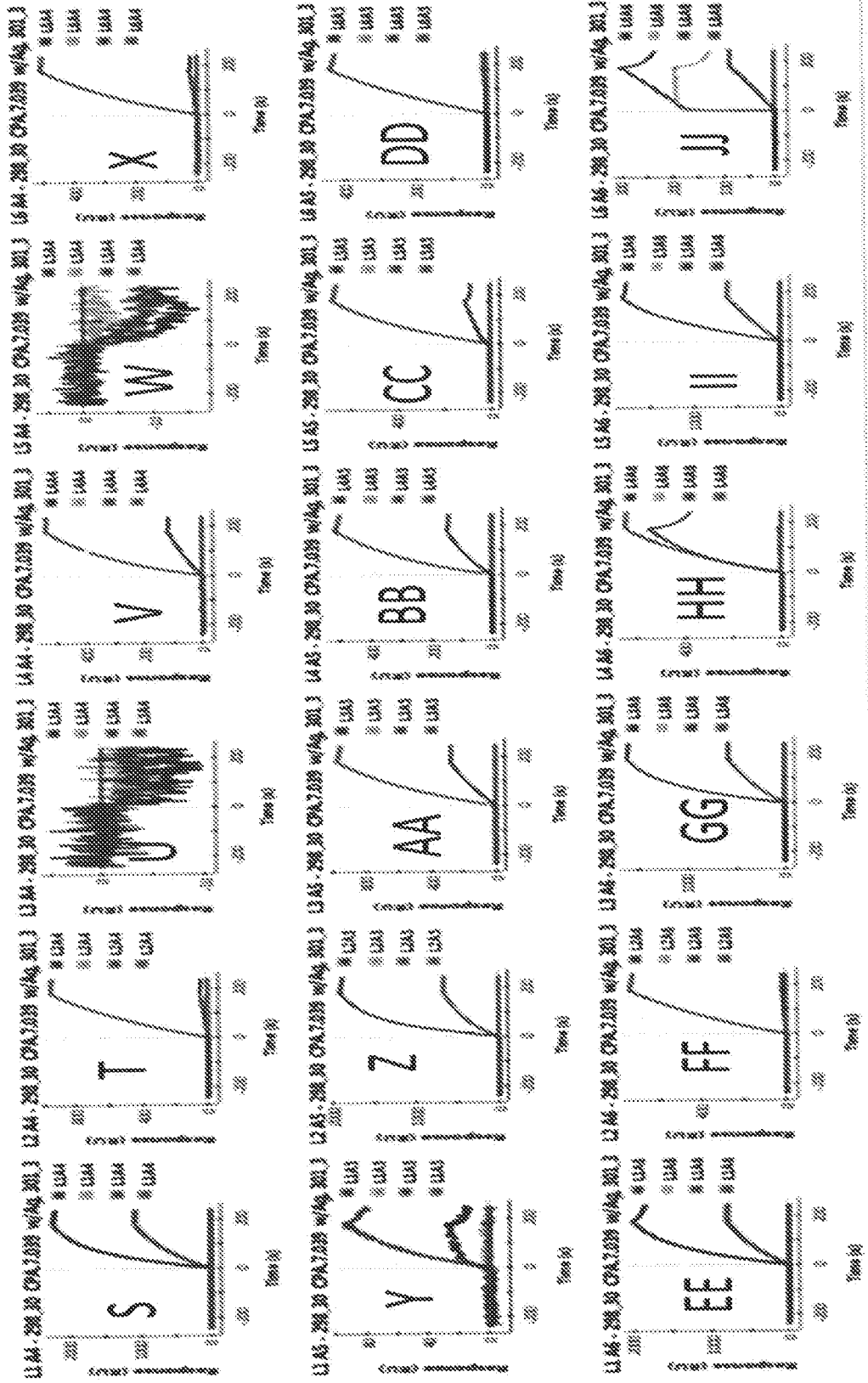
Figure 4 - 462



Figures 46S - 46JJ



Figures 47S - 47J



Figures 48S - 48JJ

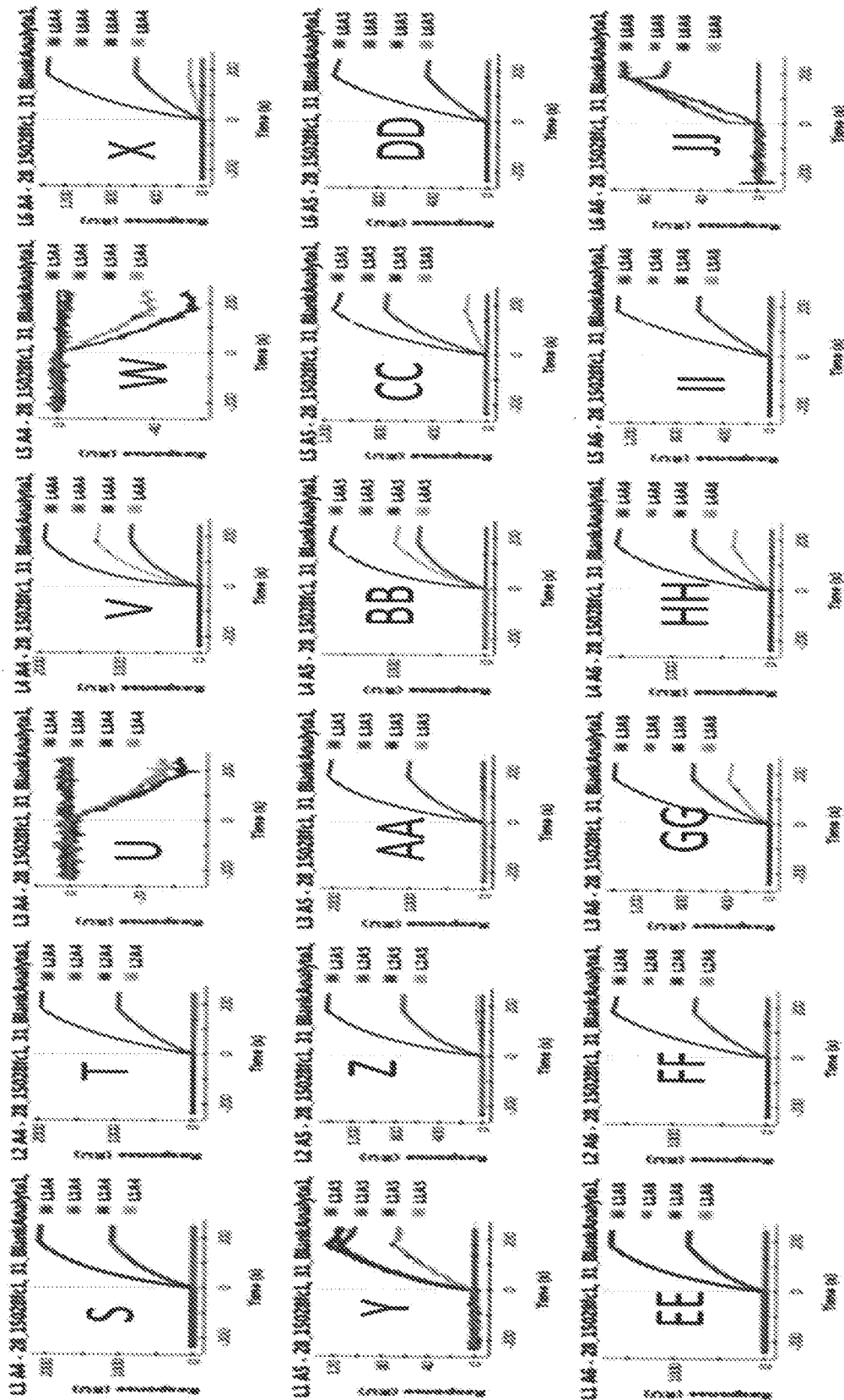


Figure 49

Table 1		k_a	k_d	K_D
Fab Clone	Figure	1/M-s	1/s	M
CPA.7.021	1A	2.20E+05	2.90E-05	1.32E-10
CPA.7.028	1B	3.08E+06	4.33E-04	1.41E-10
CPA.7.019	1C	8.59E+05	1.87E-04	2.18E-10
CPA.7.012	1D	1.51E+06	9.54E-04	6.32E-10
CPA.7.007	1E	3.24E+05	4.55E-04	1.41E-09
CPA.7.015	1F	3.13E+05	1.11E-03	3.55E-09
CPA.7.050	1G	7.08E+04	4.00E-04	5.65E-09
CPA.7.048	1H	1.35E+05	2.57E-03	1.90E-08
CPA.7.049	1I	1.51E+05	3.67E-03	2.44E-08
CPA.7.040	1J	1.33E+05	3.69E-03	2.77E-08
CPA.7.020	1K	1.34E+05	3.86E-03	2.88E-08
CPA.7.002	1L	4.59E+04	2.83E-03	6.16E-08
CPA.7.022	1M	3.55E+06	2.96E-01	8.33E-08
CPA.7.005	1N	5.22E+04	4.39E-03	8.41E-08
CPA.7.004	1O	2.12E+06	4.00E-01	1.89E-07
CPA.7.010	1P	9.06E+04	1.72E-02	1.89E-07
CPA.7.008	1Q	2.46E+04	2.03E-02	8.23E-07

Figure 50A-50Q

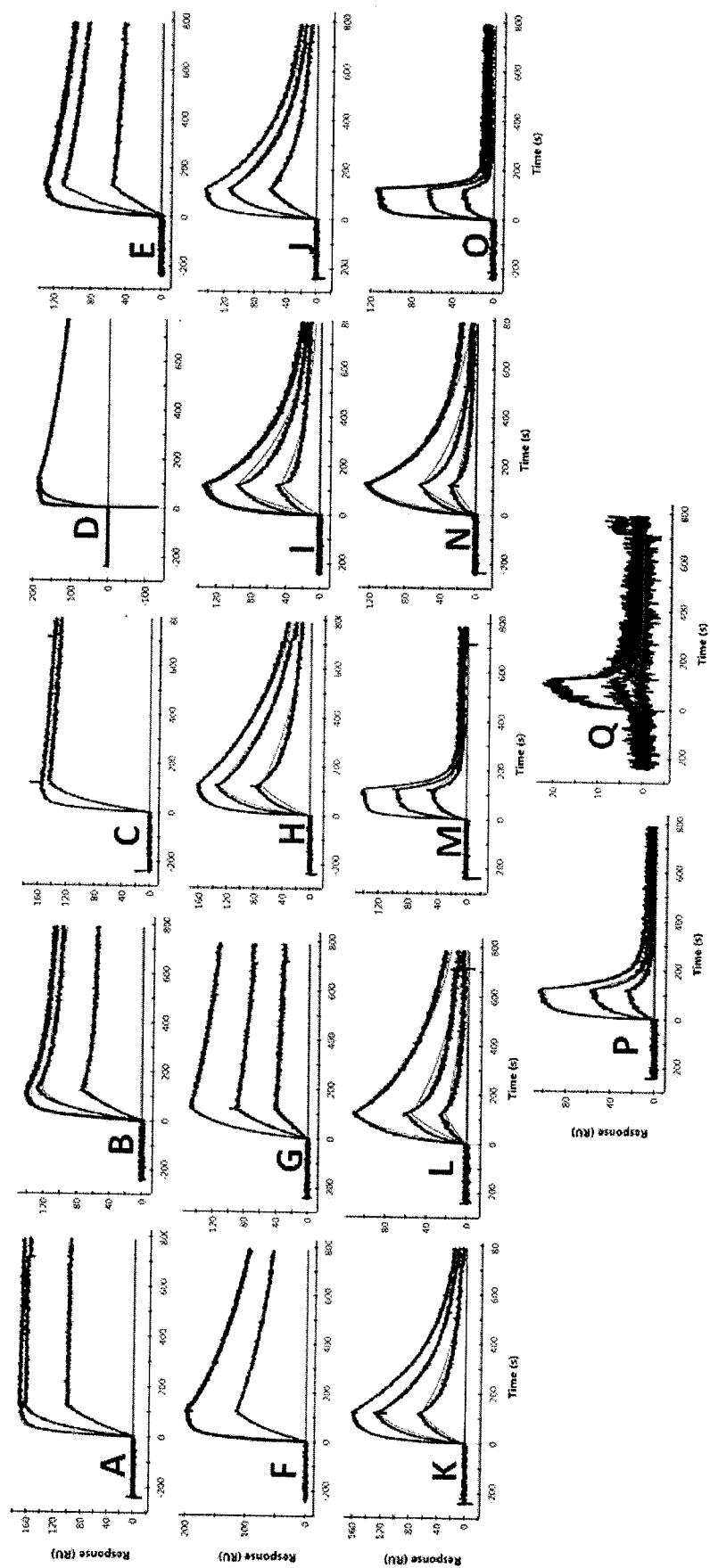


FIGURE 51A

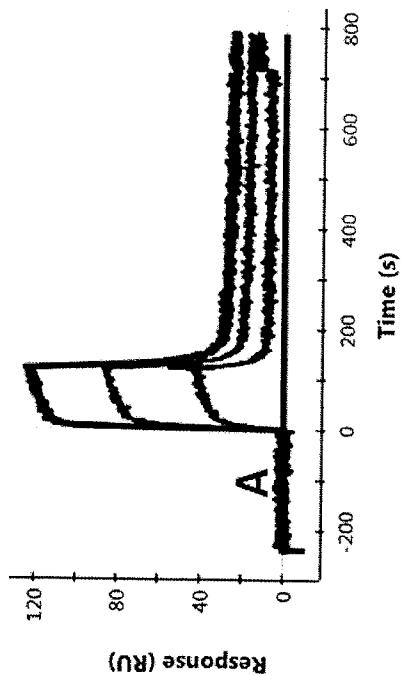


FIGURE 51B

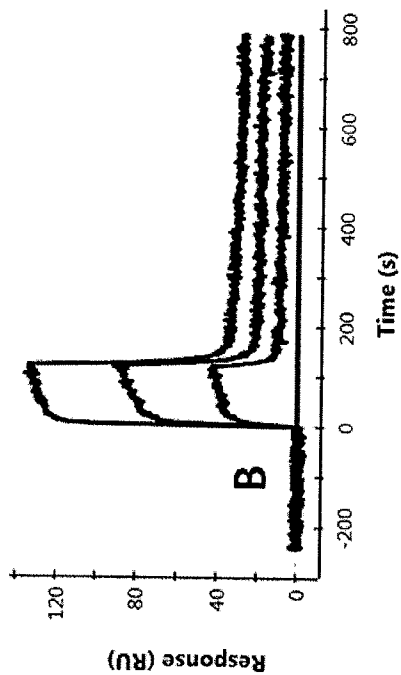


FIGURE 51C

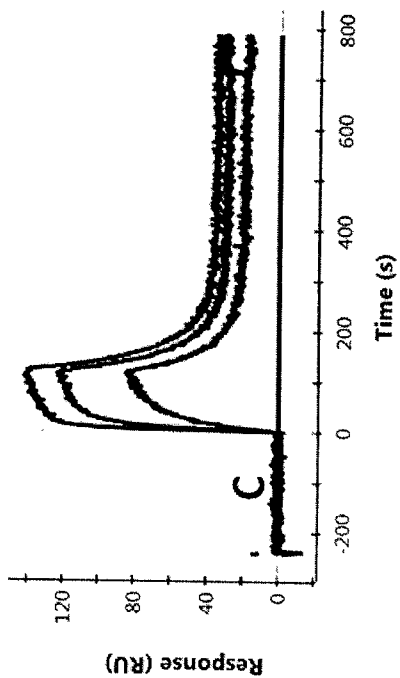


Figure 52A

Antibody	ELISA Signal	% Blocking	> 80% blocking
No mab control	1.376	0	NO
CPA.7.001	0.099	93	YES
CPA.7.002	0.558	59	NO
CPA.7.003	0.153	89	YES
CPA.7.004	0.166	88	YES
CPA.7.005	1.551	-13	NO
CPA.7.006	0.134	90	YES
CPA.7.007	0.529	62	NO
CPA.7.008	0.281	80	YES
CPA.7.009	0.05	96	YES
CPA.7.010	0.141	90	YES
CPA.7.011	0.067	95	YES
CPA.7.012	0.045	97	YES
CPA.7.013	0.045	97	YES
CPA.7.014	0.044	97	YES
CPA.7.015	0.142	90	YES
CPA.7.016	1.553	-13	NO
CPA.7.017	0.197	86	YES
CPA.7.018	0.156	89	YES
CPA.7.019	0.146	89	YES
CPA.7.020	0.728	47	NO
CPA.7.021	0.122	91	YES
CPA.7.022	0.081	94	YES
CPA.7.023	0.047	97	YES
CPA.7.024	0.048	97	YES
CPA.7.025	1.159	16	NO
CPA.7.026	1.12	19	NO
CPA.7.027	1.181	14	NO
CPA.7.028	0.702	49	NO
CPA.7.029	1.272	8	NO
CPA.7.030	1.176	15	NO
CPA.7.031	0.673	51	NO
CPA.7.032	0.427	69	NO
CPA.7.033	0.186	86	YES

Figure 52B

Antibody	ELISA Signal	% Blocking	> 80% blocking
CPA.7.034	0.26	81	YES
CPA.7.035	1.16	16	NO
CPA.7.036	0.184	87	YES
CPA.7.037	0.915	34	NO
CPA.7.038	0.301	78	NO
CPA.7.039	0.541	61	NO
CPA.7.040	0.048	97	YES
CPA.7.041	0.356	74	NO
CPA.7.042	0.307	78	NO
CPA.7.043	0.773	44	NO
CPA.7.044	1.025	26	NO
CPA.7.045	0.93	32	NO
CPA.7.046	0.104	92	YES
CPA.7.047	0.223	84	YES
CPA.7.049	0.077	94	YES
CPA.7.050	0.174	87	YES

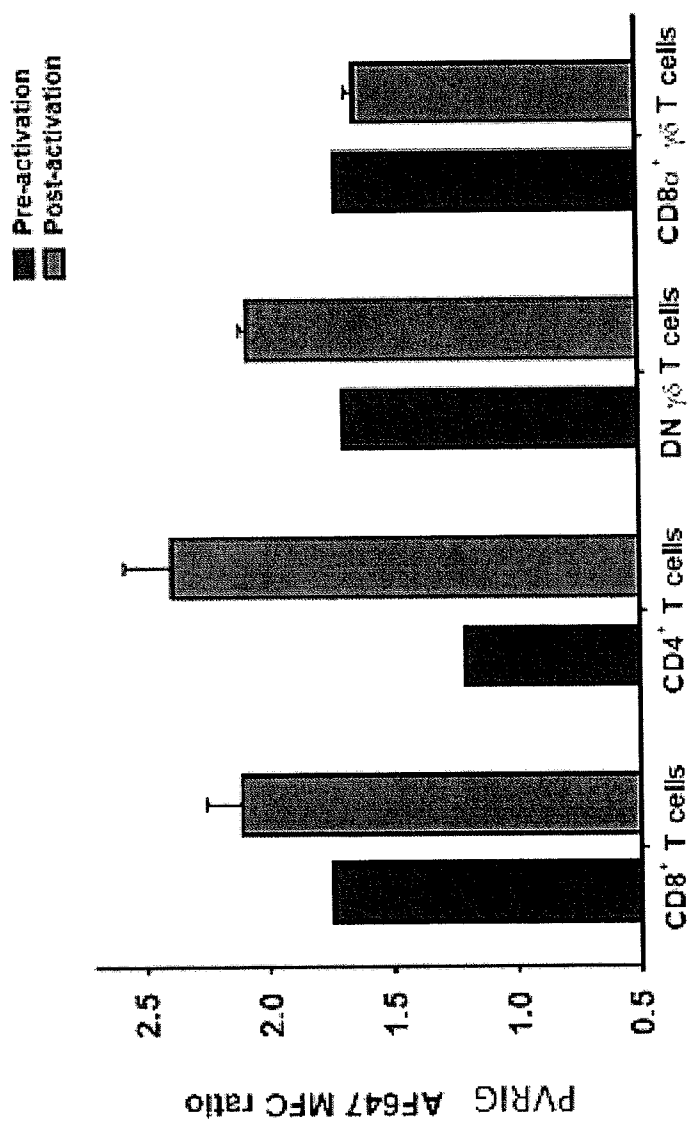


Figure 53

Figure 54

Cell line	ATCC No./CLS /ECACC No.	Morphology	Source
HL-60	ATCC, CCL-240	Leukemia	Blood
THP1	ATCC, TIB-202	Monocyte	Blood
KG-1	ATCC, CCL-246	Macrophage	Bone marrow
Jurkat	ATCC, TIB-152	T Cell	Blood
RPMI8226	ATCC, CCL-155	B lymphocyte	Blood
DAN-G	CLS, 300162	Epithelial	Pancreas
OV90	ATCC, CRL-11732	Epithelial	Ovary
NCI-H441	ATCC, CRL-174	Epithelial	Lung
TF1	ATCC, CRL-2003	Erythroblast	Bone Marrow
Capan2	ATCC, HTB-80	polygonal	Pancreas
ZR75-1	ATCC, CRL-1500	Epithelial	Mammary gland
Karpas299	ECACC, 06072604-1VL	Lymphoma	Blood
NK-YTS	ATCC, CL-273	Lymphoblast	Blood
H9	ATCC, HTB-176	Lymphoblast	Blood
K562	ATCC, CCL-243	Lymphoblast	Bone Marrow
NCI-H929	ATCC, CRL-9068	Lymphoblast	B Lymphocytes
BCP1	ATCC, CRL-2294	Lymphoblast	B Lymphoblast
HUT78	ATCC, TIB-161	Lymphoblast	Blood
HEK293	ATCC, CRL-3216	Epithelial	Kidney

Figure 55

Cell line	ATCC No.	Morphology	Source
4T1	CRL-2539	Epithelial	Mammary gland
B16-F1	CRL-6323	Spindle-shaped+epithelial-like cells	Skin
EL4	TIB-39	T-lymphoblast	blood
E.G7-OVA	CRL-2113	T-lymphoblast	blood
YAC-1	TIB-160	T-lymphoblast	blood
A20	TIB-208	B-lymphoblast	blood
P815	TIB-64	Mast cells	blood
NIH/3T3	CRL-1658	fibroblast	embryo
Sal/N	CRL-2544	fibroblast	fibrosarcoma
J774A.1	TIB-67	Macrophage	blood
LL/2	CRL-1642	Epithelial	Lung
B104-1-1	CRL-1887	Fibroblast	Glioblastoma
RAW264.7	TIB-71	Macrophage	Abelson leukemia virus-induced tumor
P388D1	CCL-46	Macrophage	Lymphoblast
KLN205	CRL-1453	Epithelial	Lung
CT26	CRL-2638	Fibroblast	Colon
Renca	CRL-2947	Epithelial	Kidney

Figure 56A

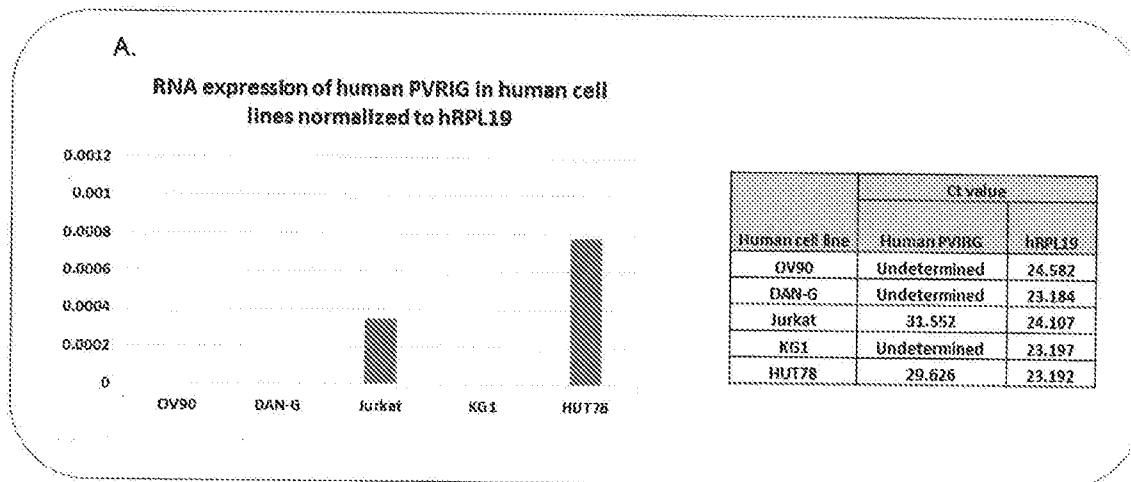


Figure 56B

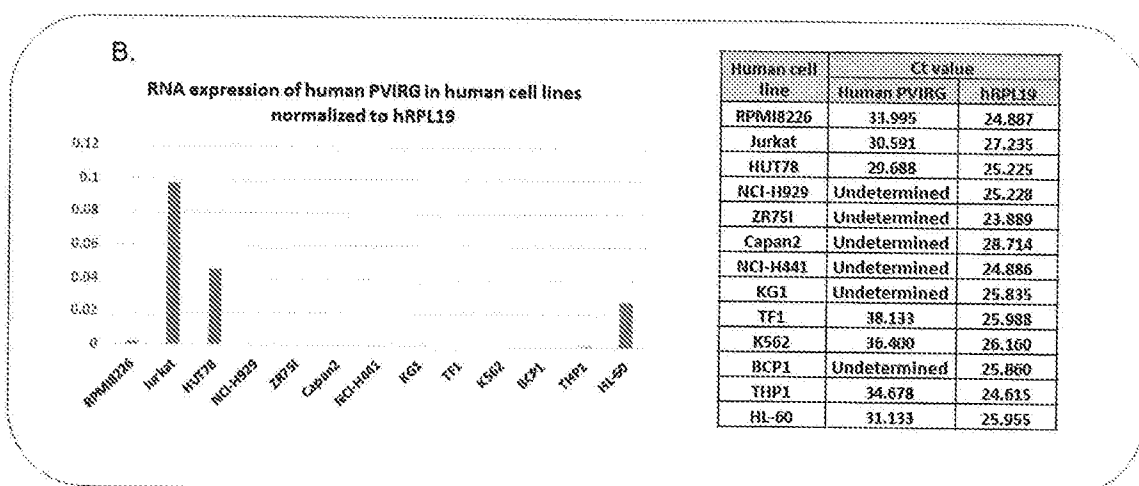


Figure 56C

C.

Human cell line	Ct value					
	Human PVIRG	hTATA box	hRPL19	hPBGD	hSDHA	hHPRT1
DAN-G	39.92	30.14	27.4	27.4	26.59	27.99
NCI-H441	39.46	28.34	26.71	26.71	26.2	27.36
KG1	38.95	28.39	26.74	28.28	26.39	26.39
TF1	39.22	29.25	27.61	33.74	36.25	36.25

Figure 57A

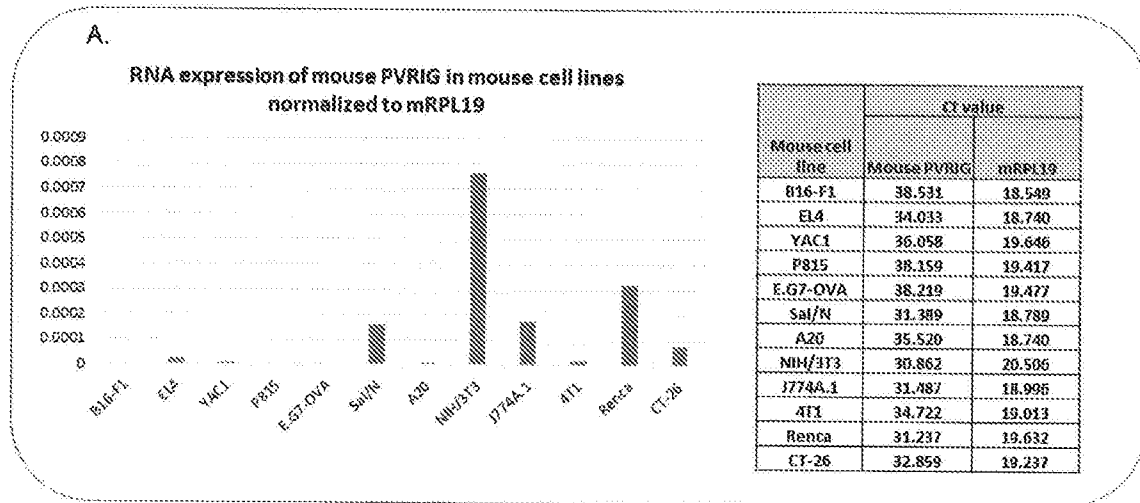


Figure 57B

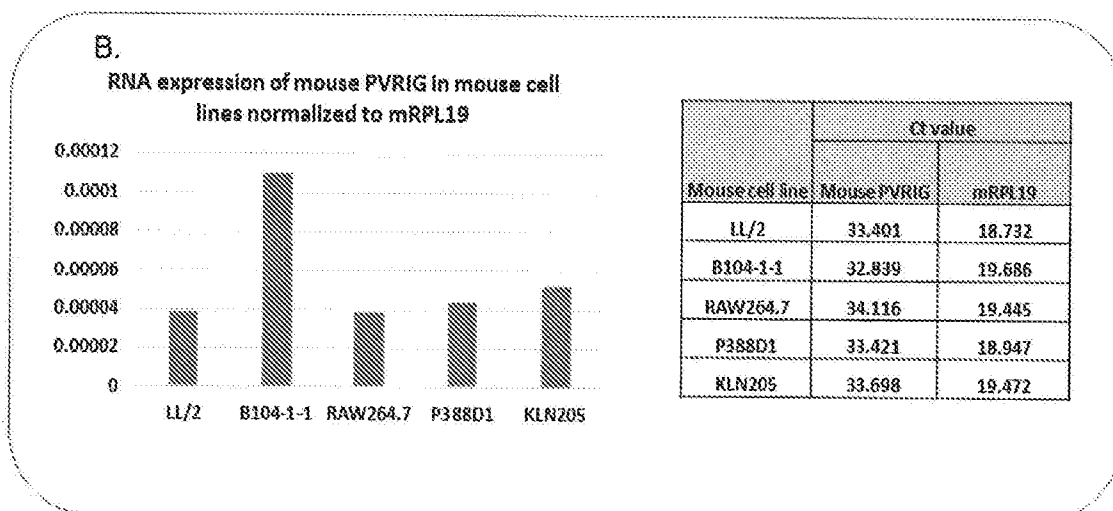


Figure 58

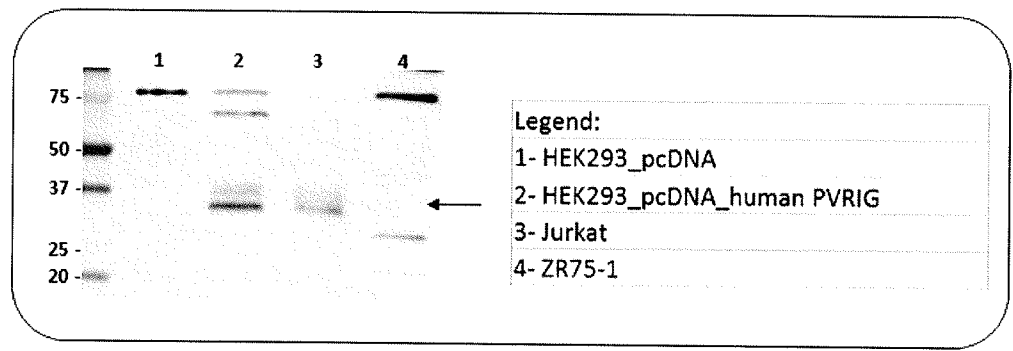


Figure 59

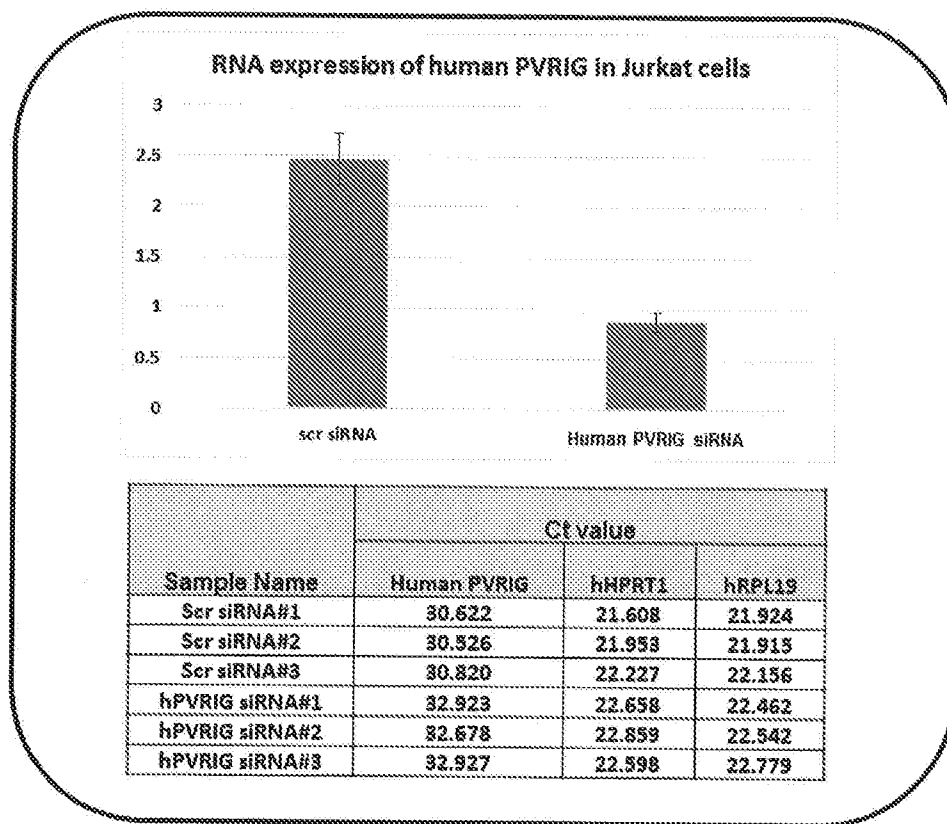


Figure 60

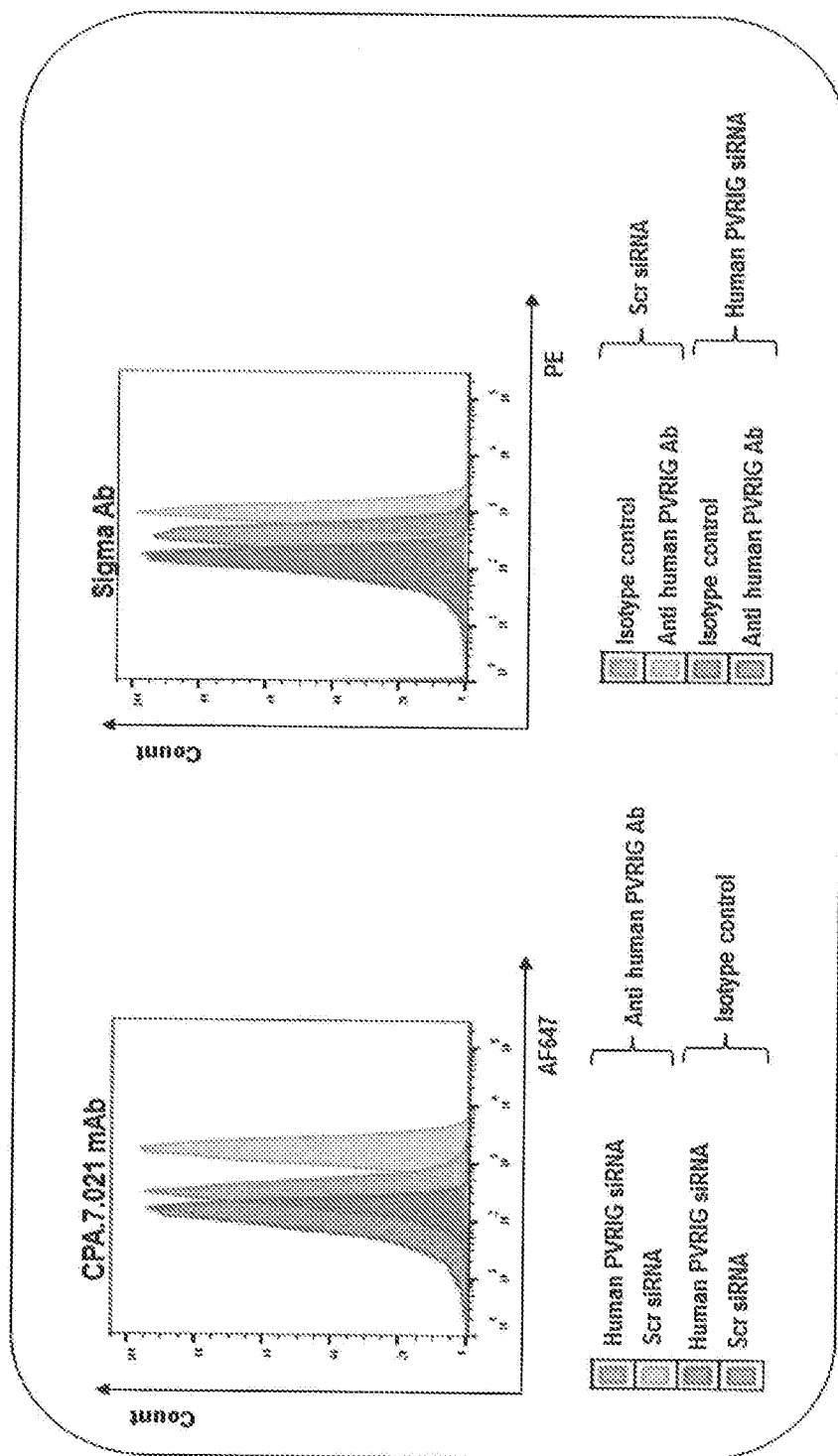


Figure 61

Cell line	qRT-PCR	FACS	WB	%KD-RNA	%KD-FACS
	(PVRIG Ct / HSKG Ct)	(fold change from isotype)			
OV90	Undetermined/24.582	NT	NT		
DAN-G	Undetermined/23.184	NT	NT		
Jurkat	31.552/24.107	14	+	65	82
KG1	Undetermined/23.197	NT	NT		
HUT78	29.626/23.192	X	NT		
RPMI8226	33.995/24.887	NT	NT		
NCI-H929	Undetermined/25.228	NT	NT		
ZR75-1	Undetermined/23.889	X	X		
Capan2	Undetermined/28.714	X	NT		
NCI-H441	Undetermined/24.886	NT	NT		
TF1	38.133/25.988	NT	NT		
K562	36.400/26.160	NT	NT		
BCP1	Undetermined/25.860	NT	NT		
THP1	34.678/24.615	NT	NT		
HL60	31.133/25.955	NT	NT		
Karpas299	NT	X	NT		
NK-YTS	NT	X	NT		

Figure 62

Cell line	qRT-PCR (PVRIG Ct / HSKG Ct)	FACS (fold change from isotype)	WB	%KD- RNA	%KD- FACS
4T1	34.722/19.013	NT	NT		
B16-F1	38.531/18.549	NT	NT		
EL4	34.033/18.740	NT	NT		
YAC-1	36.058/19.646	NT	NT		
P815	38.159/19.417	NT	NT		
E.G7-OVA	38.219/19.477	NT	NT		
NIH3T3	29.94 / 21.25	X	NT		
SAI/N	31.389/18.789	X	NT		
J774A.1	31.487/18.996	X	NT		
A20	35.520/18.740	NT	NT		
LL/2	33.401/18.732	NT	NT		
B104-1-1	32.839/19.686	NT	NT		
RAW264.7	34.116/19.445	NT	NT		
P388D1	33.421/18.947	NT	NT		
KLN205	33.698/19.472	NT	NT		
Renca	31.237/19.632	X	NT		

Figure 63A

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.001	GGTFSSYA	1060	IPIFGTA	1061	AREEVSSPYGMDV	1062	TGAVTSGHY	1063	DTG	1064	LLSYSGASWV	1065
CPA.7.002	GGTFSSSA	1066	IPIYGIT	1067	ARDDTARRVRGVPYVYV MDV	1068	QGIGNY	1069	SAS	1070	QQLKDYPT	1071
CPA.7.003	GFSLSHFS	1072	FDPEEGGT	1073	ATGIWYSSGWVVDY	1074	QSLDSSGNY	1075	LGS	1076	MQALQTPIT	1077
CPA.7.004	GYTLTELS	1078	FPEDGET	1079	ATVSRVRGVINYYVMDV	1080	QSLYRNGNYY	1081	LGS	1082	MQALQTPPT	1083
CPA.7.005	GGTFSSSA	1084	IPIYGIT	1085	ARDDTARRVRGVPYVYV MDV	1086	QSVDS	1087	DAS	1088	QYKDWPF	1089
CPA.7.006	GGTFGTYA	1090	ITPISATI	1091	ARGFEYSDGLDD	1092	QSLFYSDDGNTY	1093	RLS	1094	MQHMEFPLT	1095
CPA.7.007	GGSSSSSY	1096	IYSGST	1097	ARGAWELRLGDWFD	1098	SSNIGAGYD	1099	GNN	1100	QSYDSSLSIWW	1101
CPA.7.008	SGSISSTNW	1102	IYHSGST	1103	ARVGPAIYY	1104	SNNVGYEG	1105	RNN	1106	SAWDSSLNAV	1107
CPA.7.009	GYTLTELS	1108	FPEDGET	1109	ATAKPGIAVAGQNYVYM DV	1110	QSLYRNGNYY	1111	LGS	1112	MQALQTPPT	1113
CPA.7.010	GFTFSSYA	1114	ISYDGSNK	1115	ASSPIGYSYGWGGMDV	1116	SGIDVRTNK	1117	FQSDSK	1118	LIWHTSGWV	1119
CPA.7.011	GYTLTELS	1120	FPEDGET	1121	ATGPAAGVGYYVMDV	1122	QSLYRNGNYY	1123	LGS	1124	MQALQTPPT	1125
CPA.7.012	GFTFSSYA	1126	ISYDGSNK	1127	ARDVMVYCSSTCYFYGM DV	1128	QDIRDY	1129	DAS	1130	QQFENLPIT	1131
CPA.7.013	GYTLTELS	1132	FPEDGET	1133	ATGYSYSGFNYYVMDV	1134	QSLYRNGNYY	1135	LGS	1136	MQALQTPPT	1137
CPA.7.014	GYTLTELS	1138	FPEDGET	1139	ATGVTYYVYVMDV	1140	QSLYSNGNNF	1141	LGS	1142	MQALQTPPT	1143
CPA.7.015	GFTFSSYG	1144	IRYDGSNK	1145	ARDLDFWWDGMDV	1146	QSVSSMY	1147	GAS	1148	QQVYSSPMYT	1149

Figure 63C

Name	Hcdr1	SEQ ID No:	Hcdr2	SEQ ID No:	Hcdr3	SEQ ID No:	SEQ ID No:	Lcdr1	SEQ ID No:	Lcdr2	SEQ ID No:	Lcdr3	SEQ ID No:
CPA.7.031	GGTFSSA	1240	IPIYGIT	1241	ARDDTARRVRGVPYYYYYAMDV	1242	QSLDSDDDGNIH	1243	TLS	1244	MQRKEFPLT	1244	1244
CPA.7.032	GFTSSYG	1246	ISYDGSNK	1247	AKEDRLRFLEWLFYGMIDV	1248	HDIITY	1249	DAS	1250	QQYDNHPPEVT	1250	1250
CPA.7.033	GGTFSSA	1252	IPIYGIT	1253	ARDDTARRVRGVPYYYYYAMDV	1254	QDIDDD	1255	EAS	1256	LQHDNLPLT	1256	1256
CPA.7.034	GYLTELS	1258	FPEDGET	1259	ATEDPGPVAGPYYYYGMDV	1260	QSLLYINGVHY	1261	LGS	1262	MQALQTPPT	1262	1262
CPA.7.035	GYMFTNYP	1264	INAGTGNT	1265	AREGMFYGLESYKGGWFDP	1266	QSLLRNGYNY	1267	LAS	1268	MQALQTPPT	1268	1268
CPA.7.036	GGTFSSA	1270	IPIYGIT	1271	ARDDTARRVRGVPYYYYYAMDV	1272	QSLDSDDDGNTY	1273	TLS	1274	MQRQLFPLT	1274	1274
CPA.7.037	TYFTTYV	1276	YPSGGNT	1277	VRDQNYYSAMDV	1278	QDIRNY	1279	DAS	1280	QQFENLPIT	1280	1280
CPA.7.038	GGTFSSA	1282	IPIYGIT	1283	ARDDTARRVRGVPYYYYYAMDV	1284	RDSDS	1285	DAS	1286	HQYDNLPLT	1286	1286
CPA.7.039	GFNFRGYA	1288	ISGSGGTT	1289	AQSYAQIGYGGHIDH	1290	SGIDVATYM	1291	YKSDSDK	1292	LIWHGSHYV	1292	1292
CPA.7.040	GYLTELS	1294	FPEDGET	1295	ATGVPAAGVYYYYYAMDV	1296	QSLLYRNGYNY	1297	WGS	1298	MQAVQNPPT	1298	1298
CPA.7.041	GGTFSSA	1300	IPIYGIT	1301	ARDDTARRVRGVPYYYYYAMDV	1302	QNIDSS	1303	YAS	1304	HQSSSLPLT	1304	1304
CPA.7.042	GGTFSSA	1306	IPIYGIT	1307	ARDDTARRVRGVPYYYYYAMDV	1308	QSVYNNY	1309	DAS	1310	QQYNSWPPVT	1310	1310
CPA.7.043	GGTFSSYA	1312	IPIFGTA	1313	ARDAYYDSSGGYNPDAFDI	1314	QSLLYSNGYNY	1315	LGS	1316	MQARQTPYT	1316	1316
CPA.7.044	GGTFSSA	1318	IPIYGIT	1319	ARDDTARRVRGVPYYYYYAMDV	1320	ESVTF	1321	DAS	1322	HQHTNWPLT	1322	1322
CPA.7.045	GYLTELS	1324	FPEDGET	1325	ATEVGYCSGGSCYISYYGMDV	1326	QSLLYRNGHNF	1327	LGS	1328	MQALQTPPT	1328	1328
CPA.7.046	GGTFSSA	1330	IPIYGIT	1331	ARDDTARRVRGVPYYYYYAMDV	1332	QTMNNY	1333	DAS	1334	QQYGDWLPIIT	1335	1335

Figure 63D

Name	Hcdr1	SEQ ID NO:	Hcdr2	SEQ ID NO:	Hcdr3	SEQ ID NO:	Lcdr1	SEQ ID NO:	Lcdr2	SEQ ID NO:	Lcdr3	SEQ ID NO:
CPA.7.047	GTYLTLS	1336	FDPEDGET	1337	ATAFPEATISYYYMDV	1338	QSLLYRNGYNY	1339	WGS	1340	MQAVQNPPT	1341
CPA.7.049	GGTFSSA	1342	IPIYGIT	1343	ARDDTARRVRGVPPYYYAMDV	1344	RSLLDSDDGNTNTH	1345	SLS	1346	MQRKEFPLT	1347
CPA.7.050	GGTFSSYA	1348	IPIFGTA	1349	ARGPWYDSSSGYSSYAYYMDV	1350	QSLHSDGYNY	1351	LGS	1352	MQALHTPGVT	1353

Figure 64A

Disease	CD8A	CD4	CD3G	PD-1
Kidney renal clear cell carcinoma	0.89	0.75	0.83	0.9
Sarcoma	0.87	0.64	0.88	0.82
Liver hepatocellular carcinoma	0.86	0.6	0.78	0.63
Skin Cutaneous Melanoma	0.86	0.77	0.87	0.89
Head and Neck squamous cell carcinoma	0.85	0.72	0.82	0.86
Breast invasive carcinoma	0.84	0.67	0.81	0.85
Testicular Germ Cell Tumors	0.84	0.66	0.88	0.84
Stomach adenocarcinoma	0.8	0.66	0.78	0.78
Mesothelioma	0.79	0.63	0.8	0.67
Thyroid carcinoma	0.79	0.72	0.87	0.83
Lung squamous cell carcinoma	0.78	0.65	0.77	0.81
Kidney Chromophobe	0.77	0.6	0.7	0.71
Pancreatic adenocarcinoma	0.77	0.57	0.76	0.8
Rectum adenocarcinoma	0.77	0.69	0.68	0.72
Uterine Corpus Endometrial Carcinoma	0.77	0.68	0.75	0.7

Figure 64B

Bladder Urothelial Carcinoma	0.76	0.68	0.81	0.82
Lung adenocarcinoma	0.76	0.52	0.74	0.77
Prostate adenocarcinoma	0.76	0.73	0.72	0.76
Cervical squamous cell carcinoma and endocervical adenocarcinoma	0.74	0.64	0.76	0.7
Colon adenocarcinoma	0.74	0.71	0.75	0.67
Kidney renal papillary cell carcinoma	0.69	0.51	0.66	0.61
Uterine Carcinosarcoma	0.69	0.4	0.59	0.58
Ovarian serous cystadenocarcinoma	0.67	0.64	0.66	0.6
Esophageal carcinoma	0.63	0.57	0.66	0.65
Acute Myeloid Leukemia	0.58	-0.15	0.56	0.29
Pheochromocytoma and Paraganglioma	0.57	0.33	0.42	0.49
Adrenocortical carcinoma	0.55	0.49	0.66	0.64

Figure 65

Antibodies	Isotype	Conjugated to	Manufacturer	Catalog number	concentration (ug/ul)	Staining concentration
Anti-human PVRIG - CPA.7.021	Human IgG2	AF-647	Compugen	CPA.7.021	0.2	7.5 ug/ml
Human IgG2 isotype control	Human IgG2	AF-647	Compugen		0.2	7.5 ug/ml
Anti-CD4	mIgG1	FITC	Biolegend	300506	0.4	4 ug/ml
Anti-CD8	mIgG1	FITC	Biolegend	300906	0.15	1.5 ug/ml
Anti-CD137	mIgG1	PE	Biolegend	309804	0.2	2 ug/ml
Anti-PD1	mIgG1	APC-cy7	Biolegend	329922	0.2	4 ug/ml
isotype control	mIgG1	PE	Biolegend	400112	0.4	2 ug/ml
isotype control	mIgG1	APC-cy7	Biolegend	400128	0.2	4 ug/ml

Figure 66A

CH1																						
SEQ ID NO:	EU Index	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138
1354 (IgG1)	IgG1	A	S	T	K	G	P	S	V	F	P	L	A	P	S	S	K	S	T	S	G	G
1355 (IgG2)	IgG2	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	E	S
1356 (IgG3)	IgG3	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	G	G
1357 (IgG4)	IgG4	A	S	T	K	G	P	S	V	F	P	L	A	P	C	S	R	S	T	S	E	S

EU Index	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159
IgG1	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N
IgG2	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N
IgG3	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N
IgG4	T	A	A	L	G	C	L	V	K	D	Y	F	P	E	P	V	T	V	S	W	N

EU Index	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180
IgG1	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y
IgG2	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y
IgG3	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y
IgG4	S	G	A	L	T	S	G	V	H	T	F	P	A	V	L	Q	S	S	G	L	Y

EU Index	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201
IgG1	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	I	C	N
IgG2	S	L	S	S	V	V	T	V	P	S	S	N	F	G	T	Q	T	Y	T	C	N
IgG3	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	Q	T	Y	T	C	N
IgG4	S	L	S	S	V	V	T	V	P	S	S	S	L	G	T	K	T	Y	T	C	N

EU Index	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220
IgG1	V	N	H	K	P	S	N	T	K	V	D	K	K	V	E	P	K	S	C
IgG2	V	D	H	K	P	S	N	T	K	V	D	K	T	V	E	R	K	C	C
IgG3	V	N	H	K	P	S	N	T	K	V	D	K	R	V	E	L	K	T	P
IgG4	V	D	H	K	P	S	N	T	K	V	D	K	R	V	E	S	K	Y	G

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

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

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

Figure 66B

		CH2																											
SEQ ID NO:	EU Index	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257							
1354 (IgG1)	IgG1	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P							
1355 (IgG2)	IgG2	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P							
1356 (IgG3)	IgG3	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P							
1357 (IgG4)	IgG4	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P							
		258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278							
	IgG1	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y							
	IgG2	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	O	F	N	W	Y							
	IgG3	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	Q	F	K	W	Y							
	IgG4	E	V	T	C	V	V	V	D	V	S	O	E	D	P	E	V	Q	F	N	W	Y							
		279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299							
	IgG1	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T							
	IgG2	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	F	N	S	T							
	IgG3	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T							
	IgG4	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	F	N	S	T							
		300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320							
	IgG1	Y	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K							
	IgG2	F	R	V	V	S	V	L	T	V	V	H	Q	D	W	L	N	G	K	E	Y	K							
	IgG3	F	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K							
	IgG4	Y	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K							
		321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340								
	IgG1	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K								
	IgG2	C	K	V	S	N	K	G	L	P	A	P	I	E	K	T	I	S	K	T	K								
	IgG3	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	T	K								
	IgG4	C	K	V	S	N	K	G	L	P	S	S	I	E	K	T	I	S	K	A	K								
		CH3																											
		341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361							
	IgG1	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N							
	IgG2	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N							
	IgG3	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N							
	IgG4	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	Q	E	E	M	T	K	N							
		362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382							
	IgG1	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E							
	IgG2	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E							
	IgG3	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E							
	IgG4	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E							
		383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403							
	IgG1	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S							
	IgG2	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	M	L	D	S	D	G	S							
	IgG3	S	S	G	Q	P	E	N	N	Y	N	T	T	P	P	M	L	D	S	D	G	S							
	IgG4	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S							

Figure 66C

SEQ ID NO:	EU Index	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424
1354 (IgG1)	IgG1	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S
1355 (IgG2)	IgG2	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S
1356 (IgG3)	IgG3	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	I	F	S
1357 (IgG4)	IgG4	F	F	L	Y	S	R	L	T	V	D	K	S	R	W	Q	E	G	N	V	F	S

EU Index	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445
IgG1	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P
IgG2	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P
IgG3	C	S	V	M	H	E	A	L	H	N	R	F	T	Q	K	S	L	S	L	S	P
IgG4	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	L

EU Index	446	447
IgG1	G	K
IgG2	G	K
IgG3	G	K
IgG4	G	K

Figure 67

PVRIG ECD Fragment A TTLAVLHPERGIRQWAPARQA (SEQ ID NO: 1358)

PVRIG ECD Fragment B TTLAVLHPERGIRQWAPARQARWETQSSISLILE (SEQ ID NO: 1359)

PVRIG ECD Fragment C ARQARWETQSSISLILE (SEQ ID NO: 1360)

PVRIG ECD Fragment D TFCCKFASFPEGSWEA (SEQ ID NO: 1361)

PVRIG ECD Fragment E TFCCKFASFPEGSWEACGSLPPSS (SEQ ID NO: 1362)

PVRIG ECD Fragment F EATELSSFTIRCGFL (SEQ ID NO: 1363)

PVRIG ECD Fragment G EVVVQVRMEATELSSFTIRCGF (SEQ ID NO: 1364)

PVRIG ECD Fragment H FTIRCGFLGSGISLVTVS (SEQ ID NO: 1365)

PVRIG ECD Fragment I SISLVTVSWGPNAGAGGTTLAVLH (SEQ ID NO: 1366)

PVRIG ECD Fragment J SISLILEGSGASSPCANTTFCCKFAS (SEQ ID NO: 1367)

Figure 68

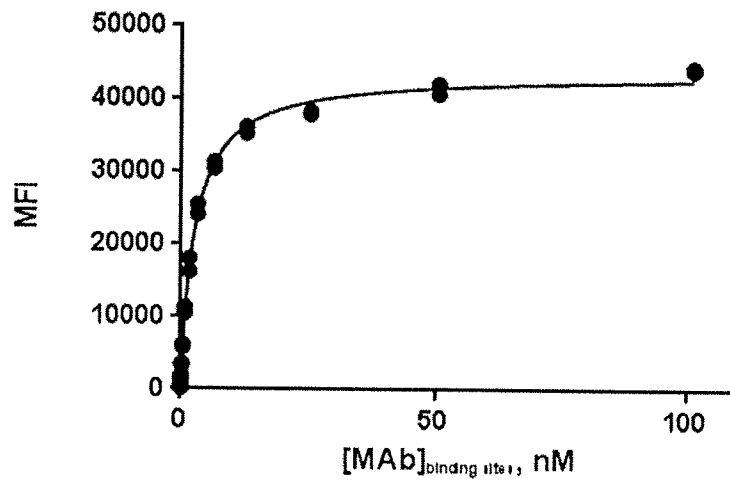


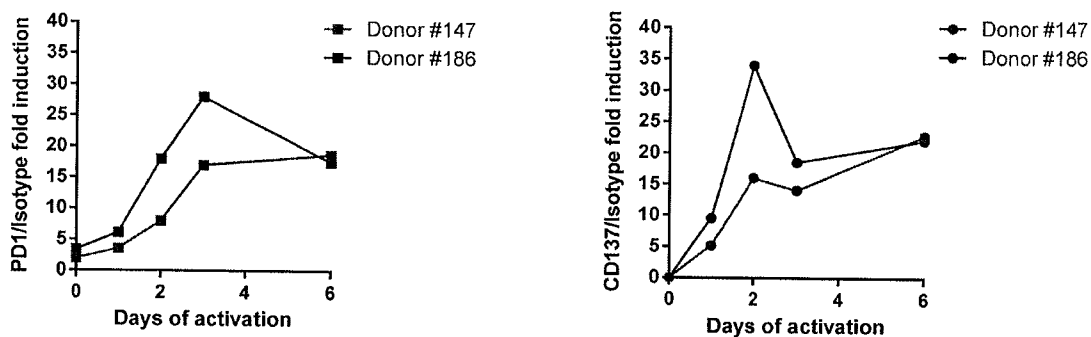
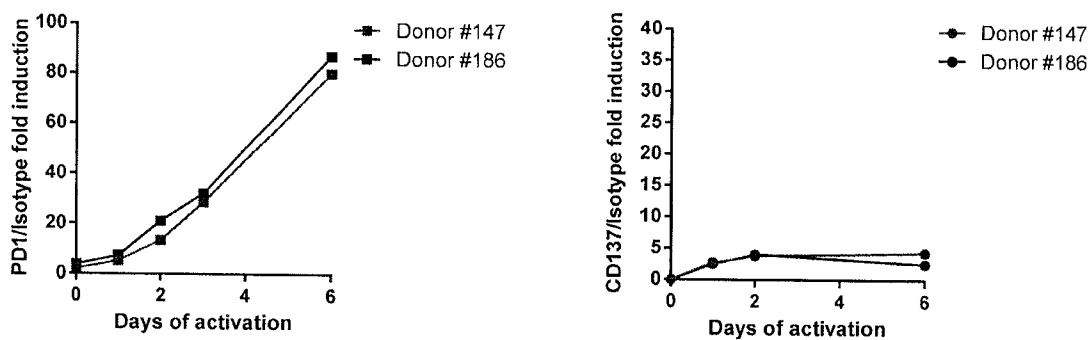
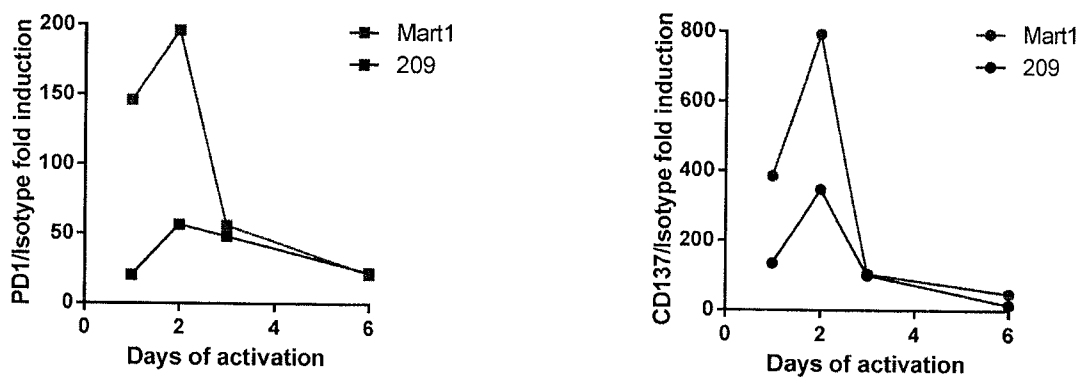
Figure 69A**Figure 69B****Figure 69C**

Figure 70A

A

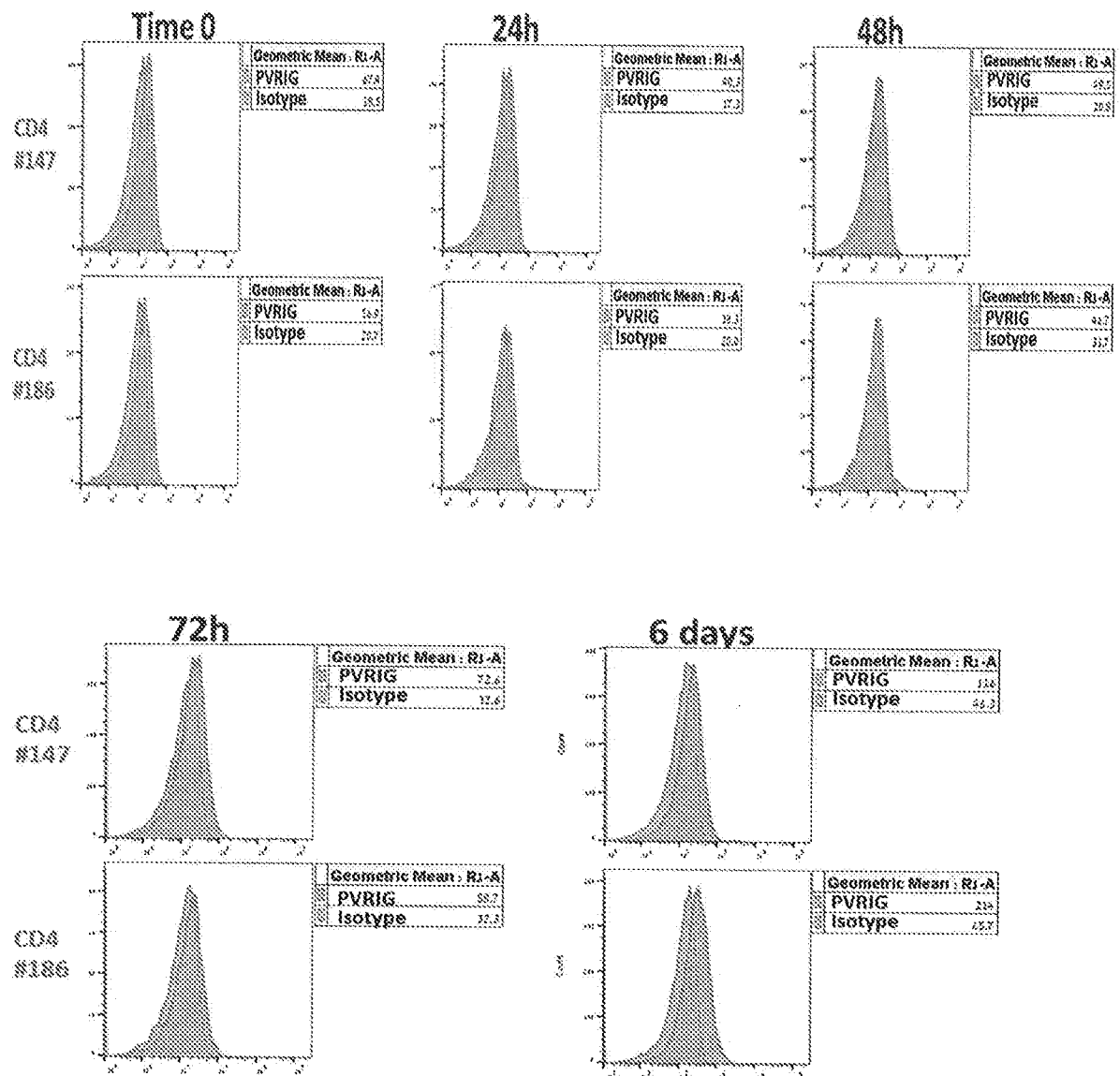


Figure 708

B

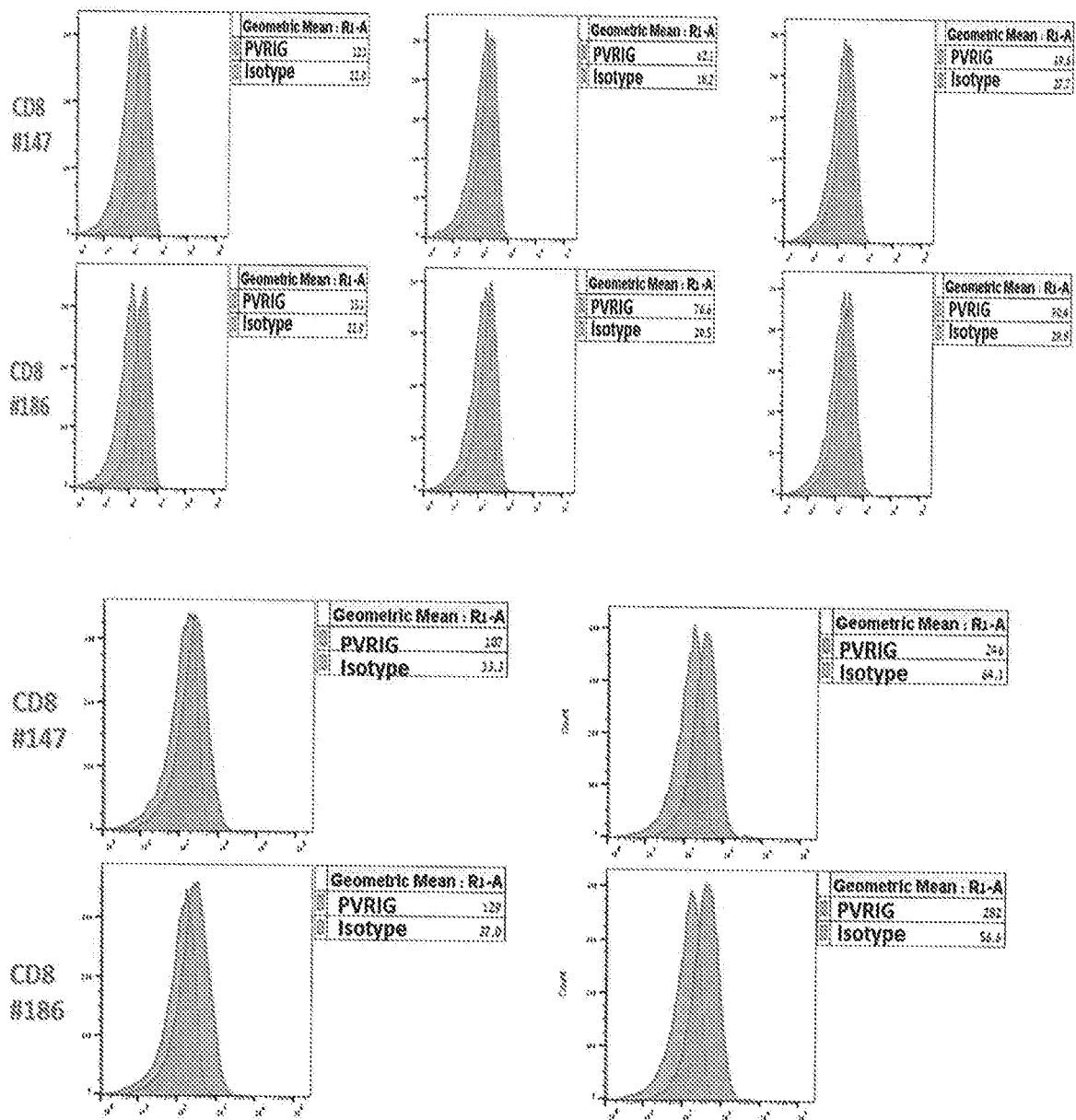


Figure 70C

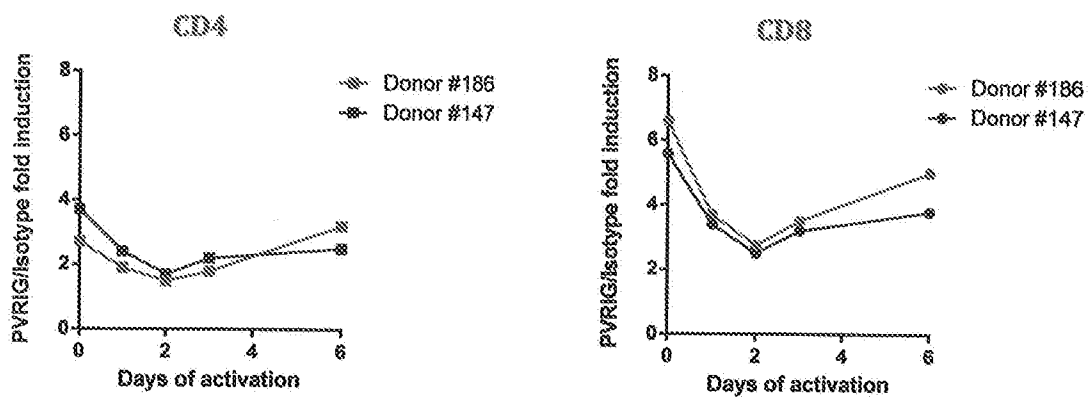


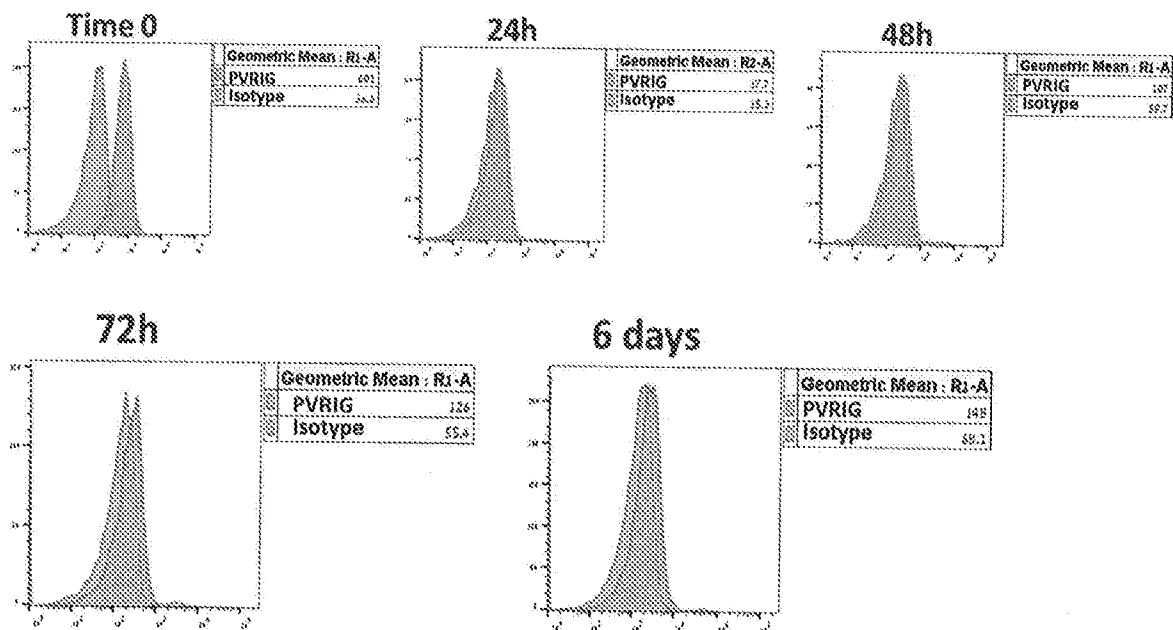
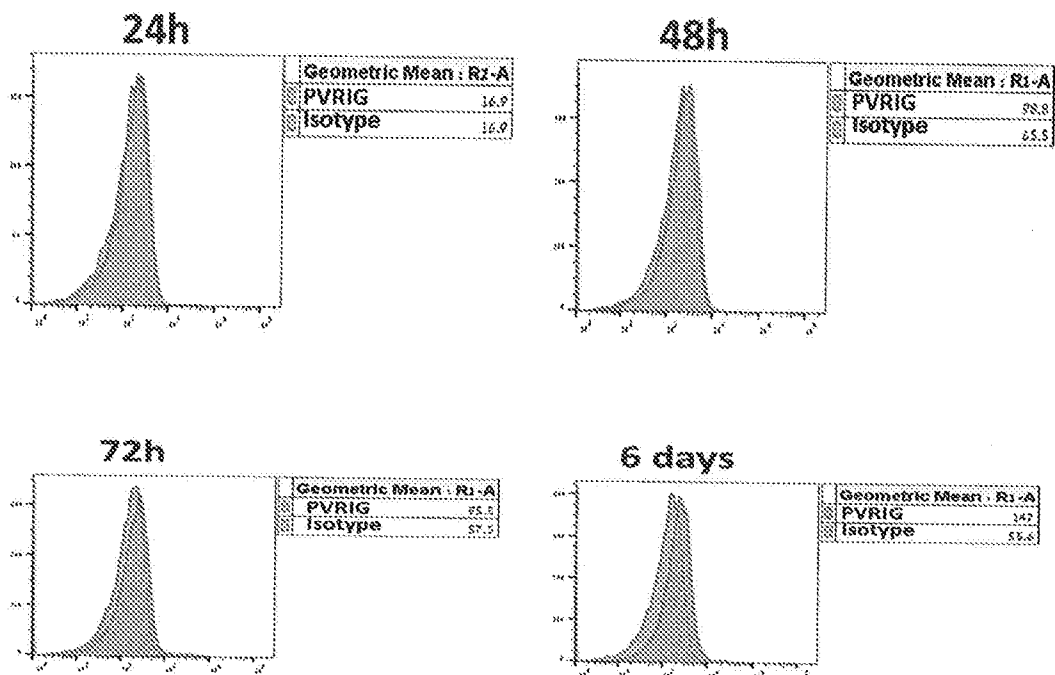
Figure 71A**Figure 71B**

Figure 71C

C

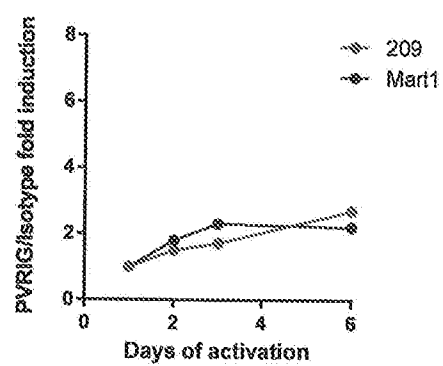


Figure 72

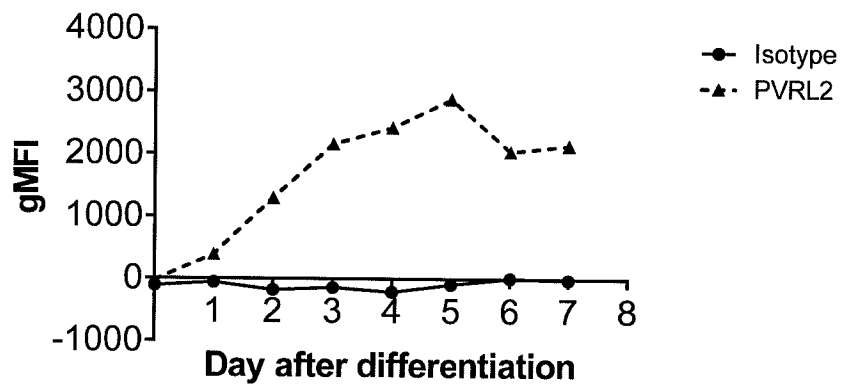
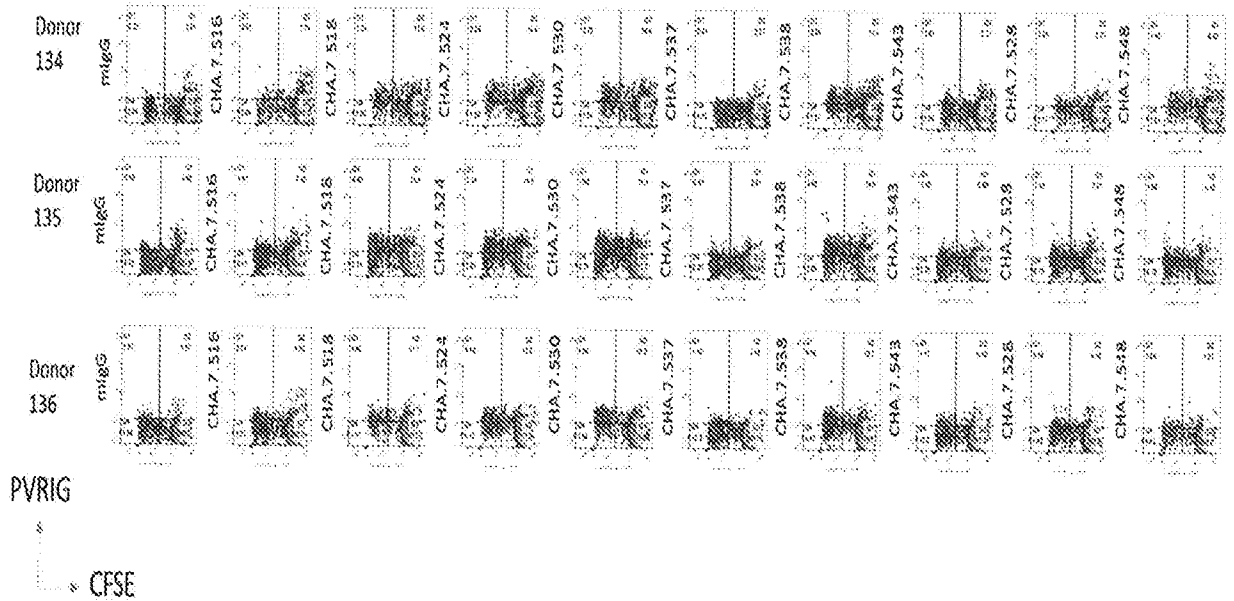


Figure 73A

A. CD4

**Figure 73B**

B. CD8

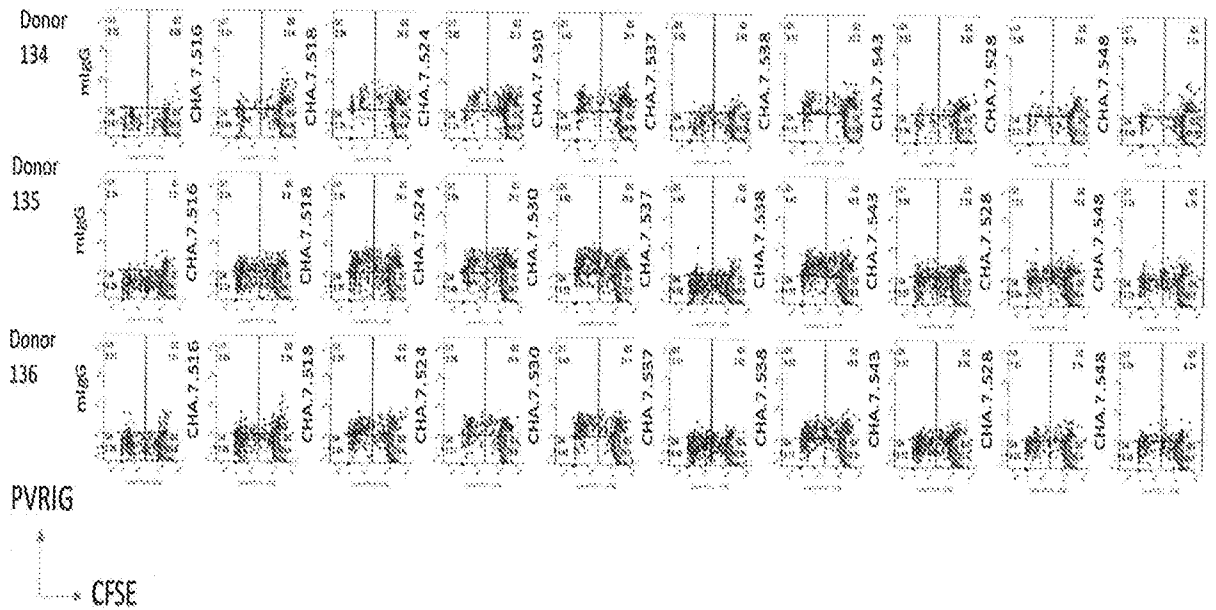


Figure 74A

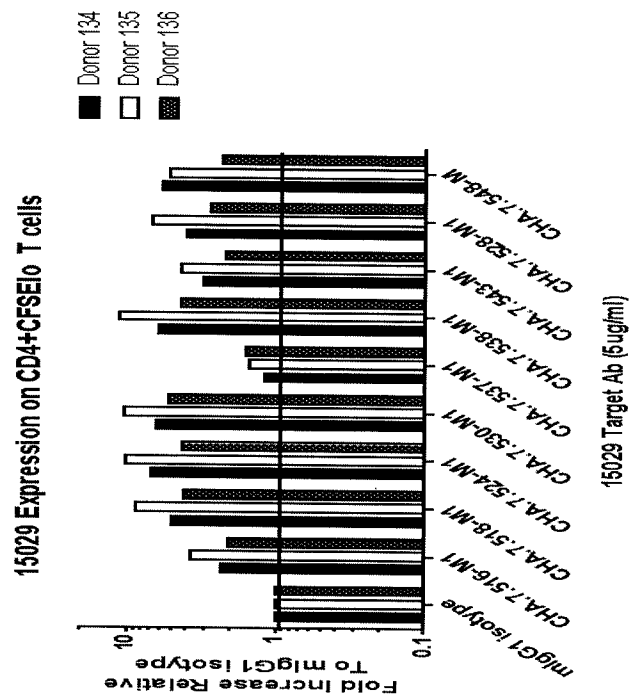


Figure 74B

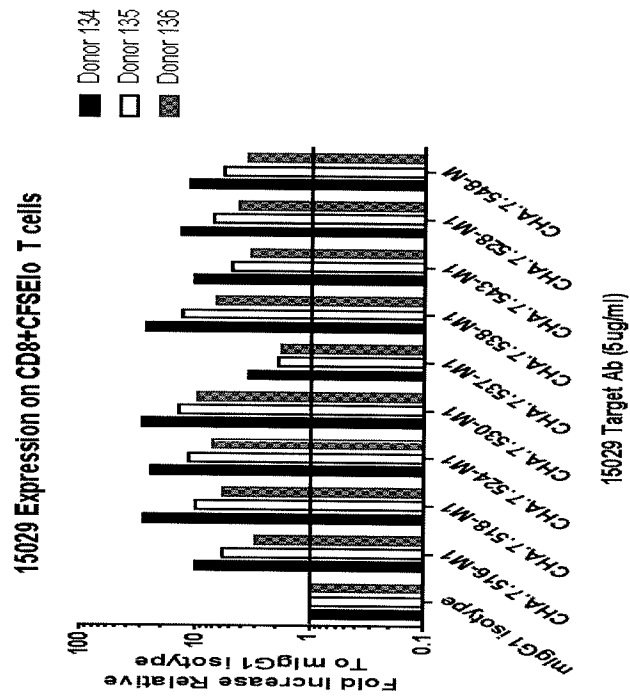


Figure 75A

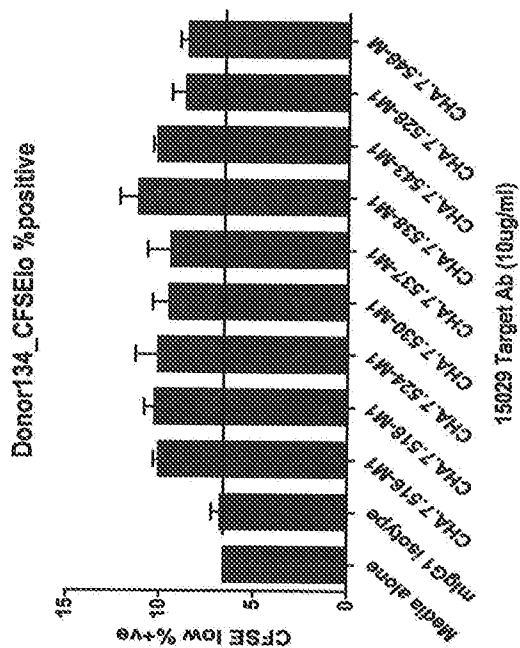


Figure 75B

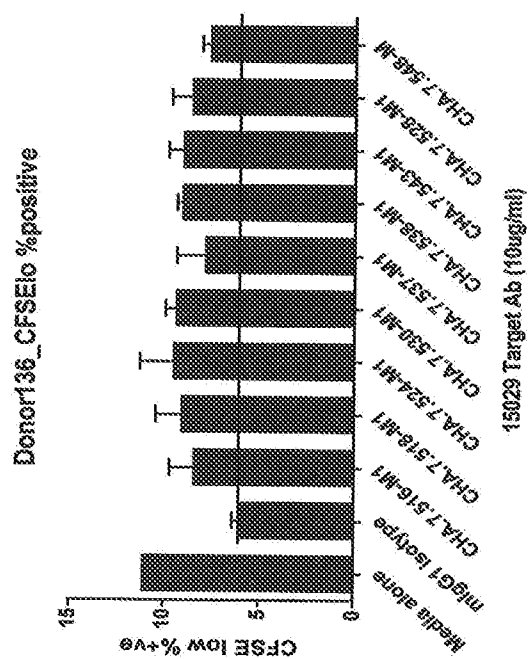


Figure 76

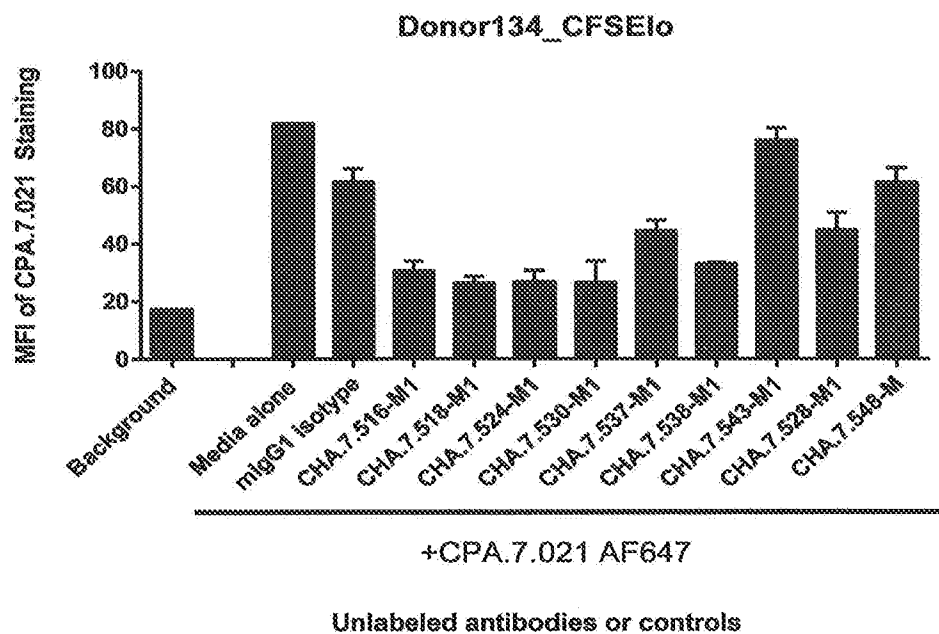


Figure 77

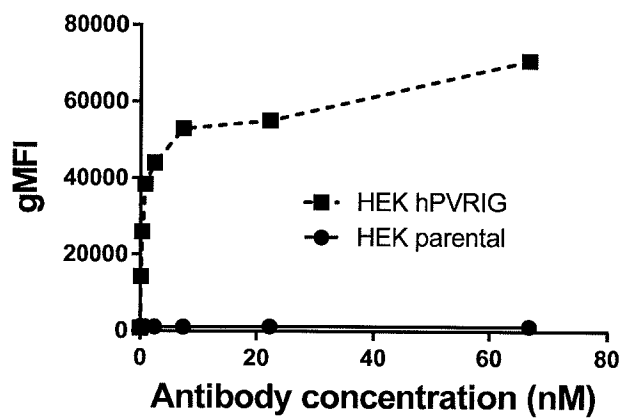


Figure 78

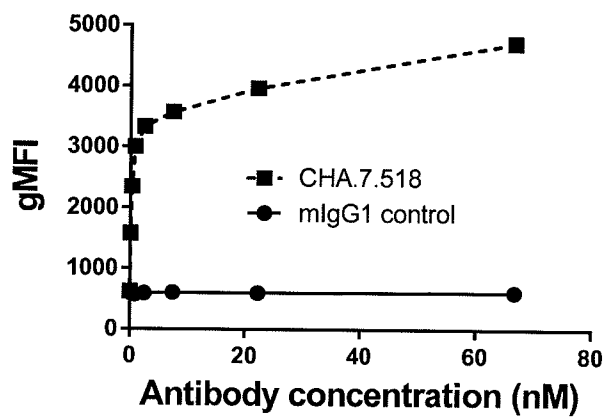


Figure 79A

Antibody (mIgG)	EC ₅₀ (HEK OE, nM)	HEK OE/par (3.3ug/ml, gMFir)	Jurkat (3.3ug/ml, gMFir)
CHA.7.502	40.18	71.99	6.21
CHA.7.503	1.05	260.98	23.59
CHA.7.506	No binding	0.76	No binding
CHA.7.508	3.30	45.86	6.50
CHA.7.510	92.81	16.32	4.19
CHA.7.512	52.99	5.12	1.47
CHA.7.514	5.31	49.82	7.67
CHA.7.516	0.79	37.90	5.73
CHA.7.518	0.36	42.24	6.58
CHA.7.520	No binding	1.01	No binding
CHA.7.522	91.44	10.12	2.99
CHA.7.524	0.46	48.33	7.87
CHA.7.525	3.05	41.86	4.30
CHA.7.526	2.99	47.28	3.98
CHA.7.527	No binding	0.98	No binding
CHA.7.528	7.31	44.88	6.17
CHA.7.530	0.33	51.14	8.04
CHA.7.534	1.87	43.72	5.05
CHA.7.535	3.67	40.44	2.79
CHA.7.537	2.47	36.61	5.53

Figure 79B

CHA.7.538	0.52	35.80	7.37
CHA.7.543	0.52	49.81	6.73
CHA.7.544	0.76	42.60	5.68
CHA.7.545	0.76	44.31	6.53
CHA.7.546	0.61	43.14	6.42
CHA.7.547	14.37	10.94	1.02
CHA.7.548	0.27	45.26	7.37
CHA.7.549	2.60	29.71	2.23
CHA.7.550	1.34	27.72	2.10

Figure 80A

Antibody (mgG)	Human CD56 int. NK (gMFir, 10ug/ml)	Human CD8+ T cells (gMFir, 10ug/ml)	Expi cyno OE/par (gMFir, 3.3ug/ml)	Cyno NK cells (gMFir, 10ug/ml)	Cyno CD8+ T cells (gMFir, 10ug/ml)
CHA.7.502	1.97	1.41	60.49	Not tested	Not tested
CHA.7.503	3.15	1.96	106.3	Not tested	Not tested
CHA.7.506	Not tested	Not tested	0.77	Not tested	Not tested
CHA.7.508	3.6	4.09	41.49	Not tested	Not tested
CHA.7.510	3.13	2.73	38.21	Not tested	Not tested
CHA.7.512	1.30	1.15	8.96	Not tested	Not tested
CHA.7.514	4.16	5.15	65.20	Not tested	Not tested
CHA.7.516	4.22	4.09	60.05	1.76	2.09
CHA.7.518	5.08	6.69	83.51	1.92	2.09
CHA.7.520	1.13	1.04	Not tested	Not tested	Not tested
CHA.7.522	2.06	1.90	27.24	Not tested	Not tested

Figure 80B

CHA.7.524	5.50	6.12	66.32	1.78	2.02
CHA.7.525	1.98	1.76	0.85	Not tested	Not tested
CHA.7.526	2.08	1.71	0.79	Not tested	Not tested
CHA.7.527	1.16	0.99	Not tested	Not tested	Not tested
CHA.7.528	3.08	3.63	12.2	1.21	1.18
CHA.7.530	6.04	6.47	60.80	1.73	1.89
CHA.7.534	2.60	1.96	46.27	Not tested	Not tested
CHA.7.535	2.24	1.28	0.82	Not tested	Not tested
CHA.7.537	3.90	3.41	1.55	1.18	1.19
CHA.7.538	6.49	6.17	15.16	1.36	1.45
CHA.7.543	4.48	4.33	0.83	1.35	1.39
CHA.7.544	2.36	2.54	61.09	Not tested	Not tested
CHA.7.545	2.54	2.82	0.91	Not tested	Not tested
CHA.7.546	2.75	2.95	0.85	Not tested	Not tested
CHA.7.547	2.21	1.13	26.65	Not tested	Not tested
CHA.7.548	3.15	3.35	4.25	1.18	1.09
CHA.7.549	3.05	1.42	1.00	Not tested	Not tested
CHA.7.550	1.60	1.29	0.90	Not tested	Not tested

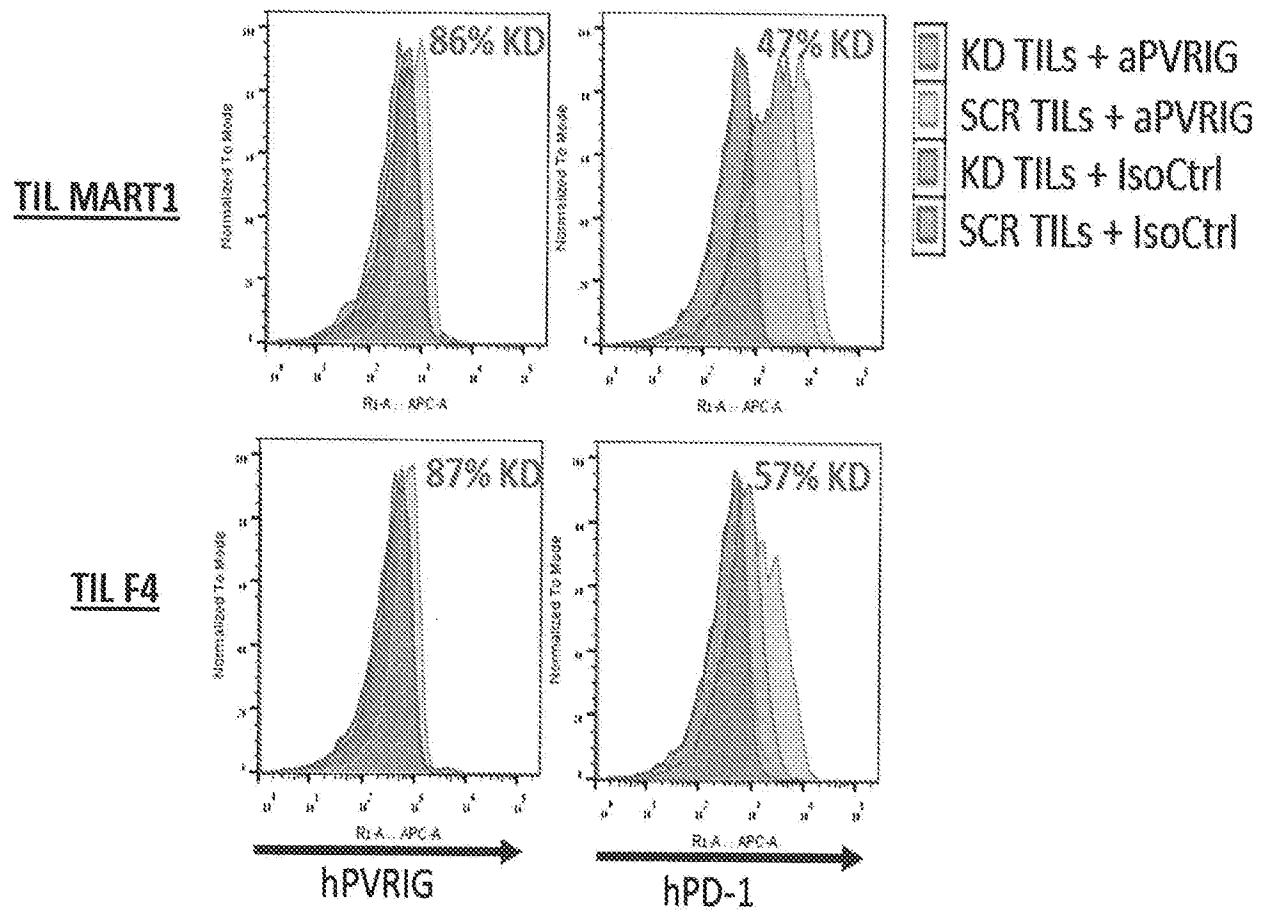
Figure 81A

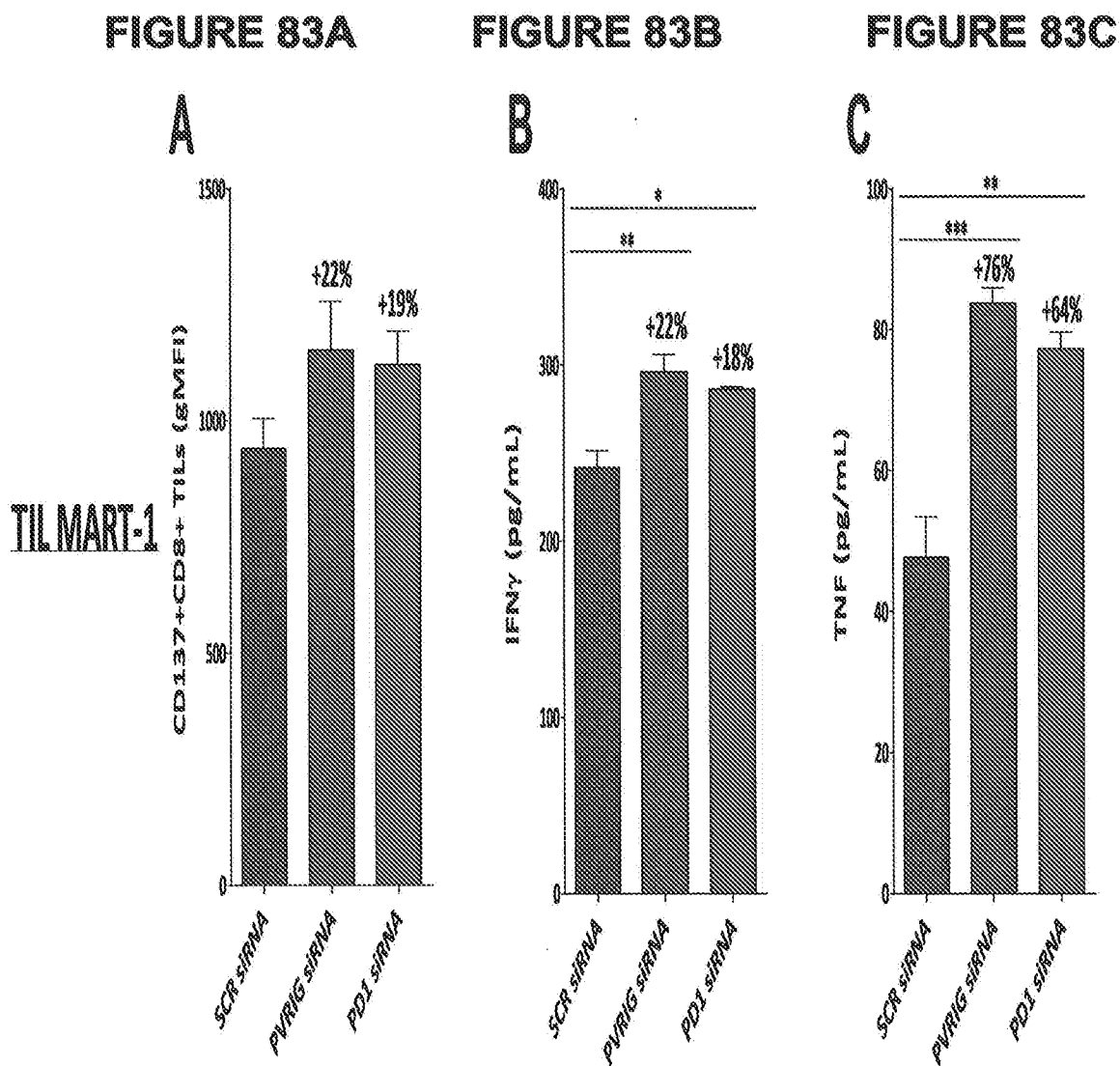
Antibody (mIgG)	IC₅₀ (nM)
CHA.7.502	39.90
CHA.7.503	No IC ₅₀
CHA.7.506	31.65
CHA.7.508	37.88
CHA.7.510	55.00
CHA.7.512	839.6
CHA.7.514	38.88
CHA.7.516	33.11
CHA.7.518	23.15
CHA.7.520	619.3
CHA.7.522	50.48
CHA.7.524	30.20
CHA.7.525	85.52
CHA.7.526	58.88
CHA.7.527	No IC ₅₀
CHA.7.528	28.88
CHA.7.530	34.56
CHA.7.534	181.4
CHA.7.535	821.1
CHA.7.537	38.95
CHA.7.538	51.87
CHA.7.543	No IC ₅₀

Figure 81B

CHA.7.544	No IC ₅₀
CHA.7.545	96.06
CHA.7.546	92.05
CHA.7.547	27.94
CHA.7.548	18.98
CHA.7.549	36.12
CHA.7.550	58.34

Figure 82





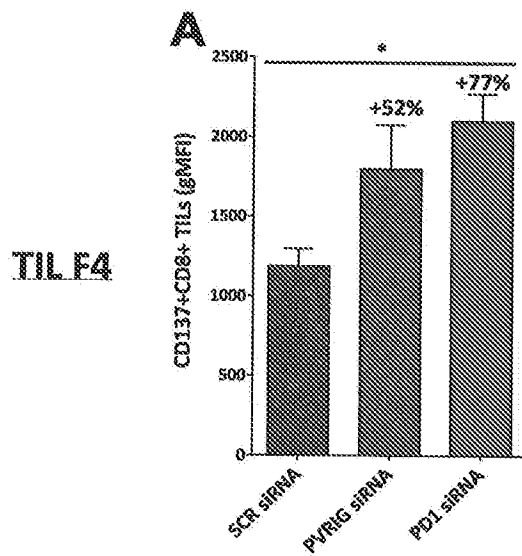


FIGURE 84A

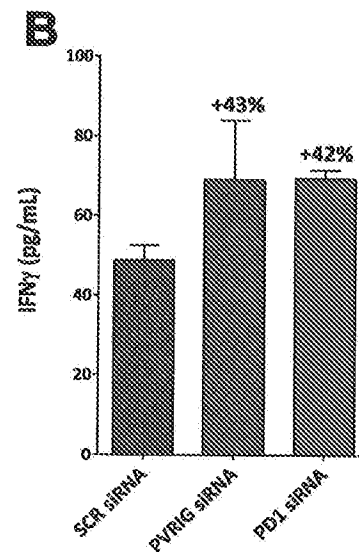


FIGURE 84B

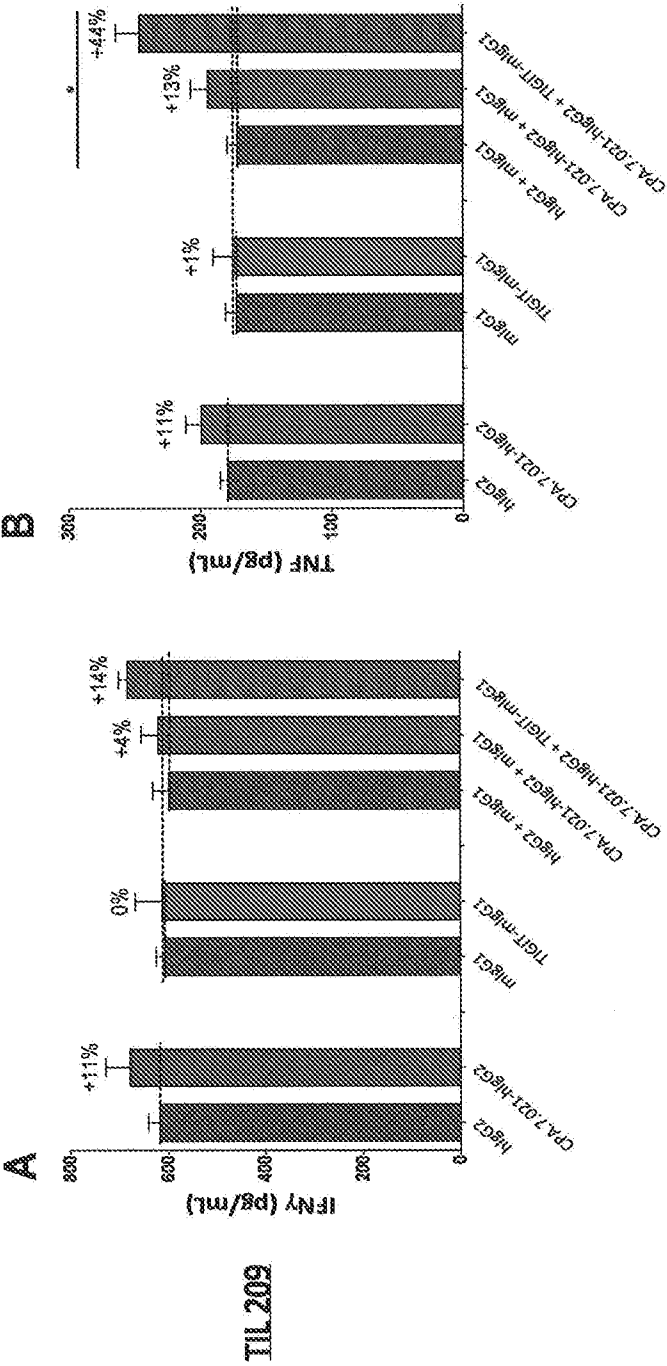
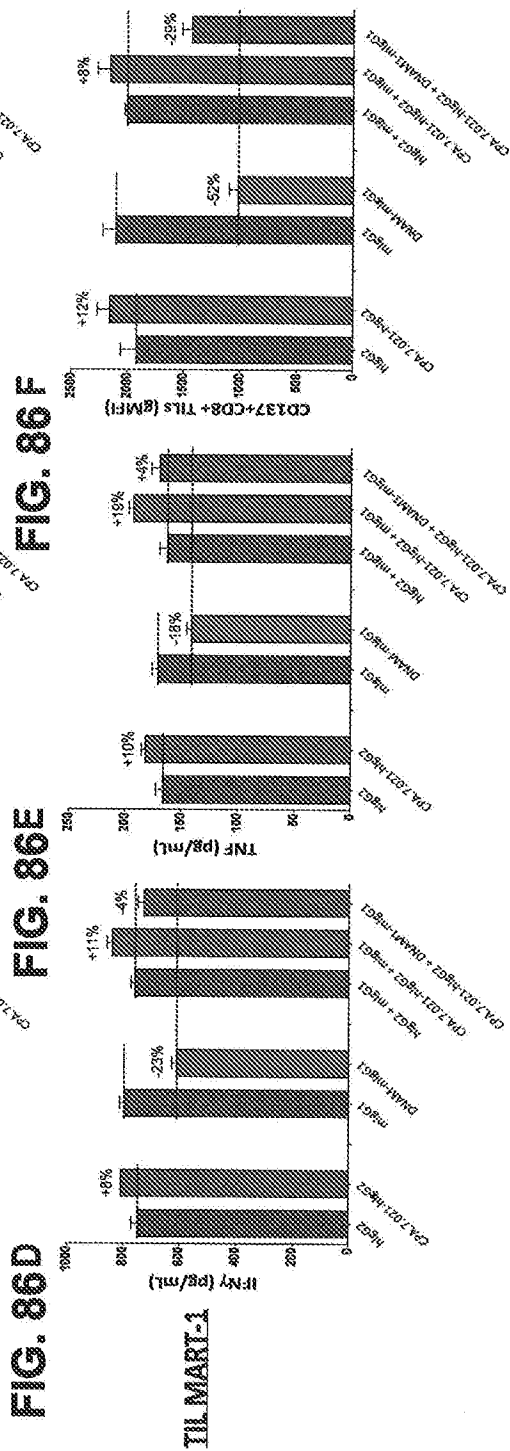
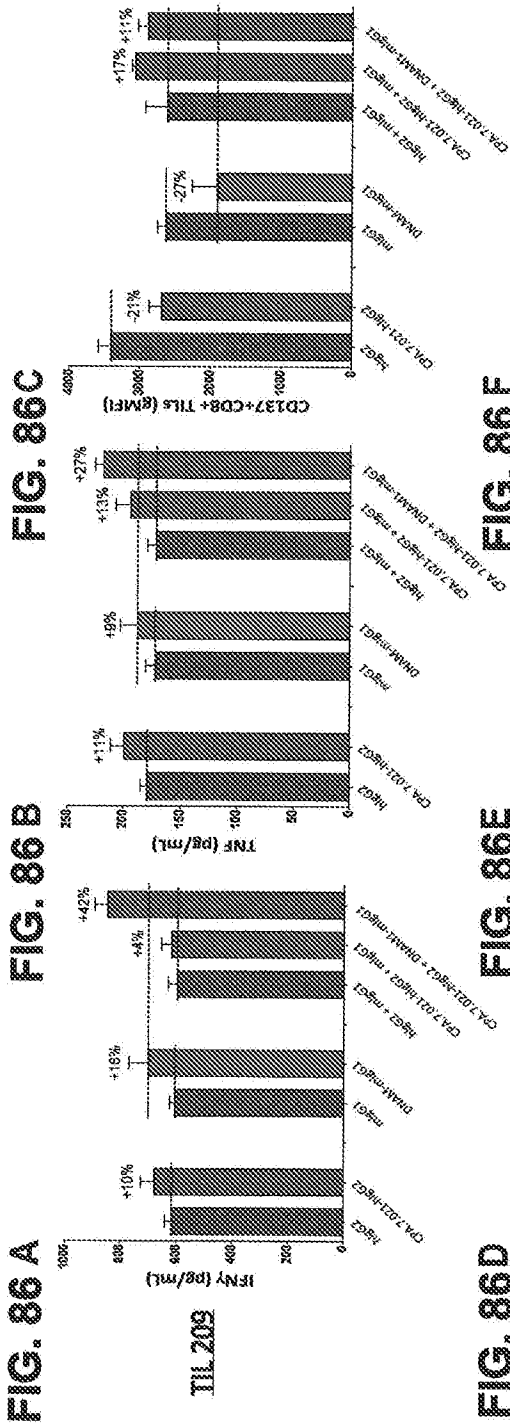
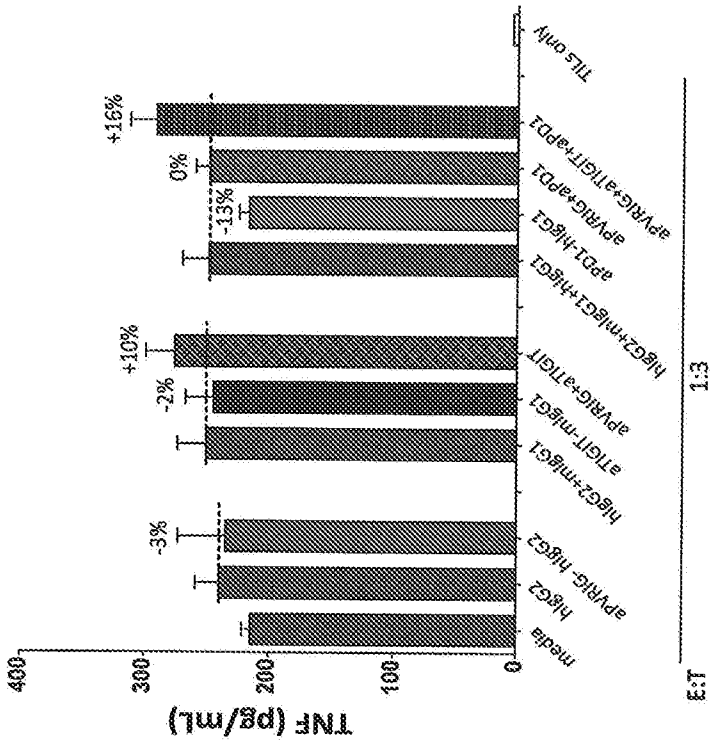


FIGURE 85B

FIGURE 85A



B



A

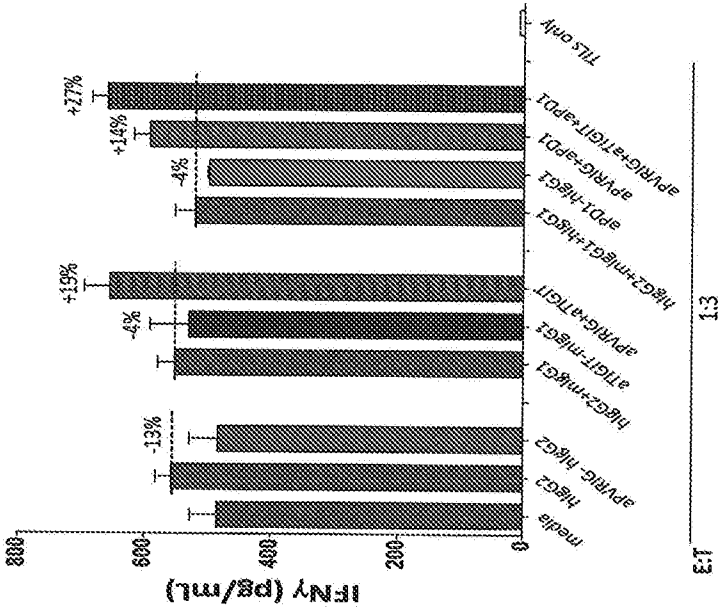


FIGURE 87A

FIGURE 87B

Figure 88A
Humanized sequences of CHA.7.518 antibody VH

Potential humanized sequence based on IMGT IGHV1-46*01 acceptor framework (AbM CDR definition)

IGHV1-46*01 QVQLVQSGAEVKKPGASVKVCKASGYFTFTSYMHVWRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTDSTSTVYMELSSLRSEDTAVYYCAR
 (SEQ ID NO: 1368)

Joining region IMGT J00256|IGHJ4*01|YFDYWGQGLVTVSS (SEQ ID NO: 1369)

seq	10	20	30	40	50	60	70	80	90
AbM	10	20	30	40	50	60	70	80	90
	b b b	p p b b	b b b	b i i	i i b b	b	i b b b x	b b b	b b i b b b
518	EVQLQSGPELVKPGASVKISCKAS	GYTFTDYNIN	WVKQSHGKSLEWIG	YIYPYIGGSG	YNQKFQGRVTMTDSTSTVYMELSSLRSEDTAVYYCAR				
	* * *	* * *	* * *	* * *	* * *				
1-46*01	QVQLVQSGAEVKKPGASVKVCKAS	GYTFTSYMH	WVRQAPGQGLEWMG	IINPSGGSTS	YAKQFQGRVTMTDSTSTVYMELSSLRSEDTAVYYCAR				
h518H1	QVQLVQSGAEVKKPGASVKVCKAS	GYTFTDYNIN	WVRQAPGQGLEWMG	YIYPYIGGSG	YAKQFQGRVTMTDSTSTVYMELSSLRSEDTAVYYCAR				
h518H2	QVQLVQSGAEVKKPGASVKVCKAS	GYTFTDYNIN	WVRQAPGQGLEWMG	YIYPYIGGSG	YAKQFQGRVTMTDSTSTVYMELSSLRSEDTAVYYCAR				
h518H3	QVQLVQSGAEVKKPGASVKISCKAS	GYTFTDYNIN	WVRQAPGQGLEWIG	YIYPYIGGSG	YAKQFQGRATLTADTSTSTAYMELSSLRSEDTAVYYCAR				
h518H4	QVQLVQSGAEVKKPGASVKISCKAS	GYTFTDYNIN	WVRQAPGQGLEWIG	YIYPYIGGSG	YAKQFQGRATLTADNSTSTAYMELSSLRSEDTAVYYCAR				

N

seq	100	110	120
AbM			
	i b b b		
518	EDKTARNAMDY	WGQGTPLTVTVSS	(SEQ ID NO: 1370)
	*		(1-46*01 disclosed as SEQ ID NO: 1371)
h518H1	EDKTARNAMDY	WGQGTPLTVTVSS	(SEQ ID NO: 1372)
h518H2	EDKTARNAMDY	WGQGTPLTVTVSS	(SEQ ID NO: 1373)
h518H3	EDKTARNAMDY	WGQGTPLTVTVSS	(SEQ ID NO: 1374)
h518H4	EDKTARNAMDY	WGQGTPLTVTVSS	(SEQ ID NO: 1375)

deamidation substitutions: Q/S/A

Figure 88B
Humanized sequences of CHA.7.518 antibody VL

Potential humanized sequence based on IMGT IGKV1-39*01 acceptor framework

IGKV1-39*01
 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQKPGKAPKLLIYAASSLQGVPSRFGSGSGTDFTLTITSSLPEDFATYYCQQSYSTPP (SEQ ID NO: 1376)

Joining region IMGT J00242|IGKJ2*01|YTFGQGTKLEIK (SEQ ID NO: 1377)

seq	10	20	30	40	50	60	70	80
AbM	10	20	30	40	50	60	70	80
	b b b	p p	p b b	b b i b i	i i i b b i	i b b	b b b	b i b b i b
518	DIQMTQSPASLSVSGEVTIIC	RVSENIYSNLA	WYQKQKSPQLLVY	EATNLAE	GVPSRFGSGSGTDYTLTITSSLPEDFGSYTC			
	* * *	* * *	* * *	* * *				
IGKV1-39	DIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQKPGKAPKLLIY	AASSLQ	GVPSRFGSGSGTDFTLTITSSLPEDFATYYC			
h518L1	DIQMTQSPSSLSASVGDRVTITC	RVSENIYSNLA	WYQKPGKAPKLLIY	EATNLAE	GVPSRFGSGSGTDFTLTITSSLPEDFATYYC			
h518L2	DIQMTQSPSSLSASVGDRVTITC	RVSENIYSNLA	WYQKPGKAPKLLIY	EATNLAE	GVPSRFGSGSGTDYTLTITSSLPEDFATYYC			
h518L3	DIQMTQSPSSLSASVGDRVTITC	RVSENIYSNLA	WYQKPGKAPKLLIY	EATNLAE	GVPSRFGSGSGTDYTLTITSSLPEDFGTYTC			

seq	90	100
AbM	90	100
	i b i	i b b b
518	QHFWGTPYT	FGGQTKLEIK (SEQ ID NO: 1378)
	*	
IGKV1-39	QQSYSTPP	(SEQ ID NO: 1379)
h518L1	QHFWGTPYT	FGGQTKLEIK (SEQ ID NO: 1380)
h518L2	QHFWGTPYT	FGGQTKLEIK (SEQ ID NO: 1381)
h518L3	QHFWGTPYT	FGGQTKLEIK (SEQ ID NO: 1382)

deamidation substitutions: Q/S/A/D
 @ tryptophan oxidation substitutions: Y/F/H

Figure 89C
Humanized sequences of CHA.7.524 antibody VH

Potential humanized sequence based on IMGT IGHV1-46*01 acceptor framework

IGHV1-46*01

QVQLVQSGAEVKKPGASVKVSCCKASGYFTFTSYMHVVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTDSTSTVYMESSLRSEDFAVYYCAR (SEQ ID NO: 1383)

Joining region IMGT J00256|IGHJ4*01|YFDYWGQGLTVTVSS (SEQ ID NO: 1384)

seq	10	20	30	40	50	60	70	80	90
AbM	10	20	30	40	50	60	70	80	90
	b b b	p p b b b	b b b	b i i	i i b b b	i b b b x	b b b x	b b b	b b b b b
524	QVQLQSGPEVVRPGVSVKISCKGS	GYKFPDYVME	WVKQSHAKSLEWIG	IISIYSGNTN	YNQFKGKATMTVDKSSSTAYMELARLTSEDSAIYYCAR				
	* * * * *	* * * * *							
1-46*01	QVQLVQSGAEVKKPGASVKVSCCKAS	GYFTTSYMH	WVRQAPGQGLEWMG	IINPSGGSTS	YAKFQGRVTMTDSTSTVYMESSLRSEDFAVYYCAR				
h524H1	QVQLVQSGAEVKKPGASVKVSCCKAS	GYKFPDYVME	WVRQAPGQGLEWMG	IISIYSGNTN	YAKFQGRVTMTDSTSTVYMESSLRSEDFAVYYCAR				
h524H2	QVQLVQSGAEVKKPGASVKVSCCKAS	GYKFPDYVME	WVRQAPGQGLEWIG	IISIYSGNTN	YAKFQGRVTMTDSTSTVYMESSLRSEDFAVYYCAR				
h524H3	QVQLVQSGAEVKKPGASVKVSCCKGS	GYKFPDYVME	WVRQAPGQGLEWIG	IISIYSGNTN	YAKFQGRATMTVDSTSTAYMELSSLRSEDFAVYYCAR				
h524H4	QVQLVQSGAEVKKPGASVKISCKGS	GYKFPDYVME	WVRQAPGQGLEWIG	IISIYSGNTN	YAKFQGRATMTVDKSTSTAYMELSSLRSEDFAVYYCAR				

V

N

I

seq	100	110
AbM	100	110
	i b b b	
524	EGDLPMFAY	WGQGLTVTVSA
		(SEQ ID NO: 1385)
		(1-46*01 disclosed as SEQ ID NO: 1386)
h524H1	EGDLPMFAY	WGQGLTVTVSS
h524H2	EGDLPMFAY	WGQGLTVTVSS
h524H3	EGDLPMFAY	WGQGLTVTVSS
h524H4	EGDLPMFAY	WGQGLTVTVSS

@

deamidation substitutions: Q/S/A

@ methionine oxidation substitutions: L/F/A

Figure 88D
humanized sequences of CHA.7.524 antibody VL

Potential humanized sequence based on IMGT IGKV3-11*01 acceptor framework
IGKV3-11*01
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRAIGIPARFSGSGGTDFTLTISSLEPEDFAVYYCQQRSNWPP (SEQ ID NO: 1391)
Joining region IMGT J00242|IGKJ2*01|YTFGGTKLEIK (SEQ ID NO: 1392)

seq	10	20	30	40	50	60	70	80
AbM	10	20	30	40	50	60	70	80
	b b b	p p	b b b	b b b	i i	b b	b b b	ib bib
524	QIVLTQSPAIMSASPGEKVTMC	NASSSVS-YMY	WYQQKPGQAPRLLIY	DTSNLA	GVPVRFSGSGGTSLTIGRMEAEADAATYYC	***	***	***
	*	***	***	***	***	***	***	***
IGKV3-11	EIVLTQSPATLSLSPGERATLSC	RASQSVSSYLA	WYQQKPGQAPRLLIY	DASNRA	GIPARFSGSGGTDFTLTISSLEPEDFAVYYC			
h524L1	EIVLTQSPATLSLSPGERATLSC	NASSSVS-YMY	WYQQKPGQAPRLLIY	DTSNLA	GIPARFSGSGGTDFTLTISSLEPEDFAVYYC			
h524L2	EIVLTQSPATLSLSPGERATLSC	NASSSVS-YMY	WYQQKPGQAPRLLIY	DTSNLA	GIPARFSGSGGTDFTLTISSLEPEDFAVYYC			
h524L3	EIVLTQSPATLSLSPGERATLSC	NASSSVS-YMY	WYQQKPGQAPRLLIY	DTSNLA	GVPARFSGSGGTDFTLTISSLEPEDFAVYYC			

seq	90	100
AbM	90	100
	ibi ibi	i b b b
524	QWSSSYPLT	FGAGTKVEIK (SEQ ID NO: 1393)
	*	***
IGKV3-11	QQRSNWPP (SEQ ID NO: 1394)	
h524L1	QWSSSYPLT	FGAGTKLEIK (SEQ ID NO: 1395)
h524L2	QWSSSYPLT	FGAGTKLEIK (SEQ ID NO: 1396)
h524L3	QWSSSYPLT	FGAGTKVEIK (SEQ ID NO: 1397)

deamidation substitutions: Q/S/A/D
@ tryptophan oxidation substitutions: Y/F/H

Figure 88E
Humanized sequences of CHA.7.530 antibody VH

Potential humanized sequence based on IMGT IGHV1-46*01 acceptor framework IGHV1-46*01

QVQLVQSGAEVKKPGASVKVSCKASGYFTSYMHVWRQAPGQGLEWMGLINPSSGGSTSYAQKFQGRVTMTTRDTSTVYMELSSLRSEDTAVYYCAR (SEQ ID NO: 1398)

Joining region IMGT J00256|IGHJ4*01|YFDYWGQGLVTVSS (SEQ ID NO: 1399)

seq	10	20	30	40	50	60	70	80	90
AbM	10	20	30	40	50	60	70	80	90
	b b b	p p b b	b b b	b i i	i i b b	i	b b b x	b b b	b b b b b b b
530	QVQLQQSGAELMRPGTSVKVSC	KAS GYAFTNHLIE	WIKQRPQG	LEWIG VINPGSDSTD	YNEKFKDKATLTADKSSSTAYMQLS	SLTSDSDSAVYFCAR			
	*	***	*	***	*		***	***	***
1-46*01	QVQLVQSGAEVKKPGASVKVSC	KAS GYTFTSYMH	WVRQAPGQ	GLEWMG IINPSSGGST	SYAQKFQGRVTMTTRDTSTVYMELSSLRSEDTAVYYCAR				
h530H1	QVQLVQSGAEVKKPGASVKVSC	KAS GYAFTNHLIE	WVRQAPGQ	GLEWMG VINPGSDSTD	SYAQKFQGRVTMTTRDTSTVYMELSSLRSEDTAVYYCAR				
h530H2	QVQLVQSGAEVKKPGASVKVSC	KAS GYAFTNHLIE	WVRQAPGQ	GLEWMG VINPGSDSTD	SYAQKFQGRVTMTTRDTSTVYMELSSLRSEDTAVYYCAR				
h530H3	QVQLVQSGAEVKKPGASVKVSC	KAS GYAFTNHLIE	WVRQAPGQ	GLEWMG VINPGSDSTD	SYAQKFQGRVTMTTRDTSTVYMELSSLRSEDTAVYYCAR				
h530H4	QVQLVQSGAEVKKPGASVKVSC	KAS GYAFTNHLIE	WVRQAPGQ	GLEWMG VINPGSDSTD	SYAQKFQGRVTMTTRDTSTVYMELSSLRSEDTAVYYCAR				
	M		#		#	N			F

seq	100	110
AbM		110
530	SLYNSWFFVY	WGQGLVTVSA (SEQ ID NO: 1400)
	i b b b	*
h530H1	SLYNSWFFVY	WGQGLVTVSS (SEQ ID NO: 1402)
h530H2	SLYNSWFFVY	WGQGLVTVSS (SEQ ID NO: 1403)
h530H3	SLYNSWFFVY	WGQGLVTVSS (SEQ ID NO: 1404)
h530H4	SLYNSWFFVY	WGQGLVTVSS (SEQ ID NO: 1405)
	# @	

deamidation substitutions: Q/S/A
 @ tryptophan oxidation substitutions: Y/F/H

Figure 88F
Humanized sequences of CHA.7.530 antibody VL

Potential humanized sequence based on IMGT IGKV1-39*01 acceptor framework (AbM CDR definition)
IGKV1-39*01
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFGSGSGTDFTLTITSSLPEDFATYYCQSYSTPP (SEQ ID NO: 1406)

Joining region IMGT J00242|IGKJ2*01|YTFGQGTKLEIK (SEQ ID NO: 1407)

seq	10	20	30	40	50	60	70	80
AbM	10	20	30	40	50	60	70	80
	b b b	p p p	b b b	b i b i	i i i b b i	i b b	b b b	b b b
530	DIQMTQSPASLSASVGETVTITC	**	RASENIYSYLA	WYQQKRGKSPQLLIVY	NAKTLVE	GVPSRFGSGSGTDFTLTITSSLPEDFATYYC	**	**
	*		*	*	*	*	*	*
IGKV1-39	DIQMTQSPSSLSASVGDRVTITC	RASQSISSYLW	WYQQKPGKAPKLLIY	AASSLQ	GVPSRFGSGSGTDFTLTITSSLPEDFATYYC			
h530L1	DIQMTQSPSSLSASVGDRVTITC	RASENIYSYLA	WYQQKPGKAPKLLIY	NAKTLVE	GVPSRFGSGSGTDFTLTITSSLPEDFATYYC			
h530L2	DIQMTQSPSSLSASVGDRVTITC	RASENIYSYLA	WYQQKPGKAPKLLIY	NAKTLVE	GVPSRFGSGSGTDFTLTITSSLPEDFATYYC			
		#		S	#			

seq	90	100
AbM	90	100
	i b i i b i	b b b
530	QHHYGTPTT	FGGQGTKLEIK
	*	
IGKV1-39	QSYSTPP	(SEQ ID NO: 1409)
h530L1	QHHYGTPTT	FGGQGTKLEIK
h530L2	QHHYGTPTT	FGGQGTKLEIK

deamidation substitutions: Q/S/A/D

Figure 88G
Humanized sequences of CHA.7.538_1 antibody VH

Potential humanized sequence based on IMGT IGHV1-46*01 acceptor framework

IGHV1-46*01
 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYMHVWRQAPGQGLEWMGIINPSSGGSYAQKFQGRVTMTTRDTSTSTVYMELSSLRSEDTAVYYCAR (SEQ ID NO: 1412)

Joining region IMGT J00256 | IGHJ4*01 | YFDYWGQGLVTVSS (SEQ ID NO: 1413)

seq	10	20	30	40	50	60	70	80	90
AbM	10	20	30	40	50	60	70	80	90
	b b b	p p b b b	b b b	b b i i	i i b b b	i b	b b b x	b b b b	b b b b b b b b
538_1	QVQLVQSGAEVKKPGASVKVSCKTS	GYAFTNYLIE	WVKORPGQGLEWIG	VINPSSGGGIY	YNDKFKVKITLTADKSSSTAYMQLSSLTSDSDSAVYFCAR	**	*****	**	**
1-46*01	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFTSYMH	WVRQAPGQGLEWMG	IINPSSGGS	YAQKFQGRVTMTTRDTSTSTVYMELSSLRSEDTAVYYCAR				
h5381H1	QVQLVQSGAEVKKPGASVKVSCKAS	GYAFTNYLIE	WVRQAPGQGLEWMG	VINPSSGGGIY	YAQKFQGRVTMTTRDTSTSTVYMELSSLRSEDTAVYYCAR				
h5381H2	QVQLVQSGAEVKKPGASVKVSCKAS	GYAFTNYLIE	WVRQAPGQGLEWMG	VINPSSGGGIY	YAQKFQGRVTMTTRDTSTSTVYMELSSLRSEDTAVYYCAR				
h5381H3	QVQLVQSGAEVKKPGASVKVSCKTS	GYAFTNYLIE	WVRQAPGQGLEWIG	VINPSSGGGIY	YAQKFQGRVTMTTRDTSTSTVYMELSSLRSEDTAVYYCAR				
h5381H4	QVQLVQSGAEVKKPGASVKVSCKTS	GYAFTNYLIE	WVRQAPGQGLEWIG	VINPSSGGGIY	YAQKFQGRVTMTTRDTSTSTVYMELSSLRSEDTAVYYCAR				

seq	100	110
AbM	110	
	i b b b	
538_1	SETHDTWFAY	WGQGLTVTVSA (SEQ ID NO: 1414)

h5381H1 SETHDTWFAY WGQGLTVTVSS * (1-46*01 disclosed as SEQ ID NO: 1415)
 h5381H2 SETHDTWFAY WGQGLTVTVSS (SEQ ID NO: 1416)
 h5381H3 SETHDTWFAY WGQGLTVTVSS (SEQ ID NO: 1417)
 h5381H4 SETHDTWFAY WGQGLTVTVSS (SEQ ID NO: 1418)
 h5381H4 SETHDTWFAY WGQGLTVTVSS (SEQ ID NO: 1419)

deamidation substitutions: Q/S/A
 @ tryptophan oxidation substitutions: Y/F/H

Figure 88H
humanized sequences of CHA.7.538_1 antibody VL

Potential humanized sequence based on IMGT IGKV1-39*01 acceptor framework
IGKV1-39*01
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQKPGKAPKLLIYAASSLQGVPSRFGSGSGTDFTLTISLQPEDFATYYCQQSYSTPP (SEQ ID NO: 1420)

Joining region IMGT J00242|IGKJ2*01|YTFGQGTKLEIK (SEQ ID NO: 1421)

IGKV1-17*02
DIQMTQSPSSLSASVGDRVTITCRASQGI RNDLGWYQKPGKAPKRLIYAASSLQGVPSRFGSGSGTDFTLTISNLPEDFATYYCLQHNSYPP (SEQ ID NO: 1422)

seq	10	20	30	40	50	60	70	80
AbM	10	20	30	40	50	60	70	80
538	b b b	p p p	b b b	b i b i	i i i b b i	i b b	b b b	b b b
	DIQMTQSQKFISTSVGDRVSI	TC	KASQSVRI	AVA	WFQKPGQSPKALII	LASTRET	GVPSRFGSGSGTDFTLTISNLPEDFATYYC	ib bib
	*	**** *	*	*	*** *	*	*	*
IGKV1-39	DIQMTQSPSSLSASVGDRVTITC	RASQSISSYL	NWYQKPGKAPKLLI	YAASSLQ	GVPSRFGSGSGTDFTLTISNLPEDFATYYC	LASTRET	GVPSRFGSGSGTDFTLTISNLPEDFATYYC	ib bib
h538L1	DIQMTQSPSSLSASVGDRVTITC	RASQSVRI	AVA	WFQKPGKAPKLLI	YAASSLQ	GVPSRFGSGSGTDFTLTISNLPEDFATYYC	LASTRET	GVPSRFGSGSGTDFTLTISNLPEDFATYYC
h538L2	DIQMTQSPSSLSASVGDRVTITC	RASQSVRI	AVA	WFQKPGKAPKALII	YAASSLQ	GVPSRFGSGSGTDFTLTISNLPEDFATYYC	LASTRET	GVPSRFGSGSGTDFTLTISNLPEDFATYYC
	I	T		S				L F

seq	90	100
AbM	90	100
538	ibi ibi	i b b b
	LQHWNYPT	FGGGTKLEIK (SEQ ID NO: 1423)
	*	
IGKV1-39	QQSYSTPP	(SEQ ID NO: 1424)
h538L1	LQHWNYPT	FGGGTKLEIK (SEQ ID NO: 1425)
h538L2	LQHWNYPT	FGGGTKLEIK (SEQ ID NO: 1426)
	@#	

deamidation substitutions: Q/S/A/D
@ tryptophan oxidation substitutions: Y/F/H

Figure 88I
humanized sequences of CHA.7.538_2 antibody VH

Potential humanized sequence based on IMGT IGHV1-46*01 acceptor framework
IGHV1-46*01
QVQLVQSGAEVKKPGASVKSCASGYFTFTSYMHVVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR (SEQ ID NO: 1427)
Joining region
IMGT J00256|IGHJ4*01|YFDYWGQGLTVTVSS (SEQ ID NO: 1428)

seq	10	20	30	40	50	60	70	80	90
AbM	10	20	30	40	50	60	70	80	90
538_2	b b b	p p b b b	b b b	b i i	i i b b b	50 a	i b b b b x	b b b b	b i b i b b
1-46*01	QVQLVQSGAEVKKPGASVKSCAS	GYFTFTSYMH	WVRQAPGQGLEWMG	IINPSGGSTS	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR
h5382H1	QVQLVQSGAEVKKPGASVKSCAS	GYFTFTSYMH	WVRQAPGQGLEWMG	IINPSGGSTS	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR
h5382H2	QVQLVQSGAEVKKPGASVKSCAS	GYFTFTSYMH	WVRQAPGQGLEWMG	IINPSGGSTS	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR
h5382H3	QVQLVQSGAEVKKPGASVKSCAS	GYFTFTSYMH	WVRQAPGQGLEWMG	IINPSGGSTS	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR	YAKFQGRVTMTDSTSTVYMESSLRSEDYAVYCAR

seq	100	110
AbM	110	
538_2	PYYGSSYGFAF	WGQGLTVTVSSA (SEQ ID NO: 1429)
h5382H1	PYYGSSYGFAF	WGQGLTVTVSS (SEQ ID NO: 1431)
h5382H2	PYYGSSYGFAF	WGQGLTVTVSS (SEQ ID NO: 1432)
h5382H3	PYYGSSYGFAF	WGQGLTVTVSS (SEQ ID NO: 1433)

deamidation substitutions: Q/S/A
@ tryptophan oxidation substitutions: Y/F/H

Figure 89A**humanized CHA.7.518 VH**

h518HH1

QVQLVQSGAEVKKPGASVKVSKKASGYTFTDYNINWVRQAPGQGLEWMGYIYPYIGGSGYAQKFQGRVTMTTRDTSTSTVY
 MELSSLRSEDTAVYYCAREDKTARNAMDYWGQGTLVTVSS (SEQ ID NO: 1434)

h518HH2

QVQLVQSGAEVKKPGASVKVSKKASGYTFTDYNINWVRQAPGQGLEWIGYIYPYIGGSGYAQKFQGRVTMTADTSTSTVY
 MELSSLRSEDTAVYYCAREDKTARNAMDYWGQGTLVTVSS (SEQ ID NO: 1435)

h518HH3

QVQLVQSGAEVKKPGASVKISKASGYTFTDYNINWVRQAPGQGLEWIGYIYPYIGGSGYAQKFQGRATLTADTSTSTAYM
 ELSSLRSEDTAVYYCAREDKTARNAMDYWGQGTLVTVSS (SEQ ID NO: 1436)

h518HH4

QVQLVQSGAEVKKPGASVKISKASGYTFTDYNINWVRQAPGQGLEWIGYIYPYIGGSGYAQKFQGRATLTADNSTSTAYM
 ELSSLRSEDTAVYYCAREDKTARNAMDYWGQGTLVTVSS (SEQ ID NO: 1437)

humanized CHA.7.524 VH

h524HH1

QVQLVQSGAEVKKPGASVKVSKKASGYKFPDYVMHWVRQAPGQGLEWMGIISIYSGNTNYAQKFQGRVTMTTRDTSTSTV
 YMELSSLRSEDTAVYYCAREGDLPMFAYWGQGTLVTVSS (SEQ ID NO: 1438)

h524HH2

QVQLVQSGAEVKKPGASVKVSKKASGYKFPDYVMHWVRQAPGQGLEWIGIISIYSGNTNYAQKFQGRVTMTVDTSTSTVY
 MELSSLRSEDTAVYYCAREGDLPMFAYWGQGTLVTVSS (SEQ ID NO: 1439)

h524HH3

QVQLVQSGAEVKKPGASVKISKKSGYKFPDYVMHWVRQAPGQGLEWIGIISIYSGNTNYAQKFQGRATMTVDTSTSTAY
 MELSSLRSEDTAVYYCAREGDLPMFAYWGQGTLVTVSS (SEQ ID NO: 1440)

Figure 89B
h524HH4

QVQLVQSGAEVKKPGASVKISKSGSYKFPDYVMHWVRQAPGQGLEWIGHIISYSGNTNYAQKFQGRATMTVDKSTSTAY
MELSSLRSEDTAVYYCAREGDLPMFAYWGQGLVTVSS (SEQ ID NO: 1441)

humanized CHA.7.530 VH

h530HH1

QVQLVQSGAEVKKPGASVKVSKASGYAFTNHLIEWVRQAPGQGLEWMGVNPGSDSTDYAQKFQGRVTMTTRDTSTSTVY
MELSSLRSEDTAVYYCARSLYYNSWVFVYWGQGLVTVSS (SEQ ID NO: 1442)

h530HH2

QVQLVQSGAEVKKPGASVKVSKASGYAFTNHLIEWVRQAPGQGLEWIGVINPGSDSTDYAQKFQGRVTMTADTSTSTVY
MELSSLRSEDTAVYYCARSLYYNSWVFVYWGQGLVTVSS (SEQ ID NO: 1443)

h530HH3

QVQLVQSGAEVKKPGASVKVSKASGYAFTNHLIEWIRQAPGQGLEWIGVINPGSDSTDYAQKFQGRATLTADTSTSTAYM
ELSSLRSEDTAVYYCARSLYYNSWVFVYWGQGLVTVSS (SEQ ID NO: 1444)

h530HH4

QVQLVQSGAEVKKPGASVKVSKASGYAFTNHLIEWIRQAPGQGLEWIGVINPGSDSTDYAQKFQGRATLTADKSTSTAYM
ELSSLRSEDTAVYYCARSLYYNSWVFVYWGQGLVTVSS (SEQ ID NO: 1445)

humanized CHA.7.538_1 VH

h5381HH1

QVQLVQSGAEVKKPGASVKVSKASGYAFTNLYIEWVRQAPGQGLEWMGVNPGSGGIYYAQKFQGRVTMTTRDTSTSTVY
MELSSLRSEDTAVYYCARSETHTDTWFAYWGQGLVTVSS (SEQ ID NO: 1446)

h5381HH2

QVQLVQSGAEVKKPGASVKVSKASGYAFTNLYIEWVRQAPGQGLEWIGVINPGSGGIYYAQKFQGRVTMTADTSTSTVY
MELSSLRSEDTAVYYCARSETHTDTWFAYWGQGLVTVSS (SEQ ID NO: 1447)

Figure 89C

h5381HH3

QVQLVQSGAEVKKPGASVKVSCKTSGYAFTNLYIEWVRQAPGQGLEWIGVINPGSGGIYYAQKFQGRVTLTADTSTSTAYM
ELSSLRSEDTAVYYCARSETHDTWFAYWGQGLVTVSS (SEQ ID NO: 1448)

h5381HH4

QVQLVQSGAEVKKPGASVKVSCKTSGYAFTNLYIEWVRQAPGQGLEWIGVINPGSGGIYYAQKFQGRVTLTADKSTSTAYM
ELSSLRSEDTAVYYCARSETHDTWFAYWGQGLVTVSS (SEQ ID NO: 1449)

humanized CHA.7.538_2 VH

h5382HH1

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYWVRQAPGQGLEWMGDIYPGGGYTNYAQKFQGRVTMTTRDTSTSTV
YMELSSLRSEDTAVYYCARPYYGSSYGFAFWGQGLVTVSS (SEQ ID NO: 1450)

h5382HH2

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYWVRQAPGQGLEWIGDIYPGGGYTNYAQKFQGRVTMTADTSTSTV
YMELSSLRSEDTAVYYCASPYYGSSYGFAFWGQGLVTVSS (SEQ ID NO: 1451)

h5382HH3

QVQLVQSGAEVKKPGASVKMSCKASGYTFTNYYWVRQAPGQGLEWIGDIYPGGGYTNYAQKFQGRATLTADTSTSTA
YMELSSLRSEDTAVYYCASPYYGSSYGFAFWGQGLVTVSS (SEQ ID NO: 1452)

humanized CHA.7.518 VL

h518HL1

DIQMTQSPSSLSASVGDRVTITCRVSENIYSNLAWYQQKPGKAPKLLIYEATNLAEGVPSRFSGSGTDFTLTISSLQPEDFA
TYYCQHFWGTPYTFGQGTKLEIK (SEQ ID NO: 1453)

h518HL2

DIQMTQSPSSLSASVGDRVTITCRVSENIYSNLAWYQQKPGKAPKLLIYEATNLAEGVPSRFSGSGTDTYTLTISSLQPEDFA
TYYCQHFWGTPYTFGQGTKLEIK (SEQ ID NO: 1454)

Figure 89D

h518HL3

DIQMTQSPSSLSASVGDRVTITCRVSENIYSLAWYQQKPGKAPKLLVYEATNLAEGVPSRFSGSGGTDYTLTISSLPEDF
 GTYYCQHFWGTPYTFGGQGTKLEIK (SEQ ID NO: 1455)

humanized CHA.7.524 VL

h524HL1

EIVLTQSPATLSLSPGERATLSCNASSSVSYMYWYQQKPGQAPRLLIYDTSNLAASGIPARFSGSGGTDFTLTISSELPEDFAVY
 YCQQWSSYPPLTFGGQGTKLEIK (SEQ ID NO: 1456)

h524HL2

EIVLTQSPATLSLSPGERATLSCNASSSVSYMYWYQQKPGQAPRLLIYDTSNLAASGIPARFSGSGGTDYTLTISSLPEDFAVY
 YCQQWSSYPPLTFGGQGTKLEIK (SEQ ID NO: 1457)

h524HL2

EIVLTQSPATLSLSPGERVTMSCNASSSVSYMYWYQQKPGQAPRLLIYDTSNLAAGVPARFSGSGGTDYTLTISSMEPEDFA
 VYYCQWSSYPPLTFGGQGTKVEIK (SEQ ID NO: 1458)

humanized CHA.7.530 VL

h530HL1

DIQMTQSPSSLSASVGDRVTITCRASENIYSYLAWYQQKPGKAPKLLIYNNAKTLVEGVPSRFSGSGGTDFTLTISLPEDFA
 TYYCQHGYGTPYTFGGQGTKLEIK (SEQ ID NO: 1459)

h530HL2

DIQMTQSPSSLSASVGDRVTITCRASENIYSYLAWYQQKPGKAPKLLVYNNAKTLVEGVPSRFSGSGGTDFTLTISLPEDFG
 TYYCQHGYGTPYTFGGQGTKLEIK (SEQ ID NO: 1460)

humanized CHA.7.538_1/538_2 VL

h538HL1

DIQMTQSPSSLSASVGDRVTITCKASQSVRIAWYQQKPGKAPKLLIYLASTRHTGVPSRFSGSGGTDFTLTISLPEDFA
 TYYCLQHWNYPTTFGGQGTKLEIK (SEQ ID NO: 1461)

Figure 89E
h538HL2

DIQMTQSPSSLSASVGDRVTITCKASQSVRIAVAWFQQKPGKAPKALIYLA STRHTGVPSRFSGSGGTDFTLTISVVQPEDFA
TYYCLQHWNPYPYTFGQGTKLEIK (SEQ ID NO: 1462)

Figure 90: humanized CHA.7 VH-VL Pairs

Antibody CHA.7.518

	VH	VL
ch518	chimVH	chimVL
h518-1	h518HH1	h518HL1 (optional)
h518-2	h518HH2	h518HL2
h518-3	h518HH3	h518HL2
h518-4	h518HH3	h518HL3
h518-5	h518HH4	h518HL3

Antibody CHA.7.524

	VH	VL
ch524	chimVH	chimVL
h524-1	h524HH1	h524HL1 (optional)
h524-2	h524HH2	h524HL2
h524-3	h524HH3	h524HL3
h524-4	h524HH4	h524HL3

Antibody CHA.7.530

	VH	VL
ch530	chimVH	chimVL
h530-1	h530HH1	h530HL1 (optional)
h530-2	h530HH2	h530HL2
h530-3	h530HH3	h530HL1
h530-4	h530HH3	h530HL2
h530-5	h530HH4	h530HL2

Antibody CHA.7.538_1

	VH	VL
ch538.1	chimVH	chimVL
h538.1-1	h538.1HH1	h538.1HL1 (optional)
h538.1-2	h538.1HH2	h538.1HL2
h538.1-3	h538.1HH3	h538.1HL2
h538.1-4	h538.1HH4	h538.1HL2

Antibody CHA.7.538_2

	VH	VL
ch538.2	chimVH	chimVL
h538.2-1	h538.2HH1	h538.1HL1 (optional)
h538.2-2	h538.2HH2	h538.1HL2
h538.2-3	h538.2HH3	h538.1HL2

Figure 91

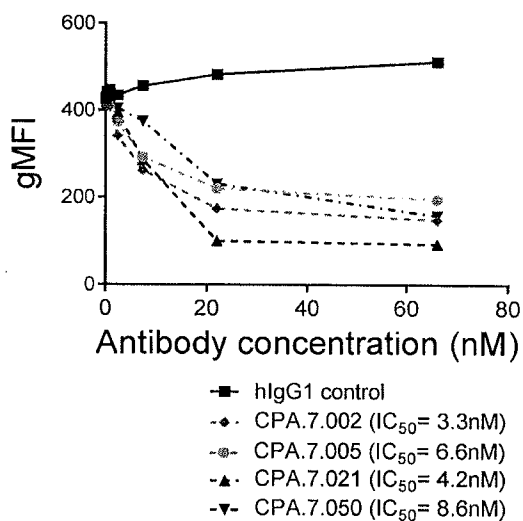
Antibody (mgG)	Human CD56 int. NK (gMFIr, 10ug/ml)	Human CD8+ T cells (gMFIr, 10ug/ml)	Expi cyno OE/par (gMFIr, 3.3ug/ml)	Cyno NK cells (gMFIr, 10ug/ml)	Cyno CD8+ T cells (gMFIr, 10ug/ml)
CHA.7.502	1.97	1.41	60.49	Not tested	Not tested
CHA.7.503	3.15	1.96	106.3	Not tested	Not tested
CHA.7.506	Not tested	Not tested	0.77	Not tested	Not tested
CHA.7.508	3.6	4.09	41.49	Not tested	Not tested
CHA.7.510	3.13	2.73	38.21	Not tested	Not tested
CHA.7.512	1.30	1.15	8.96	Not tested	Not tested
CHA.7.514	4.16	5.15	65.20	Not tested	Not tested
CHA.7.516	4.22	4.09	60.05	1.76	2.09
CHA.7.518	5.08	6.69	83.51	1.92	2.09
CHA.7.520	1.13	1.04	Not tested	Not tested	Not tested
CHA.7.522	2.06	1.90	27.24	Not tested	Not tested
CHA.7.524	5.50	6.12	66.32	1.78	2.02
CHA.7.525	1.98	1.76	0.85	Not tested	Not tested
CHA.7.526	2.08	1.71	0.79	Not tested	Not tested
CHA.7.527	1.16	0.99	Not tested	Not tested	Not tested
CHA.7.528	3.08	3.63	12.2	1.21	1.18
CHA.7.530	6.04	6.47	60.80	1.73	1.89
CHA.7.534	2.60	1.96	46.27	Not tested	Not tested
CHA.7.535	2.24	1.28	0.82	Not tested	Not tested
CHA.7.537	3.90	3.41	1.55	1.18	1.19
CHA.7.538	6.49	6.17	15.16	1.36	1.45
CHA.7.543	4.48	4.33	0.83	1.35	1.39
CHA.7.544	2.36	2.54	61.09	Not tested	Not tested
CHA.7.545	2.54	2.82	0.91	Not tested	Not tested
CHA.7.546	2.75	2.95	0.85	Not tested	Not tested
CHA.7.547	2.21	1.13	26.65	Not tested	Not tested
CHA.7.548	3.15	3.35	4.25	1.18	1.09
CHA.7.549	3.05	1.42	1.00	Not tested	Not tested
CHA.7.550	1.60	1.29	0.90	Not tested	Not tested

Figure 92

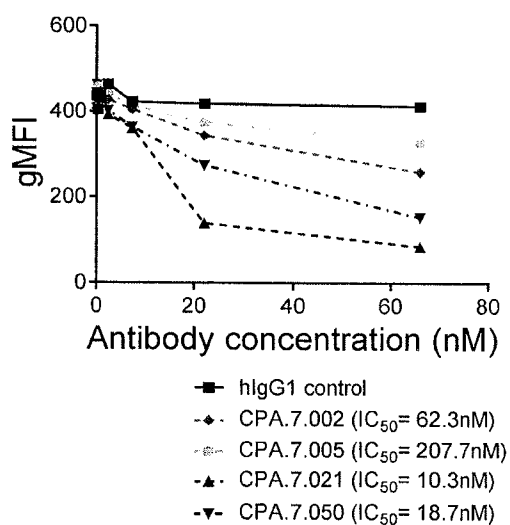
Antibody (mIgG)	IC ₅₀ (nM)
CHA.7.502	39.90
CHA.7.503	No IC ₅₀
CHA.7.506	31.65
CHA.7.508	37.88
CHA.7.510	55.00
CHA.7.512	839.6
CHA.7.514	38.88
CHA.7.516	33.11
CHA.7.518	23.15
CHA.7.520	619.3
CHA.7.522	50.48
CHA.7.524	30.20
CHA.7.525	85.52
CHA.7.526	58.88
CHA.7.527	No IC ₅₀
CHA.7.528	28.88
CHA.7.530	34.56
CHA.7.534	181.4
CHA.7.535	821.1
CHA.7.537	38.95
CHA.7.538	51.87
CHA.7.543	No IC ₅₀
CHA.7.544	No IC ₅₀
CHA.7.545	96.06
CHA.7.546	92.05
CHA.7.547	27.94
CHA.7.548	18.98
CHA.7.549	36.12
CHA.7.550	58.34

FIGURE 93A

A

**FIGURE 93B**

B



C

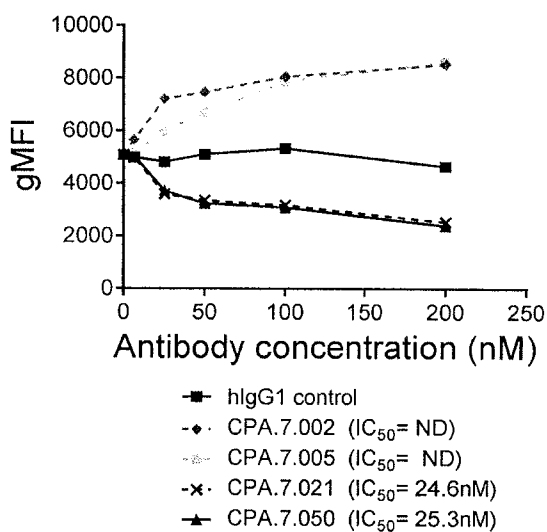
**FIGURE 93C**

FIG. 94A

a

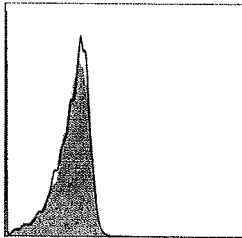


FIG. 94B

b

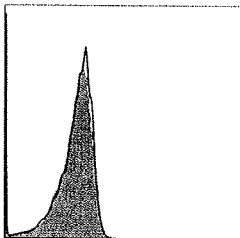


FIG. 94C

c

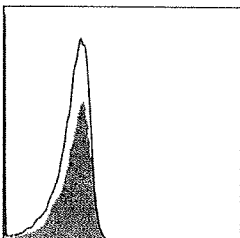
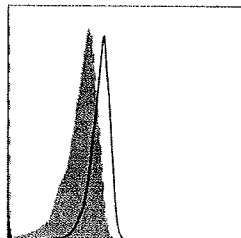
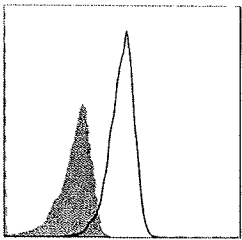


FIG. 94D

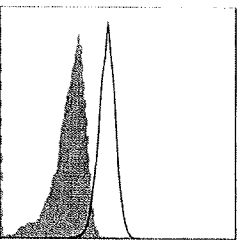
d



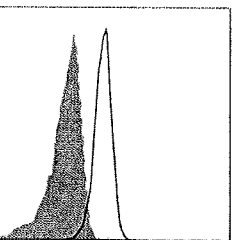
e



f



g



h

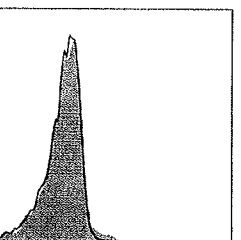


FIG. 94E

FIG. 94F

FIG. 94G

FIG. 94H

FIG. 95A

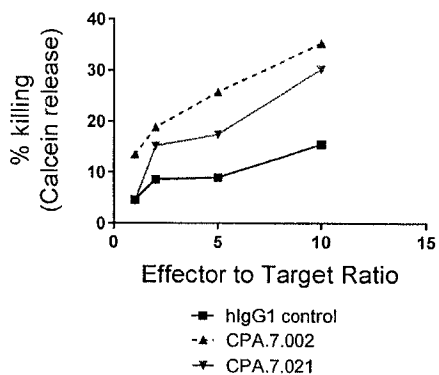


FIG. 95B

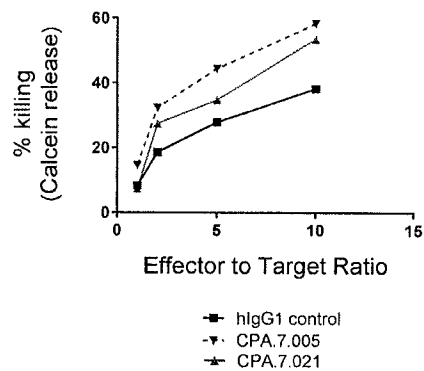


FIG. 95C

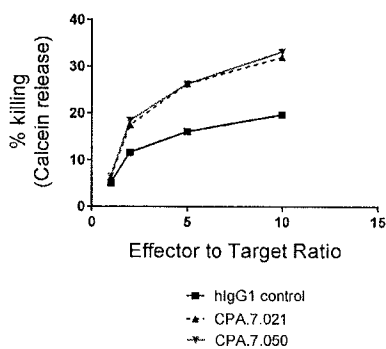


FIG. 95D

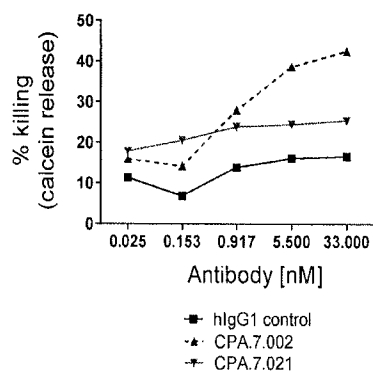


FIG. 95E

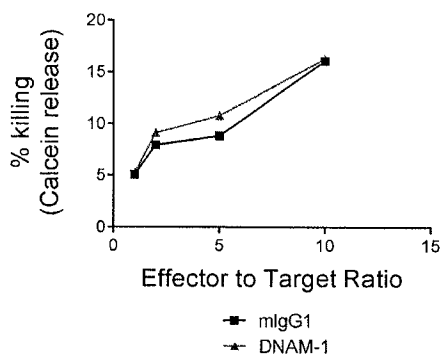


FIG. 95F

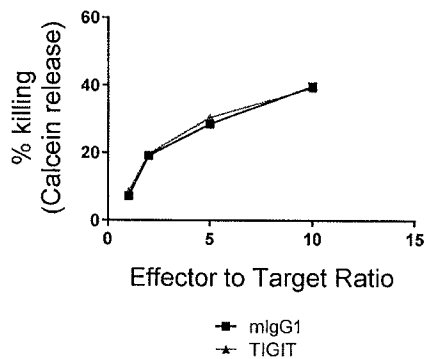
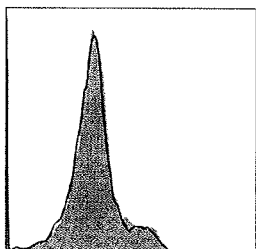
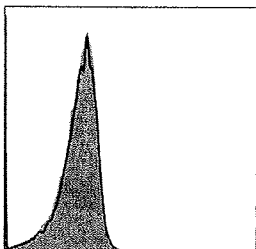
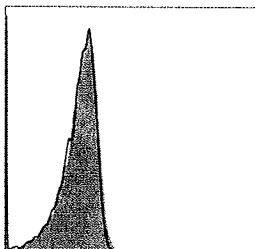
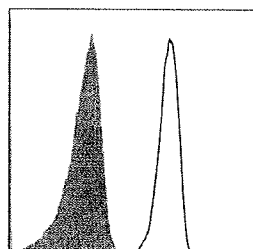
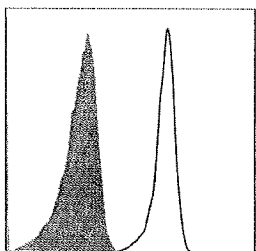
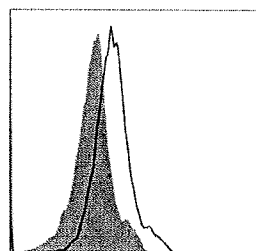
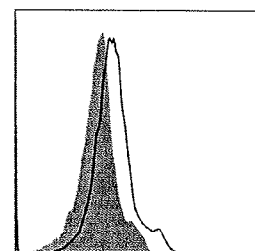
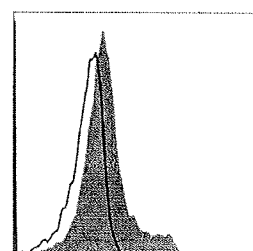
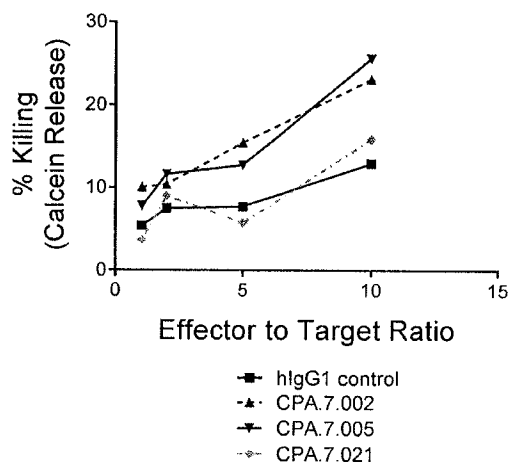


FIG. 96A**a****FIG. 96B****b****FIG. 96C****c****FIG. 96D****d****e****f****g****h****FIG. 96E****FIG. 96F****FIG. 96G****FIG. 96H**

A

FIGURE 97A

B

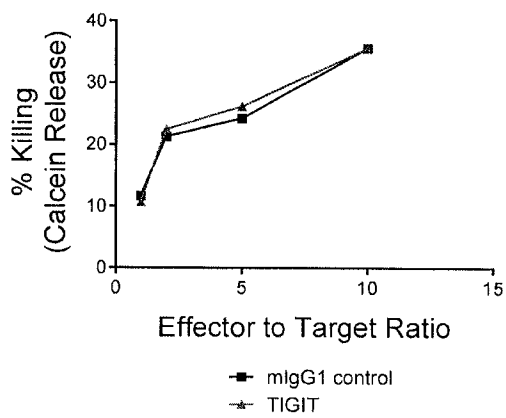
**FIGURE 97B**

Figure 98

Antibody (hIgG1)	Assay permutation and orientation	IC ₅₀ (nM)
CPA.7.002	HEK + PVRIG Fc (P)	3.3
	HEK + PVRIG Fc (NP)	62.3
	HEK hPVRIG + PVRL2 Fc	Increased binding
CPA.7.005	HEK + PVRIG Fc (P)	6.6
	HEK + PVRIG Fc (NP)	207.7
	HEK hPVRIG + PVRL2 Fc	Increased binding
CPA.7.021	HEK + PVRIG Fc (P)	4.2
	HEK + PVRIG Fc (NP)	10.3
	HEK hPVRIG + PVRL2 Fc	24.6
CPA.7.036	HEK + PVRIG Fc (P)	6.9
	HEK + PVRIG Fc (NP)	10.9
CPA.7.037	HEK + PVRIG Fc (P)	6.5
	HEK + PVRIG Fc (NP)	19.1
CPA.7.038	HEK + PVRIG Fc (P)	3.5
	HEK + PVRIG Fc (NP)	6.7
CPA.7.039	HEK + PVRIG Fc (P)	14.0
	HEK + PVRIG Fc (NP)	32.7
CPA.7.041	HEK + PVRIG Fc (P)	5.8
	HEK + PVRIG Fc (NP)	16.2
CPA.7.042	HEK + PVRIG Fc (P)	3.6
	HEK + PVRIG Fc (NP)	8.3
CPA.7.050	HEK + PVRIG Fc (P)	8.6
	HEK + PVRIG Fc (NP)	18.7
	HEK hPVRIG + PVRL2 Fc	25.3

Figure 99

Antibody (hIgG1)	Target Cell line	Fold change in cytotoxicity relative to control
CPA.7.002	Reh	2.9
	MOLM-13	1.9
CPA.7.005	Reh	1.6
	MOLM-13	1.6
CPA.7.021	Reh	1.9
	MOLM-13	0.7
CPA.7.036	Reh	0.9
CPA.7.037	Reh	1.1
CPA.7.038	Reh	0.8
CPA.7.041	Reh	0.7
CPA.7.042	Reh	0.7
CPA.7.050	Reh	1.6
TIGIT	Reh	1.1
	MOLM-13	1.1
DNAM-1	Reh	1.2

Figure 102

mAb	Cyno	Epitope bin	Epitope group
	cross-reactivity		
CPA.7.002	+	1	2
CPA.7.021	-	1	1
CPA.7.024	+++	1	3
CPA.7.028	-	1	1
CPA.7.041	++	1	2
CPA.7.050	+++	4	3

Figure 103A

>PVRIG_NP_076975_from_41_to_171_Sequence_of_Human_WT_ECD_(without_SP) SEQ ID NO: 1467
 TPEVWVQVRMEATELSSFTIRCGFLGSGSISLTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGS
 LPPSSDPGLSAPPTPAPILRAD

Figure 103B

>PVRIG_NP_076975_from_21_to_171_Sequence_of_Human_WT_ECD_(with_SP) SEQ ID NO: 1468
 MGHRTLVLPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPC
 NTTFCCKFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRAD

Figure 103C

>PVRIG_NP_076975_from_43_to_146_Sequence_of_Ig_Domain SEQ ID NO: 1469
 EVWVQVRMEATELSSFTIRCGFLGSGSISLTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEA

Figure 103D

>PVRIG_Splice_Variant_P6 SEQ ID NO: 1470
 MGHRTLVLPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPC
 NTTFCCKFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRADLAGILGVSGVLLFGCVYLLHLLRRHKHR

Figure 103E

>PVRIG_Splice_Variant_P8 SEQ ID NO: 1471
 MGHRTLVLPWVLLTLCVTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPC
 NTTFCCKFASFPEGSWEACGSLPPSSDPGLSVPPTPAPILRADLAGILGVSGVLLFDCGYLLHLLCRQKHRPAPRLQPSHTSS

Figure 103F

>PVRIG_Splice_Variant_P4 SEQ ID NO: 1472

MGHRTLVPWVLLTLCVTTAGTPEVWVQVRMEATELSSFTIRWLSAPPTPAPILRADLAGILGSGVLLFGCVLLHLLRRHKHRPAPRLQPSRTSPQAPRARAWAPSQA
SQAALHVPYATINTSCRPATLDTAHPHGGPSWWASLPTHAAHRPQGPAAWASTPIARGSFVSVENGLYAQAAGERPPHTGPGTLTFDPDPRGPRAMEGPLGVR

Figure 103G

>PVRIG_Splice_Variant_P7 SEQ ID NO: 1473

MGHRTLVPWVLLTLCVTTAGLSAPPTPAPILRADLAGILGSGVLLFGCVLLHLLRRHKHRPAPRLQPSRTSPQAPRARAWAPSQASQAALHVPYATINTSCRPATLDT
AHPHGGPSWWASLPTHAAHRPQGPAAWASTPIARGSFVSVENGLYAQAAGERPPHTGPGTLTFDPDPRGPRAMEGPLGVR

Figure 103H

>PVRIG_Splice_Variant_P14 SEQ ID NO: 1474

MGHRTLVPWVLLTLCVTTAGLSVPPTPAPILRADLAGILGSGVLLDFCGYLLHLLCRQKHRPAPRLQPSHTSS

Figure 103I

>PVRIG_Splice_Variant_P3 SEQ ID NO: 1475

MGHRTLVPWVLLTLCVTTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCA
NTTFCKFASFPEGSWEACGSLPPSSDPGGAGARGPGGQGGARELATHLILVSVPRALCPADSCPHSAGRGRDLGLRSPPLWLCLPPSSAAPT

Figure 103J

>PVRIG_Splice_Variant_P10 SEQ ID NO: 1476

MGHRTLVPWVLLTLCVTTAGTPEVWVQVRMEATELSSFTIRCGFLGSGSISLTVSWGGPNGAGGTTLAVLHPERGAQISPRGPKIGGPPCPRPGPAGKPRAASLSSW
KALGPAAPAPTPPSAASLRPSQAALHVPYATINTSCRPATLDTAHPHGGPSWWASLPTHAAHRPQGPAAWASTPIARGSFVSVENGLYAQAAGERPPHTGPGTLTFPD
PRGPRAMEGPLGVR

Figure 103K

>PVRIG_Splice_Variant_P13 SEQ ID NO: 1477

MGHRTLVLPPWVLLTLCVGTAGTPEVWVQVRMEATELSSFTIRCGFLGSGISLVTVSWGPNAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCA
NTTFCKKFASFPGCSSRPLCHYQHLPPSYFGHSSPPWGAVLVGVTPHPRCTPAPGPCRLGLHHPCTWQLCLC

Figure 103L

>PVRIG_Splice_Variant_P3_ECD SEQ ID NO: 1478

TPEVWVQVRMEATELSSFTIRCGFLGSGISLVTVSWGPNAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGS
LPPSSDPGGAGARGPGGGGARELATHLILVSVPRALCPADSCPHSAGRPRDLGGLRSPPLWLCLPPSSAAAPT

Figure 103M

>PVRIG_Splice_Variant_P10_ECD SEQ ID NO: 1479

TPEVWVQVRMEATELSSFTIRCGFLGSGISLVTVSWGPNAGAGGTTLAVLHPERGAQISPRGPKIGGPPCPRPGAPKPRAASSLSSWKALGPAAPAPTPPSAASLRPS
QAALHVPYATINTSCRPATLDTAHPHGGPSWWASLPTHAHRPQGPAAWASTPIPARGSFVSVENGLYAQAGERPPHTGPGLTLFPDPRGRAMEGPLGVR

Figure 103N

>PVRIG_Splice_Variant_P13_ECD SEQ ID NO: 1480

TPEVWVQVRMEATELSSFTIRCGFLGSGISLVTVSWGPNAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPGCSSRPLCHY
QHLPPSYFGHSSPPWGAVLVGVTPHPRCTPAPGPCRLGLHHPCTWQLCLC

Figure 103O

>PVRIG_Splice_Variant_P3_ECD_Unique SEQ ID NO: 1481

GAGARGPGGGGARELATHLILVSVPRALCPADSCPHSAGRPRDLGGLRSPPLWLCLPPSSAAAPT

Figure 103P

>PVRIG_Splice_Variant_P10_ECD_All_Unique SEQ ID NO: 1482
AQISPRGPKIGGPPCPRPGPAGKPRAASLSSWKALGPAAPAPTPPSAASLRPSQAALHVPYATINTSCRPATLDTAHPHGGPSWWASLPTHAAHRPQGGPAAWASTPIP
ARGSFVSVENGLYAQAAGERPPHTGPGLTLPDPGRPRAMEGLGVR

Figure 103Q

>PVRIG_Splice_Variant_P10_ECD_Unique_without_intracellular SEQ ID NO: 1483
AQISPRGPKIGGPPCPRPGPAGKPRAASLSSWKALGPAAPAPTPPSAASLRP

Figure 103R

>PVRIG_Splice_Variant_P13_ECD_Unique SEQ ID NO: 1484
GCCSRPLCHYQHQLPPSYFGHSSPPWGAVLVGVTPHPRCTPAPGPCRLGLHHPCTWQLCLC

Figure 103S

>PVRIG_NP_076975_from_41_to_169_variation_of_ECD_(without_SP) SEQ ID NO: 1485
TPEVWVQVRMEATELSSFTIRCGFLGSGISLVTVSWGGPNGAGGTTLAVLHHPERGIQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWACGS
LPPSSDPGLSAPPTPAPILR

Figure 103T

>PVRIG_NP_076975_from_41_to_170_variation_of_ECD_(without_SP) SEQ ID NO: 1486
TPEVWVQVRMEATELSSFTIRCGFLGSGISLVTVSWGGPNGAGGTTLAVLHHPERGIQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWACGS
LPPSSDPGLSAPPTPAPILRA

Figure 103U

>PVRIG_NP_076975_from_41_to_172_variation_of_ECD_(without_SP) SEQ ID NO: 1487
TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGPNAGAGGTTLAVLHPPERGIQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSGWEACGS
LPPSSDPGLSAPPTPAPILRADL

Figure 103V

>PVRIG_NP_076975_from_41_to_173_variation_of_ECD_(without_SP) SEQ ID NO: 1488
TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGPNAGAGGTTLAVLHPPERGIQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSGWEACGS
LPPSSDPGLSAPPTPAPILRADLA

Figure 103W

>PVRIG_NP_076975_from_41_to_144_variation_of_lg_Domain SEQ ID NO: 1489
TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGPNAGAGGTTLAVLHPPERGIQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSW

Figure 103X

>PVRIG_NP_076975_from_41_to_145_variation_of_lg_Domain SEQ ID NO: 1490
TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGPNAGAGGTTLAVLHPPERGIQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSGWE

Figure 103Y

>PVRIG_NP_076975_from_41_to_146_variation_of_lg_Domain SEQ ID NO: 1491
TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGPNAGAGGTTLAVLHPPERGIQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSGWEA

Figure 103Z

>PVRIG_NP_076975_from_41_to_147_variation_of_lg_Domain SEQ ID NO: 1492
 TPEVVVQVRMEATELSSFTIRCGFLGSGISLVTVSWGPNAGAGGTTLAVLHPERGIQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEAC

Figure 103AA

>PVRIG_NP_076975_from_41_to_148_variation_of_lg_Domain SEQ ID NO: 1493
 TPEVVVQVRMEATELSSFTIRCGFLGSGISLVTVSWGPNAGAGGTTLAVLHPERGIQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACG

Figure 103AB

>PVRIG_NP_076975_from_42_to_144_variation_of_lg_Domain SEQ ID NO: 1494
 PEVVVQVRMEATELSSFTIRCGFLGSGISLVTVSWGPNAGAGGTTLAVLHPERGIQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSW

Figure 103AC

>PVRIG_NP_076975_from_42_to_145_variation_of_lg_Domain SEQ ID NO: 1495
 PEVVVQVRMEATELSSFTIRCGFLGSGISLVTVSWGPNAGAGGTTLAVLHPERGIQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWE

Figure 103AD

>PVRIG_NP_076975_from_42_to_146_variation_of_lg_Domain SEQ ID NO: 1496
 PEVVVQVRMEATELSSFTIRCGFLGSGISLVTVSWGPNAGAGGTTLAVLHPERGIQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEA

Figure 103AE

>PVRIG_NP_076975_from_42_to_147_variation_of_lg_Domain SEQ ID NO: 1497
 PEVVVQVRMEATELSSFTIRCGFLGSGISLVTVSWGPNAGAGGTTLAVLHPERGIQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEAC

Figure 103AF

>PVRIG_NP_076975_from_42_to_148_variation_of_Ig_Domain SEQ ID NO: 1498
 PEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGPNAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACG

Figure 103AG

>PVRIG_NP_076975_from_43_to_144_variation_of_Ig_Domain SEQ ID NO: 1499
 EVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGPNAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSW

Figure 103AH

>PVRIG_NP_076975_from_43_to_145_variation_of_Ig_Domain SEQ ID NO: 1500
 EVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGPNAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWE

Figure 103AI

>PVRIG_NP_076975_from_43_to_147_variation_of_Ig_Domain SEQ ID NO: 1501
 EVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGPNAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEAC

Figure 103AJ

>PVRIG_NP_076975_from_43_to_148_variation_of_Ig_Domain SEQ ID NO: 1502
 EVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGPNAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACG

Figure 103AK

>PVRIG_NP_076975_from_44_to_144_variation_of_Ig_Domain SEQ ID NO: 1503
 VWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGPNAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSW

Figure 103AL

>PVRIG_NP_076975_from_44_to_145_variation_of_lg_Domain SEQ ID NO: 1504
 VVVQVRMEATELSSFTIRCGFLGSGSISLVTWSGGPAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWE

Figure 103AM

>PVRIG_NP_076975_from_44_to_146_variation_of_lg_Domain SEQ ID NO: 1505
 VVVQVRMEATELSSFTIRCGFLGSGSISLVTWSGGPAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEA

Figure 103AN

>PVRIG_NP_076975_from_44_to_147_variation_of_lg_Domain SEQ ID NO: 1506
 VVVQVRMEATELSSFTIRCGFLGSGSISLVTWSGGPAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEAC

Figure 103AO

>PVRIG_NP_076975_from_44_to_148_variation_of_lg_Domain SEQ ID NO: 1507
 VVVQVRMEATELSSFTIRCGFLGSGSISLVTWSGGPAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACG

Figure 103AP

>PVRIG_NP_076975_from_45_to_144_variation_of_lg_Domain SEQ ID NO: 1508
 VVVQVRMEATELSSFTIRCGFLGSGSISLVTWSGGPAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSW

Figure 103AQ

>PVRIG_NP_076975_from_45_to_145_variation_of_lg_Domain SEQ ID NO: 1509
 VVVQVRMEATELSSFTIRCGFLGSGSISLVTWSGGPAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWE

Figure 103AR

>PVRIG_NP_076975_from_45_to_146_variation_of_Ig_Domain SEQ ID NO: 1510

WVQVRMEATELSSFTIRCGFLGSGSISLVTSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEA

Figure 103AS

>PVRIG_NP_076975_from_45_to_147_variation_of_Ig_Domain SEQ ID NO: 1511

WVQVRMEATELSSFTIRCGFLGSGSISLVTSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEAC

Figure 103AT

>PVRIG_NP_076975_from_45_to_148_variation_of_Ig_Domain SEQ ID NO: 1512

WVQVRMEATELSSFTIRCGFLGSGSISLVTSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACG

Figure 103AU

>PVRIG_XP_006544085_PREDICTED_transmembrane_protein_PVRIG-like_isoform_X2_[Mus_musculus] SEQ ID NO: 1513

MRTGNTQAAHATNMGMQMQLVLFSTLLTLCVSEASPEVWVQVQMEATNLSSFVHCGVLGYSLISLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETP
NSVSVTLTMGQSKARSSLANTTFCCEFVTFPHGSRVACRDLHRSDPGLSAPTPALNLQADLVRLGTSGVFLFGFIFILRWQQRHWCLSKSQPSLTSTQAAQVETQPPHL
ASTHSSFISMENGLYALA

Figure 103AV

>PVRIG_2nd_Met_Mouse_WT SEQ ID NO: 1514

MGMQMQLVLFSTLLTLCVSEASPEVWVQVQMEATNLSSFVHCGVLGYSLISLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNVSVTLTMGQSKA
RSSLANTTFCCEFVTFPHGSRVACRDLHRSDPGLSAPTPALNLQADLVRLGTSGVFLFGFIFILRWQQRHWCLSKSQPSLTSTQAAQVETQPPHLASTHSSFISMENGLY
ALA

Figure 103AW

>PVRIG_Mouse_WT_ECD_(without_SP) SEQ ID NO: 1515
 SPEVWVQVQMEATNLSFSVHCGVLGYSLISLVTVSCGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNVSVTLTMGQSKARSSILANTTFCCEFVTFPHGSRV
 ACRDLHRSDPGLSAPTPALNLQAD

Figure 103AX

>PVRIG_Mouse_WT_ECD_(with_SP) SEQ ID NO: 1516
 MGQMQLTVLFSTLLTLCVSEASPEVWVQVQMEATNLSFSVHCGVLGYSLISLVTVSCGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNVSVTLTMGQSKA
 RSSILANTTFCCEFVTFPHGSRVACRDLHRSDPGLSAPTPALNLQAD

Figure 103A

>PVRIG_HH-1_(Human_ECD_+_human_IgG1_Fc_mutated_C220S_at_hinge)_-_without_SP SEQ ID NO: 1517
 TPEVWVQVRMEATELSFTIRCGFLGSGSISLVTVSWGPNAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGS
 LPPSSDPGLSAPPTPAPILRADEPKSSDKTHTCPAPPELLGGPSVFLPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
 VLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYVTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR
 RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 103AZ

>PVRIG_HH-1-1_(Human_ECD_with_C127F_and_C147S_mutations_+_human_IgG1_Fc_mutated_C220S_at_hinge)_-_without_SP SEQ ID NO:
 1518
 TPEVWVQVRMEATELSFTIRCGFLGSGSISLVTVSWGPNAGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEASGSL
 PPSSDPGLSAPPTPAPILRADEPKSSDKTHTCPAPPELLGGPSVFLPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
 VLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYVTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR
 WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 103BA

>PVRIG_HH-2_(Human_ECD_+_Human_IgG1_Fc_mutated_at_C220S,_C226S,_C229S_of_hinge)_-_without_SP SEQ ID NO: 1519

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGPNAGAGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGS
 LPSSDPGLSAPPTAPILRADEPKSSDKTHTSPSPAPPELLGGPSVFLPPKDKTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
 VLVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSGFFLYSKLTVDKSR
 RWQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 103BB

>PVRIG_HH-2-1-
 1_(Human_ECD_with_C127F_mutation_+_Human_IgG1_Fc_mutated_at_C220S,_C226S,_C229S_of_hinge,_encoded_by_natural_codons_instead_of_codons_optimized_for_CHO_cells)_-_without_SP SEQ ID NO: 1520

TPEVWVQVRMEATELSSFTIRCGFLGSGSISLVTVSWGPNAGAGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCANTTFCCKFASFPEGSWEACGS
 LPSSDPGLSAPPTAPILRADEPKSSDKTHTSPSPAPPELLGGPSVFLPPKDKTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
 LTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSGFFLYSKLTVDKSR
 WQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 103BC

>PVRIG_MM-1_(Mouse_ECD_+_mouse_IgG2a_Fc)_-_without_SP SEQ ID NO: 1521

SPEVWVQVRMEATNLSSFSVHCGVLGYSLISLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNVSVLTMTGQSKARSSLANTTFCCEFTFPHGSRV
 ACRDLHRSDPGLSAPTPALNLQADEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIDVLMISLSPVTCVVVDVSEDDPDVQISW FVNNVEVHTAQQTQTHREDYNSTLR
 VVSALPIQHQQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSRAPQVVVYLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTELNYKNTPEPVLDSGSGSYFMYSK
 LRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

Figure 103BD

>PVRIG_MM-1-1_(Mouse_ECD_+_mouse_igG2a_Fc_with_3_C_mutated_to_S_at_hinge)_-_without_SP SEQ ID NO: 1522

SPEVWVQVQMEATNLSFSVHCGVLGYSLSLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNVSVSVTLTMGQSKARSSLANTTFCCEVTFPHGSRV
ACRDLHRSDPGLSAPTPALNLAQADEPRGTIKPSPKSPAPNLLGGPSVFIFPPKIDVLMISLSPVTCVVDVSEDDPDVQISWVFNNEVHTAQQTQTHREDYNSTLR
VVSALPIQHQQDWMSGKEFKCKVNNKDLPAPIERTISKPGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTPEPVLDSGSGYFMYSK
LRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

Figure 103BE

>Human_Fc_(IgG1) SEQ ID NO: 1523

EPKCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWVVDGVEVHNAKTPREEQYNSTYRVSVLTVLHQDWLNGKEYCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHN
HYTQKSLSLSPGK

Figure 103BF

>Human_Fc_(IgG1)_C220S SEQ ID NO: 1524

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWVVDGVEVHNAKTPREEQYNSTYRVSVLTVLHQDWLNGKEYCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHN
HYTQKSLSLSPGK

Figure 103BG

>Human_Fc_(IgG1)_with_the_C220S_(at_hinge)_and_N297A_mutations SEQ ID NO: 1525

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWVVDGVEVHNAKTPREEQYASTYRVSVLTVLHQDWLNGKEYCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHN
HYTQKSLSLSPGK

Figure 103BH

>Human_Fc_(IgG1)_without_hinge SEQ ID NO: 1526

APELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWVVDGVEVHNAKTPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSEFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

Figure 103BI

>Mouse_Fc_(IgG2a) SEQ ID NO: 1527

EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMSISPIVTCVVVDVSEDDPDVQISWVFVNNVEVHTAQITQTHREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVN
NKDLPAPIERTISKPKGSRAPQVYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLH
NHHTTKSFSRTPGK

Figure 103BJ

>Mouse_Fc_(IgG2a)_Fc_with_the_N297A_mutation SEQ ID NO: 1528

EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMSISPIVTCVVVDVSEDDPDVQISWVFVNNVEVHTAQITQTHREDYASTLRVVSALPIQHQQDWMSGKEFKCKVN
NKDLPAPIERTISKPKGSRAPQVYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLH
NHHTTKSFSRTPGK

Figure 103BK

>Mouse_Fc_(IgG2a)_without_hinge SEQ ID NO: 1529

APNLLGGPSVFIFPPKIKDVLMSISPIVTCVVVDVSEDDPDVQISWVFVNNVEVHTAQITQTHREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLPAPIERTISKPK
GSRAPQVYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

Figure 103BL

>Human_Fc_(IgG1)_with_the_C220S_C226S_C229S_mutations_(at_hinge) SEQ ID NO: 1530
EPKSSDKTHTSPSPAPPELLGGPSVFLPFPKPKDTLMISRTPEVTCVAVDVSHEDPEVKFNWVVDGVEVHNAKTKPREEQYNSTYRVWVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 103BM

>PVRIG_PRIMER_200-554_m15029_F SEQ ID NO: 1531
CCACCAACCTCTCGTCTTTC

Figure 103BN

>PVRIG_PRIMER_200-553_m15029_R SEQ ID NO: 1532
TCATGCCAGAGCATACAG

Figure 103BO

>PVRIG_PRIMER_200-571_m15029_F SEQ ID NO: 1533
CAGTGCCTCTAACTGCTGAC

Figure 103BP

>PVRIG_PRIMER_200-572_m15029_R SEQ ID NO: 1534
TCACTGTTACCAGGGAGATGAG

Figure 103BQ

>PVRIG_PRIMER_200-549_m15029_F SEQ ID NO: 1535

CACAGGCTGCCCCATGCAAC

Figure 103BR

>PVRIG_PRIMER_200-551_m15029_R SEQ ID NO: 1536

TGCCTGGGTGCTAGTGAGAG

Figure 103BS

>PVRIG_PRIMER_200-554_m15029_F SEQ ID NO: 1537

CCACCAACCTCTCGTCTTTC

Figure 103BT

>PVRIG_PRIMER_200-546_m15029_R SEQ ID NO: 1538

GACCTGTTACCTGTCATTG

Figure 103BU

>PVRIG_flag_protein SEQ ID NO: 1539

MGHRTLVPWVLLTLCVTAGTPEWVQVRMEATELSSFTIRCGFLGSGSISLTVSWGGPNGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEGSGASSPCA
NTTFCKFASPEGSWEACGSLPPSSDPGLSAPPTPAPILRADLAGILGVSGVLLFGCVYLLHLLRRHKHRPAPRLQPSRTSPQAPRARAWAPSQASQAALHVPYATINTS
CRPATLDTAHPHGGPSWWASLPTHAAHRPQGPAAWASTPIPARGSFVSVENGLYAQAAGERPPHTGPGLTLFPDPRGRAMEGPLGVRDYKDDDDK

Figure 103BV

>PVRIG_Mouse_First_Methionine_signal_peptide-Flag-ECD SEQ ID NO: 1540

MRTGNTQAAHATNMGQMQLTLVLFSTLLTLCVSEADYKDDDDKSPEVWVQVQMEATNLSFSVHCGVLGYSLSLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPAR
QAHWETPNVSVVTLTMGQSKARSSLANTTFCCEFVTFPHGSRVACRDLHRSDPGLSAPTPALNQLADLVRLGTSGVFLFGFIFILCLRWQQRHWCLSKSQPSLTSTQAQ
VETQPPHLASTHSSFISMENGLYALA

Figure 103BW

>PVRIG_Mouse_Third_Methionine_untagged SEQ ID NO: 1541

MQTLVLFSTLLTLCVSEASPEVWVQVQMEATNLSFSVHCGVLGYSLSLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNVSVVTLTMGQSKARSSLA
NTTFCCEFVTFPHGSRVACRDLHRSDPGLSAPTPALNQLADLVRLGTSGVFLFGFIFILCLRWQQRHWCLSKSQPSLTSTQAQVETQPPHLASTHSSFISMENGLYALA

Figure 103BX

>PVRIG_Mouse_Third_Methionine_signal peptide-Flag-ECD SEQ ID NO: 1542

MQTLVLFSTLLTLCVSEADYKDDDDKSPEVWVQVQMEATNLSFSVHCGVLGYSLSLVTVSCEGFVDAGRTKLAVLHPEFGTQQWAPARQAHWETPNVSVVTLTMG
QSKARSSLANTTFCCEFVTFPHGSRVACRDLHRSDPGLSAPTPALNQLADLVRLGTSGVFLFGFIFILCLRWQQRHWCLSKSQPSLTSTQAQVETQPPHLASTHSSFISME
NGLYALA

Figure 104A Comparison of PVRIG protein isoforms

Ig→

P0 MRTEAQVPALQPPEPGLEGA TPEVWVQVRMEATELSSFTI
P1 MRTEAQVPALQPPEPGLEGAMGHRTLVLFPWVLLTLCVTAGTPEVWVQVRMEATELSSFTI
P6 MRTEAQVPALQPPEPGLEGAMGHRTLVLFPWVLLTLCVTAGTPEVWVQVRMEATELSSFTI
P8 MRTEAQVPALQPPEPGLEGAMGHRTLVLFPWVLLTLCVTAGTPEVWVQVRMEATELSSFTI
P4 MRTEAQVPALQPPEPGLEGAMGHRTLVLFPWVLLTLCVTAGTPEVWVQVRMEATELSSFTI
P7 MRTEAQVPALQPPEPGLEGAMGHRTLVLFPWVLLTLCVTAG-----
P14 MRTEAQVPALQPPEPGLEGAMGHRTLVLFPWVLLTLCVTAG-----
P3 MRTEAQVPALQPPEPGLEGAMGHRTLVLFPWVLLTLCVTAGTPEVWVQVRMEATELSSFTI
P10 MRTEAQVPALQPPEPGLEGAMGHRTLVLFPWVLLTLCVTAGTPEVWVQVRMEATELSSFTI
P13 MRTEAQVPALQPPEPGLEGAMGHRTLVLFPWVLLTLCVTAGTPEVWVQVRMEATELSSFTI

P0 R GFLGSGSISLVTVSWGGPDGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEG
P1 R GFLGSGSISLVTVSWGGPDGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEG
P6 R GFLGSGSISLVTVSWGGPDGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEG
P8 R GFLGSGSISLVTVSWGGPDGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEG
P4 R-----W-----
P7 -----
P14 -----
P3 R GFLGSGSISLVTVSWGGPDGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEG
P10 R GFLGSGSISLVTVSWGGPDGAGGTTLAVLHPERGA-QISPRGPKI-----
P13 R GFLGSGSISLVTVSWGGPDGAGGTTLAVLHPERGIRQWAPARQARWETQSSISLILEG

←Ig

P0 SGASSPCANTTF KFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRAD
P1 SGASSPCANTTF KFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRAD
P6 SGASSPCANTTF KFASFPEGSWEACGSLPPSSDPGLSAPPTPAPILRAD
P8 SGASSPCANTTF KFASFPEGSWEACGSLPPSSDPGLSVPTPAPILRAD
P4 -----LSAPPTPAPILRAD
P7 -----LSAPPTPAPILRAD
P14 -----LSVPPTPAPILRAD
P3 SGASSPCANTTF KFASFPEGSWEACGSLPPSSDPGGAGARGPG-----GQGG
P10 ---GGPPCPRPGPAGKPRAASLSSWKALGPAAPAPTPPSAASLRPS-----
P13 SGASSPCANTTF KFASFPGCSSR-----

P0 RRHKHRPAPRLQPSRTSPQAPRARAWAPSQASQAALHVPYATINTSC
P1 RRHKHRPAPRLQPSRTSPQAPRARAWAPSQASQAALHVPYATINTSC
P6 RRHKHR-----
P8 CRQKHRPAPRLQPSHTSS-----
P4 RRHKHRPAPRLQPSRTSPQAPRARAWAPSQASQAALHVPYATINTSC
P7 RRHKHRPAPRLQPSRTSPQAPRARAWAPSQASQAALHVPYATINTSC
P14 CRQKHRPAPRLQPSHTSS-----
P3 -----ARELATHLILVSVPRAL
P10 -----QAALHVPYATINTSC
P13 -----PLCHYQHQL

P0 RPATLDTAHPHG-----GPSWWASLPTHAAHRPQGPAAWASTPIPARGSFVSV
P1 RPATLDTAHPHG-----GPSWWASLPTHAAHRPQGPAAWASTPIPARGSFVSV
P6 -----
P8 -----
P4 RPATLDTAHPHG-----GPSWWASLPTHAAHRPQGPAAWASTPIPARGSFVSV
P7 RPATLDTAHPHG-----GPSWWASLPTHAAHRPQGPAAWASTPIPARGSFVSV
P14 -----
P3 CPA---DSCPHSAGRPGRDLGGLRSPPLWLCLPPSSA---APT-----
P10 RPATLDTAHPHG-----GPSWWASLPTHAAHRPQGPAAWASTPIPARGSFVSV
P13 PPSYEG---HS-----SPPWGAVLVGVTPHPRCTPA-----PGFCRLGL---

Figure 104B

P0	ENGLYAQAGERPPHTGP---GLTLFPDPRGPRAMEGPLGVR	(SEQ ID NO: 1557)
P1	ENGLYAQAGERPPHTGP---GLTLFPDPRGPRAMEGPLGVR	(SEQ ID NO: 1558)
P6	-----	(SEQ ID NO: 1559)
P8	-----	(SEQ ID NO: 1560)
P4	ENGLYAQAGERPPHTGP---GLTLFPDPRGPRAMEGPLGVR	(SEQ ID NO: 1561)
P7	ENGLYAQAGERPPHTGP---GLTLFPDPRGPRAMEGPLGVR	(SEQ ID NO: 1562)
P14	-----	(SEQ ID NO: 1563)
P3	-----	(SEQ ID NO: 1564)
P10	ENGLYAQAGERPPHTGP---GLTLFPDPRGPRAMEGPLGVR	(SEQ ID NO: 1565)
P13	-----HTHPCTWQLCLC-----	(SEQ ID NO: 1566)

P0 and P1 WT gene model (Membranal proteins), P6 and P8 ECD similar to the WT with different intracellular. P4 P7 P14 membran proteins w/o Ig domain. P3, P10 and P13 secreted isoforms having different C ends compared to the wild type.

Figure 105

Linkers

Linker	SEQ ID NO:
GS	1567
GSGS	1568
AS	1569
GGGS	1570
GGGS	1571
GGGSGGGGS	1572
GGGSGGGSGGGGS	1573
GGGSGGGSGGGSGGGGS	1574
AGAAAKGAAAKA	1575
AGAAAKGAAAKGAAAKA	1576
AGAAAKGAAAKGAAAKGAAAKA	1577
AGAAAKGAAAKGAAAKGAAAKGAAAKA	1578
AGAAAKA	1579
GSENYFQGSG	1580
AEAAAKEAAAKA	1581
AEAAAKEAAAKEAAAKEAAAKA	1582
GGEAAAKEAAAKGG	1583
GGGSGGGSGAGSGGGGS	1584
GGGGSGGGGS	1585
GGGGSG	1586
GSGG	1587
GSGGS	1588