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(54) **Title:** PASSIVE ANTIBODY DEPENDENT CELL-MEDIATED ACTIVATION

(57) **Abstract:** Disclosed herein are methods, reagents, and pharmaceutical compositions for modulating immune effector cell activation that does not requirean antigen-specific antibody. The IgG Fc region is shown herein to contain both a domain that binds an FcγR on immune effector cells and a non-overlapping region or domain that can bind Fc binding proteins on target cells, and that it is capable of bridging immune effector cells and target cells expressing Fc binding proteins without use of the antigen-binding region (Fab) of the antibody. Therefore, also disclosed are methods of enhancing or inhibiting passive ADCC in subjects.

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PASSIVE ANTIBODY DEPENDENT CELL-MEDIATED ACTIVATION

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

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BACKGROUND

Natural killer (NK) cells are innate lymphoid cells that lack the ability to rearrange germline immunoglobulin genes to generate an adaptive immune response, and can recognize virally infected cells or cancer cells without prior antigen exposure (Orr *et al.*, 2010, *Cell* 142, 847-856). The functional status of NK cells is regulated by signal inputs from a wide variety of NK cell activating/inhibitory receptors and cytokines. NK cells are also the major effector cells for antibody-dependent cell-mediated cytotoxicity (ADCC), and express the low affinity FcγRIIIA/CD16a protein (CD16a hereafter) that binds an IgG molecule at that molecule's hinge region and initiates NK cell activation via the resulting antigen-antibody complex (Sondermann *et al.*, 2000, *Nature* 406, 267-273). CD16a is coupled in NK cells with the signal transducer protein CD3ζ (Anderson *et al.*, 1990, *Proc Natl Acad Sci USA* 87, 2274-2278; Lanier *et al.*, 1989, *Nature* 342, 803-805). NK cell activation through CD16a minimally requires two CD16a binding sites physically close enough to cluster CD3ζ, whose phosphorylation in turn results in the activation of NK cells and lysis of antibody-coated target cells (O'Shea *et al.*, 1991, *Proc Natl Acad Sci USA* 88, 350-354).

There is substantial evidence in mice and in humans that NK cells can act as a first line of defense against a broad array of infectious pathogens and against malignant transformation. "First line of defense" connotes protection arising days to weeks before the adaptive, antigen-specific immune system of T-cells and B-cells can detect a pathogen and attack it, and quickly recall its response when the pathogen or tumor itself is present in the body again at a later time. The mechanisms behind this early recognition of pathogens and tumor cells by NK cells and other innate immune cells are largely but not completely unknown (Dai, *et al.*, 2017, *Immunity* 47(1), 159-

170). Understanding how NK cells and other innate immune cells can see such dangers will improve understanding of how to treat such diseases early, thus saving more lives.

As one example, NK cells constitute the first line of defense against herpesviruses infection and patients with NK cell deficiencies often suffer severe, recurrent and sometimes fatal HSV infection (Orange, 2012, *Journal of Clinical Investigation* 122, 798-801). The herpesviridae family includes many significant human pathogens that are yet to have approved vaccines (Gilden *et al.*, 2007, *Nat Clin Pract Neurol* 3, 82-94), and oncolytic HSV1 has recently been clinically approved for treating melanoma (albeit with moderate therapeutic effects; Andtbacka *et al.*, 2015, *J Clin Oncol.* Talimogene Laherparepvec Improves Durable Response Rate in Patients With Advanced Melanoma"). Thus, there remains a need in the art to develop reagents, methods, and pharmaceutical compositions for providing effective antiviral treatments and maximizing efficacy of oncolytic HSV1 therapy.

SUMMARY

The disclosure provides reagents, methods, and pharmaceutical compositions for promoting immunological activation of immune effector cells. In particular embodiments, provided herein are immunological polypeptides comprising a domain that binds an Fc gamma receptor (FcγR) on immune effector cells and a non-overlapping domain that binds Fc binding proteins on target cells. The polypeptides described herein are capable of forming a bridge between an immune effector cell and a target cell without use of the antigen-binding region (the so-called IgG Fab region) of the antibody. This type of immune effector cell activation is referred to herein as passive antibody dependent cell-mediated cytotoxicity (ADCC). Passive ADCC can have both beneficial and deleterious effects in subjects infected with pathogens that encode Fc binding proteins, subjects who express genes that encode Fc binding proteins other than FcγR, and in subjects undergoing treatment with antibody. Therefore, also disclosed are methods of enhancing or inhibiting passive ADCC in subjects.

In particular embodiments, a pharmaceutical composition for treating a subject infected with a pathogen encoding an Fc-binding protein is provided. In particular embodiments, the composition comprises an immunological polypeptide comprising a

domain that binds an Fc gamma receptor (FcγR) on an immune effector cell and a non-overlapping domain that binds the pathogen-encoded Fc binding protein. In certain embodiments the immunological polypeptide is an antibody, more specifically an IgG antibody and in particular an Fc fragment of an IgG antibody. Also within the scope of such immunological polypeptides is IgG-containing antisera. A feature of the immunological polypeptides useful in the methods and comprising the pharmaceutical compositions disclosed herein is that the efficacy and utility of said IgG antibodies is independent of their antigenic specificity.

In particular embodiments, the invention also provides reagents, methods and pharmaceutical compositions for preventing neurologic damage in a subject with HSV1 infection. In particular embodiments, the invention further provides reagents, methods and pharmaceutical compositions for preventing death in a subject with HSV1 infection. In particular embodiments, a pharmaceutical composition comprising an immunological polypeptide comprising a domain that binds an FcγR on an immune effector cell and a non-overlapping domain that binds a HSV1-encoded Fc binding protein is provided. In certain embodiments the immunological polypeptide is an antibody, more specifically an IgG antibody and in particular an Fc fragment of an IgG antibody. Also within the scope of such immunological polypeptides is IgG-containing antisera. A feature of the immunological polypeptides useful in the methods and comprising the pharmaceutical compositions disclosed herein is that the efficacy and utility of said IgG antibodies is independent of their antigenic specificity.

In some embodiments, provided herein are polypeptides comprising an Fc region of an immunoglobulin G (IgG) antibody but do not comprise an Fab region of an antibody. For example, the polypeptide can be a fragment of an IgG1, IgG2, IgG3, or IgG4 immunoglobulin. In some embodiments, the polypeptide comprises a single Fc region of an IgG immunoglobulin. In some embodiments, the polypeptide comprises two or more Fc regions of one or a plurality of IgG immunoglobulins. In some embodiments, the polypeptide comprises an Fc gamma receptor binding site that has been modified to enhance binding to an Fc gamma receptor, and in some embodiments, the polypeptide comprises an Fc gamma receptor binding site that has been modified to delete binding to an Fc gamma receptor or delete binding to an Fc binding protein other than an Fc gamma receptor.

In some embodiments, the immune effector cell is an immune cell that expresses an Fc gamma receptor. Fc gamma receptors include CD16a, CD16b, CD32, and CD64. Therefore in some embodiments, the immune effector cell is a T cell, a B cell, a natural killer (NK) cell, a monocyte, a macrophage, a granulocyte, a neutrophil, or a dendritic cell.

The disclosed reagents, methods and pharmaceutical compositions can be used in some embodiments to treat a subject infected with a pathogen expressing an Fc binding protein, wherein the methods comprise administering to the subject a therapeutically effective amount of the pharmaceutical compositions disclosed herein. In some cases, the pathogen is a virus. In a non-limiting example, the pathogen is herpes simplex virus 1 (HSV1) or HSV2 that expresses the Fc binding protein glycoprotein E (gE). In other embodiments, the pathogen is human cytomegalovirus (CMV) that expresses the Fc binding protein comprises a 68kDa-glycoprotein (gp68). In other embodiments, the pathogen is Varicella zoster virus (VZV).

In some cases, the pathogen is a bacterium, such as *Staphylococcus aureus*, *Streptococcus*, or *Escherichia coli*. In such embodiments, the Fc binding protein expressed by the pathogen comprises protein A, protein G, protein H, or M1 protein.

In particular embodiments, the methods provided herein are applied to a subject undergoing oncolytic viral therapy. While it may be advantageous to inhibit passive ADCC early after oncolytic viral infection of a tumor to allow the virus to spread to other tumor cells, the disclosed methods can also be used to enhance passive ADCC after the tumor cells are infected to enhance killing and clearing of tumor cells.

Also disclosed are reagents, methods and pharmaceutical compositions for reducing or inhibiting passive ADCC. In such embodiments, the polypeptide is a fragment of an IgG immunoglobulin modified to bind Fc binding proteins but not bind an FcγR, which will cause them to bind the Fc binding proteins of target cells and prevent them from crosslinking FcγR and activating passive ADCC. For example, the polypeptide can be an IgG fragment that lacks or has been engineered to lack a CD16a, CD32, or CD64 binding site. The Fc binding site for HSV1 gE, protein A and protein G and M1 protein of streptococcus is known to be the CH2-CH3 interface of an IgG molecule. Therefore, in some embodiments, the polypeptide is a fragment of

an IgG immunoglobulin comprising the CH2-CH3 interface of IgG but not comprising the FcγR binding region.

In other embodiments, the polypeptide can be a fragment of an IgG1, IgG2, IgG3 or IgG4 immunoglobulin. In some embodiments, the polypeptide is a fragment from more than one subclass of antibody. Regions of FcγRIIIa/CD16a involved in binding Fc are B/C loop (Trp 131 to Ala 135), F/G loop (Val 176 to Lys 179), C strand (His 137 to Thr 140) and C' strand (Asp 147 to His 153) of SEQ ID NO: 24). Additionally, Arg 173 and the connector (Ile 106 to Trp 108) region are also involved in binding. On the other hand, Cγ2 hinge (Leu 235 to Ser 239) and residue Asp 265 to Glu 269 of Fc are known to be the main contact residues for CD16a (Sondermann *et al.*, 2000, *Nature* 406, 267-273)). Thus, modification on the interacting interface can change binding between CD16a and IgG. For example, replacement of FcγRIII FG-loop with that of FcγRI (MGKHRY; SEQ ID NO: 11) resulted in a 15-fold increase in IgG1 binding affinity (Lu *et al.*, 2011, *JBC* 286, 40608 –40613). Another example is an Fc fragment comprising human IgG1 Fc amino acids 262-470 of SEQ ID NO: 10, which bind HSV1 gE, protein A and protein G, but completely failed to bind human Fcγ receptors (CD16a, CD32, CD64).

In some embodiments, the methods disclosed herein involve administering an Fc binding protein, such as protein A or protein G, which will bind antibodies and prevent bridging immune effector cells and target cells by passive ADCC.

In some embodiments, the polypeptide is a fragment of an IgG immunoglobulin modified to bind FcγR but not bind an Fc binding protein, which will bind immune effector cells and prevent them from interacting with bridging antibodies. For example, the polypeptide can be a fragment of an IgG immunoglobulin that binds FcγR but has a mutation in the CH2-CH3 interface causing it to not bind HSV1 gE, protein A and protein G. For example, human IgG 4 binds gE while IgG4 mutant H435R is unable to bind gE.

In particular embodiments, the invention provides reagents, methods and pharmaceutical compositions for reducing inflammation in a subject receiving anti-cancer therapy. These embodiments can comprise administering a therapeutically effective amount of a polypeptide comprising a region that binds to an Fc binding protein but does not comprise a region that binds to an FcγR and administering an anti-cancer therapy comprising a monoclonal antibody drug. In particular

embodiments the antibody drug is rituximab, tocilizumab, tositumomab, trastuzumab bevacizumab, brentuximab vedotin, cetuximab, daratumumab, ipilimumab, ofatumumab, panitumumab, alemtuzumab or pembrolizumab. In other embodiments, the pharmaceutical composition is administered prior to treatment with the monoclonal antibody drug.

In some embodiments, the subject is being treated with a therapeutic antibody. Most therapeutic monoclonal antibody drugs are produced from mammalian host cell lines to target specific antigens. Side effects and reduce therapeutic efficacy can be caused by sequestration of the antibody by various Fc binding proteins endogenously present in a patient, and interacting via passive ADCC with FcγR-bearing immune cells. The disclosed methods can decrease such side effects of antibody mediated infusion toxicity (the so-called first dose effect), yet enhance antibody-based immunotherapy.

The disclosed methods can also be used to prevent cytokine release from FcγR-bearing immune cells, hypotension and multiple organ failure in patients with infection from organisms that have Fc binding proteins.

In some embodiments of this use of the reagents, methods and pharmaceutical compositions disclosed herein, the subject has, or is at risk of having viremia with a virus that encodes an Fc binding protein. These disclosed methods can be used to treat or prevent viremia by enhancing viral clearance by FcγR-bearing immune cells using IgG Fc fragments or antibodies with higher-than-normal affinity for Fc binding proteins and/or FcγR, and utilizing passive ADCC for clearance.

In some embodiments provided herein, are reagents, methods and pharmaceutical compositions for treating cancer in a subject undergoing oncolytic viral therapy. The methods can be used to inhibit passive ADCC early after oncolytic viral infection of a tumor so the oncolytic virus can spread to other tumor cells. In particular embodiments, the immunological polypeptide can be administered prior to treatment with an oncolytic viral therapeutic agent, an in others the immunological polypeptide can be co-administered with an oncolytic viral therapeutic agent. In particular embodiments, a pharmaceutical composition comprising a polypeptide comprising a region that binds to an Fc binding protein on a target cell but does not comprise a region that binds to an FcγR is provided for inhibiting passive ADCC in a patient undergoing oncolytic viral therapy. A feature of the immunological

polypeptides useful in the methods and comprising the pharmaceutical compositions disclosed herein is that the efficacy and utility of said IgG antibodies is independent of their antigenic specificity.

Subsequent to viral infection of a tumor cell, reagents, methods, and pharmaceutical compositions provided herein for enhancement of passive ADCC can be used to improve destruction of virally infected tumor cells by FcγR-bearing immune cells. For example, a pharmaceutical composition comprising an immunological polypeptide comprising a domain that binds an FcγR on an immune effector cell and a non-overlapping domain that binds an Fc binding protein on a target cell can be used to enhance passive ADCC to improve destruction of virally-infected tumor cells. In certain embodiments the immunological polypeptide is an antibody, more specifically an IgG antibody and in particular an Fc fragment of an IgG antibody. Also within the scope of such immunological polypeptides is IgG-containing antisera. A feature of the immunological polypeptides useful in the methods and comprising the pharmaceutical compositions disclosed herein is that the efficacy and utility of said IgG antibodies is independent of their antigenic specificity.

Also disclosed herein is a method for identifying viral genes that modulate the interaction of a virally-infected cell and an immune effector cell. The disclosed method can involve transfecting a host cell with an expression vector comprising a candidate viral gene and a reporter gene operably linked to an expression control sequence, exposing the transfected host cells and non-transfected host cells to a cytotoxic immune effector cell, and assaying the exposed transfected host cells and non-transfected host cells to measure cell death as a function of reporter gene expression or activity. In these methods, a decrease in cell death by the transfected host cells compared to non-transfected host cells is an indication that the viral gene protected the host cell from the immune effector cell, and an increase in cell death by the transfected host cells compared to non-transfected host cells is an indication that the viral gene made the host cell susceptible to the immune effector cell. These methods can be used with any immune effector cell(s) that is/are cytotoxic. For example, the cytotoxic immune effector cell can be a CD4+ T-cell, a CD8+ T-cell, a natural killer (NK) cell, a macrophage, a granulocyte, or a dendritic cell.

In particular embodiments, the reporter gene is a fluorescence gene, wherein the exposed transfected host cells and non-transfected host cells are assayed, *inter*

alia, by flow cytometry to measure cell death as a function of fluorescence, wherein an increase in the percentage of fluorescent transfected host cells compared to non-transfected host cells is an indication that the viral gene protected the host cell from the immune effector cell, and wherein a decrease in mean fluorescence by the transfected host cells compared to non-transfected host cells is an indication that the viral gene made the host cell susceptible to the immune effector cell.

The process can be repeated for each gene in a viral genome. For example, the method can further comprising repeating the process for combinations of 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or more genes in a viral genome.

The host cell is preferably selected based on its known susceptibility or resistance to killing by an immune effector cell when virally infected.

Also disclosed herein is a recombinant oncolytic Herpes Simplex Virus (oHSV), comprising one or more activating mutations in an Us8 gene (SEQ ID NO: 18), one or more activating mutations in an UL12 gene (SEQ ID NO: 12), one or more activating mutations in an UL30 gene (SEQ ID NO: 13), one or more activating mutations in an Us3 gene (SEQ ID NO: 15), one or more activating mutations in an Us5 gene (SEQ ID NO: 14), one or more activating mutations in an Us12 gene (SEQ ID NO: 16), or any combination thereof.

Also disclosed is a recombinant HSV1 vector, comprising a CMV immediate-early enhancer upstream of the promoter for HSV1 Us7 and HSV1 Us8.

Also disclosed herein is a method for using particular IgG-binding proteins, specifically protein A and protein G, to capture monocytes and increase the efficacy of generating dendritic cells and macrophages *in vitro*. The disclosed methods can comprise coating culture plates with recombinant protein A or protein G, culturing human (or mouse) peripheral blood mononuclear cells (PBMC) or purified monocytes with cytokines in the protein A or G-coated plate to generate macrophage or dendritic cells. The disclosed methods can also include culturing human (or mouse) peripheral blood mononuclear cells (PBMC) or purified monocytes with any polymerized form of protein A, protein G, or other IgG-binding proteins. These methods can similarly comprise coating culture plates with recombinant protein A or protein G, culturing human (or mouse) peripheral blood mononuclear cells (PBMC) or purified monocytes with cytokines in the protein A or G-coated plate to generate macrophage or dendritic cells.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

Figures 1A to 1I show differential cytolysis mediated by ectopic gene expression (DC-MEGE) that identified HSV1 gE as an NK cell-activating molecule. Figure 1A is a flow diagram of the DC-MEGE assay. Figure 1B shows representative graphs of the DC-MEGE assay for representative HSV1 genes. Figure 1C is a bar graph showing DC-MEGE results for all 65 HSV1 genes ($n \geq 4$), wherein expression of HSV1 genes UL12, UL30, Us3, Us8 and Us12 promoted NK cytolysis, while expression of UL48, Us5 and Us6 inhibited NK cytolysis. Figure 1D shows cytotoxicity against transfected human glioma cell lines at the specified Effector:Target ratio (E: T, x-axis). Figure 1E shows that IFNγ is secreted by primary human NK cells after 20 hours culture with transfected glioma cells (mean of triplicates, n=5). Figures 1F and 1G show phenotypes of primary human NK cells after culturing with transfected glioma cells for 7 hours. Figure 1H shows cytotoxicity of primary human NK cells against glioma cells expressing Us8 in the presence of isotype or Us8/gE-specific antibody. Figure 1I shows phenotypes of primary human NK cells after culturing in plates precoated with inactivated pure wild type F strain or Us8 deficient F strain for 7 hours, or in the presence of isotype or gE-specific antibody (n=7). Data in Figures 1C, 1D, and 1H are set forth as mean ± sem. Each dotted line in Figure 1E, 1H and 1I links data acquired from the same donor. * $p<0.05$, ** $p<0.01$.

Figures 2A to 2G show IgG-linked gE and NK cell activation. Figure 2A shows phenotypes of primary human NK cells after culturing with transfected glioma cells for 7 hours. Percentages of NK cells gaining expression of CD69 or CD107a, or NK cells losing both CD16a and CD62L expression are summarized for 14 donors. Figure 2B shows primary human NK cells treated as in Fig 2A, wherein supernatants were collected at 20h and measured for human IFNγ (mean of triplicates, n=5). Figures 2C and 2D show human PBMC washed with pH 7.4 or pH 4 media, and subsequently stained with lineage markers and human IgG Fc antibody; mean intensity of cell markers and Fc are shown and one representative of at least five

donors is shown. Figure 2E shows the presence of human IgG on NK cells from human donors. Figure 2F shows phenotypes of NK cells from donors in Figure 2E after culturing with transfected glioma cells or K562 cells for 7 hour. Figure 2G shows the percentage of NK cells positive for CD69 or CD107a after co-culturing with glioma cells or K562, plotted against surface IgG intensity of corresponding human donors. Correlation analysis was conducted using linear regression ($n \geq 20$). Each dotted line in Figures 2A and 2B links data acquired from the same donor. * $p<0.05$, ** $p<0.01$.

Figures 3A to 3K show that IgG bridging was essential for NK cell activation by HSV gE. Figure 3A shows a model structure of a gE-IgGFc-CD16a ternary complex. Front view, side view and top view are shown respectively. CD16a is shown as a stick, gE is shown as a sphere, and two monomers of IgG Fc dimer are shown as a ribbon. Figure 3B shows binding of human IgG products (Fig. 3B) or CD16a with or without human IgG products (Fig. 3C) to transfected glioma cells. Figure 3D shows binding of CD16a to infected glioma cells in the presence of human IgG products. Figure 3E shows phosphorylation of CD3ζ following NK cell stimulation as described in 1H. Figures 3F and 3G show staining of lineage markers and protein A (Fig. 3F) or protein G (Fig. 3G) in human PBMC following a brief wash with pH 7.4 or pH 4 media. The numbers in these Figures are mean intensity of each staining. Figure 3H shows primary human NK cells that were first incubated with protein A or protein G, and then the phenotypes were assessed after culturing with transfected glioma cells, K562 or interleukins IL12+IL18 for 7h (n=7-8). Figure 3I shows primary human NK cells incubated with protein A or protein G, and subsequently assessed for cytotoxicity against glioma Us7+Us8 (Fig. 3I). Figure 3J shows NK cells treated as in (Fig. 3H), wherein IFNγ secretion was assessed at 20 hours of culture (mean of triplicates, n=5). Figure 3K shows an action model for immunostimulation mediated by IgG Fc (ADCC as an example). Each dotted line in Fig. 3H and 3J links data acquired from the same donor. Figures 3B, 3C, 3D, 3E, 3F and 3G were repeated at least 3 times. * $p<0.01$.

Figures 4A to 4F show that passive ADCC promotes viral clearance *in vivo*. Figure 4A shows cytotoxicity of primary human NK cells against infected glioma cells in the presence or absence of human IgG products. Figure 4B shows cytotoxicity of primary human NK cells against transfected glioma cells in the presence or absence

of human IgG1 Fc. Figure 4C shows cytotoxicity of primary mouse NK cells against glioma Us7+Us8 in the presence or absence of human IgG1 Fc. Figure 4D shows protection of mice against lethal HSV1 infection by human IgG products. Figures 4E and 4F show HSV1 virus load at 18h and 84h post infection (n=5). The data in Figures 4A, 4B and 4C are set forth as the mean ± sem. * $p<0.05$, ** $p<0.001$.

Figures 5A to 5H show bacterial IgG-binding proteins activate NK cells through IgG bridging. Figures 5A and 5B show phenotype (Fig. 5A) and IFN γ production (Fig. 5B) of primary human NK cells after being cultured with soluble protein A, or in plates coated with protein A, or in protein A-coated plates that were blocked with mouse serum before NK cell culture. Each dotted line in Figure 5B represents one donor (mean of triplicates, n=6). Figure 5C shows human NK cells cultured in plates coated with media or protein A for 30 min, wherein cytotoxicity was assessed against glioma cells; the results shown are mean ± sem. Figure 5D shows phosphorylation of CD3ζ after exposing primary human NK cells to stimuli indicated in the Figure for 1h. Figure 5E shows the phenotype of primary human NK cells after culture with bacteria for 7 hours. In some cases NK cells were pretreated with soluble protein A or protein G, or soluble protein A was pre-incubated with mouse serum or human IgG. Figure 5F shows IFN γ production in mouse NK cells after being cultured with bacteria (mean of triplicates, n=5), each dotted line representing one mouse. Figure 5G shows the phenotype of mouse NK cells after culturing in plates pre-coated with media or protein A. Figure 5H shows the phenotypes of NK cells from BALB/c mice that were injected with control silicone beads or protein A-conjugated silicone beads. Figures 5A, 5D, and 5E were repeated minimally on 4 donors.

Figure 6 shows the phenotype of primary human NK cells after culture for 7 hours in plates coated with inactivated pure wild type or Us8-deficient HSV1 F strain viruses. Isotype or Us8/gE-specific antibody was added to interfere with the interaction between NK cells and coated F strain viruses. Representative contour staining of 7 donors is shown.

Figure 7 shows the phenotype of primary human NK cells after being cultured for 7 hours with glioma cells expressing different HSV1 genes. A representative contour plot from one of 8 donors is shown.

Figure 8 shows the phenotype of NK cells from different donors after being cultured for 7 hours with transfected glioma cells or K562 cells.

Figures 9A and 9B show primary human NK cells treated with protein A or protein G, and subsequently cultured with K562 cells, IL12+IL18 or transfected glioma cells. Phenotyping was performed at 7 hours of culture. A representative contour plot from one of 8 donors is shown.

Figures 10A and 10B show human NK cells treated with medium alone, protein A or protein G, and subsequently cultured in plates coated with inactivated wildtype HSV1 F strain. Phenotyping was performed after 7 hours of culture. A representative contour plot from one donor (Fig. 10A) and statistical summary for 5 donors (Fig. 10B) are shown.

Figures 11A to 11D show NK cells cultured with infected glioma cells (Fig. 11A, 11B), or transfected glioma cells (Fig. 11C, 11D), in the presence of medium alone, HSV1 non-immune plasma ((-) plasma) or human IgG1 Fc, and stained after 7h of culture. A representative contour plot from one donor (Fig. 11A and 11C) and statistical summary of 7-9 donors (Fig. 11B and 11D) are shown.

Figure 12A shows serial diluted HSV1 F strain mixed with PBS or IgG products, incubated for 30 min at room temperature, and titred on Vero cells for infectivity. All numbers are normalized to PBS treatment (negative control). Human IgG contained anti-HSV1 IgG (positive control). Figure 12B shows the phenotype of NK cells from BALB/c mice that were injected with human IgG Fc or rituximab for 24 hours.

Figure 13A shows binding of mouse IgG2a to bacteria. Figure 13B shows binding of CD16a to bacteria in the presence of human IgG products.

Figures 14A and 14B show the phenotype (Fig. 14A) and IFN γ production (Fig. 14B) of primary human NK cells after culture with soluble streptococcus protein G, or in plates coated with protein G, or in protein G coated plates which were blocked with mouse serum before NK cell culture. Each dotted line in Figure 14B represents one donor (mean of triplicates, n=6). Figure 14C shows human NK cells cultured in plates coated with media or protein A for 30 min, wherein cytotoxicity was assessed against glioma cells. These results are shown as mean±sem.

Figure 15A shows the phenotype of mouse spleen NK cells from C57BL/6 and BALB/c after 24 hours culture with bacteria. This represents one of three experiments. Figure 15B shows NK cells isolated from mouse spleen cultured in plates pre-coated with media or protein A and stained at 24h of culture. CD62L and

CD27 intensity are relative to media coated plates (n=12). Figure 15C shows the phenotypes of NK cells from BALB/c mice that were injected with control silicone beads or protein A-conjugated silicone beads. A statistical summary (n=5) is shown. Figure 15D shows the phenotype of NK cells from BALB/c mice injected with soluble protein A for 24 hours. The Figures represent one of 3 experiments.

Figure 16 shows sequence alignment of protein A (UniProtKB: P99134), protein G (UniProtKB: P06654) and HSV1 gE (UniProtKB: P04488).

Figures 17A-17C show morphological and functional changes of human monocytes treated with bacterial IgG-binding proteins. Figure 17A shows the morphology of human monocytes cultured in plates pre-coated with media, protein A, or protein G for 3h or 18h. Figure17B shows the result of a monocyte respiratory burst assay, in which human primary monocytes were cultured in plates coated with bovine serum (BSA), or protein A, or stimulated with phorbol 12-myristate 13-acetate (PMA; positive control) for a specified time. Figure 17C shows intracellular IL1beta staining of monocytes as treated in Figure 17B.

Figure 18 shows that culture plates pre-coated with protein A and protein G increased efficacy of generating dendritic cells from human monocytes. One million purified human monocytes were seeded in plates on day 0 and cultured with media supplemented with 20ng/ml GM-CSF and IL4 (10ng/ml) to generate dendritic cells. Cells unattached to the plate were removed on day 1. Cells attached to plates were counted on day 1, day 4 and day 7.

Figure 19 shows that immobilized protein A and protein G induced respiration burst in primary human monocytes. Freshly isolated human monocytes were cultured under the indicated conditions in the presence of dihydrorhodamine123 (DHR123) for 10min or 30min. Cells were analyzed using flow cytometry.

Figure 20 shows that immobilized protein A and protein G induced the production of IL1β in primary human monocytes. Freshly isolated human monocytes were cultured under the indicated conditions for 6 hours and stained for IL1β.

Figure 21 shows that immobilized protein A and protein G changed the phenotype of primary human monocytes. Freshly isolated human monocytes were cultured under the indicated conditions for 6 hours and stained for HLA-DR and CD14.

Figure 22 shows that immobilized protein A and protein G induced respiration burst in primary human neutrophils. Freshly isolated human neutrophils were cultured under the indicated conditions in the presence of DHR 123 for 10min or 30min. Cells were analyzed using flow cytometry.

Figures 23A and 23B show that the binding of human Fcγ receptor CD32 and CD64 to *Staphylococcus aureus* required the presence of human IgG and protein A. Wild type (wt) or protein A deficient (Spa-) *Staphylococcus aureus* bacteria were incubated with fluorescently labeled human Fcγ receptor CD32 as show in in Figure 23A and CD64 as shown in Figure 23B in the absence or presence of humanized antibody rituximab (Ritu). Filled grey histograms represent unstained bacteria.

Figure 24 shows that immobilized protein A, protein G and human IgG changed the phenotype of monocyte-derived dendritic cells. Freshly isolated human monocytes were cultured in the presence of IL4 and GM-CSF in treated plates for specified times and stained for CD86 and CD1c.

Figure 25 shows that immobilized protein A, protein G and human IgG changed the function of monocyte-derived dendritic cells. Dendritic cells were generated from monocytes cultured in plates for 5 days, activated and loaded with Epstein-Barr virus (EBV) replication activator BamHI Z leftward reading frame 1 (BZLF1) peptide for 24 hours, and cultured with autologous T cells for 10 days. EBV-specific cytotoxic T cells were determined by tetramer staining.

Figures 26A and 26B show binding of human and pathogen IgG-binding proteins to IgG molecules from different species. The experimental procedures used are shown in Figure 26A and representative results are shown in Figure 26B for determining interactions between different IgG and IgG-binding proteins using flow cytometry. IgG-binding proteins were cloned individually to fuse with 2A protein from the *Thosea asigna* virus and green fluorescent protein (GFP), wherein GFP reports expression of IgG-binding protein. To determine interactions between IgG and IgG-binding proteins, glioma cells were transfected with IgG binding protein and incubated with fluorescently labeled IgG from different species. Samples were read by flow cytometry, and GFP^+ (heavy black line) and GFP^- cells (filled gray histogram) were analyzed for fluorescent signal intensity and overlaid with each other.

Figures 27A and 27B show that human primary NK cells, B cells, monocytes and granulocytes are naturally coated with human IgG molecules, which is accessible for protein A binding. Human PBMC were washed with pH7.4 or pH4 media, and subsequently stained with fluorescently labeled lineage markers and mouse anti-human IgG Fc antibody as shown in Figure 27A, or fluorescently labeled lineage markers and protein A as shown in Figure 27B. Mean intensity of cell markers, human Fc and protein A are shown.

Figures 28A-28E show that CMV IgG binding protein gp68 is capable of forming a ternary complex with human IgG1 Fc and CD16a. Figure 28A illustrates the results obtained from glioma cells transfected with individual IgG binding proteins and incubated with fluorescently labeled rituximab. Figure 28B illustrates the results obtained from glioma cells transfected with individual IgG binding proteins and incubated with human IgG1 Fc. Figures 28C, 28D, and 28E illustrate the results obtained from transfected glioma cells incubated with fluorescently labeled CD16a alone (Figure 28C) or in the presence of rituximab (Figure 28D) or human IgG1 Fc (Figure 28E). Filled gray areas represent cells not expressing IgG binding proteins. Heavy black lines represent cells expressing IgG binding proteins.

Figures 29A and 29B show the phenotypes of primary human NK cells after being cultured for 7 hours with transfected glioma cells. Figure 29A shows the percentages of NK cells gaining expression of CD69 or CD107a and cells losing both CD16a and CD62L. Figure 29B is a graphical summary of the results for 6 donors. * $p<0.05$.

Figure 30 demonstrates that mouse cytomegalovirus infection allows 3T3 cells to bind non-immune mouse IgG. 3T3 cells were not infected (filled grey) or infected with murine cytomegalovirus (MCMV; heavy black) for 24h, and incubated with fluorescent labeled IgG. Cells were collected using flow cytometry.

DETAILED DESCRIPTION

Definitions

In this specification and in the claims that follow, reference will be made to a number of terms, which shall be defined to have the following meanings:

As used in the description and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise.

Thus, for example, reference to "a composition" includes mixtures of two or more such compositions, reference to "the compound" includes mixtures of two or more such compounds, reference to "an agent" includes mixture of two or more such agents, and the like.

"Optional" or "optionally" means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not.

Ranges can be expressed herein and when such a range is expressed, another aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, it will be understood that the particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that when a value is disclosed, then "less than or equal to" the value, "greater than or equal to the value," and possible ranges between values are also disclosed, as appropriately understood by the skilled artisan. It is also understood that throughout the application data are provided in a number of different formats and that this data represent endpoints and starting points and ranges for any combination of the data points. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

The term "subject" or "patient" refers to any individual who is the target of administration of a pharmaceutical composition of the invention or treatment using a method as disclosed herein. The subject can be a vertebrate, for example, a mammal. Thus, the subject can be a human or veterinary patient. The term "patient" specifically refers to a subject under the treatment of a clinician, e.g., physician.

By "prevent" or other forms of the word, such as "preventing" or "prevention," is meant to stop a particular event or characteristic, to stabilize or delay the development or progression of a particular event or characteristic, or to minimize the chances that a particular event or characteristic will occur. Prevent does not require comparison to a control as it is typically more absolute than, for example, the term "reduce." As used herein, something could be reduced but not prevented, but something that is reduced could also be prevented. Likewise, something could be prevented but not reduced, but something that is prevented could also be reduced. It is

understood that where reduce or prevent are used, unless specifically indicated otherwise, the use of the other word is also expressly disclosed.

The term "treatment" or "treating" refers to the medical management of a subject with the intent to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or disorder. This term includes active treatment, that is, treatment directed specifically toward the improvement of a disease, pathological condition, or disorder, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological condition, or disorder. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, or disorder; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, or disorder; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, or disorder.

As used herein, a "target cell" refers to a target of an immune effector cell that expresses an Fc binding protein. This includes virally infected cells and also microorganisms, such as bacteria and fungi.

A "host cell" includes an individual cell or cell culture which can be or has been a recipient of a virus and/or viral vector. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or of total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change.

The term "gene" is well understood in the art to mean a polynucleotide encoding a polypeptide. In addition to the polypeptide coding regions, a gene can include non-coding regions including, but not limited to, introns, transcribed but untranslated segments, and regulatory elements upstream and downstream of the coding segments.

The terms "polypeptide," "peptide" and "protein" are used interchangeably to refer to polymers of amino acids of any length. These terms also include proteins that are post-translationally modified through reactions that include glycosylation, acetylation, myristoylation, and phosphorylation.

The term "antibody" specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity.

A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature (e.g., SEQ ID NO: 1), with the caveat that naturally occurring Fc regions and fragments can be heterogeneous in amino acid sequence as the consequence, *inter alia*, of population-related genetic heterogeneity (although species-specific "canonical" sequences have been derived. Human immunoglobulins, specifically IgG embodiments thereof, are known to exhibit sequence polymorphisms classically termed allotypes. *See*, Jefferis and Lefranc, 209, Human immunoglobulin allotypes: Possible implications for immunogenicity, *mAbs* 1: 1-7. As used herein, IgG isotypes (IgG1, IgG2, IgG3, and IgG4) comprise such allotypes throughout the scope of naturally occurring variability, including combinations and mixtures thereof as well as isolated and purified allotypes of such isotypes.

A "variant Fc region" as appreciated by one of ordinary skill in the art comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one "amino acid modification." Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g., from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and more preferably at least about 90% sequence identity therewith, more preferably at least about 95% sequence identity therewith, even more preferably, at least about 99% sequence identity therewith.

The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody. An FcγR is a receptor that binds to the Fc region of an IgG antibody. FcγRs includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") (e.g., SEQ ID

NO: 21) and FcγRIIB (an "inhibiting receptor") (e.g., SEQ ID NO: 22), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain.

The term "Fc binding protein" refers to any protein that binds IgG Fc region outside of the FcγR binding sites. In particular embodiments, the Fc binding protein binds a region of the Fc region of IgG without interfering with the binding of FcγR to the IgG Fc.

By "effective amount" is meant an amount sufficient to bring about a beneficial or desired clinical result (*e.g.* improvement in clinical condition).

A "promoter" is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A "promoter" contains core elements required for basic interaction of RNA polymerase and transcription factors and can contain upstream elements and response elements.

"Enhancer" generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' or 3' to the transcription unit. Furthermore, enhancers can be within an intron as well as within the coding sequence itself. They are usually between 10 and 300 bp in length, and they function in cis. Enhancers function to increase transcription from nearby promoters. Enhancers, like promoters, also often contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression.

Enhancing Passive ADCC

Disclosed herein are reagents, methods, and pharmaceutical compositions for enhancing passive ADCC in a subject. In particular embodiments, provided herein are methods for treating a subject infected with a pathogen encoding a Fc-binding protein wherein the subject is administered a pharmaceutical composition of the invention comprising a domain that binds an FcγR on an immune effector cell and a non-overlapping domain that binds the pathogen-encoded Fc binding protein. In other embodiments, reagents, methods, and pharmaceutical compositions for preventing neurologic damage in a subject with HSV1 infection and for preventing death in a subject with HSV1 infection are provided. In certain embodiments the immunological

polypeptide is an antibody, more specifically an IgG antibody and in particular an Fc fragment of an IgG antibody. Also within the scope of such immunological polypeptides is IgG-containing antisera. A feature of the immunological polypeptides useful in the methods and comprising the pharmaceutical compositions disclosed herein is that the efficacy and utility of said IgG antibodies is independent of their antigenic specificity.

Fc Fragments

In some embodiments, the immunological polypeptide comprises an Fc region of an immunoglobulin G (IgG) antibody but does not comprise the antigen-binding region of an antibody, *e.g.*, Fab region. For example, the immunological polypeptide can be a fragment of an IgG1 (e.g., SEQ ID NO: 6), IgG2 (e.g., SEQ ID NO: 7), IgG3 (e.g., SEQ ID NO: 8) or IgG4 (e.g., SEQ ID NO: 9) immunoglobulin; the sequences set forth herein are exemplary, and the skilled worker will recognize that the claimed reagents, methods, and pharmaceutical compositions comprise allotypic variation of IgG isotyopes. In some embodiments, the immunological polypeptide comprises an Fc region of an IgG immunoglobulin, or a fragment thereof capable of simultaneously binding an FcγR and an Fc binding protein, or a fragment thereof capable of binding either an FcγR or an Fc binding protein, but not both (e.g., IgG3) and does not comprise the antigen-binding region of an antibody, *e.g.*, Fab region. In particular embodiments, the immunological polypeptides of the disclosure comprise a domain that has been altered (naturally, by genetic engineering, or otherwise) to bind the FcγR on an immune effector cell with a higher affinity than IgG found in nature and/or a non-overlapping domain that binds the pathogen-encoded Fc binding protein with a higher affinity than an IgG found in nature. The immunological polypeptide can be a recombinant protein, containing fragments of human IgG1 (S6B291; SEQ ID NO: 10). For example, in particular embodiments the recombinant protein comprises residue 235-466 of human IgG1 (S6B291) (SEQ ID NO: 2), or equivalent homologue sequence of IgG2, IgG3, or IgG4. The immunological polypeptide also can be made by papain or plasmin digestion of human IgG1, IgG2, IgG3 or IgG4 as known in the art (*see, for example,* Goding, J. (1983). Monoclonal Antibodies: Principles and Practice. Academic Press Inc., London, U.K).

The polypeptide can be a recombinant protein, containing residue 262-466 of human IgG1 (S6B291) (SEQ ID NO: 1), or equivalent homologue sequence of IgG2, IgG3, or IgG4.

Fc Variants

Also disclosed are synthetic or recombinant polypeptides capable of simultaneously binding an FcγR and an Fc binding protein. In some embodiments, the immunological polypeptide comprises two or more Fc regions of an IgG immunoglobulin. In particular embodiments, the Fc region is modified by for example PEGylation or myrisoylation.

In some embodiments, the immunological polypeptide comprises an Fc gamma receptor binding site that has been modified to enhance binding to an Fc gamma receptor. In some embodiments, this involves a structure-guided design of the IgG-Fcγ receptor interface to produce a higher binding affinity. In some embodiments, this involves removal of the fucose linked to Asn297 of an IgG molecule. In some embodiments, this involves chemically modifying the polypeptide to enhance Fcγ receptor binding (see, for example, Konno *et al.* (2010) Controlling Fucosylation Levels of Antibodies with Osmolality during Cell Culture. In: Kamihira M., Katakura Y., Ito A. (eds) Animal Cell Technology: Basic & Applied Aspects. Animal Cell Technology: Basic & Applied Aspects, vol 16. Springer, Dordrecht).

In some embodiments, the immune effector cell is an immune cell that expresses an Fc gamma receptor. Fc gamma receptors include CD16a, CD16b, CD32, and CD64. Therefore in some embodiments, the immune effector cell is a T-cell, a B cell, a natural killer (NK) cell, a monocyte, a macrophage, a neutrophil or granulocyte, or a dendritic cell.

Diseases/disorders

The methods disclosed herein are broadly applicable to any disease or condition in which killing or interruption of target cells or pathogens expressing Fc binding proteins is desirable.

In some embodiments, a virus or an infected target cell infected with a virus expresses an Fc binding protein. For example, the herpes simplex viruses 1 (HSV1) and HSV2 expresses the Fc binding protein glycoprotein E (gE)(SEQ ID NO: 5) that can induce passive ADCC. The herpes virus cytomegalovirus (CMV) expresses the Fc binding protein 68kDa-glycoprotein (gp68) that can induce passive ADCC. Note that

CMV gp32 also binds IgG Fc, but it competes with the same binding site on IgG Fc as CD16a, so it does not induce passive ADCC. Additionally, Varicella zoster virus (VZV) expresses the IgG binding protein gE, which is a homologue of HSV1 gE (reference PMC241147 and PMID: 2167554).

Viruses that can be targeted by the reagents, methods, and pharmaceutical compositions of the invention in general include but are not limited to those in the following families: picornaviridae; caliciviridae; togaviridae; flaviviridae; coronaviridae; rhabdoviridae; filoviridae; paramyxoviridae; orthomyxoviridae; bunyaviridae; arenaviridae; reoviridae; retroviridae; hepadnaviridae; parvoviridae; papovaviridae; adenoviridae; herpesviridae; and poxyviridae.

In some embodiments, the pathogen is a bacterium, such as *Staphylococcus aureus*, *Streptococcus*, or *Escherichia coli*. *Staphylococcus aureus* expresses the Fc binding protein A, *Streptococcus* expresses the Fc binding proteins protein G, protein H, and M1 protein, and *Escherichia coli* expresses the Fc binding protein M1 protein. Therefore, in some embodiments, the Fc binding protein comprises protein G, protein H, or M1 protein.

Bacteria in general include but are not limited to: *P. aeruginosa; E. coli, Klebsiella* sp.; *Serratia* sp.; *Pseudomanas* sp.; *P. cepacia; Acinetobacter* sp.; *S. epidermis; E. faecalis; S. pneumonias; S. aureus; Haemophilus* sp.; *Neisseria* Sp.; *N. meningitidis;Bacteroides* sp.; *Citrobacter* sp.; *Branhamella* sp.; *Salmonelia* sp.; *Shigel la* sp.; *S. pyogenes;*
Proteus sp.; *Clostridium* sp.; *Erysipelothrix* sp.; *Lesteria* sp.; *Pasteurella multocida; Streptobacillus* sp.; *Spirillum* sp.; *Fusospirochetas*p.; *Treponema pallidum; Borrelia* sp.; *Actinomycetes;*
Mycoplasma sp.; *Chlamydia* sp.; *Rickettsia* sp.; *Spirochaeta;*
Legionella sp.; *Mycobacteria* sp.; *Ureaplasma* sp.; *Streptomyces* sp.; *Trichomoras* sp.; and *P. mirabilis.*

Parasites include but are not limited to: *Plasmodium falciparum, P. vivax, P. ovale, P. malaria; Toxoplasma gondii; Leishmania mexicana, L. tropica, L. major, L. aethiopica, L. donovani, Trypanosoma cruzi, T. brucei, Schistosoma mansoni, S. haematobium, S. japonium; Trichinella spiralis; Wuchereria bancrofti; Brugia malayli; Entamoeba histolytica; Enterobius vermiculoarus; Taenia solium, T. saginata, Trichomonas vaginatis, T. hominis, T. tenax; Giardia lamblia;*

Cryptosporidium parvum; Pneumocytis carinii, Babesia bovis, B. divergens, B. microti, Isospore belli, L hominis; Dientamoeba fragiles; Onchocerca volvulus; Ascaris lumbricoides, Necator americanis; Ancylostoma duodenale; Strongyloides stercoralis; Capillaria philippinensis; Angiostrongylus cantonensis; Hymenolepis nana; Diphyllobothrium latum; Echinococcus granulosus, E. multilocularis; Paragonimus westermani, P. caliensis; Chlonorchis sinensis; Opisthorchis felineas, G. Viverini, Fasciola hepatica Sarcoptes scabiei, Pediculus humanus; Phthirius pubis; and *Dermatobia hominis.*

Fungi in general include but are not limited to: *Cryptococcus neoformans*; *Blastomyces dermatitidis*; *Aiellomyces dermatitidis*; *Histoplasfria capsulatum*; *Coccidioides immitis*; *Candids* species, including *C. albicans, C. tropicalis, C. parapsilosis, C. guilliermondii and C. krusei, Aspergillus* species, including A. *fumigatus, A. flavus* and *A. niger, Rhizopusspecies*; *Rhizomucor* species; *Cunninghammella s*pecies; *Apophysomyces* species, including *A. saksenaea, A. mucor* and *A. absidia*; *Sporothrix schenckii, Paracoccidioides brasiliensis; Pseudallescheria boydii, Torulopsis glabrata*; and *Dermatophyres* species.

Inhibiting Passive ADCC

Also disclosed are methods, reagents, and pharmaceutical compositions for inhibiting or reducing passive ADCC. These methods reduce the cytotoxicity of immune effector cells in the subject by inhibiting passive ADCC.

In some embodiments, the immunological polypeptide is a fragment of an IgG immunoglobulin modified to not bind an Fc gamma receptor (FcγR). For example, the immunological polypeptide does not comprise amino acids 235-262 of SEQ ID No. 10, or functional equivalent thereof. For example, the polypeptide can be an IgG fragment that lacks a CD16a, CD32, or CD64 binding site. For example, this can be is a fragment of an IgG1, IgG2, IgG3 or IgG4 immunoglobulin. In some embodiments, the immunological polypeptide is a fragment from more than one subclass of antibody.

In some embodiments provided herein, are reagents, methods, and pharmaceutical compositions for reducing inflammation in a subject receiving anti-cancer therapy. The methods disclosed herein comprise administering a therapeutically effective amount of an immunological polypeptide comprising a

region that binds to an Fc binding protein but does not comprise a region that binds to an Fc gamma receptor (FcγR); and administering an anti-cancer therapy comprising a monoclonal antibody drug, wherein the immunological polypeptide does not comprise amino acids 235-262 of SEQ ID No. 10, or functional equivalent thereof.

In some embodiments, the subject is being treated with a therapeutic antibody such as rituximab, tocilizumab, tositumomab, trastuzumab bevacizumab, brentuximab vedotin, cetuximab, daratumumab, ipilimumab, ofatumumab, panitumumab, alemtuzumab or pembrolizumab. Most therapeutic monoclonal antibody drugs are produced from mammalian host cell lines to target specific antigens. Side effects and reduced therapeutic efficacy can result from sequestration of the antibody by various native Fc binding proteins. The disclosed methods can decrease side effects of antibody mediated infusion toxicity (the so-called "first dose effect"), yet enhance antibody-based immunotherapy.

In some embodiments, provided herein are reagents, methods, and pharmaceutical compositions for treating a subject undergoing oncolytic HSV1 viral therapy. In other embodiments, provided herein are methods, reagents, and pharmaceutical compositions for enhancing oncolytic viral therapy in a subject, comprising administering a pharmaceutical composition to a subject comprising an polypeptide comprising a region that binds to a binds a Fc binding protein on a target cell but does not comprise a region that binds to a FcγR. For example, wherein the immunological polypeptide does not comprise amino acids 235-262 of SEQ ID No. 10, or functional equivalent thereof.

. The methods can be used to inhibit passive ADCC early after oncolytic viral infection of a tumor so the virus can spread to other tumor cells. While it may be advantageous to inhibit passive ADCC early after oncolytic viral infection of a tumor so the virus can spread to other tumor cells, the disclosed methods can also be used to enhance passive ADCC after the tumor cells are infected to enhance killing of tumor cells.

Thus, in some embodiments, the methods disclosed herein provide a pharmaceutical composition comprising an immunological polypeptide comprising a domain that binds an FcγR on an immune effector cell and a non-overlapping domain that binds n Fc binding protein on a target cell. In certain embodiments the immunological polypeptide is an antibody, more specifically an IgG antibody and in

particular an Fc fragment of an IgG antibody. Also within the scope of such immunological polypeptides is IgG containing antisera. A feature of the immunological polypeptides useful in the methods and comprising the pharmaceutical compositions disclosed herein is that the efficacy and utility of said IgG antibodies is independent of their antigenic specificity.

As above, the immune effector cell can be any immune cell that expresses an Fc gamma receptor. Fc gamma receptors include CD16a, CD16b, CD32, and CD64. Therefore in some embodiments, the immune effector cell is a T-cell, a B cell, a natural killer (NK) cell, a monocyte, a macrophage, a neutrophil or granulocyte, or a dendritic cell.

Differential Cytolysis Mediated by Ectopic Gene Expression (DC-MEGE)

Also disclosed herein are methods for identifying genes that modulate interaction of a virally infected cell and an immune effector cell. The method is referred to herein as differential cytolysis mediated by ectopic gene expression (DC-MEGE). This method provides for a comprehensive understanding of the interaction between human lymphocytes and each gene expressed by target cells infected with a virus.

The disclosed methods can comprise transfecting a host cell with an expression vector comprising a candidate viral gene and a reporter gene operably linked to an expression control sequence, exposing the transfected host cells and non-transfected host cells to a cytotoxic immune effector cell, and assaying the exposed transfected host cells and non-transfected host cells to measure cell death as a function of reporter gene expression or activity. In these methods, a decrease in cell death by the transfected host cells compared to non-transfected host cells is an indication that the viral gene suppressed the immune effector cell or in other words, protected the transfected host cell from the immune effector cell; and an increase in cell death by the transfected host cells compared to non-transfected host cells is an indication that the viral gene activated the immune effector cell or in other words, made the transfected host cell susceptible to the immune effector cell.

In particular embodiments, the reporter gene is a fluorescence gene, wherein the exposed transfected host cells and non-transfected host cells are assayed by flow cytometry to measure cell death as a function of fluorescence, wherein an increase in

the percentage of fluorescent transfected host cells compared to non-transfected host cells is an indication that the viral gene protected the transfected host cell from the immune effector cell, and wherein a decrease in mean fluorescence by the transfected host cells compared to non-transfected host cells is an indication that the viral gene made the transfected host cell susceptible to the immune effector cell.

For example, a fluorescence reading where only non-infected, non-fluorescent cells are killed, and fluorescence increases, is an indication that the transfected target cell was protected from killing. A fluorescence reading where only infected, fluorescent cells are killed, and fluorescence decreases, is an indication that the transfected target cell was susceptible to killing. A fluorescence reading where there is no change in fluorescence is an indication that the target cell remained unchanged to the cytotoxic lymphocyte killing.

Examples of fluorescent protein genes includes: AcGFP1, Azami-Green, Azurite BFP, BFP, CFP, Citrine, Clover, CopGFP, Cycle 3 GFP, CyOFP1, CyPet, d1EGFP, d2ECFP, d2EGFP, d2EYFP, d4EGFP, daGFP, Dendra2, dKeima-Red, dKeima570, Dronpa-Green1, Dronpa-Green3, DsRed-Express, DsRed-Express2, DsRed-Max, DsRed-Monomer, DsRed.T3, DsRed1, DsRed2, dTomato, E2-Crimson, E2-Orange, E2-Red/Green, EBFP, EBFP2, ECFP, ecliptic pHluorin, EGFP, Emerald GFP, EosFP, EYFP, Fast-FT, Fluorescent Timer, FusionRed, GFP, GFPuv, HcRed1, hdKeima-Red, hdKeima570, hKikGR1, hKO, hmAzami-Green, hMGFP, hmKeima-Red, hmKeima8.5, hmKikGR1, hmKO, hmKO2, hmMiCy1, hmUkG1, hrGFP, IFP1.1, IFP1.4, IFP2.0, iRFP670, iRFP682, iRFP702, iRFP713, iRFP720, Kaede, KikGR1, KillerRed, Kohinoor, Kusabira-Orange, LanYFP, LSSmKate1, LSSmKate2, LSSmOrange, mAmetrine, mAmetrine1.1, mApple, mAzami-Green, mCardinal, mCerulean, mCherry, mCherry2, mClavGR2, mClover2, mClover3, mECFP, Medium-FT, mEGFP, mEmerald, mEos2, mEos3.2, mEos4a, mEos4b, mEYFP, mgfp5, mHoneydew, MiCy, mIFP, miniSOG, mKalama1, mKate2, mKeima-Red, mKikGR1, mKO, mKO2, mMaple, mMiCy1, mNectarine, mNeonGreen, mNeptune, mNeptune2, mNeptune2.5, mOrange, mOrange2, mPapaya1, mPlum, mRaspberry, mRFP1, mRuby, mRuby2, mRuby3, mseCFP, mTagBFP2, mTangerine, mTFP1, mTurquoise, mTurquoise2, mUkG1, mVenus, mWasabi, PA-GFP, PA-TagRFP, pAcGFP1, pAcGFP1-1, pAcGFP1-C1, pAcGFP1-C2, pAcGFP1-C3, pAcGFP1-C In-Fusion Ready, pAcGFP1-N1, pAcGFP1-N2, pAcGFP1-N3, pAcGFP1-N In-Fusion

Ready, pAG-S1, PAmCherry, PAmCherry1, pAmCyan, pAmCyan1-C1, pAmCyan1-N1, PAmKate, pAsRed2, pAsRed2-C1, pAsRed2-N1, pd1EGFP-N1, pd2ECFP-N1, pd2EGFP-N1, pd2EYFP-N1, pd4EGFP-N1, pDendra2, pDendra2-C, pDendra2-N, pDG1-S1, pDG3-S1, pdKeima-Red-S1, pdKeima570-S1, pDsRed-Express, pDsRed-Express-1, pDsRed-Express-C1, pDsRed-Express-N1, pDsRed-Express2, pDsRed-Express2-1, pDsRed-Express2-C1, pDsRed-Express2-N1, pDsRed-Monomer-C1, pDsRed-Monomer-C In-Fusion Ready, pDsRed-Monomer-N1, pDsRed-Monomer-N In-Fusion Ready, pDsRed2, pDsRed2-1, pDsRed2-C1, pDsRed2-N1, pE2-Crimson, pE2-Crimson-C1, pE2-Crimson-N1, pECFP, pECFP-1, pECFP-C1, pECFP-N1, pEGFP, pEGFP-1, pEGFP-C1, pEGFP-C2, pEGFP-C3, pEGFP-N1, pEGFP-N2, pEGFP-N3, pEYFP, pEYFP-1, pEYFP-C1, pEYFP-N1, pFusionRed-B, pFusionRed-C, pFusionRed-N, pGFP, pGFPuv, pGLO, pHcRed1, pHcRed1-1, pHcRed1-C1, pHcRed1-N1_1, phdKeima-Red-S1, phdKeima570-S1, phKikGR1-S1, phKO1-S1, phmAG1-S1, phMGFP, phmKeima-Red-S1, phmKO1-S1, phmUkG1-S1, pHTomato, pHuji, pKaede-S1, pKikGR1-S1, pKillerRed-B, pKillerRed-C, pKillerRed-N, pKindling-Red-B, pKindling-Red-N, pKO1-S1, pLSSmOrange-C1, pLSSmOrange-N1, pmAG1-S1, pmBanana, pmCherry, pmCherry-1, pmCherry-C1, pmCherry-N1, pMiCy1-S1, pmKate2-C, pmKate2-N, pmKeima-Red-S1, pmKikGR1-S1, pmKO1-S1, pmKO2-S1, pmMiCy1-S1, pmOrange, pmOrange2, pmOrange2-C1, pmOrange2-N1, pmPlum, pmRaspberry, pmStrawberry, pmUkG1-S1, pNirFP-C, pNirFP-N, pPA-TagRFP-C, pPA-TagRFP-N, pPAmCherry-C1, pPAmCherry-N1, pPAmCherry1-C1, pPAmCherry1-N1, pPhi-Yellow-B, pPhi-Yellow-C, pPhi-Yellow-N, pPhi-Yellow-PRL, pPS-CFP2-C, pPS-CFP2-N, pPSmOrange-C1, pPSmOrange-N1, pRSET-BFP, pRSET-CFP, pRSET-EmGFP, PS-CFP2, PSmOrange, PSmOrange2, pTagBFP-C, pTagBFP-N, pTagCFP-C, pTagCFP-N, pTagGFP2-C, pTagGFP2-N, pTagRFP-C, pTagRFP-N, pTagYFP-C, pTagYFP-N, ptd-Tomato-N1, ptdTomato, ptdTomato-C1, pTimer, pTimer-1, pTurboFP602-B, pTurboFP602-C, pTurboFP602-N, pTurboFP602-PRL, pTurboGFP-B, pTurboGFP-C, pTurboGFP-N, pTurboGFP-PRL, pTurboRFP-B, pTurboRFP-C, pTurboRFP-N, pTurboRFP-PRL, pTurboYFP-B, pTurboYFP-C, pTurboYFP-N, pTurboYFP-PRL, pZsGreen, pZsGreen1-1, pZsGreen1-C1, pZsGreen1-N1, pZsYellow, pZsYellow1-C1, pZsYellow1-N1, ratiometric pHluorin, Rhacostoma GFP, rsEGFP, rsEGFP2, rsTagRFP, Slow-FT, super-ecliptic pHluorin, superfolder GFP, TagBFP, TagCFP, TagGFP2, TagRFP,

TagRFP-T, TagRFP657, TagYFP, tdTomato, TurboFP602, TurboFP635, TurboGFP, TurboRFP, TurboYFP, yeGFP, YFP, YPet, ZsGreen, ZsGreen1, and ZsYellow1. In particular embodiments, the gene encodes Green Fluorescent Protein (*see*, Chalfie *et al.*, 1994, *Science* 263, 802–805).

The process can be repeated for each gene in a viral genome. For example, the method can further comprise of repeating the process for each gene individually, or for combinations of 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or more genes in a viral genome.

These methods can be used with any immune effector cell(s) that is/are cytotoxic or secrete some biomarker (e.g., a cytokine) indicative of activation or suppression. For example, the cytotoxic immune effector cell can be a CD4+ T-cell, a CD8+ T-cell, a natural killer (NK) cell, a macrophage, a granulocyte, or a dendritic cell. The method described above can be repeated for each candidate immune effector cell alone, or in combination with other immune cells or bioactive agents.

The disclosed methods can also be used to assay the effects of candidate drugs on a gene's ability to affect cytolysis. For example, if a gene is found to protect a cell from killing by immune effector cells, a series of candidate drugs can be added in future assays to find a drug that inhibits the gene's protection.

A host cell is preferably selected based on its known susceptibility or resistance to killing by an immune effector cell when virally infected, expressing exogenous proteins, or in native status. Host cells can be primary cells isolated from an animal or a human subject. The host cells can be a cell line, such as an immortalized cell line. Host cells can include a single cell type, or a mixture of cells. Host cells can be cultured in suspension, on a surface (two-dimensional), or in a three-dimensional matrix.

The methods disclosed herein comprise exposing transfected host cells and non-transfected host cells to a cytotoxic immune effector cell. This step can involve co-culturing the host cells and cytotoxic immune effector cells under standard culture condition (37°C with 5% CO_2) or relevant experimental settings that enhance or inhibit the function of immune cells and host cells.

Recombinant Oncolytic HSV

Glioblastoma multiforme (GBM) is a uniformly fatal disease despite the application of available combination therapies. Replication-competent viruses including oncolytic HSV ("oHSV") vectors, represent a promising therapeutic alternative.

As disclosed herein, the HSV Us8, UL12, UL30, US3, and Us12 genes make glioma cells more susceptible to killing by NK cells. Therefore, also disclosed herein is a recombinant oncolytic Herpes Simplex Virus (oHSV), comprising one or more activating mutations in an Us8 gene, one or more activating mutations in an UL12 gene, one or more activating mutations in an UL30 gene, one or more activating mutations in an Us3 gene, one or more activating mutations in an Us12 gene, or any combination thereof. Activating mutations of HSV genes are known in the art. *See, for example*, US 8,092,791; US 9,623,059; WO 2007052029; WO 2009052426; WO 2017013419; WO 2017132552; Varghese & Rabkin, 2002, *Cancer Gene Ther*. 9, 967-978; Grandi et al., 2009, *Expert Rev Neurother*. 9, 505-517; and Sokolowski et al., 2015, *Oncolytic Virother*. 4, 207-219. These modifications to HSV oncolytic viruses can enhance tumor killing of the oncolytic viruses.

The disclosed oHSV may be derived from several different types of herpes viruses. The Herpesviridae are a large family of DNA viruses that cause diseases in humans and animals. Herpes virus is divided into three subfamilies, alpha, beta, and gamma. Herpes viruses all share a common structure and are composed of relatively large double-stranded, linear DNA genomes encoding 100-200 genes encased within an icosahedral protein cage called the capsid which is itself wrapped in a lipid bilayer membrane called the envelope. The large genome provides many non-essential sites for introducing one or more transgenes without inactivating the virus (e.g., without completely inhibiting infection or replication). However, it should be appreciated that viral vectors are preferably modified (e.g., replication conditional, attenuated) so that they do not have undesirable effects (e.g., killing normal cells, causing disease).

As used herein, oncolytic Herpes virus refers to any one of a number of therapeutic viruses having a Herpes virus origin that are useful for killing cancer cells, particularly cancer stem cells, and/or inhibiting growth of a tumor, for example by killing cancer stem cells in the tumor. Typically, an oncolytic Herpes virus is a mutant version of a wild-type Herpes virus. In some cases, when the wild-type Herpes virus is of the subfamily alpha (i.e., is a Herpes simplex virus) the oncolytic Herpes viruses

may be referred to as an oncolytic Herpes Simplex virus (oHSV). In some cases, the oHSV is a replication-conditional Herpes virus. Replication-conditional Herpes viruses are designed to preferentially replicate in actively dividing cells, such as cancer cells, in particular cancer stem cells. Thus, these replication-conditional viruses target cancer cells for oncolysis, and replicate in these cells so that the virus can spread to other cancer cells. In preferred embodiments, replication conditional Herpes viruses target cancer stem cells for oncolysis, and replicate in these cells so that the virus can spread to other cancer stem cells.

The disclosed oHSV may comprise any one of a number of mutations that affect expression of a viral gene. In most cases, a mutation is in virulence gene that contributes to the pathogenicity of the virus to a host organism. The mutation may be a point mutation, a deletion, an inversion, or an insertion. Typically the mutation is an inactivating mutation. As used herein, the term "inactivating mutation" is intended to broadly indicate a mutation or alteration to a gene wherein the expression of that gene is significantly decreased, or wherein the gene product is rendered nonfunctional, or its ability to function is significantly decreased.

Several types of replication-conditional herpes virus mutants have been developed and are useful in aspects of the methods disclosed herein. For example, one aspect involves viral mutants with defects in the function of a viral gene needed for nucleic acid metabolism, such as thymidine kinase (Martuza *et al.*, 1991, *Science* 252:854-856), ribonucleotide reductase (RR) (Goldstein & Weller, 1988, *J. Virol.* 62:196-205; Boviatsis *et al.*, 1994, *Gene Ther.* 1:323-331; Boviatsis *et al.*, 1994, *Cancer Res.* 54:5745-5751; Mineta *et al.*, 1994, *Cancer Res.* 54:3363-3366), or uracil-N-glycosylase (Pyles and Thompson, 1994, *J. Virol.* 68:4963-4972). Another aspect involves viral mutants with defects in the function of the γ-34.5 gene (Chambers *et al.*, 1995, *Proc. Natl. Acad. Sci. USA* 92:1411-1415), which functions as a virulence factor by markedly enhancing the viral burst size of infected cells through suppression of the shutoff of host protein synthesis (Chou *et al.*, 1990, *Science* 250:1262-1266; Chou and Roizman, 1992, *Proc. Natl. Acad. Sci. USA* 89:3266-3270). Other examples include G207 (Mineta *et al.*, 1995, *Nat. Med* 1:938-943; U.S. Pat. No. 5,585,096, issued Dec. 17, 1996 to Martuza *et al.*), and MGH1 (Kramm *et al.*, 1997, *Hum. Gene Ther.* 8:2057-2068), which possess deletions of both copies of γ-34.5 and an insertional mutation of RR.

The disclosed oHSV can comprise viruses that are based on herpes viruses, such as herpes simplex viruses (HSV), for example, HSV-1 (e. g., HSV-1 strain F or strain Patton) or HSV-2, that include an inactivating mutation in a virulence gene. In the case of herpes simplex viruses, this mutation can be an inactivating mutation in the γ-34.5 gene, which is the major HSV neurovirulence determinant.

Any of the viruses described above and herein and elsewhere can include an additional mutation or modification that is made to prevent reversion of the virus to wild type. For example, the virus can include a mutation in the ICP6 gene (SEQ ID NO: 26), which encodes the large subunit of ribonucleotide reductase.

The disclosed oHSV can also include sequences encoding a heterologous gene product, such as a vaccine antigen or an immunomodulatory protein. Virus carrying heterologous gene products may also be referred to as augmented viruses

The effects of the disclosed oHSV can be augmented if the viruses also contain a heterologous nucleic acid sequence encoding one or more therapeutic agents, for example, a cytotoxin, an immunomodulatory protein (i.e., a protein that either enhances or suppresses a host immune response to an antigen), a tumor antigen, small interfering nucleic acid, an antisense RNA molecule, or a ribozyme.

Examples of immunomodulatory proteins include, e. g., cytokines (e. g., interleukins, alpha-interferon, beta-interferon, gamma-interferon, tumor necrosis factor, granulocyte macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), and granulocyte colony stimulating factor (G-CSF)), chemokines (e.g., neutrophil activating protein (NAP), macrophage chemoattractant and activating factor (MCAF), RANTES, and macrophage inflammatory peptides MIP-l a and MIP-lb), complement components and their receptors, immune system accessory molecules (e.g., B7.1 and B7.2), adhesion molecules (e.g., ICAM-1, 2, and 3), and adhesion receptor molecules.

Examples of tumor antigens that can be produced using the present methods include, in non-limiting examples, the E6 and E7 antigens of human papillomavirus, EBV-derived proteins (Van der Bruggen *et al.*, 1991, *Science* 254: 1643-1647), mucins (Livingston *et al.*, 1992, *Curr. Opin. Immun.* 4 (5): 624-629), such as MIJC1 (Burchell *et al.*, 1989, *Int. J. Cancer* 44: 691-696), melanoma tyrosinase, and MZ2-E (Van der Bruggen *et al., supra*).

Therapeutic agents can also be an RNA molecule, such as an antisense RNA molecule that, by hybridization interactions, can be used to block expression of a cellular or pathogen mRNA. Alternatively, the RNA molecule can be a ribozyme (e.g., a hammerhead or a hairpin-based ribozyme) designed either to repair a defective cellular RNA, or to destroy an undesired cellular or pathogen-encoded RNA (*see, e.g.*, Sullenger, 1995, *Chem. Biol.* 2 (5): 249-253; Czubayko *et al.*, 1997, *Gene Ther.* 4 (9): 943-949; Rossi, 1997, *Ciba Found. Symp.* 209: 195-204; James *et al.*, 1998, *Blood* 91 (2): 371-382; Sullenger, 1996, *Cytokines Mol. Ther.* 2 (3): 201-205; Hampel, 1998, *Prog. Nucleic Acid Res. Mol. Bio.* 58: 1-39; and Curcio *et al.*, 1997, *Pharmacol. Ther.* 74 (3): 317-332).

In some embodiments, the therapeutic agent can be a small interfering nucleic acid molecule capable of inhibiting expression of a gene associated with the cancer, such as an oncogene. Small interfering nucleic acids (e.g., shRNAs, miRNAs) that inhibit the expression of these genes and their homologues are useful as therapeutic agents in certain embodiments of the methods. Oncogenes associated with various cancers are well known in the art and disclosed, in non-limiting examples, in Cooper, 1995, Oncogenes. Jones and Bartlett Publishers. and Vogelstein and Kinzler, 1998, The Genetic Basis of Human Cancer. McGraw-Hill the contents are incorporated herein by reference in their entirety.

A heterologous nucleic acid sequence can be inserted into the disclosed oHSV in a location that renders it under the control of a regulatory sequence of the virus. Alternatively, the heterologous nucleic acid sequence can be inserted as part of an expression cassette that includes regulatory elements, such as promoters or enhancers. Appropriate regulatory elements can be selected by those of ordinary skill in the art based on, for example, the desired tissue-specificity and level of expression. For example, a cell-type specific or tumor-specific promoter can be used to limit expression of a gene product to a specific cell type. This is particularly useful, for example, when a cytotoxic, immunomodulatory, or tumor antigenic gene product is being produced in a tumor cell in order to facilitate its destruction. In addition to using tissue-specific promoters, local administration of the viruses of the invention can result in localized expression and effect.

Examples of non-tissue specific promoters that can be used in the disclosed oHSV include the early Cytomegalovirus (CMV) promoter (U.S. Pat. No. 4,168,062)

and the Rous Sarcoma Virus promoter (Norton *et al.*, 1985, *Molec. Cell. Biol.* 5: 281). Also, HSV promoters, such as HSV-1 IE and IE 4/5 promoters, can be used.

Examples of tissue-specific promoters that can be used in the disclosed oHSV include, for example, prostate-specific antigen (PSA) promoter, which is specific for cells of the prostate; desmin promoter, which is specific for muscle cells (Li *et al.*, 1989, *Gene* 78: 243; Li *et al.*, 1991, *J. Biol. Chem.* 266: 6562; Li *et al.*, 1993, *J. Biol. Chem.* 268: 10403); enolase promoter, which is specific for neurons (Forss-Petter *et al.*, 1986, *J. Neuroscience Res.* 16 (1): 141-156); beta-globin promoter, which is specific for erythroid cells (Townes *et al.*, 1985, *EMBO J.* 4: 1715); tau-globin promoter, which is also specific for erythroid cells (Brinster *et al.*, 1980, *Nature* 283: 499); growth hormone promoter, which is specific for pituitary cells (Behringer *et al.*, 1988, *Genes Dev.* 2: 453); insulin promoter, which is specific for pancreatic beta cells (Selden *et al.*, 1986, *Nature* 321: 545); glial fibrillary acidic protein promoter, which is specific for astrocytes (Brenner *et al.*, 1994, *J. Neurosci.* 14: 1030); tyrosine hydroxylase promoter, which is specific for catecholaminergic neurons (Kim *et al.*, 1993, *J. Biol. Chem.* 268: 15689); amyloid precursor protein promoter, which is specific for neurons (Salbaum et al., 1988, *EMBO J.* 7: 2807); dopamine beta-hydroxylase promoter, which is specific for noradrenergic and adrenergic neurons (Hoyle *et al.*, 1994, *J. Neurosci.* 14: 2455); tryptophan hydroxylase promoter, which is specific for serotonin/pineal gland cells (Boularand *et al.*, 1995, *J. Biol. Chem.* 270: 3757); choline acetyltransferase promoter, which is specific for cholinergic neurons (Hersh *et al.*, 1993, *J. Neurochem.* 61: 306); aromatic L-amino acid decarboxylase (AADC) promoter, which is specific for catecholaminergic/5-HT/D-type cells (Thai *et al.*, 1993, *Mol. Brain Res.* 17: 227); proenkephalin promoter, which is specific for neuronal/spermatogenic epididymal cells (Borsook *et al.*, 1992, *Mol. Endocrinol.* 6: 1502); reg (pancreatic stone protein) promoter, which is specific for colon and rectal tumors, and pancreas and kidney cells (Watanabe *et al.*, 1990, *J Biol. Chem.* 265: 7432); and parathyroid hormone-related peptide (PTHrP) promoter, which is specific for liver and cecum tumors, and neurilemoma, kidney, pancreas, and adrenal cells (Campos *et al.*, 1992, *Mol. Endocrinol.* 6: 1642).

Examples of promoters that function specifically in tumor cells include the stromelysin 3 promoter, which is specific for breast cancer cells (Basset *et al.*, 1990, *Nature* 348: 699); the surfactant protein A promoter, which is specific for non-small

cell lung cancer cells (Smith *et al.*, 1994, *Hum. Gene Ther.* 5: 29-35); the secretory leukoprotease inhibitor (SLPI) promoter, which is specific for SLPI-expressing carcinomas (Garver *et al.*, 1994, *Gene Ther.* 1: 46-50); the tyrosinase promoter, which is specific for melanoma cells (Vile *et al.*, 1994, *Gene Therapy* 1: 307; WO 94/16557); the stress inducible grp78/BiP promoter, which is specific for fibrosarcoma/tumorigenic cells (Gazit *et al.*, 1995, *Cancer Res.* 55 (8): 1660); the AP2 adipose enhancer, which is specific for adipocytes (Graves, 1992, *J. Cell. Biochem.* 49: 219); the a-1 antitrypsin transthyretin promoter, which is specific for hepatocytes (Grayson *et al.*, 1988, *Science* 239: 786); the interleukin-10 promoter, which is specific for glioblastoma multiform cells (Nitta *et al.*, 1994, *Brain Res.* 649: 122); the c-erbB-2 promoter, which is specific for pancreatic, breast, gastric, ovarian, and non-small cell lung cells (Harris *et al.*, 1994, *Gene Ther.* 1: 170); the a-B-crystallin/heat shock protein 27 promoter, which is specific for brain tumor cells (Aoyama *et al.*, 1993, *Int. J. Cancer* 55: 760); the basic fibroblast growth factor promoter, which is specific for glioma and meningioma cells (Shibata *et al.*, 1991, *Growth Fact.* 4: 277); the epidermal growth factor receptor promoter, which is specific for squamous cell carcinoma, glioma, and breast tumor cells (Ishii *et al.*,1993, *Proc. Natl. Acad. Sci. U.S.A.* 90: 282); the mucin-like glycoprotein (DF3, MUC1) promoter, which is specific for breast carcinoma cells (Abe *et al.*,1993, *Proc. Natl. Acad. Sci. U.S.A.* 90: 282); the mtsl promoter, which is specific for metastatic tumors (Tulchinsky *et al.*, 1992, *Proc. Natl. Acad. Sci. U.S.A.* 89: 9146); the NSE promoter, which is specific for small-cell lung cancer cells (Forss-Petter *et al.*, 1990, *Neuron* 5: 187); the somatostatin receptor promoter, which is specific for small cell lung cancer cells (Bombardieri *et al.*, 1995, *Eur. J. Cancer* 31A: 184; Koh *et al.*,1995, *Int. J. Cancer* 60: 843); the c-erbB-3 and c-erbB-2 promoters, which are specific for breast cancer cells (Quin *et al.*, 1994, *Histopathology* 25: 247); the c-erbB4 promoter, which is specific for breast and gastric cancer cells (Rajkumar *et al.*, 1994, *Breast Cancer Res. Trends* 29: 3); the thyroglobulin promoter, which is specific for thyroid carcinoma cells (Mariotti *et al.*, 1995, *J. Clin. Endocrinol. Meth.* 80: 468); the a-fetoprotein promoter, which is specific for hepatoma cells (Zuibel *et al.*, 1995, *J. Cell. Phys.* 162: 36); the villin promoter, which is specific for gastric cancer cells (Osborn *et al.*, 1988, *Virchows Arch. A. Pathol. Anat. Histopathol.* 413: 303); and the albumin

promoter, which is specific for hepatoma cells (Huber, 1991, *Proc. Natl. Acad. Sci. U.S.A.* 88: 8099).

The disclosed oHSV can be used to treat a subject having (*e.g.*, harboring) or at risk of having a cancer stem cell (CSC) and/or a tumor having CSCs (e.g., a tumor for which sustained growth is dependent on CSCs; such tumors may also be referred to as a CSC-dependent tumor). Whether a subject is deemed "at risk" of having a CSC or a tumor having CSCs is a determination that may be within the discretion of the skilled practitioner caring for the subject. Any suitable diagnostic test and/or criteria can be used. For example, a subject may be considered "at risk" of having a CSC or a tumor having CSCs if (i) the subject has a mutation, genetic polymorphism, gene or protein expression profile, and/or presence of particular substances in the blood, associated with increased risk of developing or having cancer relative to other members of the general population not having mutation or genetic polymorphism; (ii) the subject has one or more risk factors such as having a family history of cancer, having been exposed to a carcinogen or tumor-promoting agent or condition, e.g., asbestos, tobacco smoke, aflatoxin, radiation, chronic infection/inflammation, etc., advanced age; (iii) the subject has one or more symptoms of cancer, etc.

In some embodiments, the cancer is a colon carcinoma, a pancreatic cancer, a breast cancer, an ovarian cancer, a prostate cancer, a squamous cell carcinoma, a cervical cancer, a lung carcinoma, a small cell lung carcinoma, a bladder carcinoma, a squamous cell carcinoma, a basal cell carcinoma, an adenocarcinoma, a sweat gland carcinoma, a sebaceous gland carcinoma, a papillary carcinoma, a papillary adenocarcinoma, a cystadenocarcinoma, a medullary carcinoma, a bronchogenic carcinoma, a renal cell carcinoma, a hepatocellular carcinoma, a bile duct carcinoma, a choriocarcinoma, a seminoma, an embryonal carcinoma, a Wilms' tumor, melanoma, or a testicular tumor. In one embodiment, the cancer is a glioma. In one embodiment, the cancer is a breast or prostate carcinoma. Other cancers will be known to one of ordinary skill in the art.

In particular embodiments, the cancer is a brain cancer. In some embodiments, the cancer is a glioma. A glioma is a type of primary central nervous system (CNS) tumor that arises from glial cells. In addition to the brain, gliomas can also affect the spinal cord or any other part of the CNS, such as the optic nerves. The gliomas for which the methods of the invention are useful to treat include ependymomas,

astrocytomas, oligodendrogliomas, and mixed gliomas, such as oligoastrocytomas. In some embodiments, the gliomas contain cancer stem cells that are CD133+. In some embodiments, the glioma is a glioblastoma.

Gliomas are further categorized according to their grade, which is determined by pathologic evaluation of the tumor. Low-grade gliomas are well-differentiated (not anaplastic); these are benign and portend a better prognosis for the patient. High-grade gliomas are undifferentiated or anaplastic; these are malignant and carry a worse prognosis. Of numerous grading systems in use, the most common is the World Health Organization (WHO) grading system for astrocytoma. The WHO system assigns a grade from 1 to 4, with 1 being the least aggressive and 4 being the most aggressive. Various types of astrocytomas are given corresponding WHO grades. WHO Grade 1 includes, for example, pilocytic astrocytoma; WHO Grade 2 includes, for example, diffuse or low-grade astrocytoma; WHO Grade 3 includes, for example, anaplastic (malignant) astrocytoma; and WHO Grade 4 includes, for example, glioblastoma multiforme (most common glioma in adults). Accordingly, in some embodiments the methods of the invention are useful for treating patients (subjects) with WHO Grade 1, Grade 2, Grade 3, or Grade 4 gliomas.

Also disclosed are methods of inducing a systemic immune response to cancer in a subject, which involve administering to the subject an oHSV disclosed herein. The herpes virus can be administered, for example, to a tumor of the subject. In addition, the patient can have or be at risk of developing metastatic cancer, and the treatment can be carried out to treat or prevent such cancer.

Recombinant HSV vaccine

Also as disclosed herein, HSV gE and gI enhance passive ADCC and promote clearance of HSV1 infection by FcγR-bearing immune cells. Therefore, disclosed is an HSV vaccine that comprises a viral vector comprising the HSV Us7 and Us8 genes that encode gE and gI. These genes can be operably connected, collectively or independently, to an expression control sequence that promotes earlier and/or higher expression of gE and gI in infected cells to promote passive ADCC.

In some embodiments, the vector is an attenuated HSV vector. Methods to construct expression vectors containing genetic sequences and appropriate transcriptional and translational control elements are well established and described

previously (Kambara, *et al.*, 2005, *Cancer Res.* 65, 2832-9). These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described in Sambrook et al., Molecular Cloning, A Laboratory Manual (Cold Spring Harbor Press, Plainview, N.Y., 1989), and Ausubel et al., Current Protocols in Molecular Biology (John Wiley & Sons, New York, N.Y., 1989).

Expression vectors generally contain regulatory sequences necessary elements for the translation and/or transcription of the inserted coding sequence. For example, the coding sequence is preferably operably linked to a promoter and/or enhancer to help control the expression of the desired gene product.

Promoters used in biotechnology are of different types according to the intended type of control of gene expression. They can be generally divided into constitutive promoters, tissue-specific or development-stage-specific promoters, inducible promoters, and synthetic promoters. An enhancer is a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' or 3' to the transcription unit. Furthermore, enhancers can be within an as well as within the coding sequence itself. They are usually between 10 and 300 bp in length, and they function in *cis*. Enhancers function to increase transcription from nearby promoters. Enhancers also often contain response elements that mediate the regulation of transcription. Promoters can also contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression of a gene. While many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin), typically one will use an enhancer from a eukaryotic cell virus for general expression. Preferred examples are the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

In preferred embodiments, the promoter is an immediate-early (IE) promoter, such as the cytomegalovirus (CMV) promoter/enhancer, or EF1a, CAG, SV40, PGK1, Ubc, human beta actin promoter, etc.

Treatment

The compositions disclosed can be used therapeutically in combination with a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, *i.e.*, the material may be administered to a subject without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier is selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

When the polypeptide or viral vector disclosed herein is prepared for administration, it can be combined with a pharmaceutically acceptable carrier, diluent or excipient to form a pharmaceutical formulation, or unit dosage form. The total active ingredients in such formulations include from 0.1 to 99.9% by weight of the formulation. A "pharmaceutically acceptable" substance is a carrier, diluent, excipient, and/or salt that is compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof. The active ingredient for administration may be present as a powder or as granules; as a solution, a suspension or an emulsion.

The vectors or polypeptides (active ingredients) can be formulated and administered to treat a variety of disease states by any means that produces contact of the active ingredient with the agent's site of action in the body of the organism. They can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic active ingredients or in a combination of therapeutic active ingredients. They can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

In general, water, suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration contain the active ingredient, suitable stabilizing agents and, if necessary, buffer substances. Antioxidizing agents such as sodium bisulfate, sodium sulfite or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used are citric acid and its salts and sodium ethylenediaminetetraacetic acid (EDTA). In addition, parenteral solutions can contain preservatives such as benzalkonium chloride, methyl-

or propyl-paraben and chlorobutanol. Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, a standard reference text in this field.

Additionally, standard pharmaceutical methods can be employed to control the duration of action. These are well known in the art and include control release preparations and can include appropriate macromolecules, for example polymers, polyesters, polyamino acids, polyvinyl, pyrolidone, ethylenevinylacetate, methyl cellulose, carboxymethyl cellulose or protamine sulfate. The concentration of macromolecules as well as the methods of incorporation can be adjusted in order to control release. Additionally, the agent can be incorporated into particles of polymeric materials such as polyesters, polyamino acids, hydrogels, poly (lactic acid) or ethylenevinylacetate copolymers. In addition to being incorporated, these agents can also be used to trap the compound in microcapsules.

Pharmaceutical formulations containing the therapeutic agents disclosed herein can be prepared by procedures known in the art using well known and readily available ingredients. The therapeutic agents can also be formulated as solutions appropriate for parenteral administration, for instance by intramuscular, subcutaneous or intravenous routes. The pharmaceutical formulations of the therapeutic agents can also take the form of an aqueous or anhydrous solution or dispersion, or alternatively the form of an emulsion or suspension.

The herein disclosed compositions, including pharmaceutical composition, may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. These parenteral (including subcutaneous, intravenous, intramedullary, intraarticular, intramuscular, or intraperitoneal injection), topical, transdermal, and oral. Administration may occur in a single dose or in repeat administrations. The vectors or polypeptides disclosed herein may be administered in combination with other therapeutic agents such as monoclonal antibodies and intraveneous IgG.

As used herein the terms treatment, treat, or treating refers to a method of reducing the effects of a disease or condition or symptom of the disease or condition. Thus in the disclosed method, treatment can refer to a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% reduction in the severity of an established disease or condition or symptom of the disease or condition. For example, a method for treating a disease is considered to be a treatment if there is a 10% reduction in one or more

symptoms of the disease in a subject as compared to a control. Thus the reduction can be a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any percent reduction in between 10% and 100% as compared to native or control levels. It is understood that treatment does not necessarily refer to a cure or complete ablation of the disease, condition, or symptoms of the disease or condition.

As used herein, the terms prevent, preventing, and prevention of a disease or disorder refers to an action, for example, administration of a therapeutic agent, that occurs before or at about the same time a subject begins to show one or more symptoms of the disease or disorder, which inhibits or delays onset or exacerbation of one or more symptoms of the disease or disorder. As used herein, references to decreasing, reducing, or inhibiting include a change of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater as compared to a control level. Such terms can include but do not necessarily include complete elimination.

The pharmaceutical compositions may be given following, preceding, in lieu of, or in combination with, other therapies in the subject. The subject may have been administered a vaccine or other composition in order to stimulate an immune response.

Methods of Making Polypeptides

A cell engineered to express the polypeptides disclosed herein is provided. The engineered cell can be propagated in cell culture (e.g., as opposed to being a part of a living animal ("*in vivo*")). For example, the cell may be a mammalian cell, e.g., a CHO cell or a human cell or a mouse hybridoma cell. Examples of other types of cells that may be used for expression the polypeptides disclosed herein include mouse myeloma cells (e.g., NSO), human embryonic kidney cells (e.g., HEK293), monkey kidney cells (e.g., COS), human epithelial carcinoma cells (e.g., HeLa), human fibrosarcoma cells (e.g., HT-1080), baby hamster kidney cells, yeast cells, insect cells, and others (see, e.g., Fernandez et al. (eds.) Gene Expression Systems, Academic Press, 1999). Any cell compatible with the disclosed polypeptides and appropriate culture conditions may be used.

Methods of making polypeptides, such as those that simultaneously bind an FcγRand an Fc binding domain protein, are known in the art. One method which may be employed is the method of Kohler, G. et al. (1975) *Continuous Cultures Of Fused Cells Secreting Antibody Of Predefined Specificity* " *Nature* 256:495-497 or a

modification thereof. In one embodiment, the desired polypeptide which interacts with the immune effector cell or a protein present on the surface of an immune effector cell that expresses such an activating receptor are obtained using host cells that over-express such molecules.

Also disclosed are modifications to disclosed polypeptides that do not significantly affect their properties and variants that have enhanced or decreased activity. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs. Amino acid residues which can be conservatively substituted for one another include but are not limited to: glycine/alanine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid/glutamic acid; serine/threonine; lysine/arginine; and phenylalanine/tryosine. These polypeptides also include glycosylated and nonglycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Preferably, the amino acid substitutions would be conservative, i.e., the substituted amino acid would possess similar chemical properties as that of the original amino acid. Such conservative substitutions are known in the art, and examples have been provided above. Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay, such as the attachment of radioactive moieties for radioimmunoassay. Modified polypeptides are made using established procedures in the art and can be screened using standard assays known in the art.

The invention also encompasses fusion proteins comprising one or more fragments or regions from the disclosed polypeptides. In one embodiment, a fusion polypeptide is provided that comprises at least 10 contiguous amino acids of an IgG Fc region.

Polypeptides of the invention may be conveniently prepared using solid phase peptide synthesis (Merrifield, B. (1986) "Solid Phase Synthesis," *Science* 232(4748):341- 347; Houghten, R.A. (1985) General Method For The Rapid Solid-

Phase Synthesis Of Large Numbers Of Peptides: Specificity Of Antigen-Antibody Interaction At The Level Of Individual Amino Acids" *Proc. Natl. Acad. Sci. (U.S.A.)* 82(15):5131- 135; Ganesan, A. (2006) "Solid-Phase Synthesis In The Twenty-First Century " *Mini Rev. Med. Chem.* 6(1):3- 10).

Vectors containing polynucleotides that encode the disclosed polypeptides can be introduced into a host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (e.g., where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

Any host cells capable of over-expressing heterologous DNAs can be used for the purpose of isolating the genes encoding the disclosed polypeptides. Non-limiting examples of suitable mammalian host cells include but are not limited to COS, HeLa, and CHO cells.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

EXAMPLES

Example 1: A novel function of immunoglobulin G in immune recognition.

Materials and Methods

Viruses, bacteria, antibody and proteins. HSV1 F strain was purchased from ATCC, Manassas, VA. Generating Us8 deficient HSV1 F was described previously (Suenaga *et al.*, 2014, *Microbiology and Immunology* 58, 513-522). R8411, a HSV1 F strain which expresses luciferase was provided by Bernard Roizman (Zerboni *et al.*, 2013, *J Virol* 87, 2791-2802). Wild type (WT) newman strain (ATCC, 25904) and protein A deficient (Spa-) newman were a gift from Dr Timothy Frost (Dublin, Ireland), and grew in tryptic soy broth (Patel *et al.*, 1987, *Infect Immun* 55, 3103-3110). Antibody specific for CD3(HIT3a), CD14(M5E2), CD19(HIB19), CD56 (N901), CD16a (3G8), CD253 (RIK2), CD69 (FN50), CD62L (DREG56), CD107a (H4a3), CD3ζ (pY142) (K25-407.69), CD3ζ (6B10.2), CD3 (17A2), CD62L (MEL-

14), CD27 (LG.3A10) CD69 (H1.2F3), NKp46 (29A1.4), Anti-HSV1 gE (9H3), Anti-HSV1 gC (1C8), and Anti-HSV1 gB (T111) were purchased from BD Biosciences , Franklin Lakes, NJ; Biolegend, San Diego, CA; Beckman Coulter, Brea, CA; Abcam, Cambridge, MA; R&D Systems, Minneapolis, MN; Sigma-Aldrich, St. Louis, MO; and Millipore, Burlington, VA. Anti-HSV1 gD (ID3) was provided by Roselyn J. Eisenberg and Gary Cohen. Anti-HSV1 gE (9H3) was purchased from Abcam. Biotinylated CD16a and HuIgG1 Fc was purchased from Sino Biological, Beijing, China, IgG1Fc(ΔCD16) was made by cloning human IgG1 Fc aa262-466 (SEQ ID NO: 2) into a pFuse vector (InvivoGen, San Diego,CA) after IL2 signal peptide, expressed in BHK cells and purified using protein A agarose beads (Thermofisher). Pooled human IgG (GamaStan, Grifols USA, Los Angeles, CA), which contains HSV1 specific antibody, was purchased from Ohio State University pharmacy, Columbus, OH.

Human IgG Fc (I2724, Scripps Laboratories, San Diego, CA) was validated as containing no Fab, and did not bind cells infected with Us8- HSV1 viruses. Rituximab (Genentech, South San Francisco, CA) and Darzalex (Janssen Pharmaceuticals, Fremont, CA) were purchased from Ohio State University pharmacy.

Cloning of HSV1 genes. Individual HSV1 genes were amplified from HSV1 F strain DNA (sequence accession number: GU734771) with gene specific primers, which are flanked with SpeI site at the 5' and PacI site at the 3' end, respectively (e.g., SEQ ID NOs: 25, 27-170), and cloned into a pCDH vector (System Bioscience, Palo Alto, CA, CD510B) using conventional methods.

Culture and transfection of human glioma spheres. Glioma cells were derived from primary human brain tumors and grown in DMEM/F12 (Life Technology, Carlsbad, CA) supplemented with B27 (1:50), heparin (5 ug/mL), basic FGF (bFGF) (20 ng/ mL), and EGF (20 ng/mL) as described previously (Mao *et al.*, 2013, *Proc Natl Acad Sci U S A* 110, 8644-8649). Except as noted otherwise, #83 glioma cells were used throughout this study (Mao *et al.*, 2013, *Proc Natl Acad Sci U S A* 110, 8644-8649). For one single transfection, ten million glioma cells were washed once with DMEM/F12 media, and resuspended in 100 µl of basic nucleofector solution (Lonza Inc., Allendale, NJ). Subsequently, the cell suspension was mixed with 6 µg of plasmid expressing HSV1 genes and nucleofected using program A33 (Amaxa GmBH, Koeln, Germany). Following nucleofection, cells were immediately mixed

with 1 ml media and transferred into one well of 6-well plates containing 4 ml DMEM/F12 with all supplements.

Differential cytolysis mediated by ectopic gene expression. 24h after transfection, glioma cells were resuspended and centrifuged down at 50g for 5 min to remove cell debris and dead cells. Subsequently, 1 x 10^4 glioma cells were resuspended with 100 µl DMEM/F12 media and seeded into each well of a U bottom 96-well plate. Purified human NK cells were resuspended in RPMI media (Life Technology, Carlsbad, CA) supplemented with 10% heat inactivated FBS (Sigma-Aldrich, St. Louis, MO) to a final concentration of 5 × 10^6/ml of media, and 100 µl of NK cells were added to culture with transfected glioma cells. In a parallel control experiment, 100 µl RPMI 1640 media supplemented with 10% FBS, instead of human NK cells, was added to the seeded glioma cells. Culture samples were collected using LSRII (BD Biosciences, Franklin Lakes, NJ) at 5h of culture. Living glioma cells were gated in based on their forward scatter (FSC) and side scatter (SSC) and measured for the percentage of GFP+ cells.

Differential cytotoxicity contributed by the expression of individual viral gene was calculated with the formula:

$$\Delta GFP = \frac{GFP\%(+nk) - GFP\%(-nk)}{GFP\%(-nk)} * 100\%,$$

wherein ΔGFP = change in GFP;

$GFP\%(+nk)$ = percentage of GFP in presence of NK cells;

$GFP\%(-nk)$ = percentage of GFP in absence of NK cells

Virus production, purification and inactivation. Vero cells were seeded at a density of 7×10^6 cells per 100 mm dish and inoculated with 2.5 pfu per cell of HSV-1 F strain or #30 mutants (Suenaga *et al.* 2014, *Microbiology and Immunology* 58, 513-522). At 24 hours post-inoculation, culture media and cell debris were collected. After three freeze-thaw cycles to release virus, cell debris was removed by low speed centrifugation (2,000×g for 5 minutes), samples were loaded on a 5 ml 35% sucrose gradient and centrifuged in a Beckman SW27 rotor at 25,000 rpm for 1 hour. Virus pellet was collected, washed and concentrated in PBS. To inactivate viruses, purified HSV1 viruses were treated with 0.2% Trition-100 for 30min. Inactivated viruses were diluted to 0.1ug/ml for coating plates.

Plaque assay. Briefly, sequentially diluted viruses were loaded on single layer of Vero cells and incubated at 37°C; pooled human IgG (final concentration 0.1%) was added 1h later to restrain viral spread. Plaques were counted after 48h culture. To determine the effect of human IgG3, human IgG Fc, Rituximab, daratumumab, and human IgG on the infectivity of HSV1, 1ug/ml of these reagents were added into sequentially diluted viruses and incubated at room temperature for 30min prior to a plaque assay. Treated viruses were tittered with standard plaque assay and all results were normalized to PBS control.

Human NK cell Isolation and stimulation condition. All NK cells used herein were freshly enriched from peripheral blood leukopacks of healthy donors (American Red Cross, Columbus, OH) using RosetteSep cocktail (StemCell Technologies, Cambridge, MA) as previously described (Yu *et al.* 2010, *Blood* 115, 274-281). A half million isolated human NK cells were incubated with media, or media supplement with 5ug/ml protein A or protein G for 30min prior to stimulation. 1×10^5 infected or transfected glioma cells, or K562 cells were used in the culture with 5×10^5 NK cells. For all CD107a staining, CD107a antibody was added at the beginning of the cell culture. Flat 96 well plates (MaxiSorp, Thermo Fisher Scientific, Waltham, MA) were coated with 50ul protein A (0.1ug/ul), protein G (0.1ug/ul), or wt or Us8-HSV1 F (0.1ug/ul) overnight at 4 C°.

Chromium release cytotoxicity assay. Glioma cells were labeled by incubating 5×10^5 cells in 50 μCi ^{51}Cr for 90min at 37°C. Radiolabeled cells were washed 3 times and resuspended in complete RPMI 1640 media, and seeded in U bottom 96-well plates in triplicates at a concentration of 5×10^4 cells/ml. In some cases, antibodies or IgG products (5ug/ml) were added into radiolabeled target cells and incubated on ice for 30 minutes for binding or blocking certain interactions. Effector cells were added in specified effector-to-target ratios (E:T, x-axis on Figure 1D) and incubated at 37°C for 4-6 hours. Cytolysis was then measured and calculated as previously described (Dai *et al.*, 2017, *Immunity*, 47, 159-170)).

CD3ζ phosphorylation staining. One-half million NK cells were rested at 37°C for 1h and then stimulated with H_2O_2 (11mM), IL12(10ng/ml)+IL18(10ng/ml), 2×10^5 transfected glioma cells, or 1×10^8 cfu of bacteria for 1h. NK cells were fixed using Phosflow Fix Buffer I (BD), permeabilized with Phosflow™ Perm Buffer III (BD Biosciences, Franklin Lakes, NJ), blocked with normal mouse immunoglobulin,

and then stained with anti- CD 3ζ (pY142) and anti CD3ζ antibodies. Phosphatase inhibitor (Roche, South San Francisco, CA) was supplemented in all the staining steps.

Modeling structure of gE-Fc-CD16a complex and protein A-Fc-CD16a complex. Docking predictions for gE-Fc (RCSB Protein Data Bank ID: PDB ID: 2GJ7) and CD16a-Fc (RCSB Protein Data Bank ID: PDB ID: IE4K) were conducted on the ZDOCK online server. For gE-Fc, only the gE subunits were uploaded to the server, and residues 225, 245–247, 249–250, 256, 258, 311, 316, 318–322, 324, and 338–342 were specified as contact residues (Patel *et al.*, 1987, *Infect Immun* 55, 3103-3110). In the case of CD16a-Fc, residues 252–258, 307, 309–311, 314–315, 382, 428, and 433–436 on Fc (SEQ ID NO: 11) were specified as contact residues (Patel *et al.*, 1987, *Infect Immun* 55, 3103-3110).

CD16a binding. Transfected glioma cells or bacteria were first incubated for 30min on ice with PBS with or without IgG1Fc(ΔCD16), IgG1Fc (Scripps Laboratories, San Diego, CA), rituximab, or hu IgG (GamaStan, Grifols), respectively. After one wash with FACS buffer, samples were incubated on ice with biotinylated CD16a, and 20 minutes later apc-streptavidin (BD Biosciences, Franklin Lakes, NJ) was added and samples were kept on ice for another 20min. After two washes with FACS buffer, cells or bacteria were immediately checked on LSRII flow cytometer (BD Biosciences, Franklin Lakes, NJ).

Mouse experiments. 8-to-12 weeks old female C57BL/6 and BALB/c mice (Jackson Laboratory, CITY) were used for all the studies. For survival studies, BALB/c mice were injected intraperitoneally (i.p.) with 3×10^6 pfu HSV1 F strain virus. PBS, 200μg human IgG3 (Sigma-Aldrich, , St. Louis, MO), 200ug human IgG Fc (Scripps Laboratories, San Diego, CA), 200μg Rituximab or 200μg Darzalex were given via i.p. injection at 4 hours prior to virus challenge, and at 24 hours and 72 hours after virus challenge. For bioluminescence imaging to track virus load, BALB/c mice were injected i.p with 1.2×10^5 pfu of R8411 virus (Zerboni, L. *et al. J Virol* 87, 2791-2802 (2013)). To study the clearance of HSV1 by human IgG1, BALB/c mice were injected i.p with 200μg rituximab at 4 hours prior to virus challenge, and at 24 hours after virus challenge. Each mouse was given 3mg luciferin potassium 10 minutes prior to isoflurane anesthesia to ensure consistent photon flux. Images were taken using an IVIS Spectrum (Perkin Elmer, Waltham, MA) at 18h and 84h post

infection. Each group was recorded for 4 sections of 2 minute exposure. Bioluminescence values were measured from the whole mouse and calculated as photon flux (photons/s) using Living Image 4.0 (Perkin Elmer, Waltham, MA). To study the effect of protein A on NK cells *in vivo*, mice were injected intravenously (i.v.) with 40μg silicone beads and protein A conjugated beads (AlphaBio, Racho Santa Margarita, CA). 24-48h after beads completed this inoculation, blood, spleen and lung were collected and mononuclear cells were isolated from these tissues and stained using antibodies against mouse antigens. For *in vitro* mouse NK cell stimulation and cytotoxicity, NK cells were enriched from spleens of 8 to 12 weeks old C57BL/6 and BALB/c mice using an NK cell isolation kit (Miltenyi Biotec, Cambridge, MA) following manufacturer's instruction.

Statistics. Two-sample *t* test was used to compare two independent groups and paired *t* test was used to compare two paired groups. Data transformation was performed if the original distribution was non-normal. Linear mixed models were used to account for the covariance structure due to repeated measures from the same donor when three or more groups were compared. *P* values were adjusted for multiple comparisons by Holm's procedure. A p value of < 0.05 was considered significant. Experiments were repeated at least three or more times. Data are displayed as mean ± SEM.

Results

Differential Cytolysis Mediated by Ectopic Gene Expression (DC-MEGE) identified HSV1 gE as a human NK cell activator.

The HSV1 genome contains 84 open reading frames, encoding 74 unique viral proteins (Szpara et al., 2010, *J Virol* 84, 5303-5313), however very few of them have been studied for their roles in immune recognition or evasion (Imai et al., 2013, *PLoS One* 8, e72050; Chisholm *et al.*, 2007, *The Journal of Infectious Diseases* 195, 1160-1168; Huard & Fruh, 2000, *Eur J Immunol* 30, 509-515). To gain a comprehensive understanding of the interaction between human NK cells and HSV1, DC-MEGE were developed to measure how NK cells respond to glioma cells expressing a single HSV1 gene (Fig. 1A). Each HSV1 gene was cloned upstream of the "self-cleaving" T2a sequence and green fluorescence protein (GFP); as a consequence, fluorescence reveals expression of viral proteins (Szymczak *et al.*, 2004, *Nat Biotechnol* 22, 589-594). Glioma cells were transfected with an individual HSV1 gene, and subsequently

cultured with or without fresh human NK cells. The percentage of GFP+ living glioma cells were recorded in parallel as GFP(+NK)% when NK cells are present, or GFP(-NK)% when glioma cells are cultured alone. When expression of a HSV1 viral protein made glioma cells susceptible to NK cell cytolysis, GFP+ glioma cells were preferably killed by NK cells, therefore, GFP(+NK)% would be less than GFP(-NK)%, or *vice versa* when HSV1 viral protein made glioma cells resistant to NK cell cytolysis (Fig. 1A). Applying the DC-MEGE assay, 65 HSV1 genes were screened, demonstrating that glioma cells expressing UL12, UL30, Us3, Us8 and Us12 were more susceptible to NK cell cytolysis, while expression of UL48, Us5, or Us6 made glioma cells resistant to NK cell cytolysis (Fig. 1B and 1C).

HSV1 Us8 encodes gE, which alone is a low affinity human IgG Fc receptor, binding human IgG1, IgG2 and IgG4 at the CH2-CH3 interface (Sprague *et al.*, 2006, *PLoS Biol* 4, e148). DC-MEGE results were validated using a ^{51}Cr release assay against a human mesenchymal glioma cell line #1123 and a human proneural glioma cell line #84 (Mao *et al.*, 2013, *Proc Natl Acad Sci U S A* 110, 8644-8649), shown in Fig. 1D. Glioma cells expressing Us8 (referred as glioma Us8 hereafter) also induced human primary NK cells to secret IFN-γ (Fig. 1E), express CD69 and CD107a (Fig. 1F), and cleave CD62L and CD16a (Fig. 1G), which are characteristic phenotypes of activated human NK cells. NK cytotoxicity towards glioma Us8 was attenuated by a gE specific mouse monoclonal antibody (Abcam) (Fig. 1h). Wild type (wt) HSV1 F strain viruses (of which gE is a major protein component) and a mutant HSV1 F strain with targeted-deletion of Us8 (Us8$^-$) (Suenaga *et al.*, 2014, *Microbiology and Immunology* 58, 513-522) were purified. NK cells were cultured in plates coated with inactivated pure viruses. NK cells were only activated by wild-type (wt) but not Us8$^-$ F strain viruses (Figs. 1I, 6), and activation of NK cells by wt HSV1 was also inhibited by the anti-Us8 antibody (Figs. 1I, 6). Taken together, these results demonstrated that direct interaction between gE and human NK cells contributed to the functional enhancement of NK cells.

Human IgG links gE and NK cell activation. HSV1 gE can form heterodimers with glycoprotein I (gI), encoded by HSV1 Us7, and the resultant gE/gI complex is the high affinity viral Fc receptor for human IgG (Sprague *et al.*, 2006, *PLoS Biol* 4, e148; Johnson *et al.*, 1988, *J Virol* 62, 1347-1354). Glioma cells expressing Us7 (glioma Us7, hereafter) did not activate NK cells (Figs. 1B, 1C, 2A, 2B); however,

glioma cells expressing both Us7 and Us8 (glioma Us7+Us8) activated NK cells much more potently than glioma Us8 (Figs. 2A 2B, 7), suggesting IgG-binding function of gE may be involved in NK cell activation.

Although human IgG was not supplemented in the glioma-NK cell co-culture, IgG molecules were shown to be naturally present on the surface of primary human NK cells (Fig. 2C). Washing NK cells briefly with acidic media (RPMI1640 plus 10% FBS) adjusted to pH4.0 with acetic acid) decreased surface IgG (Fig. 2C) and increased binding of an anti-human CD16 antibody (3G8) (Perussia *et al.*, 1984, *J Immunol* 133, 180-189) that competes with human IgG for the same binding site on CD16a (Fig. 2D), demonstrating IgG molecules are anchored on human NK cells via CD16a. In addition to NK cells, B cells, monocytes and granulocytes were shown to be naturally coated with human IgG molecules, via non-covalent binding, revealed by acidic media treatment which removed most of the IgG from the cell surface (Fig. 27A). These surface IgG molecules provide interaction sites for protein A binding (Fig. 27B). Primary NK cells from a variety of healthy human donors had very different levels of surface IgG (Fig. 2E) and their response to stimulus varied (Figs. 2F and 8). The response (measured by the percentage of CD69+ or CD107a+ NK cells) of human NK cells towards glioma Us8 correlated with the level of surface IgG; and the correlation became even stronger when NK cells were cultured with glioma Us7+Us8 (Fig. 2G). In contrast, the response of NK cells to K562 cells, which are leukemia cells negative for MHC I molecule and widely used as an activating control for NK cells, showed no correlation with surface IgG (Fig. 2G). Taken together, human IgG was shown to link gE and NK cell activation.

CD16a, IgG Fc and HSV1 gE form a ternary complex essential for NK cell activation. Human CD16a binding sites on IgG Fc are located far apart from the CH2-CH3 interface where gE binds IgG (Sondermann *et al.*, 2000, *Nature* 406, 267-273; Sprague *et al.* 2006, *PLoS Biol* 4, e148), leading to the hypothesis that IgG, gE and CD16a could form a ternary complex. Structure modeling using the known gE-IgG Fc and CD16a-IgG Fc crystal structure supported the conclusion that gE and CD16a could bind the same IgG Fc molecule without interfering with each other (Fig. 3A). To validate the existence of such a CD16a-IgGFc-gE complex, experiments were conducted to determine whether the extracellular domain of CD16a could bind glioma Us7+Us8 in the presence of different human IgG products. IgG1 Fc(ΔCD16) is a

recombinant human IgG1 Fc fragment without the CD16a binding sites; while IgG1 Fc has intact CD16a binding sites. Consistent with the crystal structure (Fig. 3A), CD16a binding sites played no role in the binding between Fc and HSV1 gE as both IgG1 Fc(ΔCD16) and IgG1 Fc bound to glioma Us7+Us8 efficiently (Fig. 3B, left and middle left). Although no direct interaction existed between CD16a and glioma Us7+Us8 (Fig. 3C left), CD16a was found to bound glioma Us7+Us8 when IgG1Fc was present and this interaction was dependent on the CD16a binding sites on IgG1Fc (Fig. 3C middle left and middle), thus proving the formation of CD16a-IgGFc-gE complex.

Human HSV1 specific IgG contains antibodies that specifically recognize gE or gI (Fig. 3B, middle right), and its presence allowed CD16a to bind glioma cells expressing an HSV1 gene via the classical interaction between CD16a and antigen-antibody complex (Fig. 3C, middle right). Similar CD16a binding tests were also performed using Rituximab (a humanized IgG1 monoclonal antibody against human CD20) (Edwards *et al.*, 2004, *N Engl J Med* 350, 2572-2581) (Fig. 3B and 3C, right), or glioma cells infected with Us8- HSV1 or wild type HSV1 (Fig. 3D), which again confirmed CD16a, IgG Fc and HSV1 gE formed a ternary complex. Furthermore, phosphorylation of CD3ζ specifically occurred when NK cells were stimulated with H_2O_2 (positive control) or in culture with glioma Us7+Us8, but not with IL12+IL18 (Fig. 3E), demonstrating gE activated NK cells through CD16a-CD3ζ axis.

IgG-binding proteins, protein A from Staphylococcus aureus and protein G from group G streptococcus, bind IgG mainly at the CH2-CH3 interface (Sauer-Eriksson *et al.*, 1995, *Structure* 3, 265-278; Deis *et al.*, 2015, *Proc Natl Acad Sci U S A* 112, 9028-9033). Protein A and protein G were shown to also bind primary human NK cells via IgG present on the membrane of NK cells (Fig. 3F and 3G), which also suggested that surface IgG coated on primary NK cells were fully accessible for gE to bind. To test the essentiality of IgG to NK cell activation by HSV1 gE, primary human NK cells were incubated with an excess of protein A or protein G to occupy all CH2-CH3 interface prior to culturing the NK cells with different stimuli. Pre-incubation with protein A or protein G completely inhibited all functional enhancements of NK cell by gE (Figs. 3H-3J and 9). This treatment did not change human NK cells' responses to K562 cells or IL12+IL18 (Fig. 9). Additionally, plates coated with pure and inactivated wt HSV1 virus could no longer activate NK cells

that were pre-incubated with protein A or protein G (Figs. 10A and 10B). Taken together, these results supported the conclusion that IgG Fc bridged the interaction between NK cells and target cells expressing HSV1 gE, and resulted in NK cell activation.

Passive ADCC promotes the clearance of HSV1 infection in vivo. Human NK cell activation by gE represents a previously unappreciated immunostimulation mechanism which is solely bridged by IgG Fc, and differs from classical IgG functions by requiring no antigen-specific antibody (Fig 3K). This type of NK cell activation was named passive ADCC (Fig 3K). The above experiments were all conducted without adding human IgG to the interaction, and NK cell activation by gE was mediated by IgG already present on primary NK cells (Figs. 3H-3J). During primary HSV1 infection *in vivo*, infected cells are likely to be coated with human IgG due to the expression of gE and the abundance of IgG in human serum. To test whether the non-immune IgG coating on infected cells could provide additional anchoring/activating sites for CD16a(+) NK cells, non-HSV1 non-immune plasma [(-) plasma] or IgG1 Fc fragments were added in culture. Both (-) plasma and IgG1 Fc further enhanced activation of NK cells by infected or transfected glioma cells in a gE dependent manner (Figs. 11A-11D). NK cytotoxicity toward glioma cells infected with wt or $us8^{-}$ F was enhanced by HSV1-specific IgG (classical ADCC, Fig 3K), however human IgG1 Fc and an antibody targeting an irrelevant antigen (i.e rituximab) also enhanced NK cell cytotoxicity to glioma cells infected with wt F but not $Us8^{-}$ F strain (Fig. 4A), or glioma cells expressing gE (Fig. 4B).

HSV1 gE does not bind mouse IgG (Chapman *et al.*, 1999, *J Biol Chem* 274, 6911-6919), however mouse FcγR binds human IgG with high affinity (Ober *et al.*, 2001, *Int Immunol* 13, 1551-1559), and thus supplementing human IgG should be able to bridge mouse NK cells and HSV1 infected cells, promote immune activation and clearance of HSV1 infection. Consistent with this hypothesis, NK cells isolated from C57BL/6 and BALB/c mice displayed enhanced cytotoxicity towards glioma Us7+Us8 in the presences of human IgG Fc fragments (Fig. 4C). To demonstrate that passive ADCC could be an important mechanism for clearing HSV1 infection *in vivo*, BALB/c mice were injected with PBS, human IgG3, human IgG Fc fragments, daratumumab (a human IgG1 antibody directed against human CD38) or rituximab, four hours before and at 24h and 72h after virus challenge (Fig. 4D). Each of these

reagents did not affect the infectivity of HSV1 viruses when NK cells were absent (Fig 12A), nor their *in vivo* administration alone changed phenotypes of NK cells *in vivo* (Fig. 12B), human IgGFc fragments, daratumumab and rituximab alleviated HSV1 infection symptoms and provided mice complete protection from lethal HSV1 infection (Fig. 4D). HSV1 gE is required for the cell-to-cell spread of HSV1 *in vivo* (Polcicova *et al.*, 2005, *J Virol* 79, 11990-12001), therefore the $Us8^{-}$ F strain was not used *in vivo* to confirm dependence on gE for this kind of protection. However, human IgG3, which does not bind HSV1 gE (Sprague *et al.*, 2006, *PLoS Biol* 4, e148), failed to provide any protection against HSV1 infection (Fig. 4F), suggesting an gE-IgG interaction is critical for protection against lethal HSV1 infection by human IgG products. Furthermore, tracking virus infection *in vivo* using a HSV1 F strain expressing luciferase (Zerboni *et al.*, 2013, *J Virol* 87, 2791-2802) revealed that rituximab increased clearance of HSV1 infection (Fig. 4E, 4F). Taken together, IgG Fc bridging viral Fc receptor and immune Fc receptor provides robust protection against HSV1 infection when pathogen-specific antibody is not available.

Bacterial IgG binding proteins activate NK cells through the IgG Fc-mediated bridging. Experiments were conducted to test whether IgG binding proteins from other pathogens can activate NK cell through the same mechanism. Although protein A bound IgG Fc presented on the surface of human NK cells (Fig. 3F and 3G), adding pure protein A directly in culture failed to activate human NK cells (Fig. 5A), because monomeric forms of protein A did not cause accumulation of CD16a prerequisite for CD3ζ auto phosphorylation. However, primary human NK cells were activated after culturing in protein A-coated plates (Fig. 5A), produced IFN-γ (Fig. 5B) and showed enhanced NK cytotoxicity (Fig. 5C). These NK cell functional enhancements were abrogated when protein A coated plates were blocked with mouse serum (Fig. 5A), because mouse IgG blocked all potential interactions between human IgG and protein A/G.

The formation of a CD16a-Fc-protein A complex was tested using wild type *Staphylococcus aureus* (S.A.) newman strain (wt) and a protein A deficient newman strain (Spa) (Patel *et al.*, 1987, *Infect Immun* 55, 3103-3110) (Fig. 13A). CD16a bound S.A when either full human IgG (rituximab) or IgG1 Fc fragments were present and protein A was indispensable for this interaction (Fig. 13B). Phosphorylation of CD3ζ by S.A depended on protein A as protein A deficiency or

blocking of protein A with mouse serum canceled CD3ζ phosphorylation. Furthermore, pre-incubating S.A with human IgG slightly enhanced CD3ζ phosphorylation (Fig. 5D). Phenotypes of NK cells cultured with S.A mirrored the result of CD3ζ phosphorylation. Activation of NK cells by wt S.A was inhibited by pre-incubating wt S.A with mouse serum, and enhanced by human IgG (Fig. 5E). NK cell activation by wt S.A was also abolished if primary human NK cells were pretreated with either soluble monomeric protein A or protein G (Fig. 5E). Furthermore, human NK cells were activated by streptococcus protein G in a similar fashion (Figs. 14A-14C). Taken together, these results demonstrate that bacterial IgG binding proteins activated human NK cells through Fc bridge and CD16a.

Additionally, mouse NK cells cultured with wildtype S.A produced more IFNγ than spa- S.A (Fig. 5F), and expressed more early activation marker CD27 and CD62L (in contrast to a loss of CD62L in activated human NK cells) (Peng, H. *et al. J Immunol* 190, 4255-4262 (2013); Hayakawa & Smyth, 2006, *J Immunol* 176, 1517-1524) (Fig. 15A). A similar phenotype was observed when mouse NK cells were cultured in protein A-coated plates (Figs. 5G and 15B). S.A produces many inflammatory factors (Fournier & Philpott, 2005, *Clin Microbiol Rev* 18, 521-540 (2005)). To avoid confounding of results by bystander activation, mice were injected with protein A-coated silicone beads to study if oligomeric protein A could activate NK cell *in vivo*. NK cells from mice injected with protein A conjugated silicone beads adopted a more activating phenotype compared with NK cells from mice injected with control silicon beads (Fig. 5H and 15C). However, injection of soluble protein A did not cause any phenotypic changes on mouse NK cells compared to PBS control (Fig. 15D). Taken together these results demonstrate that protein A activated mouse NK cells *in vitro* and *in vivo*.

Discussion

As demonstrated by the results set forth above, an unbiased cytotoxicity assay, DC-MEGE, illustrated interactions of human NK cells and host tumor cells following HSV1 infection (Fig. 1A). With the exception of Us12 (Huard & Fruh, 2000, *Eur J Immunol* 30, 509-515) and Us3 (Imai *et al.*, 2013, *PLoS One* 8, e72050), this is the first time the remaining viral genes have been reported as significant for their regulation of NK cell cytotoxicity. DC-MEGE is therefore useful to study how NK cells interact with other pathogens.

HSV1 gE/gI complexes have been shown to participate in "antibody bipolar bridging", whereby a single HSV1-specific IgG antibody simultaneously binds to a HSV1-antigen using its Fab region and to gE/gI via its Fc region (Frank & Friedman, 1989, *Journal of virology* 63, 4479-4488). It has been proposed that such antibody bipolar bridging could block access of the Fc portion of an antibody to FcγR expressed on innate immune effector cells, and thereby reduce classical ADCC and presumably provide a mechanism for immune evasion following HSV1 infection (Dubin *et al.*, 1991, *Journal of virology* 65, 7046-7050; Corrales-Aguilar *et al.*, 2014, *PLoS Pathog* 10, e1004131). This seems to contradict the disclosed finding that gE or the gE/gI complex promotes activation and cytotoxicity of human NK cells (Figs. 2A, 2B). However previous studies suggesting an NK-inhibition of HSV1 gE/gI were all conducted in the presence of HSV1-specific antibody (Frank & Friedman, 1989, *Journal of virology* 63, 4479-4488; Dubin *et al.*, 1991, *Journal of virology* 65, 7046-7050; Corrales-Aguilar *et al.*, 2014, *PLoS Pathog* 10, e1004131); thus these results were all relative to classical ADCC, whereas the actual function of HSV1 gE binding non-immune IgG under the condition of primary viral infection was not assessed.

Disclosed herein is an unappreciated immunostimulatory role of HSV1 gE/gI. Crystal structure, *in vitro* and *in vivo* functional validation demonstrated that IgG Fc bridged gE and CD16a (Fig 3C), and the resultant ternary complex transduced an intracellular signal that activated NK cells (Fig. 3E), and promoted clearance of HSV1 infection. The disclosed work suggests that during primary HSV1 infection when anti-HSV1 antibody is not yet available, NK cells can utilize "passive ADCC" to clear HSV1-infected cells (Fig. 4D-4F). This result is consistent with the observation that most primary HSV1 infections in man are clinically asymptomatic. It is also highly likely that passive ADCC is at least in part responsible for the rapid NK cell clearance of oncolytic HSV1 in the setting of malignant glioma (Alvarez-Breckenridge *et al.*, 2012, *Nat Med* 18, 1827-1834 (2012)).

This disclosure has also established a functional role for surface IgG, anchored by its Fc domain to CD16a expressed on the NK cell surface, and a new mechanism by which NK cells are able to recognize pathogens in the absence of specific antigen recognition. As demonstrated herein, HSV1 infected host cells, as well as protein A and protein G, are capable of activating human NK cells by binding NK cell surface IgG. Protein A has long been proposed as a virulent factor for Staphylococcus aureus

newman strain, and Spa⁻ Staphylococcus aureus newman strain. causes milder symptoms in mice than wt S.A (Palmqvist *et al.*, 2002, *Microb Pathog* 33, 239-249). The disclosed findings that coated protein A and wt S.A. activated NK cells, and Spa⁻ S.A. did not activate NK cells, provide a mechanistic explanation for this phenotype. This new mechanism of innate immune cell activation has broad implications for clinical toxicity observed during infection, given that many viruses and bacteria encode proteins capable of binding the Fc domain of human IgG (Litwin *et al.*, 1992, *J Virol* 66, 3643-3651; Sprague *et al.*, 2008, *Journal of Virology* 82, 3490-3499; Loukas *et al.*, 2001, *Infect Immun* 69, 3646-3651; De Miranda-Santos & Campos-Neto, 1981, *J Exp Med* 154, 1732-1742).

Example 2: Use of IgG-binding proteins protein A and protein G to capture monocytes and increase the efficacy of generating dendritic cells and macrophages in vitro.

Dendritic cells and macrophages are highly specialized antigen-presenting cells (APC), which account for a very small percentage (~0.2%) of human blood mononuclear cells. Accordingly, dendritic cells and macrophages are generated from *in vitro* culture of monocytes for numerous therapeutic purposes.

Conventional procedures for generating dendritic cells and macrophages involve: (1) plating PBMC or monocytes on culture dishes, (2) incubating cells at 37^0C for few hours to allow monocytes to attached to the plate, (3) removing non-adherent cells by vigorously washing the plates with media, and (4) treating adherent cells with GM-CSF (for macrophage) or GMCSF and IL4 (for dendritic cells) for one week. While this protocol yields consistent results, cells were lost during step 2 and 3, and a relative large amount of monocytes or PBMC were required for generating enough dendritic cells and macrophages for downstream uses.

Like natural killer cells, primary monocytes are also coated on the surface with IgG molecules, which are anchored on monocytes by Fcγ receptors, including CD64, CD32 and CD16a and provide interaction sites for protein A to bind. Binding of human Fcγ receptor CD32 and CD64 to staphylococcus aureus (S.A) required the presence of human IgG and protein A. Wild type (wt) or protein A deficient (Spa-) S.A bacteria were incubated with fluorescent labeled human Fcγ receptor CD32 and CD64 in the absence or presence of humanized antibody rituximab (Ritu) (Figs. 23A

and 23B). These results confirmed that protein A, IgG and CD32/CD64 formed ternary complexes in a way similar to protein A/IgG/CD16 complex. CD64, the high affinity FcγR, is majorly expressed by monocytes, macrophages and dendritic cells.

Protein A and protein G can bind IgG coated on NK cells and monocytes. Therefore, protein A or protein G molecule coated on a plate should be able to bind surface IgG of monocytes and thus increase adhesion of monocytes. By plating the same amount of monocytes in different plates, monocytes were found to attach more firmly to protein A or protein G treated plates than to bovine serum treated plates during the first few hours, and monocytes culture in plate A or protein G coated plates started to form colonies, an indication of activation (Fig. 17A). Further experiments using protein A coated plate showed that other than increasing adhesion of monocytes, protein A coated plates also increased metabolic activity of monocytes, induced monocytes to produce IL1β and changed the phenotype of primary human monocytes after 6 hours (Fig. 17B, 17C, Fig. 19, Fig. 20, and Fig. 21). Similarly, immobilized protein A and protein G induced respiration burst in primary human neutrophils (Fig. 22). Therefore, instead of culturing monocytes in regular plates, monocytes or PBMC were cultured in plates coated with protein A or protein G for generating dendritic cells or macrophages. This reduced loss of cells at steps 2 and 3 and required much less starting monocytes and PBMC for generating equal amount of dendritic cells and macrophage (Fig. 18).

Dendritic cells generated from plates pre-coated with protein A, protein G or human IgG expressed a higher amount of costimulator molecule CD86 (Fig. 24). Upon loading with Epstein Barr Virus (EBV) antigenic peptide and coculturing with autologous T cells, these dendritic cells also tended to induce more EBV specific cytotoxic T cells (Fig. 25).

Previous studies reported that protein A binds TNFR1 and activates epithelial cells, and protein G does not bind TNFR1 and not activate epithelial cells (reference PMID: 15247912). While it is possible that monocyte attachment and activation by protein A may partially contribute to binding TNFR1 which is expressed on monocytes, protein G increased monocyte attachment can be explained by binding surface IgG on monocytes because the existence of the ternary complex of protein G-IgG-CD16 and the present data showing protein G activates NK cells through binding surface IgG.

Example 3: Activation of NK cells using CMV

Both CMV gp34 and gp68 are IgG-binding proteins capable of binding both humanized antibody rituximab and human IgG through portions of their Fc (Figs. 28A and 28B). CD16a does not directly interact with glioma cells expressing either gp34 or gp68 (Fig. 28C), however it can bind glioma cells expressing gp68 in the presence of rituximab or human IgG1 Fc fragment, but does not bind to the glioma cells expressing gp34 even when human IgG Fc is present (Figs. 28D and 28E). Therefore, gp68 is capable of forming a ternary complex with human IgG1 Fc and CD16a. Additionally, primary human NK cells cultured with glioma cells expressing gp68 showed activated phenotypes, which was represented by the increase of CD69 and CD107a, and the decrease of CD62L and CD16a (Fig. 29A and 29B).

MCMV infection also allowed 3T3 to bind non immune mouse IgG (Fig. 30), indicating that MCMV produce IgG-binding protein(s).

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs. Publications cited herein and the materials for which they are cited are specifically incorporated by reference.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

SEQ ID NO: 1: IgG1 Fc (binds both human FcγR and pathogen IgG binding protein)	EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSP GK
SEQ ID NO: 2: IgG1 Fc (binds only pathogen IgG binding protein)	FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG K
SEQ ID NO: 3: IgG binding	MKKKNIYSIR KLGVGIASVT LGTLLISGGV TPAANAAQHD EAQQNAFYQV LNMPNLNADQ RNGFIQSLKD

protein A	DPSQSANVLG EAQKLNDSQA PKADAQQNNF NKDQQSAFYE ILNMPNLNEA QRNGFIQSLK DDPSQSTNVL GEAKKLNESQ APKADNNFNK EQQNAFYEIL NMPNLNEEQR NGFIQSLKDD PSQSANLLSE AKKLNESQAP KADNKFNKEQ QNAFYEILHL PNLNEEQRNG FIQSLKDDPS VSKEILAEAK KLNDAQAPKE EDNKKPGKED GNKPGKEDGN KPGKEDNKKP GKEDGNKPGK EDNNKPGKED GNKPGKEDNN KPGKEDGNKP GKEDGNKPGK EDGNGVHVVK PGDTVNDIAK ANGTTADKIA ADNKLADKNM IKPGQELVVD KKQPANHADA NKAQALPETG EENPFIGTTV FGGLSLALGA ALLAGRRREL
SEQ ID NO: 4: IgG binding protein G	MEKEKKVKYF LRKSAFGLAS VSAAFLVGST VFAVDSPIED TPIIRNGGEL TNLLGNSETT LALRNEESAT ADLTAAAVAD TVAAAAAENA GAAAWEAAAA ADALAKAKAD ALKEFNKYGV SDYYKNLINN AKTVEGIKDL QAQVVESAKK ARISEATDGL SDFLKSQTPA EDTVKSIELA EAKVLANREL DKYGVSDYHK NLINNAKTVE GVKELIDEIL AALPKTDTYK LILNGKTLKG ETTTEAVDAA TAEKVFKQYA NDNGVDGEWT YDDATKTFTV TEKPEVIDAS ELTPAVTTYK LVINGKTLKG ETTTKAVDAE TAEKAFKQYA NDNGVDGVWT YDDATKTFTV TEMVTEVPGD APTEPEKPEA SIPLVPLTPA TPIAKDDAKK DDTKKEDAKK PEAKKDDAKK AETLPTTGEG SNPFFTAAAL AVMAGAGALA VASKRKED
SEQ ID NO: 5: Glycoprotein E	MARGAGLVFFVGVWVVSCLAAAPRTSWKRVTSGEDVVLL PAPAGPEERTRAHKLLWAAEPLDACGPLRPSWVALWPPRR VLETVVDAACMRAPEPLAIAYSPPFPAGDEGLYSELAWRDR VAVVNESLVIYGALETDSGLYTLSVVGLSDEARQVASVVLV VEPAPVPTPTPDDYDEEDDAGVSERTPVSVPPPTPPRRPPVA PPTHPRVIPEVSHVRGVTVHMETPEAILFAPGETFGTNVSIHA IAHDDGPYAMDVVWMRFDVPSSCAEMRIYEACLYHPQLPE CLSPADAPCAVSSWAYRLAVRSYAGCSRTTPPPRCFAEARM EPVPGLAWLASTVNLEFQHASPQHAGLYLCVVYVDDHIHA WGHMTISTAAQYRNAVVEQHLPQRQPEPVEPTRPHVRAPPP APSARGPLRLGAVLGAALLLAALGLSAWACMTCWRRRSW RAVKSRASATGPTYIRVADSELYADWSSDSEGERDGSLWQ DPPERPDSPSTNGSGFEILSPTAPSVYPHSEGRKSRRPLTTFGS GSPGRRHSQASYSSVLW
SEQ ID NO: 6: IgG1 (GenBank Accession No: AAC82527.1)	ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV

	LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
SEQ ID NO: 7: IgG2 (GenBank Accession No: AAB59393.1)	ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSNFGTQT YTCNVDHKPS NTKVDKTVER KCCVECPPCP APPVAGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTFR VVSVLTVVHQ DWLNGKEYKC KVSNKGLPAP IEKTISKTKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDISVEW ESNGQPENNY KTTPPMLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK
SEQ ID NO: 8: IgG3 (GenBank Accession No: AA52805.1)	ASTKGPSVFP LAPCSRSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YTCNVNHKPS NTKVDKRVEL KTPLGDTTHT CPRCPEPKSC DTPPPCPRCP EPKSCDTPPP CPRCPEPKSC DTPPPCPRCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFKWYVD GVEVHNAKTK PREEQYNSTF RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESSGQPENN YNTTPPMLDS DGSFFLYSKL TVDKSRWQQG NIFSCSVMHE ALHNRFTQKS LSLSPGK
SEQ ID NO: 9:IgG4 (GenBank Accession No: AAB5934.1)	ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRVES KYGPPCPSCP APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS LSLSLGK
SEQ ID NO: 10: IgG1 (S6B291)	MEFGLSWLFLVAILKGVQCEVQLLESGGDLVQPGGSLRLSC AASGFTFSTYAMSWVRQAP GKGLEWVSGIGDSGHSIYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCATGSQ WPGDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES

	NGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK
SEQ ID NO: 11: FcγRI FG-loop	MGKHRY
SEQ ID NO: 12: UL12	MESTVGPACP PGRTVTKRPW ALAEDTPRGP DSPPKRPRPN SLPLTTTFRP LPPPPQTTSA VDPSSHSPVN PPRDQHATDT ADEKPRAASP ALSDASGPPT PDIPLSPGGT HARDPDADPD SPDLDSMWSA SVIPNALPSH ILAETFERHL RGLLRGVRAP LAIGPLWARL DYLCSLAVVL EEAGMVDRGL GRHLWRLTRR GPPAAADAVA PRPLMGFYEA ATQNQADCQL WALLRRGLTT ASTLRWGPQG PCFSPQWLKH NASLRPDVQS SAVMFGRVNE PTARSLLFRY CVGRADDGGE AGADTRRFIF HEPSDLAEEN VHTCGVLMDG HTGMVGASLD ILVCPRDIHG YLAPVPKTPL AFYEVKCRAK YAFDPMDPSD PTASAYEDLM AHRSPEAFRA FIRSIPKPSV RYFAPGRVPG PEEALVTQDQ AWSEAHASGE KRRCSAADRA LVELNSGVVS EVLLFGAPDL GRHTISPVSW SSGDLVRREP VFANPRHPNF KQILVQGYVL DSHFPDCPPH PHLVTFIGRH RTSAEEGVTF RLEDGAGALG AAGPSKASIL PNQAVPIALI ITPVRIDPEI YKAIQRSSRL AFDDTLAELW ASRSPGPGPA AAETTSSSPT TGRSSR
SEQ ID NO: 13: UL30	MFSGGGGPLS PGGKSAARAA SGFFAPAGPR GASRGPPPCL RQNFYNPYLA PVGTQQKPTG PTQRHTYYSE CDEFRFIAPR VLDEDAPPEK RAGVHDGHLK RAPKVYCGGD ERDVLRVGSG GFWPRRSRLW GGVDHAPAGF NPTVTVFHVY DILENVEHAY GMRAAQFHAR FMDAITPTGT VITLLGLTPE GHRVAVHVYG TRQYFYMNKE EVDRHLQCRA PRDLCERMAA ALRESPGASF RGISADHFEA EVVERTDVYY YETRPALFYR VYVRSGRVLS YLCDNFCPAI KKYEGGVDAT TRFILDNPGF VTFGWYRLKP GRNNTLAQPA APMAFGTSSD VEFNCTADNL AIEGGMSDLP AYKLMCFDIE CKAGGEDELA FPVAGHPEDL VIQISCLLYD LSTTALEHVL LFSLGSCDLP ESHLNELAAR GLPTPVVLEF DSEFEMLLAF MTLVKQYGPE FVTGYNIINF DWPFLLAKLT DIYKVPLDGY GRMNGRGVFR VWDIGQSHFQ KRSKIKVNGM VNIDMYGIIT DKIKLSSYKL NAVAEAVLKD KKKDLSYRDI PAYYAAGPAQ RGVIGEYCIQ DSLLVGQLFF KFLPHLELSA VARLAGINIT RTIYDGQQIR VFTCLLRLAD QKGFILPDTQ GRFRGAGGEA PKRPAAARED EERPEEEGED EDEREEGGGE REPEGARETA GRHVGYQGAR VLDPTSGFHV NPVVVFDFAS LYPSIIQAHN LCFSTLSLRA DAVAHLEAGK DYLEIEVGGR RLFFVKAHVR ESLLSILLRD

	WLAMRKQIRS RIPQSSPEEA VLLDKQQAAI KVVCNSVYGF TGVQHGLLPC LHVAATVTTI GREMLLATRE YVHARWAAFE QLLADFPEAA DMRAPGPYSM RIIYGDTDSI FVLCRGLTAA GLTAVGDKMA SHISRALFLP PIKLECEKTF TKLLLIAKKK YIGVIYGGKM LIKGVDLVRK NNCAFINRTS RALVDLLFYD DTVSGAAAAL AERPAEEWLA RPLPEGLQAF GAVLVDAHRR ITDPERDIQD FVLTAELSRH PRAYTNKRLA HLTVYYKLMA RRAQVPSIKD RIPYVIVAQT REVEETVARL AALRELDAAA PGDEPAPPAA LPSPAKRPRE TPSPADPPGG ASKPRKLLVS ELAEDPAYAI AHGVALNTDY YFSHLLGAAC VTFKALFGNN AKITESLLKR FIPEVWHPPD DVAARLRTAG FGAVGAGATA EETRRMLHRA FDTLA
SEQ ID NO: 14: Us5	MSLRAVWHLG LLGSLVGAVL AATHRGPAAN TTDPLTHAPV SPHPSPLGGF AVPLVVGGLC AVVLGAACLL ELLRRTCRGW GRYHPYMDPV VV
SEQ ID NO: 15: Us3	MKPVLVLAIL AVLFLRLADS VPRPLDVVVS EIRSAHFRVE ENQCWFHMGM LYFKGRMSGN FTEKHFVNVG IVSQSYMDRL QVSGEQYHHD ERGAYFEWNI GGHPVTHTVD MVDITLSTRW GDPKKYAACV PQVRMDYSSQ TINWYLQRSM RDDNWGLLFR TLLVYLFSLV VLVLLTVGVS ARLRFI
SEQ ID NO: 16: Us12	MVQIQFHQGE PLGHKKEKPP PVSPPSPPPI RRVTVITKDE DTLRSVQHFL WMVRLYGTVV FQTSATIATT ILFMLIPWRV TTPYLRDTLP FWSTLLPCAL RCHAYWLERQ RRPGTLMLVM VYTTLTTISV STIGLCFDRT VVIQAYVLSS MLCVWCTGLA WLMAWNMQRR LAILCLLSFM LPILWLFIAV QSWEPYQRII LALTVSFIYG LKIVLIRDTL TVLYRSPSNC YTDGDLLRTA MLLYMDQVIM FLLVVVPLTA PIWYPNYAGA LGRTAHWLFH K
SEQ ID NO: 17: Us7	MRIQLLLVST LVASIVATRV EDMATFRTEK QWQQDLQYRR EFVKRQLAPK PKSNIVVSHT VSCVIDGGNM TSVWRFEGQF NPHIASEVIL HDTSGLYNVP HEVQNDGQVL TVTVKRSAPA DIAKVLISLK PVQLSSGQYE CRPQLQLPWV PRPSSFMYDS YRLWYEKRWL TIILYVFMWT YLVTLLQYCI VRFIGTRLFY FLQRNITIRF TGKPTYNLLT YPVKG
SEQ ID NO: 18:Us8	MRRWLRLLVG LGCCWVTLAH AGNPYEDDDY YYYREDEPRQ HGEPNYVAPP ARQFRFPPLN NVSSYQASCV VKDGVLDAVW RVQGTFYPEK GIVARVGWSG RRGRKWGRLH APECLVETTE AVFRLRQWVP TDLDHLTLHL VPCTKCKPMW CQPRYHIRYF SYGNSVDNLR RLHYEYRHLE LGVVIAIQMA MVLLLGYVLA RTVYRVSSAY YLRWHACVPQ KCEKSLC
SEQ ID NO: 19: Us6	MDLLIRLGFL LMCALPTPGE RSSRDPKTLL SLSPRQQACV PRTKSHRPVC YNDTGDCTDA DDSWKQLGED FAHQCLQAAK KRPKTHKSRP NDRNLEGRLT

	CQRVRRLLPC DLDIHPSHRL LTLMNNCVCD GAVWNAFRLI ERHGFFAVTL YLCCGITLLV VILALLCSIT YESTGRGIRR CGS
SEQ ID NO: 20: UL48	MDLLVDELFA DMNADGASPP PPRPAGGPKN TPAAPPLYAT GRLSQAQLMP SPPMPVPPAA LFNRLLDDLG FSAGPALCTM LDTWNEDLFS ALPTNADLYR ECKFLSTLPS DVVEWGDAYV PERTQIDIRA HGDVAFPTLP ATRDGLGLYY EALSRFFHAE LRAREESYRT VLANFCSALY RYLRASVRQL HRQAHMRGRD RDLGEMLRAT IADRYYRETA RLARVLFLHL YLFLTREILW AAYAEQMMRP DLFDCLCCDL ESWRQLAGLF QPFMFVNGAL TVRGVPIEAR RLRELNHIRE HLNLPLVRSA ATEEPGAPLT TPPTLHGNQA RASGYFMVLI RAKLDSYSSF TTSPSEAVMR EHAYSRARTK NNYGSTIEGL LDLPDDDAPE EAGLAAPRLS FLPAGHTRRL STAPPTDVSL GDELHLDGED VAMAHADALD DFDLDMLGDG DSPGPGFTPH DSAPYGALDM ADFEFEQMFT DALGIDEYGG
SEQ ID NO: 21: FcγRIIA	MTMETQMSQN VCPRNLWLLQ PLTVLLLLAS ADSQAAAPPK AVLKLEPPWI NVLQEDSVTL TCQGARSPES DSIQWFHNGN LIPTHTQPSY RFKANNNDSG EYTCQTGQTS LSDPVHLTVL SEWLVLQTPH LEFQEGETIM LRCHSWKDKP LVKVTFFQNG KSQKFSHLDP TFSIPQANHS HSGDYHCTGN IGYTLFSSKP VTITVQVPSM GSSSPMGIIV AVVIATAVAA IVAAVVALIY CRKKRISANS TDPVKAAQFE PPGRQMIAIR KRQLEETNND YETADGGYMT LNPRAPTDDD KNIYLTLPPN DHVNSNN
SEQ ID NO: 22: FcγRIIB	MGILSFLPVL ATESDWADCK SPQPWGHMLL WTAVLFLAPV AGTPAAPPKA VLKLEPQWIN VLQEDSVTLT CRGTHSPESD SIQWFHNGNL IPTHTQPSYR FKANNNDSGE YTCQTGQTSL SDPVHLTVLS EWLVLQTPHL EFQEGETIVL RCHSWKDKPL VKVTFFQNGK SKKFSRSDPN FSIPQANHSH SGDYHCTGNI GYTLYSSKPV TITVQAPSSS PMGIIVAVVT GIAVAAIVAA VVALIYCRKK RISALPGYPE CREMGETLPE KPANPTNPDE ADKVGAENTI TYSLLMHPDA LEEPDDQNRI
SEQ ID NO: 23: gI	MPCRPLQGLV LVGLWVCATS LVVRGPTVSL VSNSFVDAGA LGPDGVVEED LLILGELRFV GDQVPHTTYY DGGVELWHYP MGHKCPRVVH VVTVTACPRR PAVAFALCRA TDSTHSPAYP TLELNLAQQP LLRVQRATRD YAGVYVLRVW VGDAPNASLF VLGMAIAAEG TLAYNGSAYG SCDPKLLPSS APRLAPASVY QPAPNQASTP STTTSTPSTT IPAPSTTIPA PQASTTPFPT GDPKPQPPGV NHEPPSNATR ATRDSRYALT VTQIIQIAIP ASIIALVFLG SCICFIHRCQ RRYRRSRRPI YSPQMPTGIS CAVNEAAMAR LGAELKSHPS TPPKSRRRSS RTPMPSLTAI AEESEPAGAA GLPTPPVDPT TPTPTPPLLV
SEQ ID NO:	MWQLLLPTAL LLLVSAGMRT EDLPKAVVFL EPQWYRVLEK DSVTLKCQGA YSPEDNSTQW

24: FcγRIIIa	FHNESLISSQ ASSYFIDAAT VDDSGEYRCQ TNLSTLSDPV QLEVHIGWLL LQAPRWVFKE EDPIHLRCHS WKNTALHKVT YLQNGKGRKY FHHNSDFYIP KATLKDSGSY FCRGLVGSKN VSSETVNITI TQGLAVSTIS SFFPPGYQVS FCLVMVLLFA VDTGLYFSVK TNIRSSTRDW KDHKFKWRKD PQDK
SEQ ID NO: 25; UL1 forward	gtctacacta gtatggggat tttgggttgg gtcggg
SEQ ID NO: 26; ICP6	MASRPAASSP VEARAPVGGQ EAGGPSAATQ GEAAGAPLAH GHHVYCQRVN GVMVLSDKTP GSASYRISDS NFVQCGSNCT MIIDGDVVRG RPQDPGAAAS PAPFVAVTNI GAGSDGGTAV VAFGGTPRRS AGTSTGTQTA DVPAEALGGP PPPPRFTLGG GCCSCRDTRR RSAVFGGEGD PVGPAEFVSD DRSSDSDSDD SEDTDSETLS HASSDVSGGA TYDDALDSDS SSDDSLQIDG PVCRPWSNDT APLDVCPGTP GPGADAGGPS AVDPHAPTTG AGAGLAADPA VARDDAEGLS DPRPRLGTGT AYPVPLELTP ENAEAVARFL GDAVNREPAL MLEYFCRCAR EETKRVPPRT FCSPPRLTED DFGLLNYALV EMQRLCLDVP PVPPNAYMPY YLREYVTRLV NGFKPLVSRS VRLYRILGVL VHLRIRTREA SFEEWLRSKE VALDFGLTER LREHEAQLVI LAQALDHYDC LIHSTPHTLV ERGLQSALKY EEFYLKRFGG HYMESVFQMY TRIAGFLACR ATRGMRHIAL GREGSWWEMF KFFFHRLYDH QIVPSTPAML NLGTRNYYTS SCYLVNPQAT TNKATLRAIT SNVSAILARN GGIGLCVQAF NDSGPGTASV MPALKVLDSL VAAHNKESAR PTGACVYLEP WHTDVRAVLR MKGVLAGEEA QRCDNIFSAL WMPDLFFKRL IRHLDGEKNV TWTLFDRDTS MSLADFHGEE FEKLYQHLEV MGFGEQIPIQ ELAYGIVRSA ATTGSPFVMF KDAVNRHYIY DTQGAAIAGS NLCTEIVHPA SKRSSGVCNL GSVNLARCVS RQTFDFGRLR DAVQACVLMV NIMIDSTLQP TPQCTRGNDN LRSMGIGMQG LHTACLKLGL DLESAEFQDL NKHIAEVMLL SAMKTSNALC VRGARPFNHF KRSMYRAGRF HWERFPDARP RYEGEWEMLR QSMMKHGLRN SQFVALMPTA ASAQISDVSE GFAPLFTNLF SKVTRDGETL RPNTLLLKEL ERTFSGKRLL EVMDSLDAKQ WSVAQALPCL EPTHPLRRFK TAFDYDQKLL IDLCADRAPY VDHSQSMTLY VTEKADGTLP ASTLVRLLVH AYKRGLKTGM YYCKVRKATN SGVFGGDDNI VCTSCAL
SEQ ID NO: 27 UL1 forward	gtctacacta gtatggggat tttgggttgg gtcggg

SEQ ID NO: 28: UL1 reverse	gtctacttaa ttaagatgcg ccgggagtgg ggtcgtc
SEQ ID NO: 29: UL2 forward	gtctacacta gtatgaagcg ggcctgcagc cgaag
SEQ ID NO: 30: UL2 reverse	gtctacttaa ttaaaaccga ccagtcgatg ggtg
SEQ ID NO: 31: UL3 forward	gtctacacta gtatggttaa acctctggtc tcatac
SEQ ID NO: 32: UL3 reverse	gtctacttaa ttaactcggc ccccgaggcc agcatg
SEQ ID NO: 33: UL4 forward	gtctacacta gtatgtccaa tccacagacg accatc
SEQ ID NO: 34: UL4 reverse	gtctacttaa ttaaggaccc caaaagtttg tctgcg
SEQ ID NO: 35: UL5 forward	gtctacacta gtatggcggc ggccggcggg gag
SEQ ID NO: 36: UL5 reverse	gtctacttaa ttaaatatac aatgaccacg ttcggatcg
SEQ ID NO: 37: UL6 forward	gtctacacta gtatgaccgc accacgctcg cgg
SEQ ID NO: 38: UL6 reverse	gtctacttaa ttaatcgtcg gccgtcgcgg cggccatcc
SEQ ID NO: 39: UL7 forward	gtctacacta gtatggccgc cgcgacggcc gac
SEQ ID NO: 40: UL7 reverse	gtctacttaa ttaaacaaaa ctgataaaac agcgacg
SEQ ID NO: 41: UL8 forward	gtctacacta gtatggacac cgcagatatc gtgtgg
SEQ ID NO: 42: UL8 reverse	gtctacttaa ttaaggcaaa cagaaacgac atcttg
SEQ ID NO: 43: UL9 forward	gtctacacta gtatgccttt cgtgggggggc gcggag
SEQ ID NO:	gtctacttaa ttaatagggt gctaaagttc accg

44: UL9 reverse	
SEQ ID NO: 45: UL10 forward	gtctacacta gtatgggacg cccggccccc ag
SEQ ID NO: 46: UL10 reverse	gtctacttaa ttaaccaacg gcggacggtg ctgtac
SEQ ID NO: 47: UL11 forward	gtctacacta gtatgggcct ctcgttctcc ggggc
SEQ ID NO: 48: UL11 reverse	gtctacttaa ttaattcgct atcggacatg gggggtg
SEQ ID NO: 49: UL12 forward	gtctacacta gtatggagtc cacggtaggc cc
SEQ ID NO: 50: UL12 reverse	gtctacttaa ttaagcgaga cgacctcccc gtcg
SEQ ID NO: 51: UL13 forward	gtctacacta gtatggatga gtcccgcaga cagcg
SEQ ID NO: 52: UL13 reverse	gtctacttaa ttaacgacag cgcgtgccgc gcgcac
SEQ ID NO: 53: UL14 forward	gtctacacta gtatggaccg agatgccgcc cacg
SEQ ID NO: 54: UL14 reverse	gtctacttaa ttaattcgcc atcgggatag tcccg
SEQ ID NO: 55: UL15 forward	gtctacacta gtatgtttgg tcagcagctg gcgtc
SEQ ID NO: 56: UL15 reverse	gtctacttaa ttaacgaaac gcgtgtgatg ggagcg
SEQ ID NO: 57: UL16 forward	gtctacacta gtatggcgca gctgggaccc cggcg
SEQ ID NO: 58: UL16 reverse	gtctacttaa ttaattcggg atcgcttgag gaggcccg
SEQ ID NO: 59: UL17 forward	gtctacacta gtatgaacgc gcacttggcc aacgaggtc
SEQ ID NO: 60: UL17	gtctacttaa ttaagcgaga acggccgttc ccgga

reverse	
SEQ ID NO: 61: UL18 forward	gtctacacta gtatgctggc ggacggcttt gaaac
SEQ ID NO: 62: UL18 reverse	gtctacttaa ttaagggata gcgtataacg gg
SEQ ID NO: 63: UL19 forward	gtctacacta gtatggccgc tcccaaccgc gaccc
SEQ ID NO: 64: UL19 reverse	gtctacttaa ttaacagagc cagtcccttg agcggggatg
SEQ ID NO: 65: UL20 forward	gtctacacta gtatgaccat gcgggatgac cttcctc
SEQ ID NO: 66: UL20 reverse	gtctacttaa ttaagaacgc gacgggtgca ttcaag
SEQ ID NO: 67: UL21forward	gtctacacta gtatggagct tagctacgcc acc
SEQ ID NO: 68: UL21 reverse	gtctacttaa ttaacacaga ctgtccgtgt ttgg
SEQ ID NO: 69: UL22 forward	gtctacacta gtatggggaa tggtttatgg ttcg
SEQ ID NO: 70: UL22 reverse	gtctacttaa ttaattcgcg tctccaaaaa aacgggacac
SEQ ID NO: 71: UL23 forward	gtctacacta gtatggcttc gtacccctgc catc
SEQ ID NO: 72: UL23 reverse	gtctacttaa ttaagttagc ctcccccatc tcccgggcaa acg
SEQ ID NO: 73: UL24forward	gtctacacta gtatggccgc gagaacgcgc agc
SEQ ID NO: 74: UL24 reverse	gtctacttaa ttaattcgga ggcggctcgg ggtttg
SEQ ID NO: 75: UL25 forward	gtctacacta gtatggaccc gtactgccca tttg
SEQ ID NO: 76: UL25 reverse	gtctacttaa ttaaaaccgc cgacaggtac tgtgg

SEQ ID NO: 77: UL26 forward	gtctacacta gtatggcagc cgatgccccg ggag
SEQ ID NO: 78: UL26 reverse	gtctacttaa ttaagcgggc ccccatcatc tgagag
SEQ ID NO: 79: UL26.5 forward	gtctacacta gtatgaaccc cgttccggca tcgggc
SEQ ID NO: 80: UL26.5 reverse	gtctacttaa ttaagcgggc ccccatcatc tgagag
SEQ ID NO: 81: UL27 forward	gtctacacta gtatgcgcca gggcgccccc gc
SEQ ID NO: 82: UL27 reverse	gtctacttaa ttaacaggtc gtcctcgtcg gcgtc
SEQ ID NO: 83: UL28 forward	gtctacacta gtatggccgc cccggtgtcc gagccc
SEQ ID NO: 84: UL28 reverse	gtctacttaa ttaacggggg cccgtcgtgc cccc
SEQ ID NO: 85: UL29 forward	gtctacacta gtatggagac aaagcccaag acggc
SEQ ID NO: 86: UL29 reverse	gtctacttaa ttaacagcat atccaacgtc aggtctc
SEQ ID NO: 87: UL30 forward	gtctacacta gtatgttttc cggtggcggc gg
SEQ ID NO: 88: UL30 reverse	gtctacttaa ttaatgctag agtatcaaag gctc
SEQ ID NO: 89: UL31forward	gtctacacta gtatgtatga caccgacccc catc
SEQ ID NO: 90: UL31 reverse	gtctacttaa ttaacggcgg aggaaactcg tcgaatg
SEQ ID NO: 91: UL32forward	gtctacacta gtgcccagcc atggcaactt cg
SEQ ID NO: 92: UL32 reverse	gtctacttaa ttaatacata ggtacacagg gtgtgc
SEQ ID NO:	gtctacacta gtgaagttgc catggctggg c

93: UL33 forward	
SEQ ID NO: 94: UL33 reverse	gtctacttaa ttaagccccg cagaatctgg tgcaggtc
SEQ ID NO: 95: UL34 forward	gtctacacta gtatggcggg actgggcaag ccc
SEQ ID NO: 96: UL34 reverse	gtctacttaa ttaataggcg cgcgccagca ccaac
SEQ ID NO: 97: UL35 forward	gtctacacta gtatggccgt cccgcaattt cac
SEQ ID NO: 98: UL35 reverse	gtctacttaa ttaacggggt cccgggcgtc gaagg
SEQ ID NO: 99: UL36 forward	gtctacacta gtatgatcgc gggcacccca ccgcac
SEQ ID NO: 100: UL36 reverse	gtctacttaa ttaagcccag taacatgcgc acgtgatg
SEQ ID NO: 101: UL37 forward	gtctacacta gtatggcaga ccgcggtctc ccgtccg
SEQ ID NO: 102: UL37 reverse	gtctacttaa ttaattggta actcgttaac ggcaagtc
SEQ ID NO: 103: UL38 forward	gtctacacta gtatgaagac caatccgcta cccg
SEQ ID NO: 104: UL38reverse	gtctacttaa ttaacgcgca tgcccgccac tcgcc
SEQ ID NO: 105: UL39 forward	gtctacacta gtatggccag ccgcccagcc gc
SEQ ID NO: 106: UL39 reverse	gtctacttaa ttaacagcgc gcagctcgtg cagac
SEQ ID NO: 107: UL40 forward	gtctacacta gtatggattc cgcggcccca g
SEQ ID NO: 108: UL40 reverse	gtctacttaa ttaacagatc gttgacgacc gc
SEQ ID NO: 109: UL41	gtctacacta gtatgggttt gttcgggatg atgaag

forward	
SEQ ID NO: 110: UL41 reverse	gtctacttaa ttaactcgtc ccagaatttg gccag
SEQ ID NO: 111: UL42 forward	gtctacacta gtatgacgga ttcccctggc ggtg
SEQ ID NO: 112: UL42 reverse	gtctacttaa ttaaggggaa tccaaaacca gac
SEQ ID NO: 113: UL43 forward	gtctacacta gtatgctccg caacgacagc cacc
SEQ ID NO: 114: UL43 reverse	gtctacttaa ttaaatcgcc cgaccgcccg cccgttg
SEQ ID NO: 115: UL44 forward	gtctacacta gtgctttgcc gggaacgcta gc
SEQ ID NO: 116: UL44 reverse	gtctacttaa ttaaccgccg atgacgctgc cgcgac
SEQ ID NO: 117: UL45 forward	gtctacacta gtatgcctct gcgggcatcg gaac
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SEQ ID NO: 119: UL46 forward	gtctacacta gtctggacgc ggcataactc cgac
SEQ ID NO: 120: UL46 reverse	gtctacttaa ttaaccggct ccggcgtcct tcgcgtttaa g
SEQ ID NO: 121: UL47 forward	gtctacacta gtatgtcggc tcgcgaaccc gc
SEQ ID NO: 122: UL47 reverse	gtctacttaa ttaatgggcg tggcgggcct cccag
SEQ ID NO: 123: UL48 forward	gtctacacta gtatggacct cttggtcgac gagctg
SEQ ID NO: 124: UL48 reverse	gtctacttaa ttaacccacc gtactcgtca attccaag
SEQ ID NO: 125: UL49 forward	gtctacacta gtatgacctc tcgccgctcc gtgaag

SEQ ID NO: 126: UL49 reverse	gtctacttaa ttaactcgac gggccgtctg g
SEQ ID NO: 127: UL49A forward	gtctacacta gtctcatctt cctgttaggg acgatg
SEQ ID NO: 128: UL49A reverse	gtctacttaa ttaaggcgtg cccggcagcc agtag
SEQ ID NO: 129: UL50 forward:	gtctacacta gtgtccctaa caggaagatg agtcag
SEQ ID NO: 130: UL50 reverse	gtctacttaa ttaaaatacc ggtagagcca aaacc
SEQ ID NO: 131: UL51 forward	gtctacacta gtatggcttc tcttctcggg gc
SEQ ID NO: 132: UL51 reverse	gtctacttaa ttaattgacc caaaacacac ggagctgc
SEQ ID NO: 133: UL52 forward	gtctacacta gtatggggca ggaagacggg aac
SEQ ID NO: 134: UL52 reverse	gtctacttaa ttaaagacga cggttgagag gtgctgc
SEQ ID NO: 135: UL53 forward	gtctacacta gtatgctcgc cgtccgttcc ctgcag
SEQ ID NO: 136: UL53 reverse	gtctacttaa ttaatacatc aaacaggcgc ctctggatc
SEQ ID NO: 137: UL54 forward	gtctacacta gtatggcgac tgacattgat atgctaattg
SEQ ID NO: 138: UL54 reverse	gtctacttaa ttaaaaacag ggagttgcaa taaaaatatt tgc
SEQ ID NO: 139: UL55 forward	gtctacacta gtcttttgca ctatgacagc gacc
SEQ ID NO: 140: UL55 reverse	gtctacttaa ttaacgcctt aattttaatc ttgac
SEQ ID NO: 141: UL56 forward	gtctacacta gtcatccatg gcttcggagg cggcgc
SEQ ID NO:	gtctacttaa ttaaccgcca caggaatacc agaataatg

142: UL56 reverse	
SEQ ID NO: 143: RS1 forward	gtctacacta gtatggcgtc ggagaacaag cagcg
SEQ ID NO: 144: RS1 reverse	gtctacttaa ttaacagcac cccgtccccc tcgaacgcg
SEQ ID NO: 145: US1 forward	gtctacacta gtatggccga catttcccca gg
SEQ ID NO: 146 US1 reverse:	gtctacttaa ttaacggccg gagaaacgtg tcgctg
SEQ ID NO: 147: US2 forward	gtctacacta gtatgggcgt tgttgtcgtc aacg
SEQ ID NO: 148: US2 reverse	gtctacttaa ttaacagggt ggtaaccgga tagcagatg
SEQ ID NO: 149: US3 forward	gtctacacta gtatggcctg tcgtaagttt tgtcg
SEQ ID NO: 150: US3 reverse	gtctacttaa ttaatttctg ttgaaacagc ggcaaac
SEQ ID NO: 151: US4 forward	gtctacacta gtcatcatgt cgccgggcgc catg
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SEQ ID NO: 155: US6 forward	gtctacacta gtgtgtggtg cgttccggta tg
SEQ ID NO: 156: US6 reverse	gtctacttaa ttaagtaaaa caagggctgg tgcgag
SEQ ID NO: 157: US7 forward	gtctacacta gtgtcccgtt ccgggatgcc gtg
SEQ ID NO: 158: US7	gtctacttaa ttaataccaa caggggaggc gttg

reverse	
SEQ ID NO: 159: US8 forward	gtctacacta gtgacatgga tcgcggggcg gtg
SEQ ID NO: 160: US8 reverse	gtctacttaa ttaaccagaa gacggacgaa tcgg
SEQ ID NO: 161: US8A forward	gtctacacta gtatggatcc ggctttgaga tc
SEQ ID NO: 162: US8A reverse	gtctacttaa ttaatgcgcc tcgggcaatt gacgtc
SEQ ID NO: 163: US9 forward	gtctacacta gtatgacgtc ccggctctcc g
SEQ ID NO: 164: US9 reverse	gtctacttaa ttaagcggag cagccacatc aggag
SEQ ID NO: 165: US10 forward	gtctacacta gtgtgataat gatcaagcgg cgg
SEQ ID NO: 166: US10 reverse	gtctacttaa ttaagcacag gggtggggtt agg
SEQ ID NO: 167: US11 forward	gtctacacta gtgtggctct cgagatgagc cag
SEQ ID NO: 168: US11 reverse	gtctacttaa ttaatacaga cccgcgagcc gtacgtg
SEQ ID NO: 169: US12 forward	gtctacacta gtatgtcgtg ggccctggaa atggc
SEQ ID NO: 170: US12 reverse	gtctacttaa ttaaacgggt taccggatta cggggac

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for treating a subject infected with a pathogen encoding an Fc-binding protein, the composition comprising an immunological polypeptide comprising a domain that binds an FcγR on an immune effector cell and a non-overlapping domain that binds the pathogen-encoded Fc binding protein.
2. The pharmaceutical composition of claim 1, wherein the immune effector cell is a B cell, a natural killer (NK) cell, a monocyte, a macrophage, a neutrophil or granulocyte, T cell or a dendritic cell.
3. The pharmaceutical composition of claim 1, wherein the pathogen encoding an Fc-binding protein is herpes simplex virus (HSV), cytomegalovirus, or Varicella zoster virus (VZV).
4. The pharmaceutical composition of claim 3, wherein the pathogen-encoded Fc binding protein comprises a herpesvirus glycoprotein E (gE) or a cytomegalovirus 68kDa-glycoprotein (gp68).
5. The pharmaceutical composition of claim 1, wherein the immunological polypeptide comprising a domain that binds an FcγR on an immune effector cell is an Fc fragment of an IgG antibody.
6. A method of treating a subject infected with a pathogen encoding an Fc-binding protein comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of claim 1.
7. The method of claim 6, wherein the immunological polypeptide comprising a domain that binds an FcγR on an immune effector cell is an Fc fragment of an IgG antibody.
8. A pharmaceutical composition for preventing neurologic damage in a subject with HSV1 infection, the composition comprising an immunological polypeptide comprising a domain that binds an FcγR on an immune effector cell and a non-overlapping domain that binds a herpes simplex virus-encoded Fc binding protein.
9. A method of for preventing neurologic damage in a subject with HSV1 infection comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of claim 8.

10. A pharmaceutical composition for preventing death in a subject with HSV1 infection, the composition comprising an immunological polypeptide comprising a domain that binds an FcγR on an immune effector cell and a non-overlapping domain that binds a herpes simplex virus-encoded Fc binding protein.
11. A method of for preventing death in a subject with HSV1 infection comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of claim 10.
12. A pharmaceutical composition for treating cancer in a subject undergoing oncolytic viral therapy, the composition comprising an immunological polypeptide comprising a domain that binds an FcγR on an immune effector cell and a non-overlapping domain that binds a Fc binding protein on a target cell.
13. A method for treating cancer in a subject undergoing oncolytic viral therapy comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of claim 12.
14. A pharmaceutical composition for enhancing oncolytic viral therapy in a subject, the composition comprising an polypeptide comprising a region that binds to a binds a Fc binding protein on a target cell but does not comprise a region that binds to a Fc gamma receptor (FcγR).
15. A method enhancing oncolytic viral therapy in a subject comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition of claim 14.
16. The method of claim 15, wherein the pharmaceutical composition is administered prior to treatment with an oncolytic viral therapeutic agent.
17. A method of reducing inflammation in a subject receiving anti-cancer therapy comprising:
    (a) administering a therapeutically effective amount of a polypeptide comprising a region that binds to a Fc binding protein but does not comprise a region that binds to a Fc gamma receptor (FcγR); and
    (b) administering an anti-cancer therapy comprising a monoclonal antibody drug.

18. The method of claim 17 wherein the monoclonal antibody drug is rituximab, tocilizumab, tositumomab, trastuzumab bevacizumab, brentuximab vedotin, cetuximab, daratumumab, ipilimumab, ofatumumab, panitumumab, alemtuzumab or pembrolizumab.
19. The method of claim 17, wherein the pharmaceutical composition is administered prior to treatment with the monoclonal antibody drug.
20. The method of claim 17, wherein the pharmaceutical composition comprises an immunological polypeptide comprising a domain that binds an FcγR on an immune effector cell that is an Fc fragment of an IgG antibody.
21. A method increasing efficiency of generating dendritic cells or macrophages from PBMC or monocytes comprising culturing PBMC or monocytes in plates pre-coated with protein A or protein G, or with polymerized protein A or protein G.

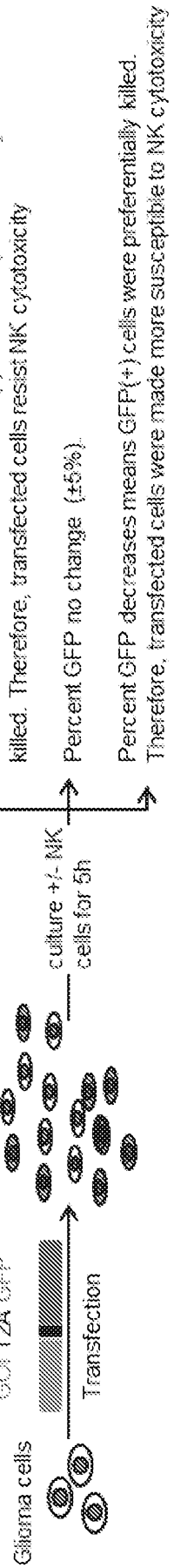

Glioma cells
GOI T2A GFP
Transfection
culture +/- NK cells for 5h
Percent GFP increases means GFP(-) cells were preferentially killed. Therefore, transfected cells resist NK cytotoxicity
Percent GFP no change (±5%).
Percent GFP decreases means GFP(+) cells were preferentially killed. Therefore, transfected cells were made more susceptible to NK cytotoxicity

FIG. 1A

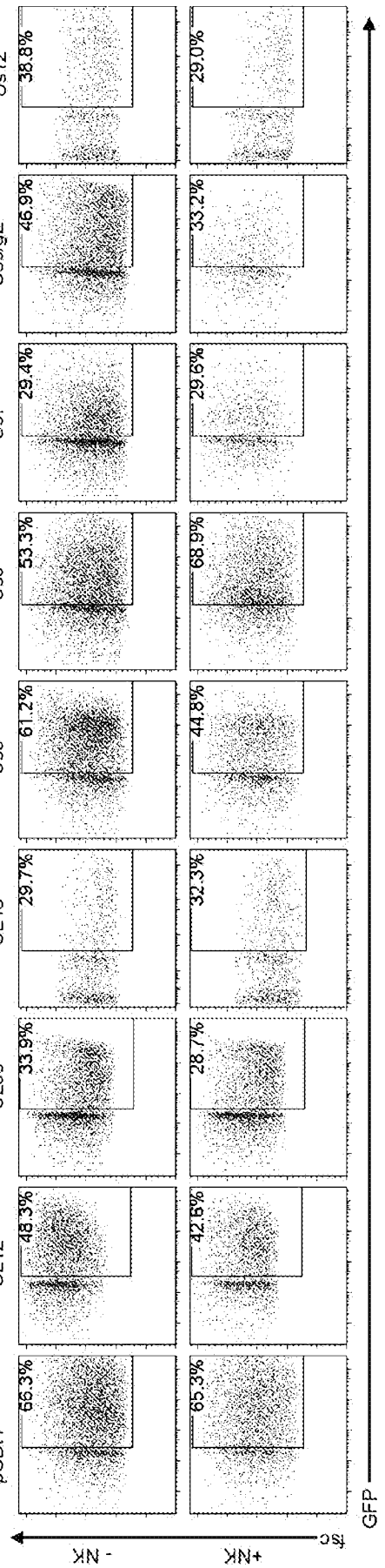

pCDH
UL12
UL30
UL48
Us3
Us6
Us7
Us8/gE
Us12
66.3%
48.3%
33.9%
29.7%
61.2%
53.3%
29.4%
46.9%
38.8%
65.3%
42.6%
28.7%
32.3%
44.8%
68.9%
29.6%
33.2%
29.0%
- NK
+NK
GFP

FIG. 1B

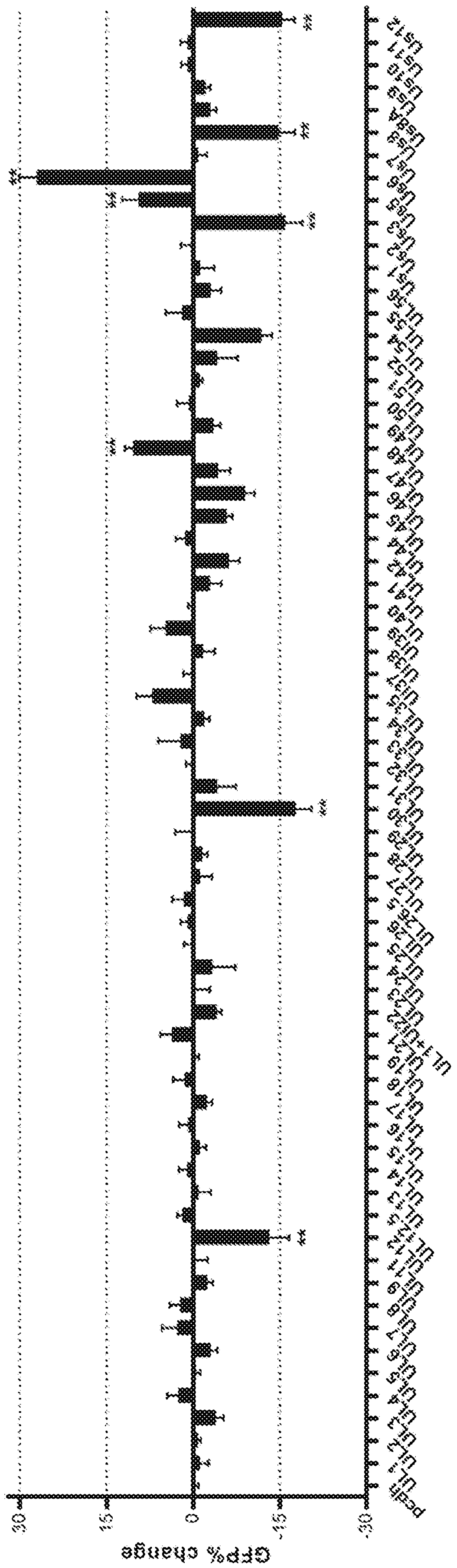


FIG. 1C

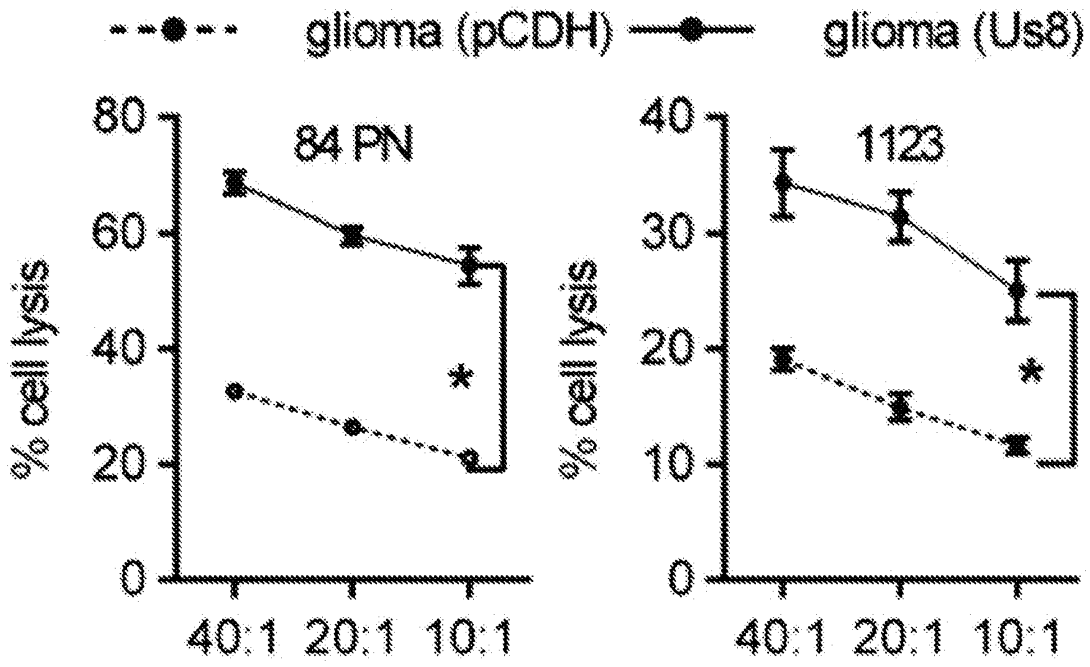

glioma (pCDH)
glioma (Us8)
84 PN
1123
% cell lysis
% cell lysis
80
60
40
20
0
40
30
20
10
0
*
*
40:1 20:1 10:1
40:1 20:1 10:1

FIG. 1D

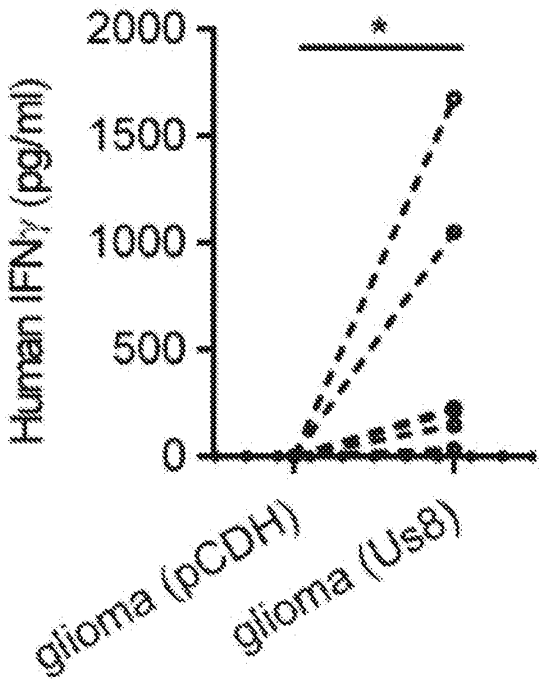

Human IFNγ (pg/ml)
2000
1500
1000
500
0
*
glioma (pCDH)
glioma (Us8)

FIG. 1E

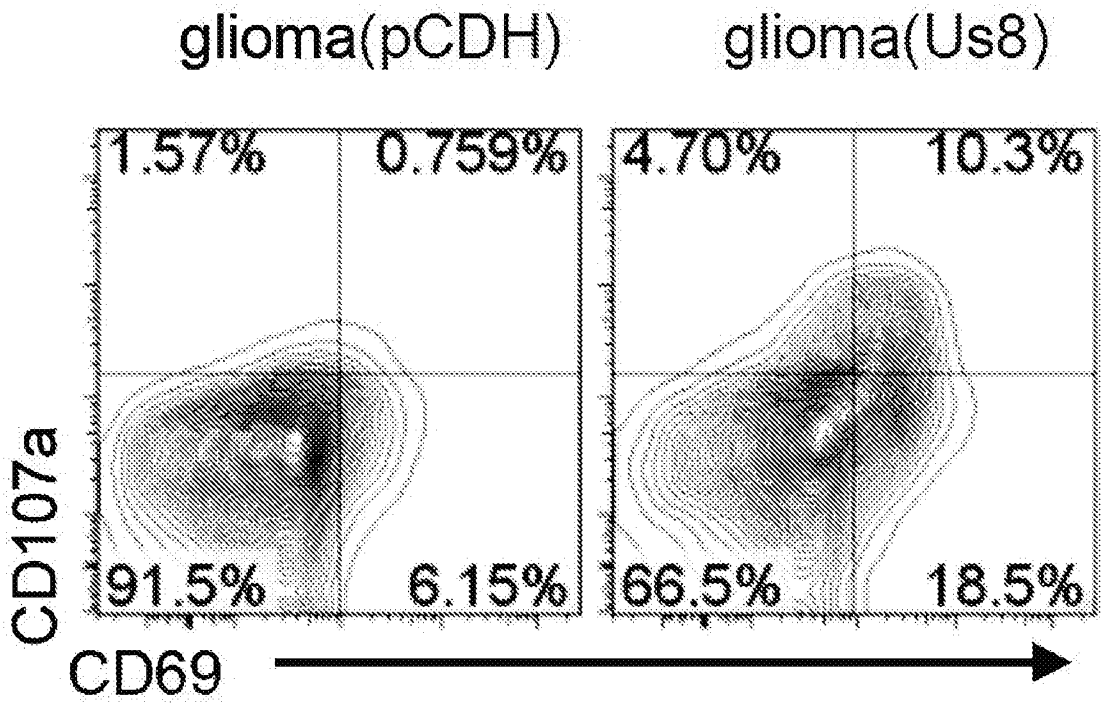

glioma(pCDH)
glioma(Us8)
1.57%
0.759%
4.70%
10.3%
91.5%
6.15%
66.5%
18.5%
CD107a
CD69

FIG. 1F

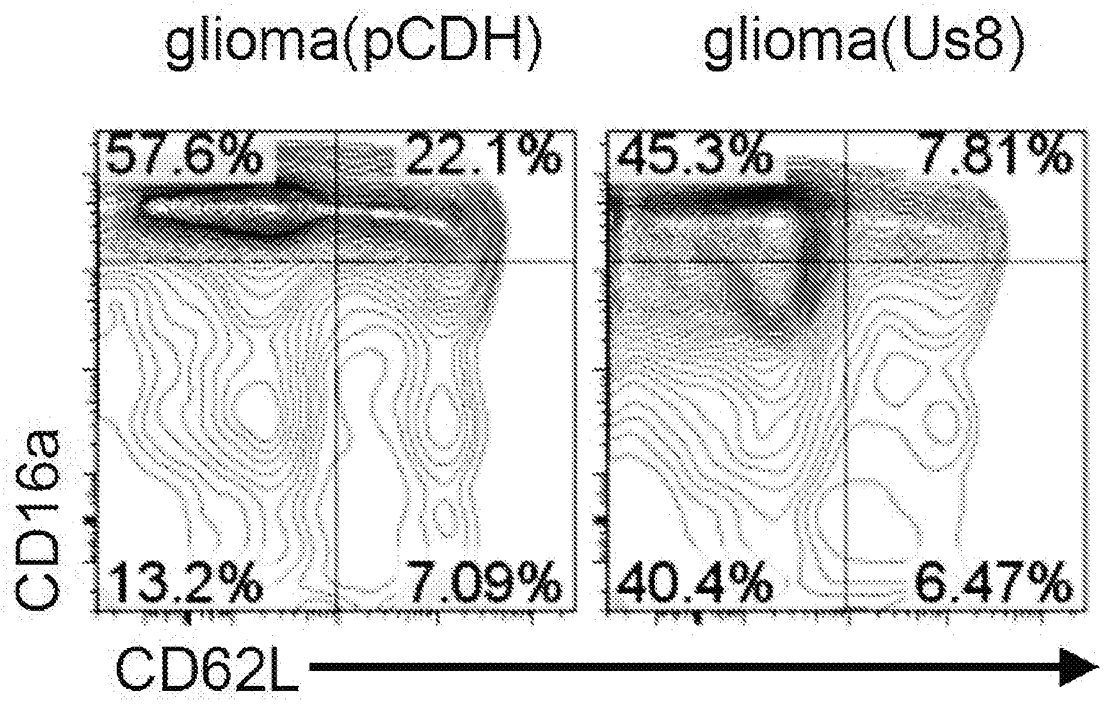

glioma(pCDH)
glioma(Us8)
57.6%
22.1%
45.3%
7.81%
13.2%
7.09%
40.4%
6.47%
CD16a
CD62L

FIG. 1G

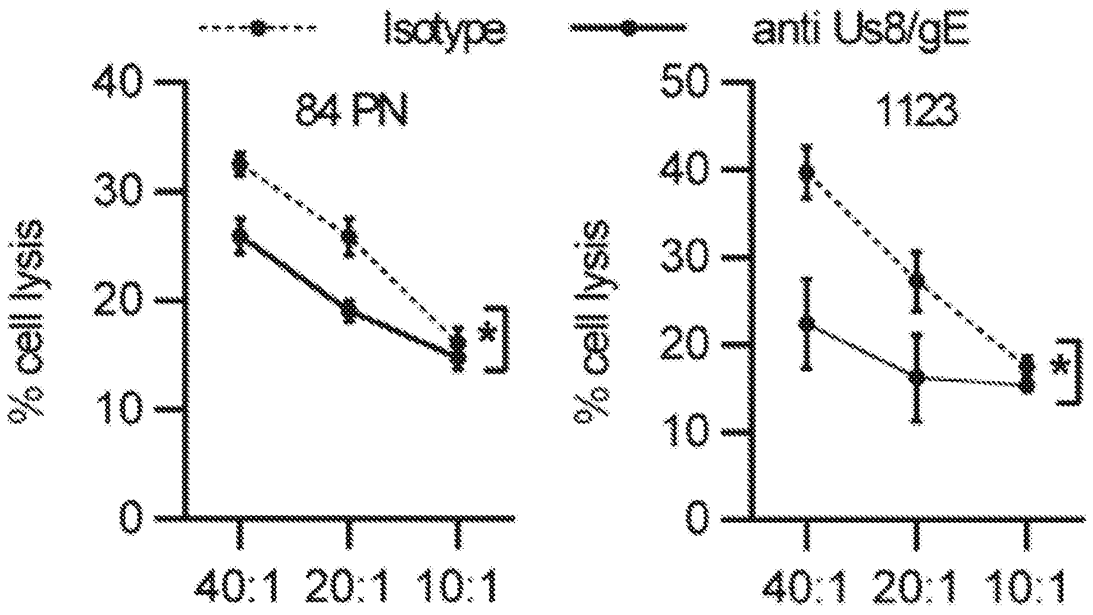

Isotype
anti Us8/gE
84 PN
1123
% cell lysis
% cell lysis
40
30
20
10
0
50
40
30
20
10
0
*
*
40:1 20:1 10:1
40:1 20:1 10:1

FIG. 1H

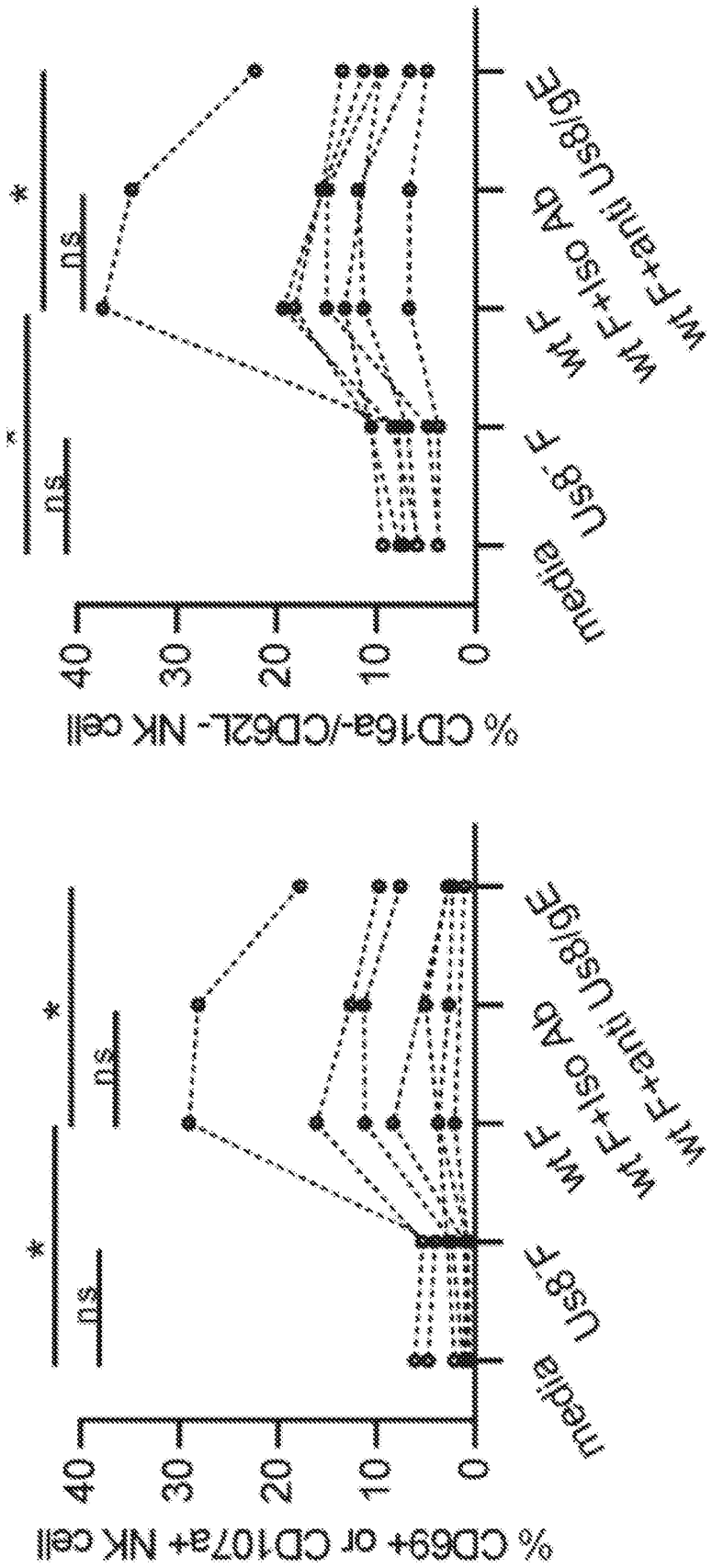

% CD16a-/CD62L- NK cell
0
10
20
30
40
media
Usb F
Wt F
Wt F+Iso Ab
Wt F+anti Usb/gE
ns
ns
*
*
% CD69+ or CD107a+ NK cell
0
10
20
30
40
media
Usb F
Wt F
Wt F+Iso Ab
Wt F+anti Usb/gE
ns
ns
*
*

FIG. 11

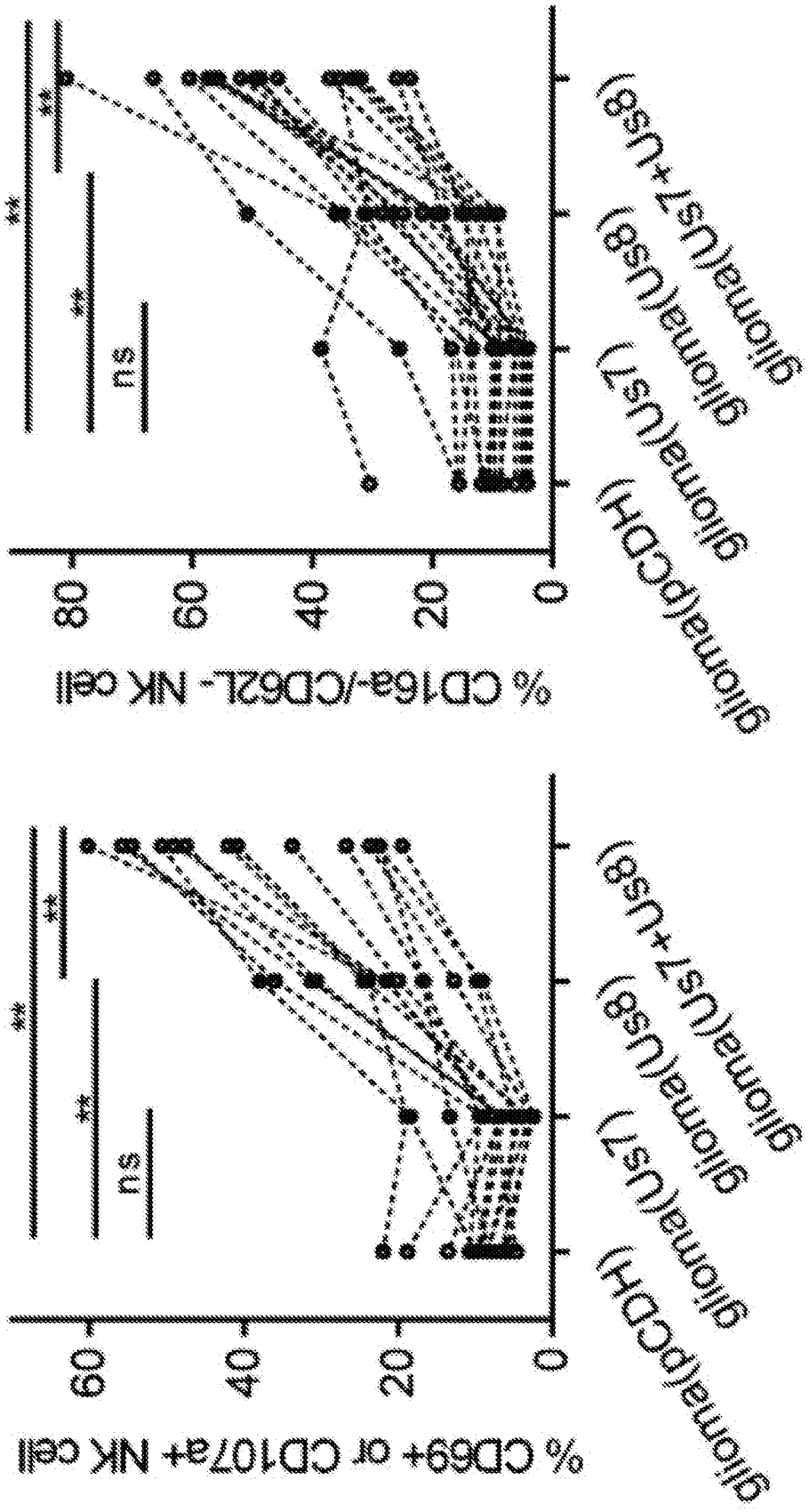

% CD16a-/CD62L- NK cell
80
60
40
20
0
**
**
ns
glioma(pCDH)
glioma(US7)
glioma(US8)
glioma(US7+US8)
% CD69+ or CD107a+ NK cell
60
40
20
0
**
**
ns
glioma(pCDH)
glioma(US7)
glioma(US8)
glioma(US7+US8)

FIG. 2A

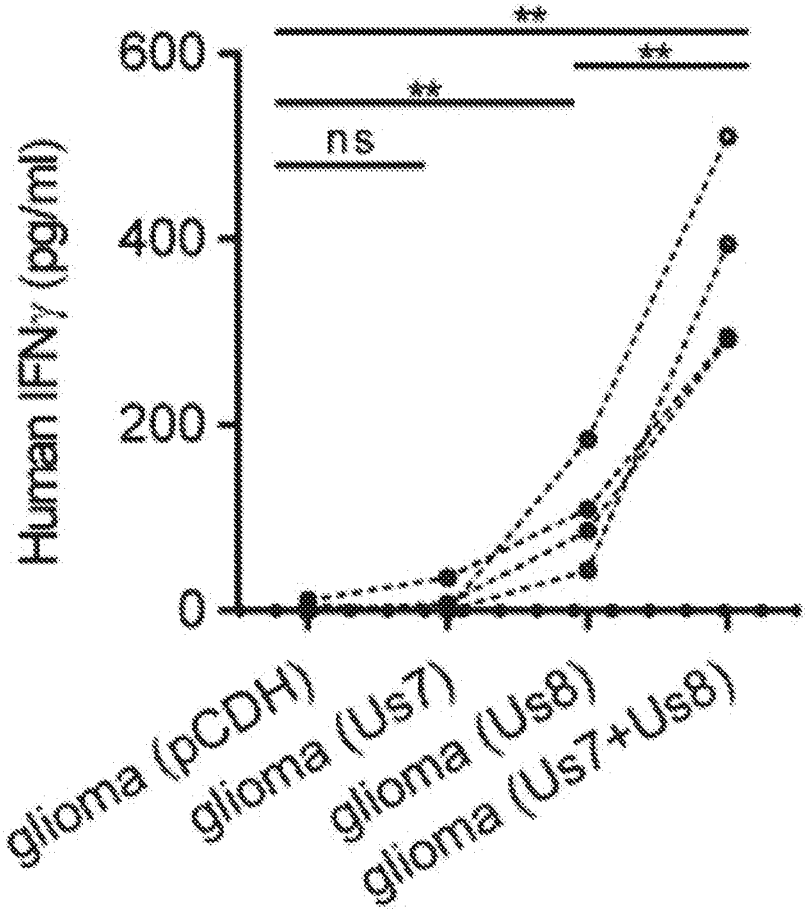

600
400
200
0
Human IFNγ (pg/ml)
**
**
**
ns
glioma (pCDH)
glioma (Us7)
glioma (Us8)
glioma (Us7+Us8)

FIG. 2B

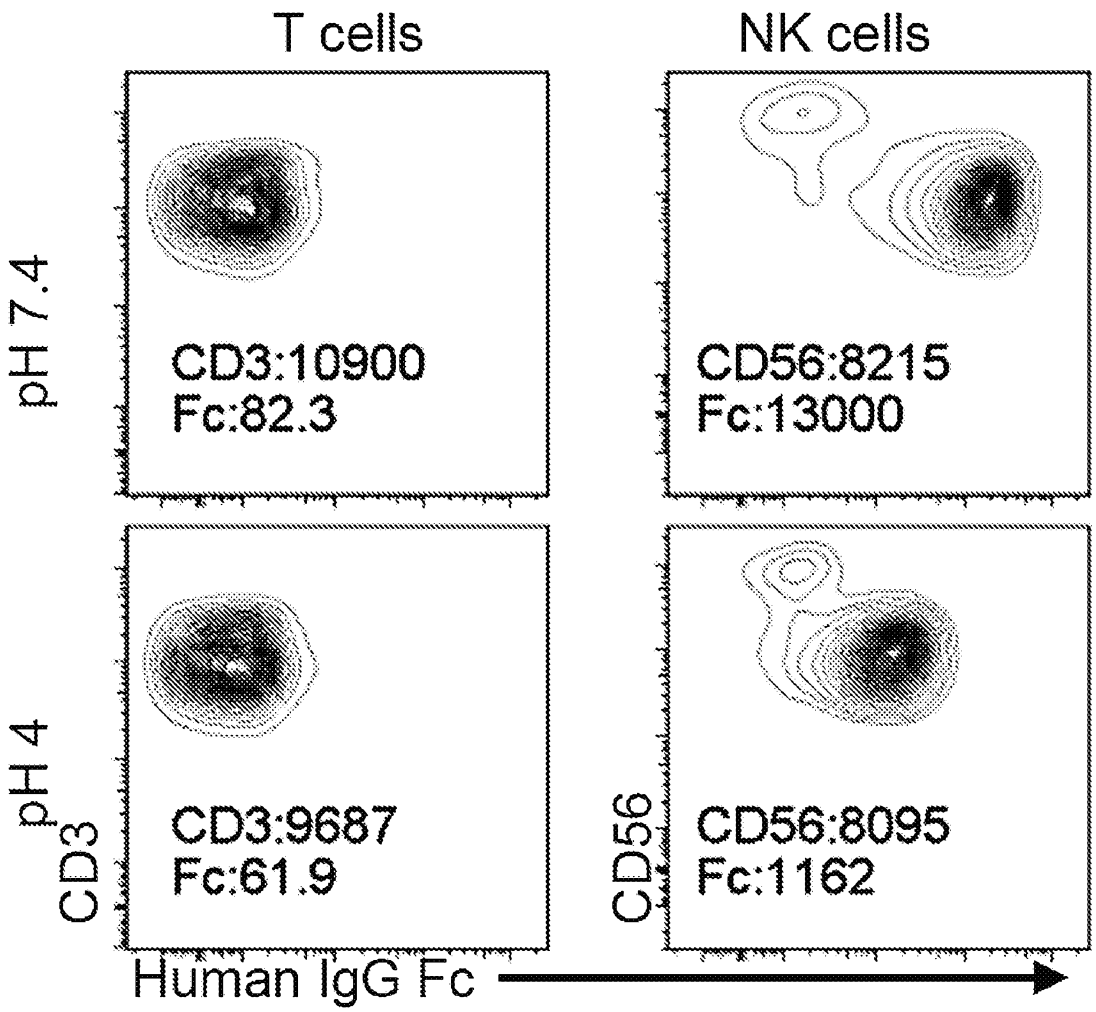

T cells
NK cells
pH 7.4
pH 4
CD3
CD56
CD3:10900
Fc:82.3
CD56:8215
Fc:13000
CD3:9687
Fc:61.9
CD56:8095
Fc:1162
Human IgG Fc

FIG. 2C

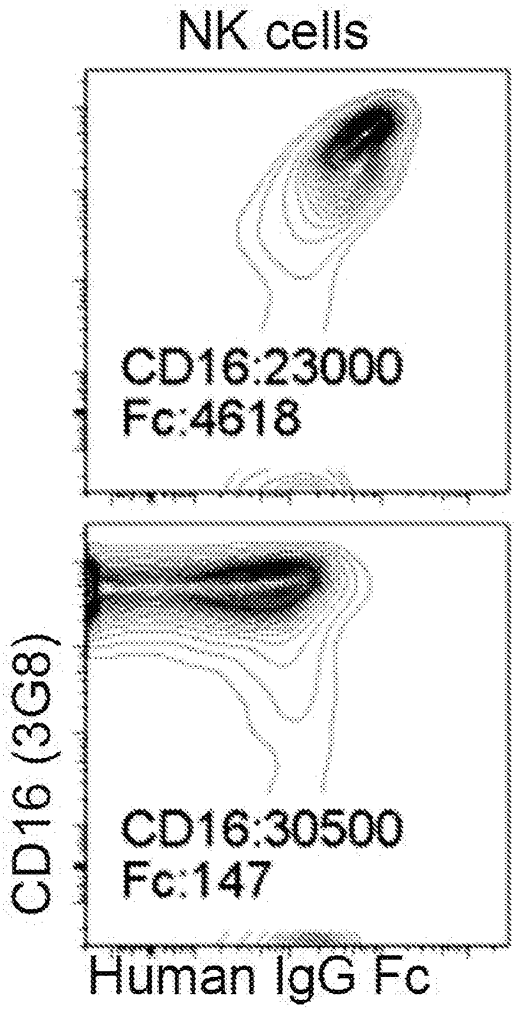

NK cells
CD16:23000
Fc:4618
CD16:30500
Fc:147
CD16 (3G8)
Human IgG Fc

FIG. 2D

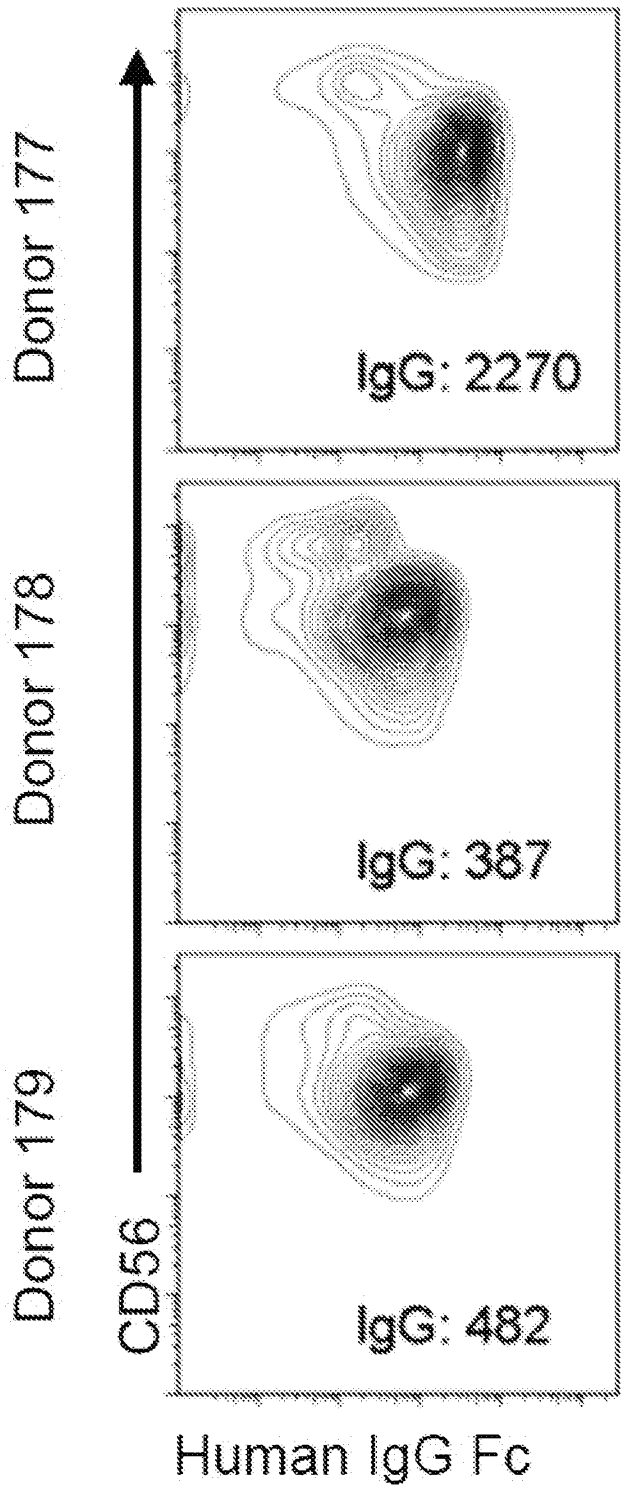

Donor 177
IgG: 2270
Donor 178
IgG: 387
Donor 179
IgG: 482
CD56
Human IgG Fc

FIG. 2E

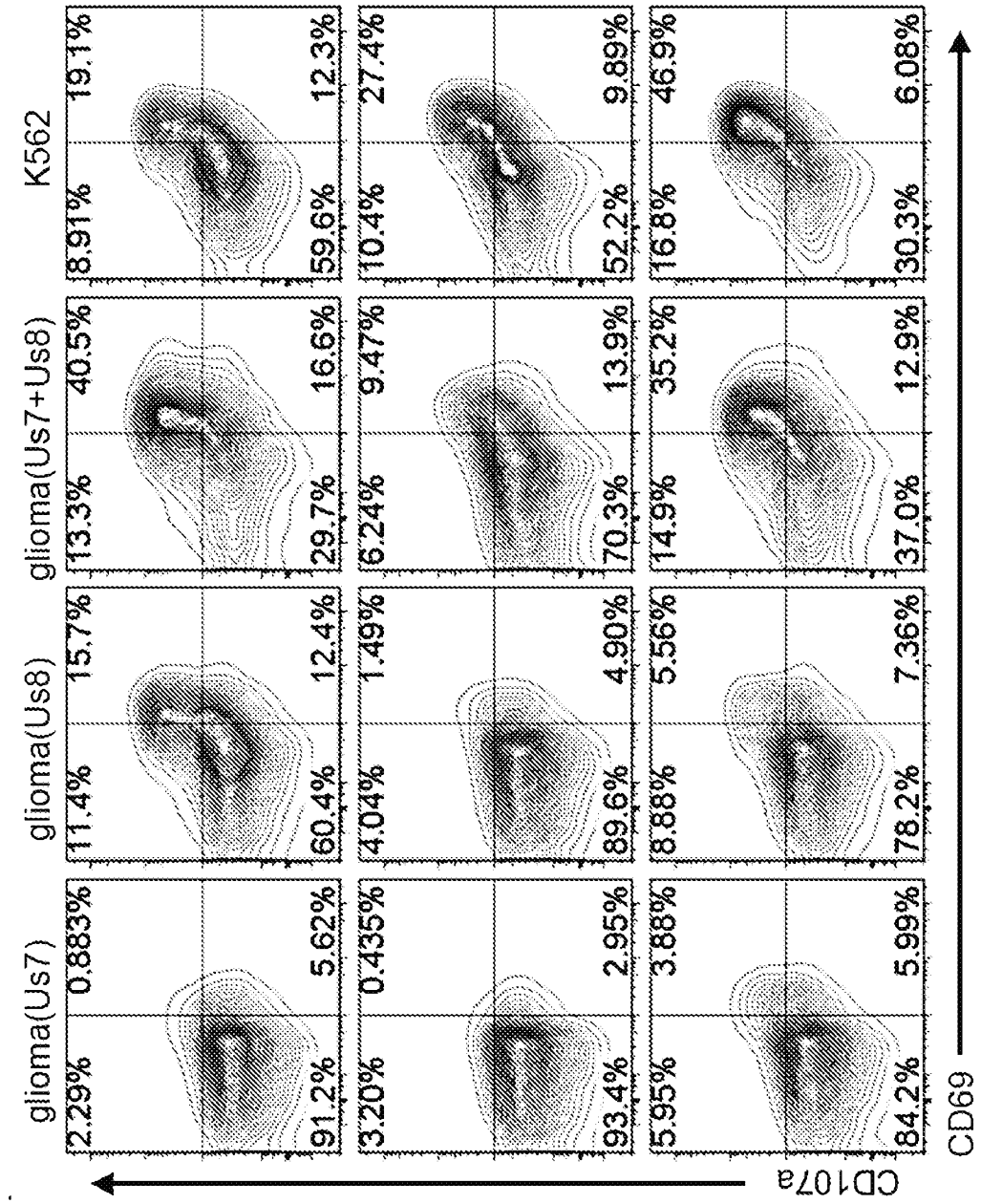

glioma(Us7)
glioma(Us8)
glioma(Us7+Us8)
K562
2.29%
0.883%
91.2%
5.62%
11.4%
15.7%
60.4%
12.4%
13.3%
40.5%
29.7%
16.6%
8.91%
19.1%
59.6%
12.3%
3.20%
0.435%
93.4%
2.95%
4.04%
1.49%
89.6%
4.90%
6.24%
9.47%
70.3%
13.9%
10.4%
27.4%
52.2%
9.89%
5.95%
3.88%
84.2%
5.99%
8.88%
5.56%
78.2%
7.36%
14.9%
35.2%
37.0%
12.9%
16.8%
46.9%
30.3%
6.08%
CD107a
CD69

FIG. 2F

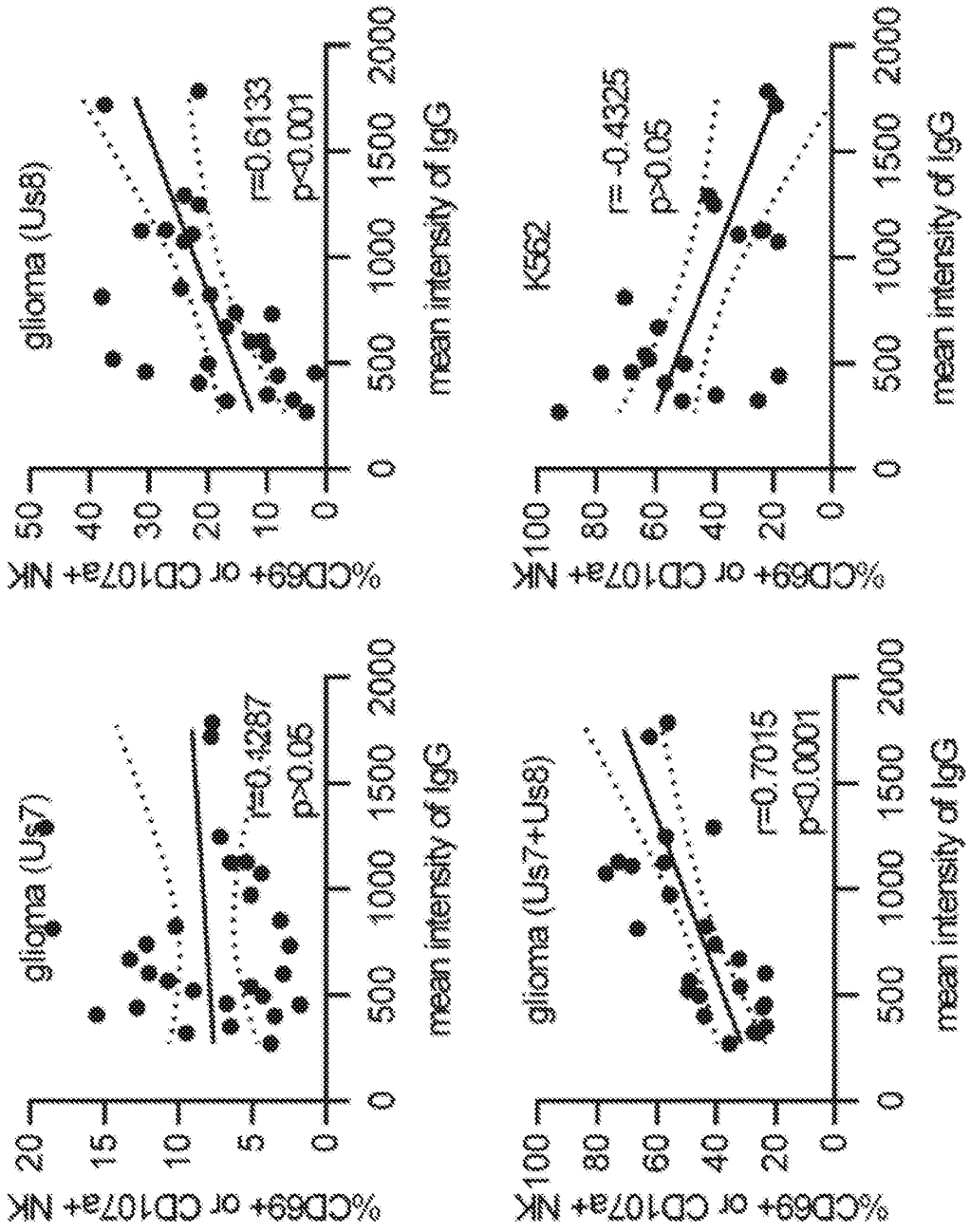

glioma (Us8)
r=0.6133
p<0.001
%CD69+ or CD107a+ NK
mean intensity of IgG
K562
r= -0.4325
p>0.05
glioma (Us7)
r=0.1287
p>0.05
glioma (Us7+Us8)
r=0.7015
p<0.0001

FIG. 2G

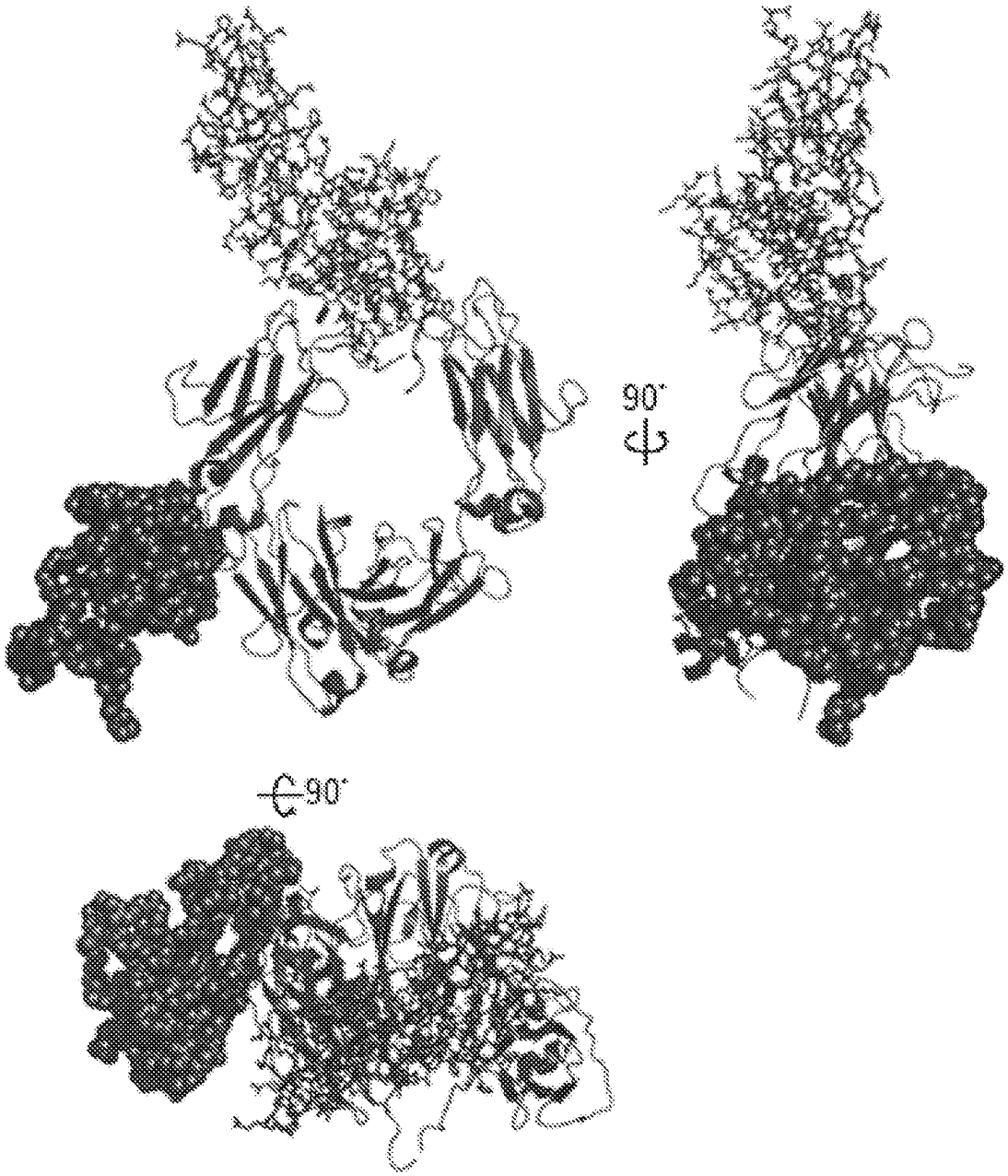


FIG. 3A

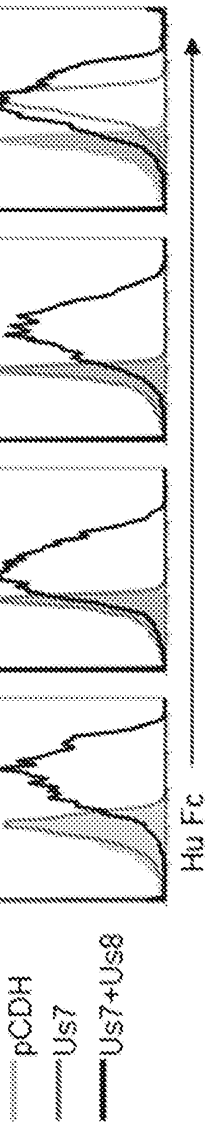

Hu Fc(ΔCD16)
Hu IgG1 Fc
rituximab
anti HSV1 IgG
pCDH
Us7
Us7+Us8
Hu Fc

FIG. 3B

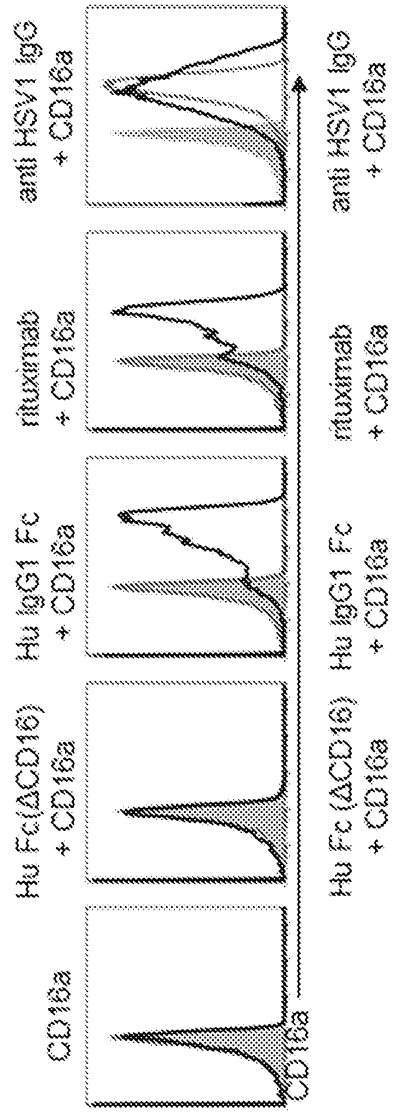

Hu Fc(ΔCD16) + CD16a
Hu IgG1 Fc + CD16a
rituximab + CD16a
anti HSV1 IgG + CD16a
CD16a
Hu Fc (ΔCD16) + CD16a
Hu IgG1 Fc + CD16a
rituximab + CD16a
anti HSV1 IgG + CD16a
CD16a

FIG. 3C

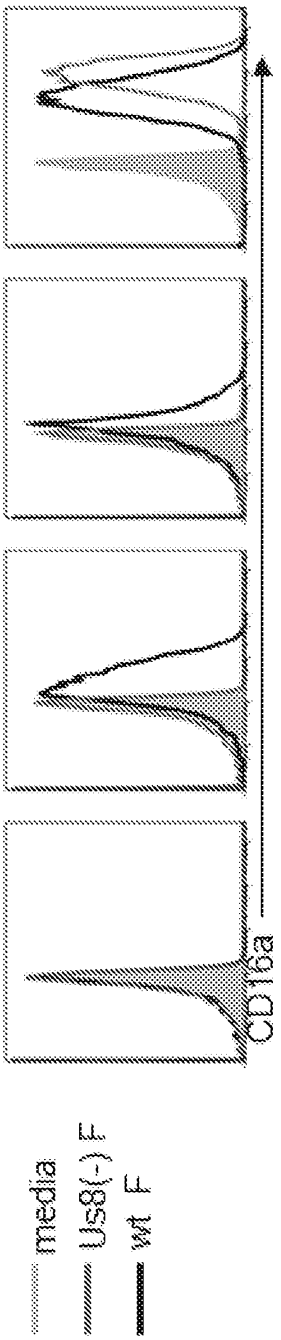

media
Us8(-) F
wt F
CD16a

FIG. 3D

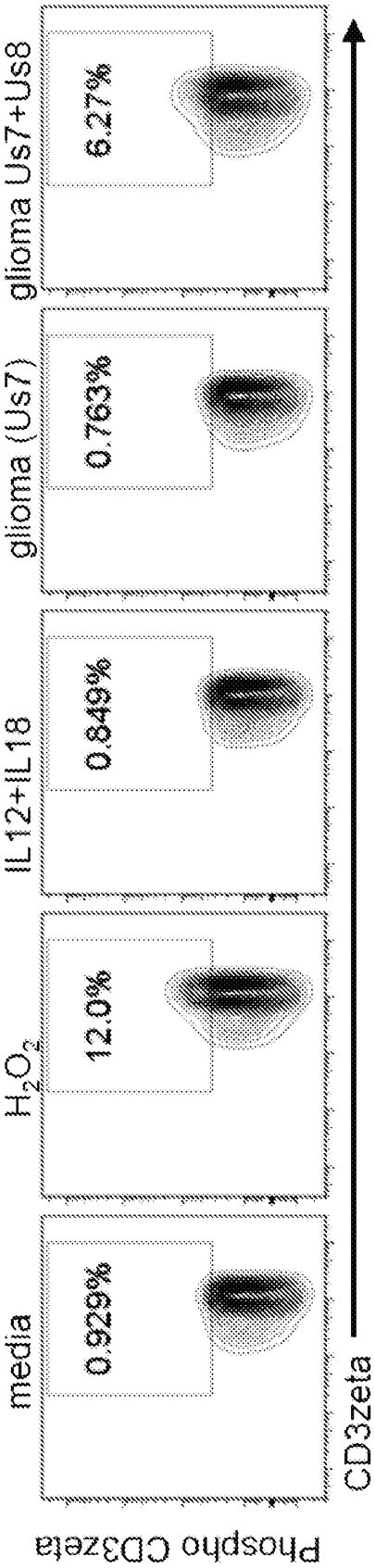

media
0.929%
H2O2
12.0%
IL12+IL18
0.849%
glioma (Us7)
0.763%
glioma Us7+Us8
6.27%
Phospho CD3zeta
CD3zeta

FIG. 3E

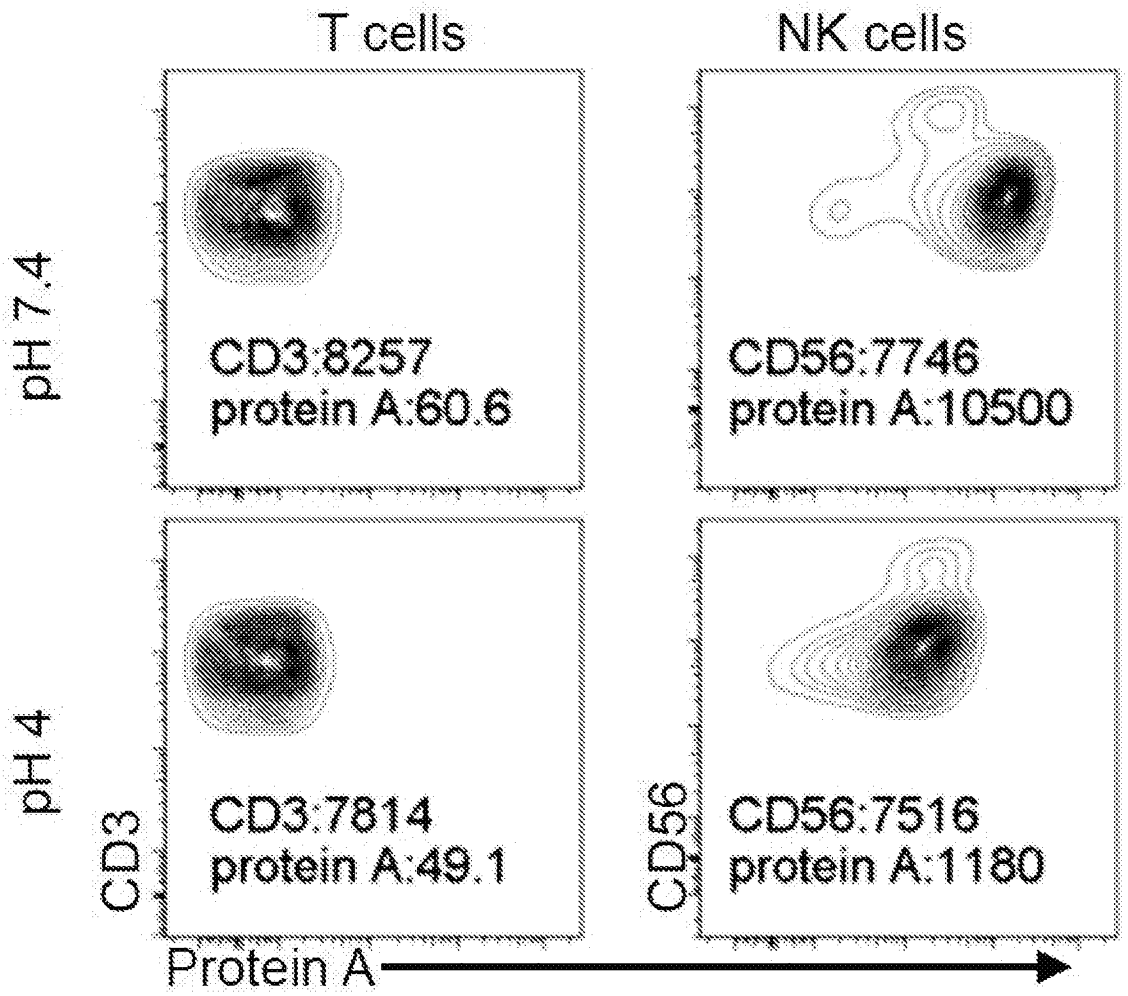

T cells
NK cells
pH 7.4
pH 4
CD3
CD56
CD3:8257
protein A:60.6
CD56:7746
protein A:10500
CD3:7814
protein A:49.1
CD56:7516
protein A:1180
Protein A

FIG. 3F

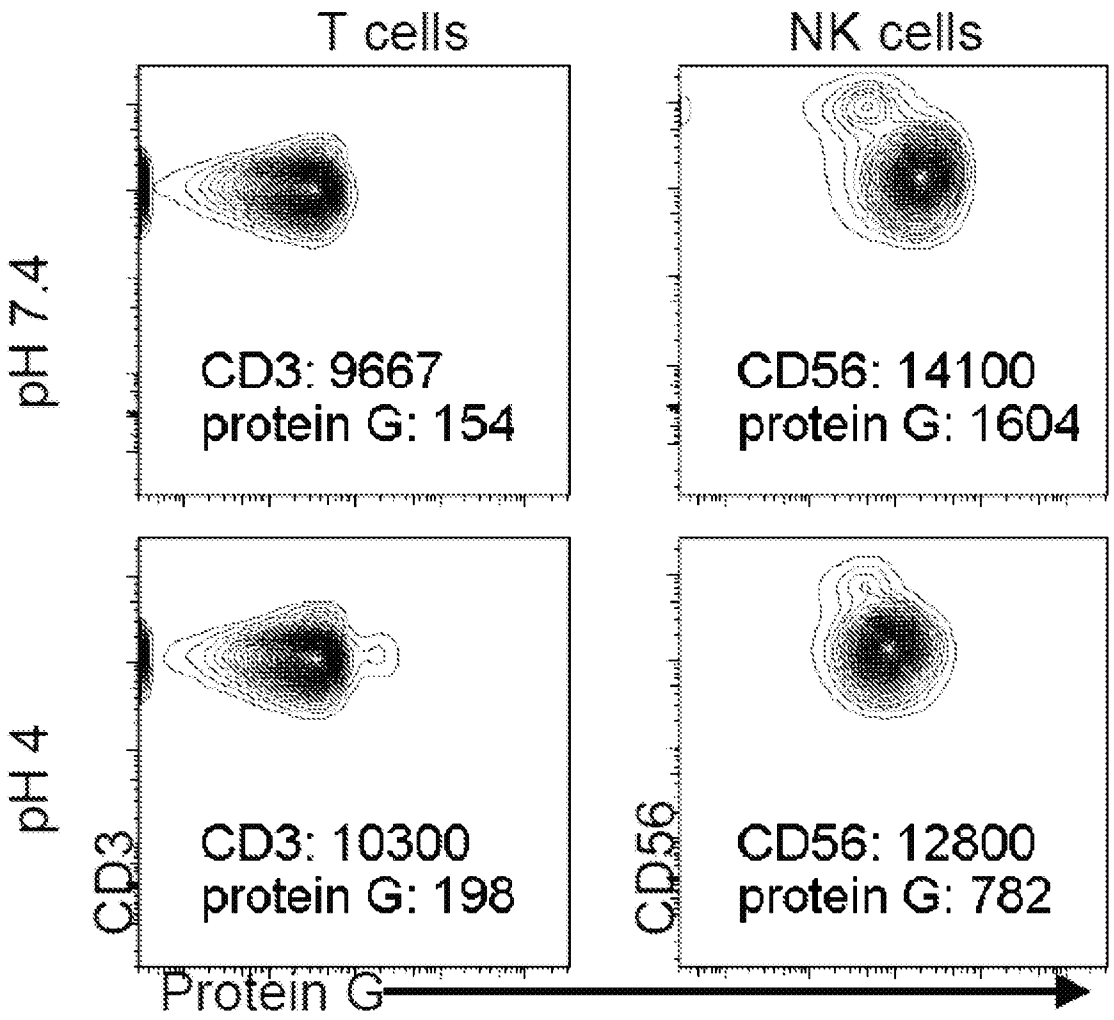

T cells
NK cells
pH 7.4
pH 4
CD3: 9667
protein G: 154
CD56: 14100
protein G: 1604
CD3: 10300
protein G: 198
CD56: 12800
protein G: 782
CD3
CD56
Protein G

FIG. 3G

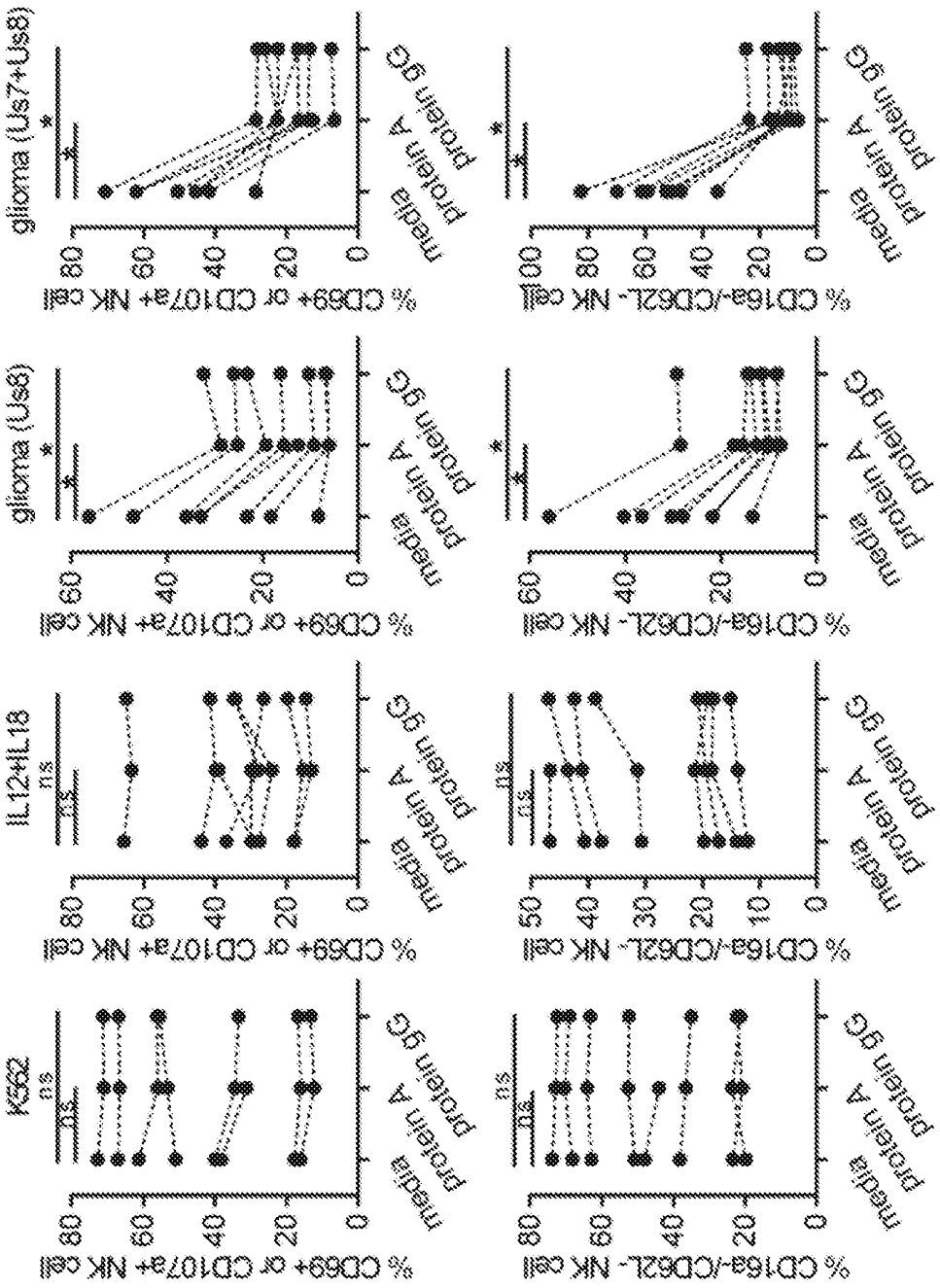

K562
IL12+IL18
glioma (Us8)
glioma (Us7+Us8)
% CD69+ or CD107a+ NK cell
% CD16a-/CD62L- NK cell
media
protein A
protein gG
ns
*

FIG. 3H

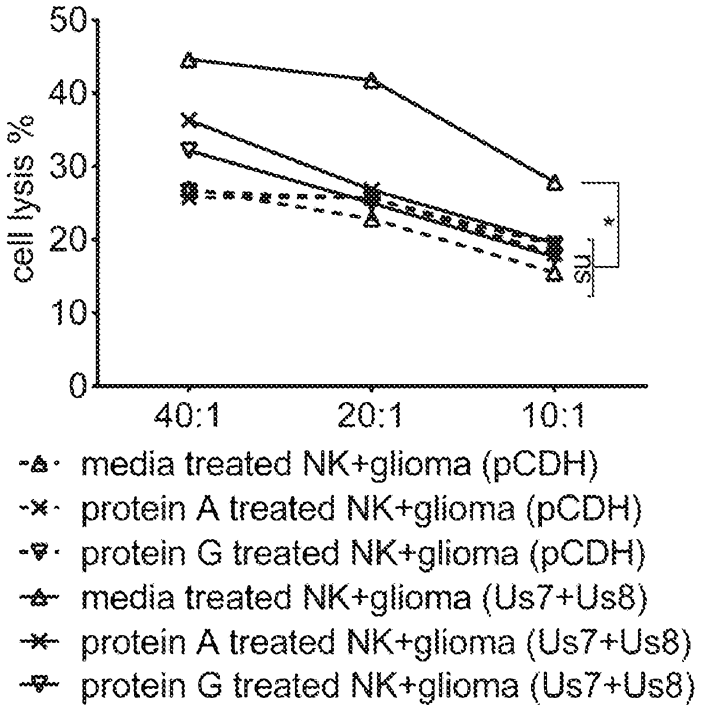

cell lysis %
50
40
30
20
10
0
40:1
20:1
10:1
*
media treated NK+glioma (pCDH)
protein A treated NK+glioma (pCDH)
protein G treated NK+glioma (pCDH)
media treated NK+glioma (Us7+Us8)
protein A treated NK+glioma (Us7+Us8)
protein G treated NK+glioma (Us7+Us8)

FIG. 3I

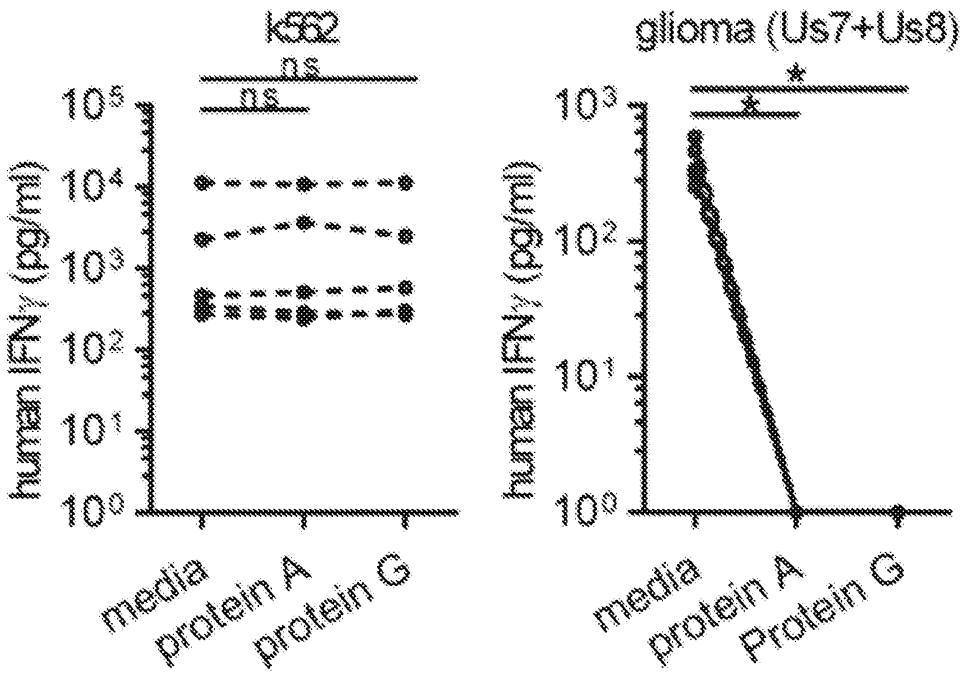

K562
ns
ns
glioma (Us7+Us8)
*
*
human IFNγ (pg/ml)
media
protein A
protein G
Protein G

FIG. 3J

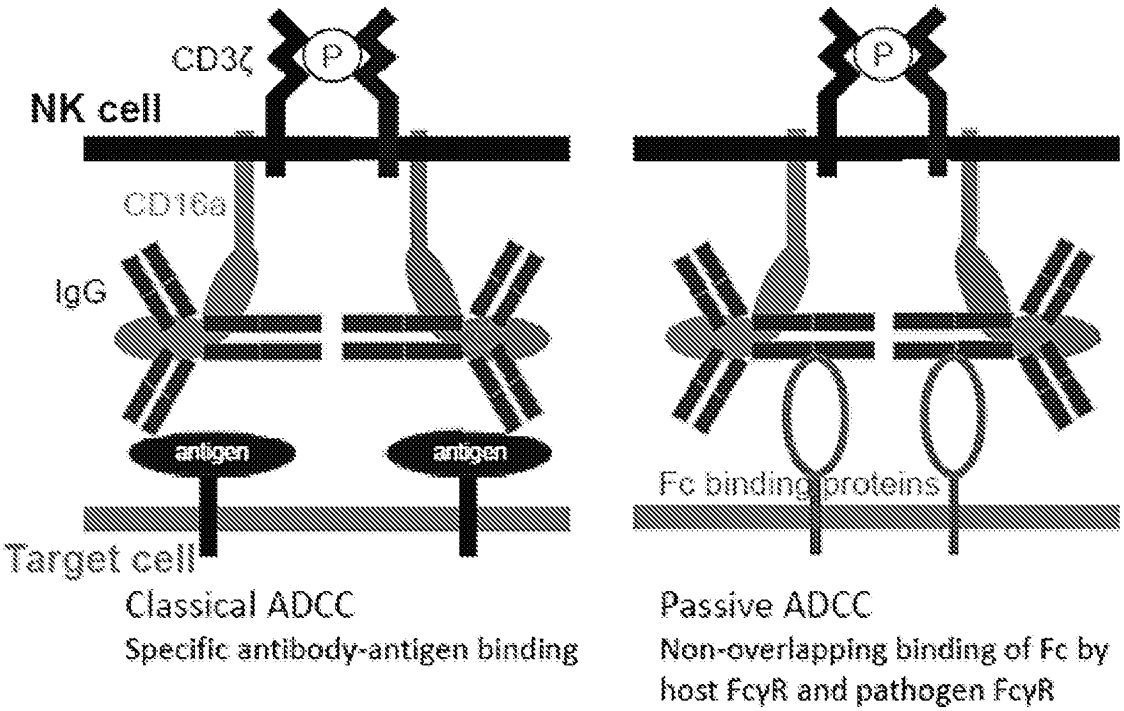

CD3ζ
P
NK cell
CD16a
IgG
antigen
antigen
Target cell
Classical ADCC
Specific antibody-antigen binding
P
Fc binding proteins
Passive ADCC
Non-overlapping binding of Fc by host FcγR and pathogen FcγR

FIG. 3K

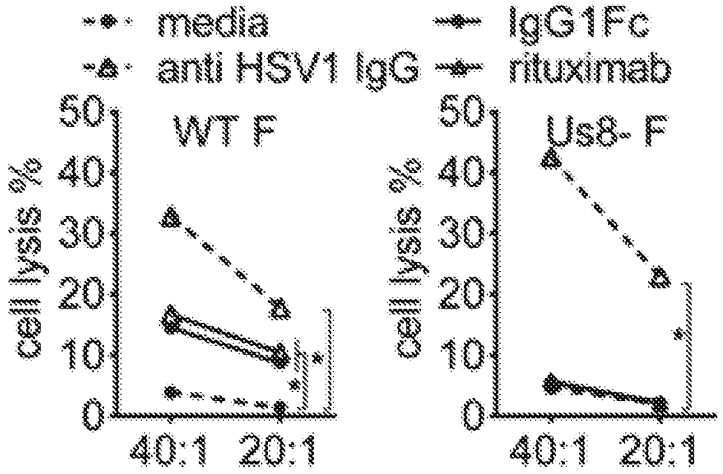

media
anti HSV1 IgG
IgG1Fc
rituximab
WT F
Us8- F
cell lysis %
50
40
30
20
10
0
40:1
20:1

FIG. 4A

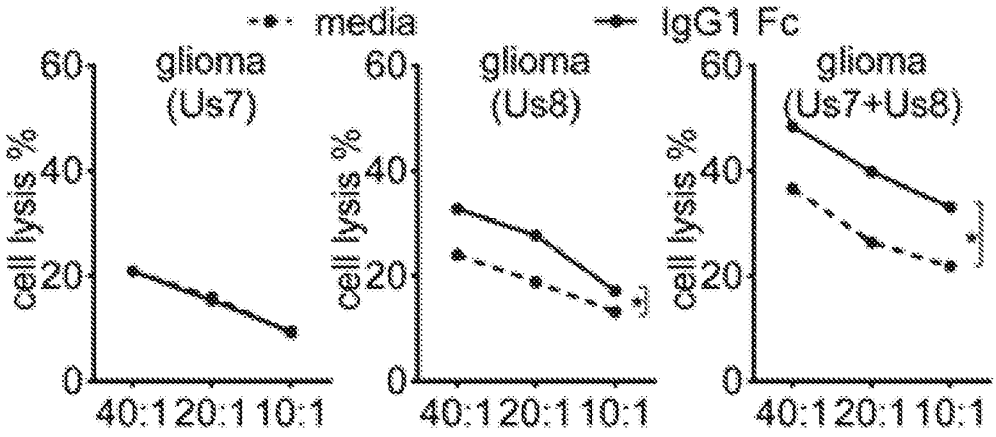

media
IgG1 Fc
glioma (Us7)
glioma (Us8)
glioma (Us7+Us8)
cell lysis %
60
40
20
0
40:1
20:1
10:1

FIG. 4B

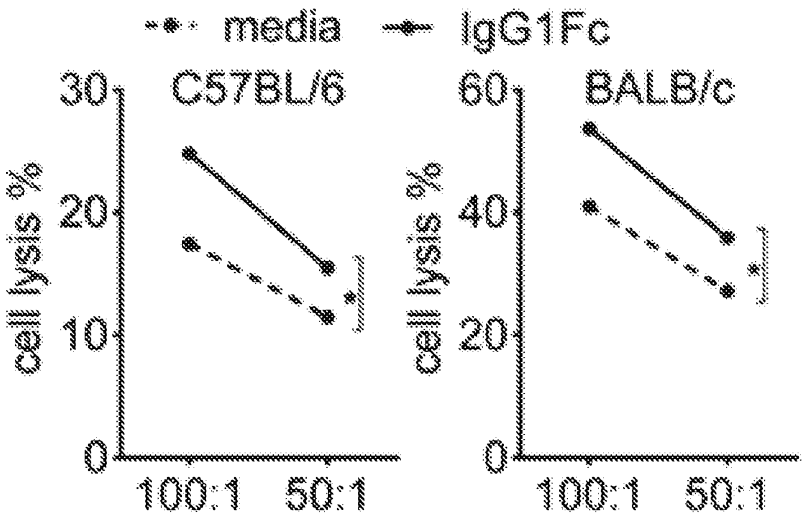

media
IgG1Fc
C57BL/6
BALB/c
cell lysis %
30
20
10
0
60
40
20
0
100:1
50:1
*

FIG. 4C

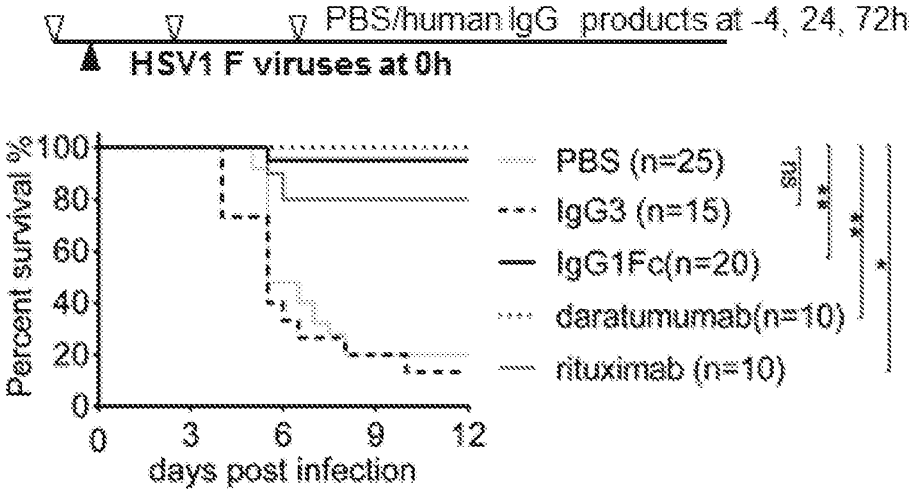

PBS/human IgG products at -4, 24, 72h
HSV1 F viruses at 0h
Percent survival %
100
80
60
40
20
0
0
3
6
9
12
days post infection
PBS (n=25)
IgG3 (n=15)
IgG1Fc(n=20)
daratumumab(n=10)
rituximab (n=10)
NS
**
**
*

FIG. 4D

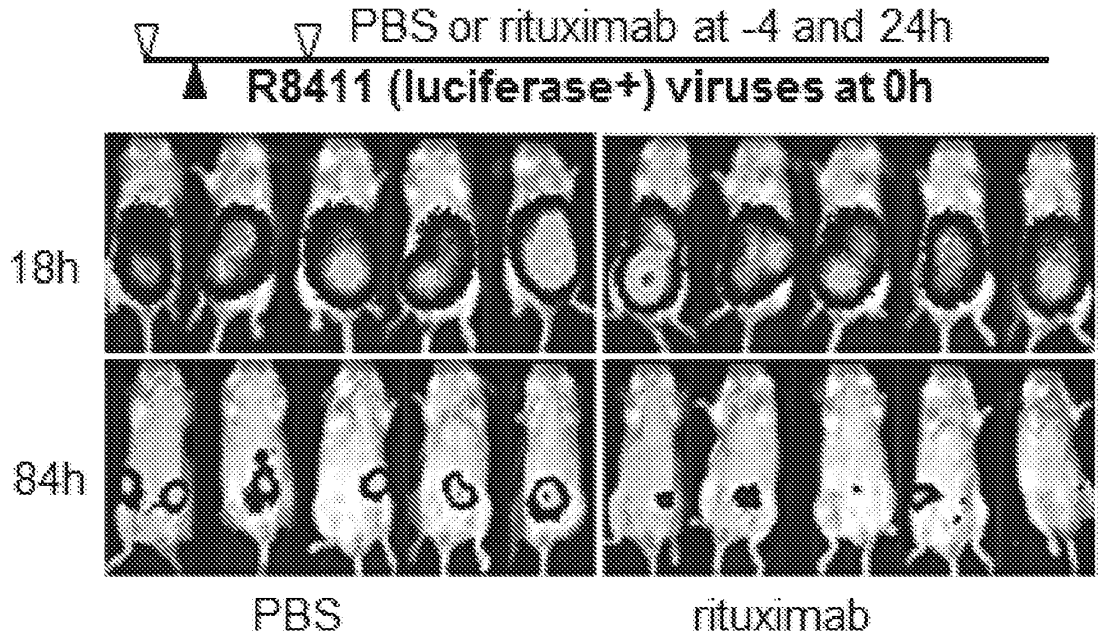

PBS or rituximab at -4 and 24h
R8411 (luciferase+) viruses at 0h
18h
84h
PBS
rituximab

FIG. 4E

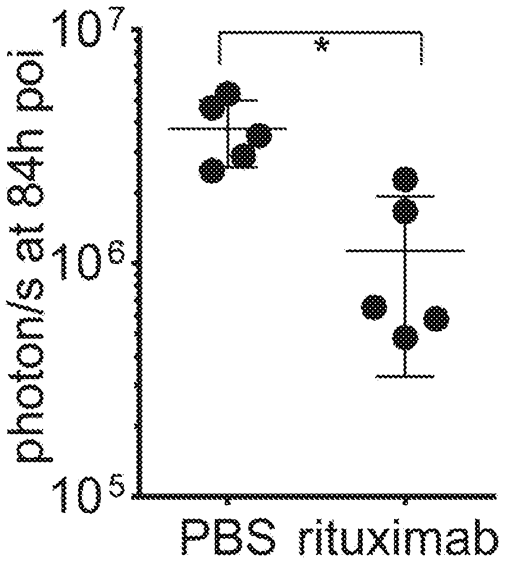

photon/s at 84h poi
10^7
10^6
10^5
*
PBS rituximab

FIG. 4F

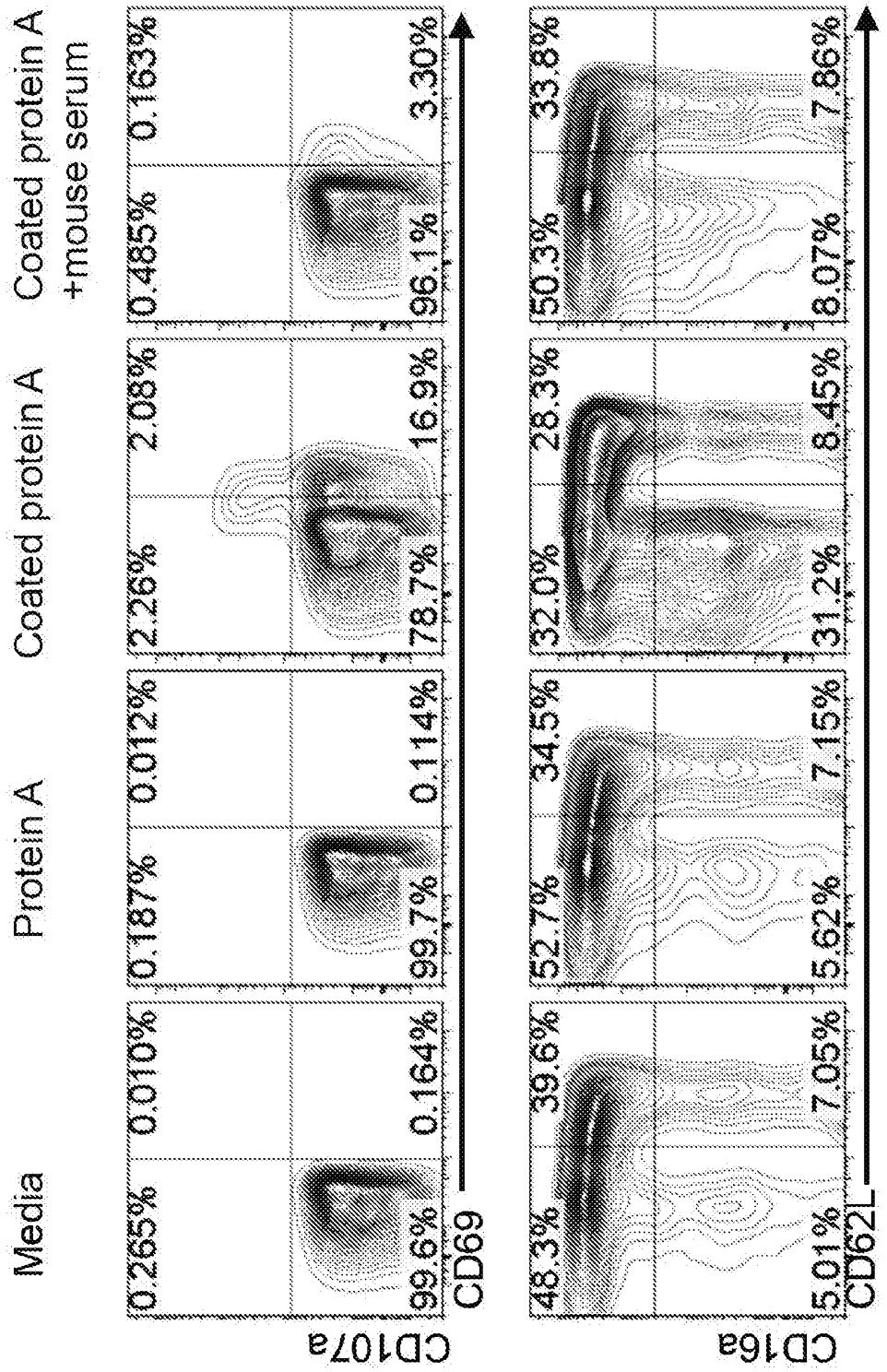

Media
Protein A
Coated protein A
Coated protein A
+mouse serum
0.265%
0.010%
0.187%
0.012%
2.26%
2.08%
0.485%
0.163%
99.6%
0.164%
99.7%
0.114%
78.7%
16.9%
96.1%
3.30%
CD107a
CD69
48.3%
39.6%
52.7%
34.5%
32.0%
28.3%
50.3%
33.8%
5.01%
7.05%
5.62%
7.15%
31.2%
8.45%
8.07%
7.86%
CD16a
CD62L

FIG. 5A

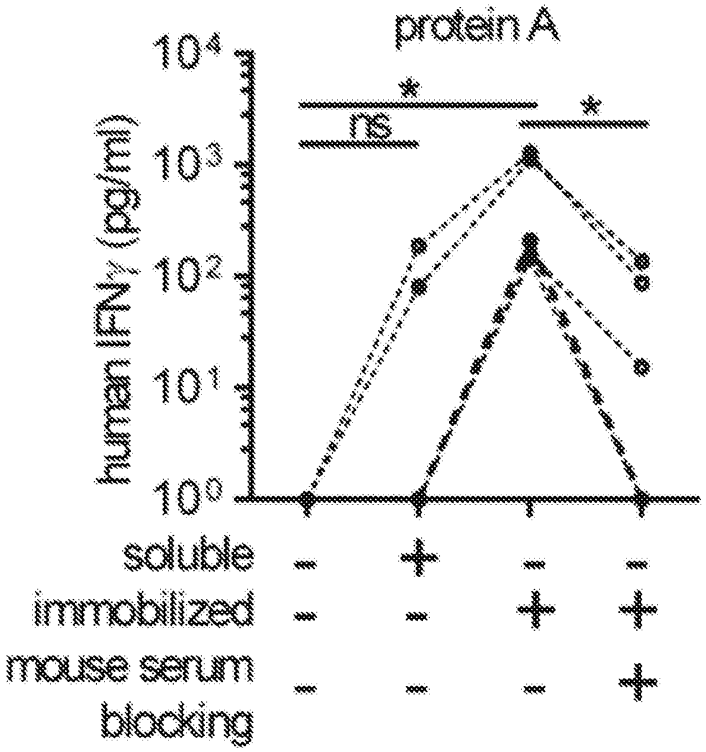

protein A
human IFNγ (pg/ml)
10^4
10^3
10^2
10^1
10^0
*
ns
*
soluble - + - -
immobilized - - + +
mouse serum - - - +
blocking

FIG. 5B

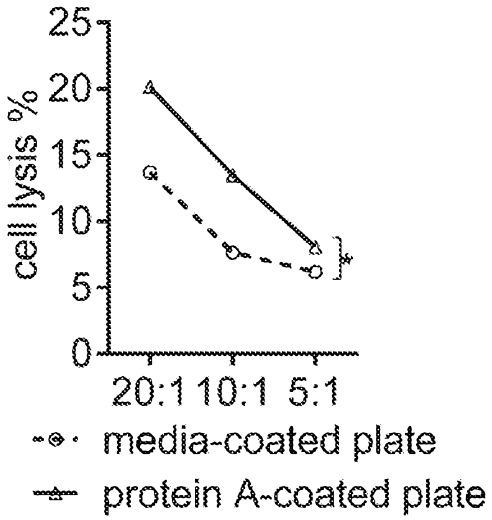

cell lysis %
25
20
15
10
5
0
20:1 10:1 5:1
media-coated plate
protein A-coated plate

FIG. 5C

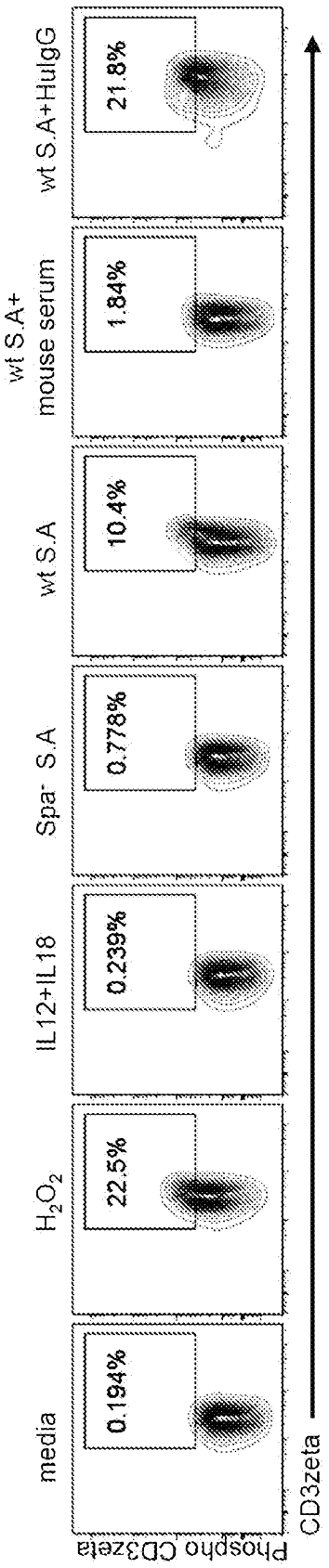

media
H_2O_2
IL12+IL18
Spar S.A
wt S.A
wt S.A+ mouse serum
wt S.A+HulgG
0.194%
22.5%
0.239%
0.778%
10.4%
1.84%
21.8%
Phospho CD3zeta
CD3zeta

FIG. 5D

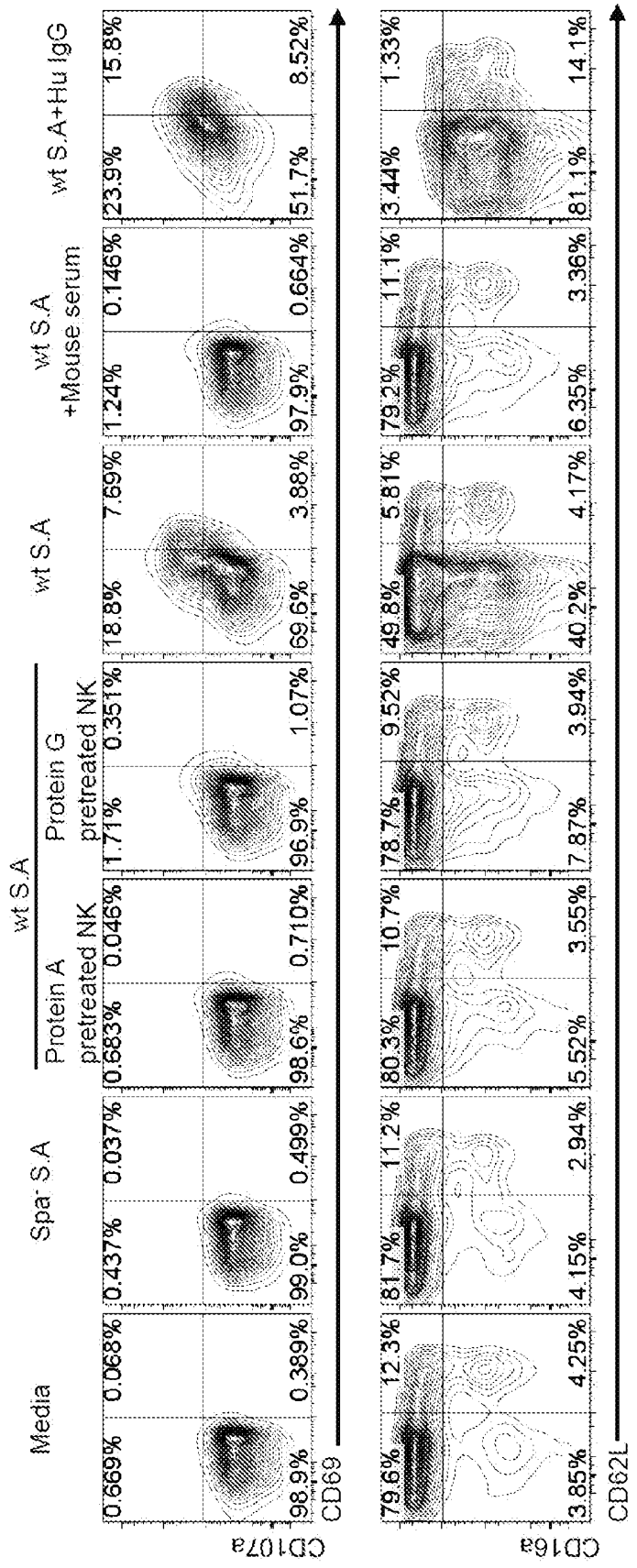

Media
Spa S.A
wt S.A
Protein A pretreated NK
Protein G pretreated NK
wt S.A
wt S.A +Mouse serum
wt S.A+Hu IgG
0.669%
0.068%
0.437%
0.037%
0.683%
0.046%
1.71%
0.351%
18.8%
7.69%
1.24%
0.146%
23.9%
15.8%
98.9%
0.389%
99.0%
0.499%
98.6%
0.710%
96.9%
1.07%
69.6%
3.88%
97.9%
0.664%
51.7%
8.52%
CD107a
CD69
79.6%
12.3%
81.7%
11.2%
80.3%
10.7%
78.7%
9.52%
49.8%
5.81%
79.2%
11.1%
3.44%
1.33%
3.85%
4.25%
4.15%
2.94%
5.52%
3.55%
7.87%
3.94%
40.2%
4.17%
6.35%
3.36%
81.1%
14.1%
CD16a
CD62L

FIG. 5E

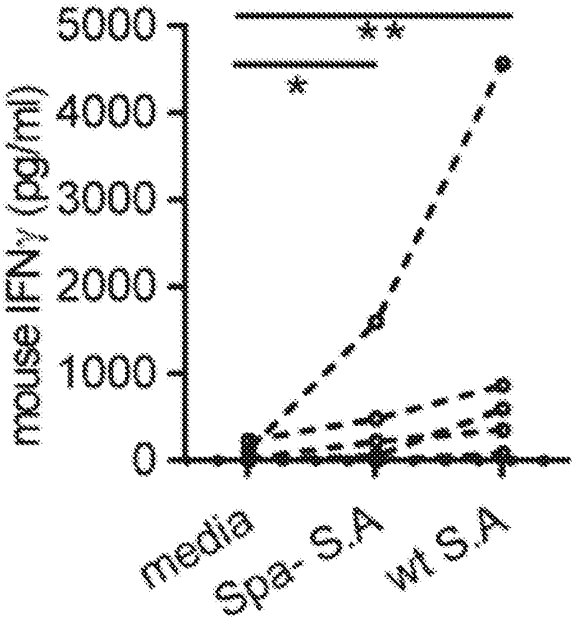

5000
4000
3000
2000
1000
0
mouse IFNγ (pg/ml)
**
*
media
Spa- S.A
wt S.A

FIG. 5F

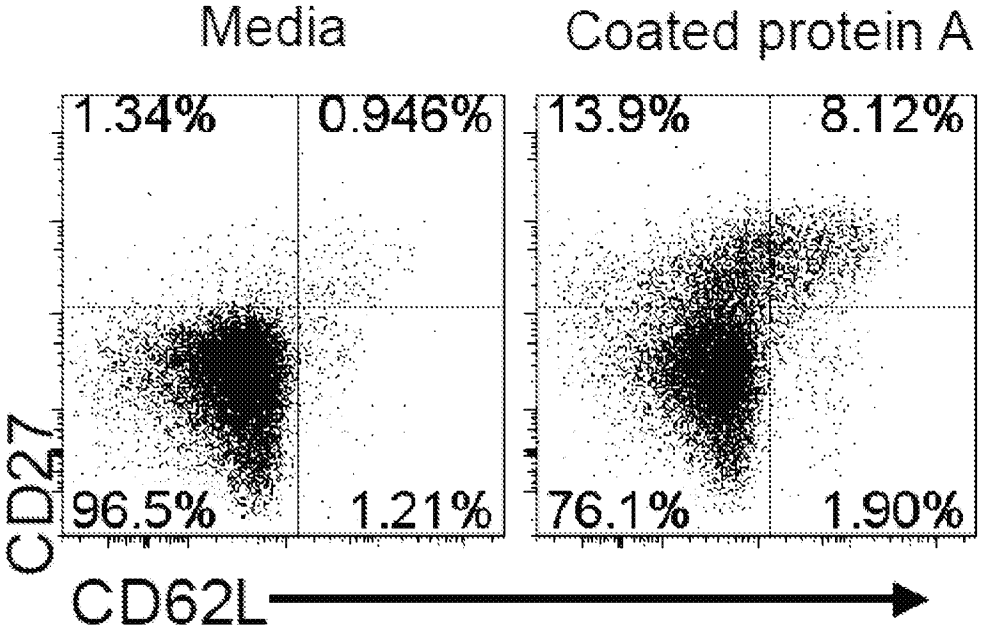

Media
Coated protein A
1.34%
0.946%
13.9%
8.12%
96.5%
1.21%
76.1%
1.90%
CD27
CD62L

FIG. 5G

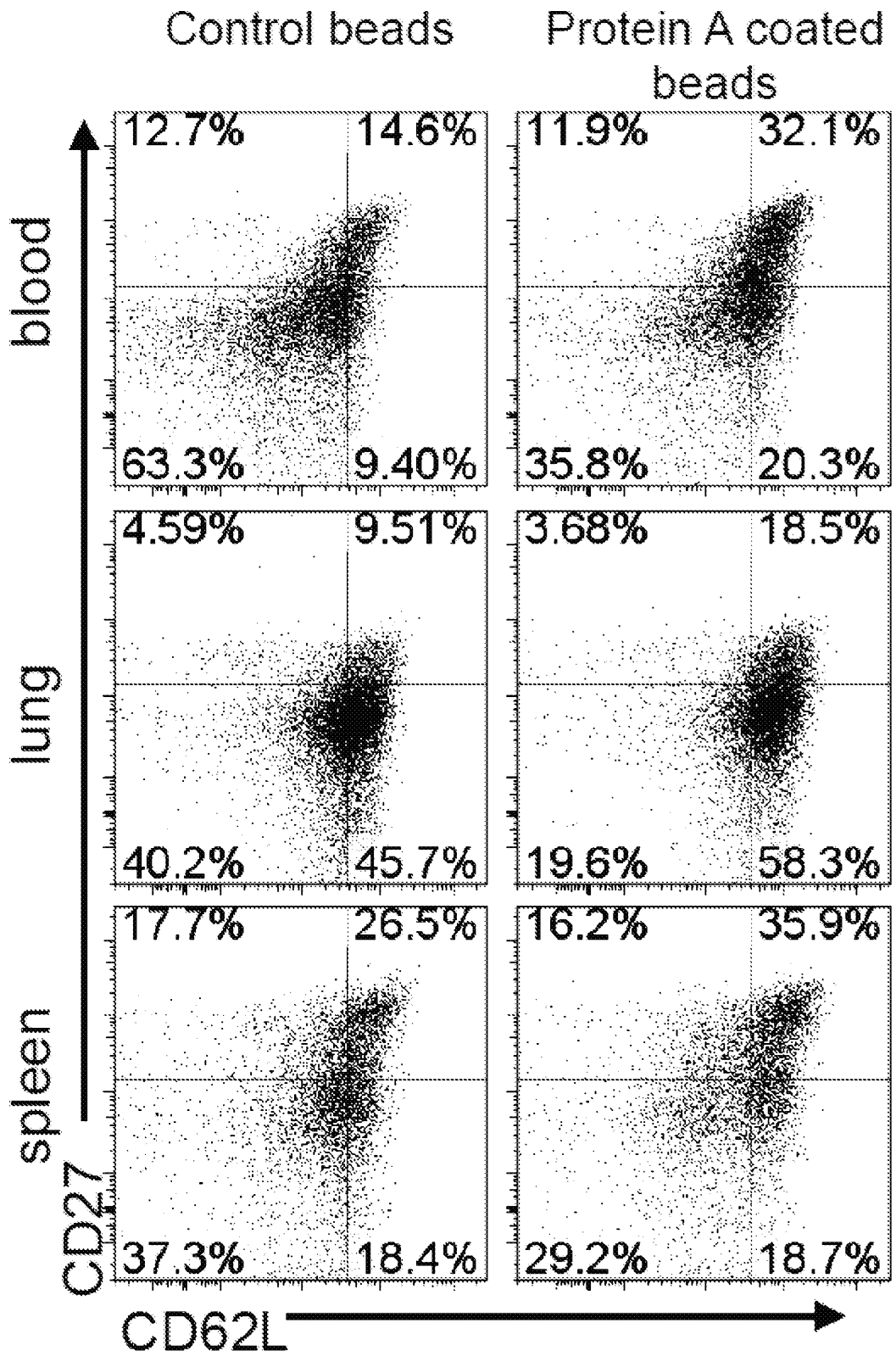

Control beads
Protein A coated beads
blood
lung
spleen
CD27
CD62L
12.7%
14.6%
63.3%
9.40%
11.9%
32.1%
35.8%
20.3%
4.59%
9.51%
40.2%
45.7%
3.68%
18.5%
19.6%
58.3%
17.7%
26.5%
37.3%
18.4%
16.2%
35.9%
29.2%
18.7%

FIG. 5H

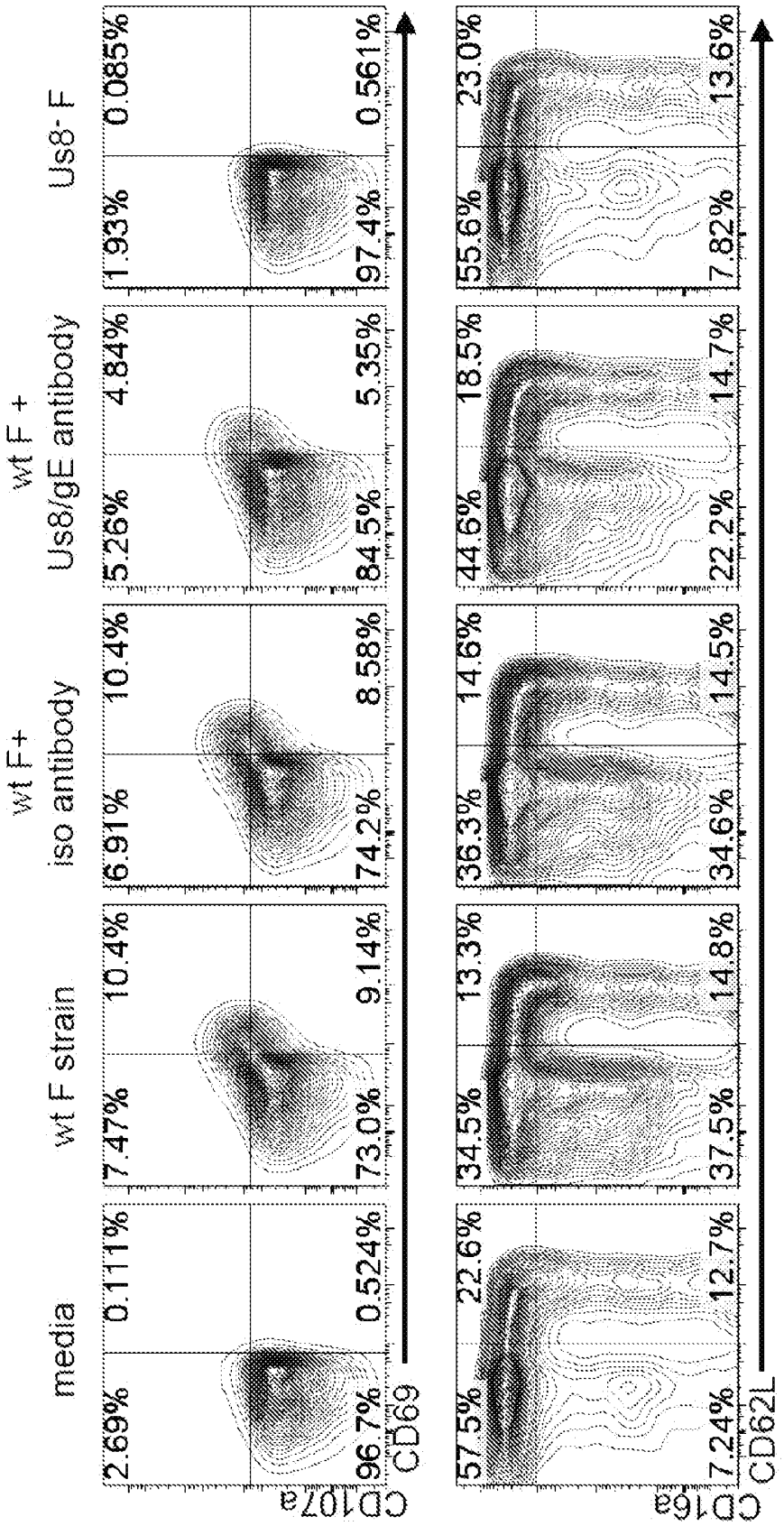

media
wt F strain
wt F+ iso antibody
wt F+ Us8/gE antibody
Us8- F
CD107a
CD69
CD16a
CD62L
2.69%
0.111%
96.7%
0.524%
7.47%
10.4%
73.0%
9.14%
6.91%
10.4%
74.2%
8.58%
5.26%
4.84%
84.5%
5.35%
1.93%
0.085%
97.4%
0.561%
57.5%
22.6%
7.24%
12.7%
34.5%
13.3%
37.5%
14.8%
36.3%
14.6%
34.6%
14.5%
44.6%
18.5%
22.2%
14.7%
55.6%
23.0%
7.82%
13.6%

FIG. 6

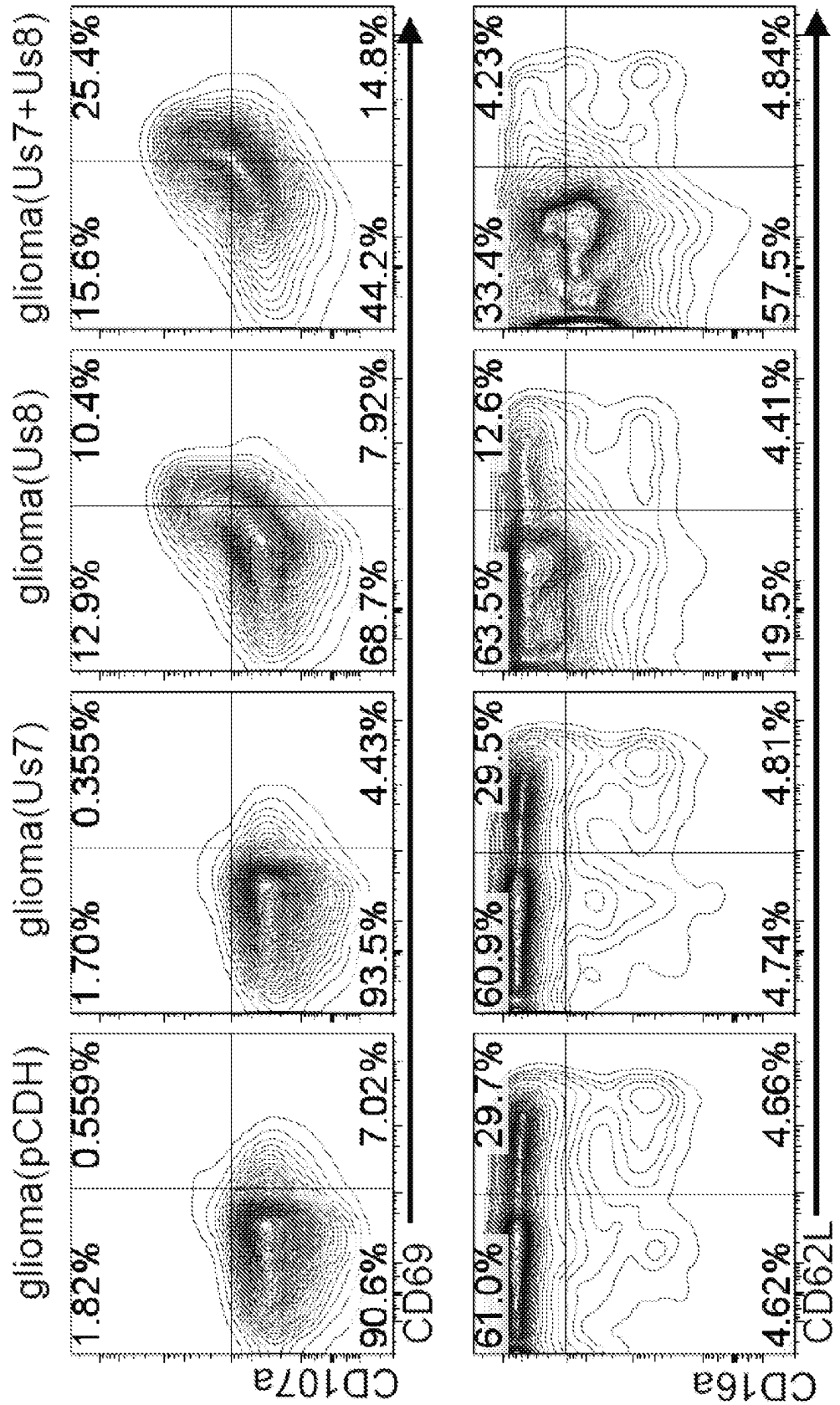

glioma(pCDH)
glioma(Us7)
glioma(Us8)
glioma(Us7+Us8)
1.82%
0.559%
90.6%
7.02%
1.70%
0.355%
93.5%
4.43%
12.9%
10.4%
68.7%
7.92%
15.6%
25.4%
44.2%
14.8%
61.0%
29.7%
4.62%
4.66%
60.9%
29.5%
4.74%
4.81%
63.5%
12.6%
19.5%
4.41%
33.4%
4.23%
57.5%
4.84%
CD107a
CD69
CD16a
CD62L

FIG. 7

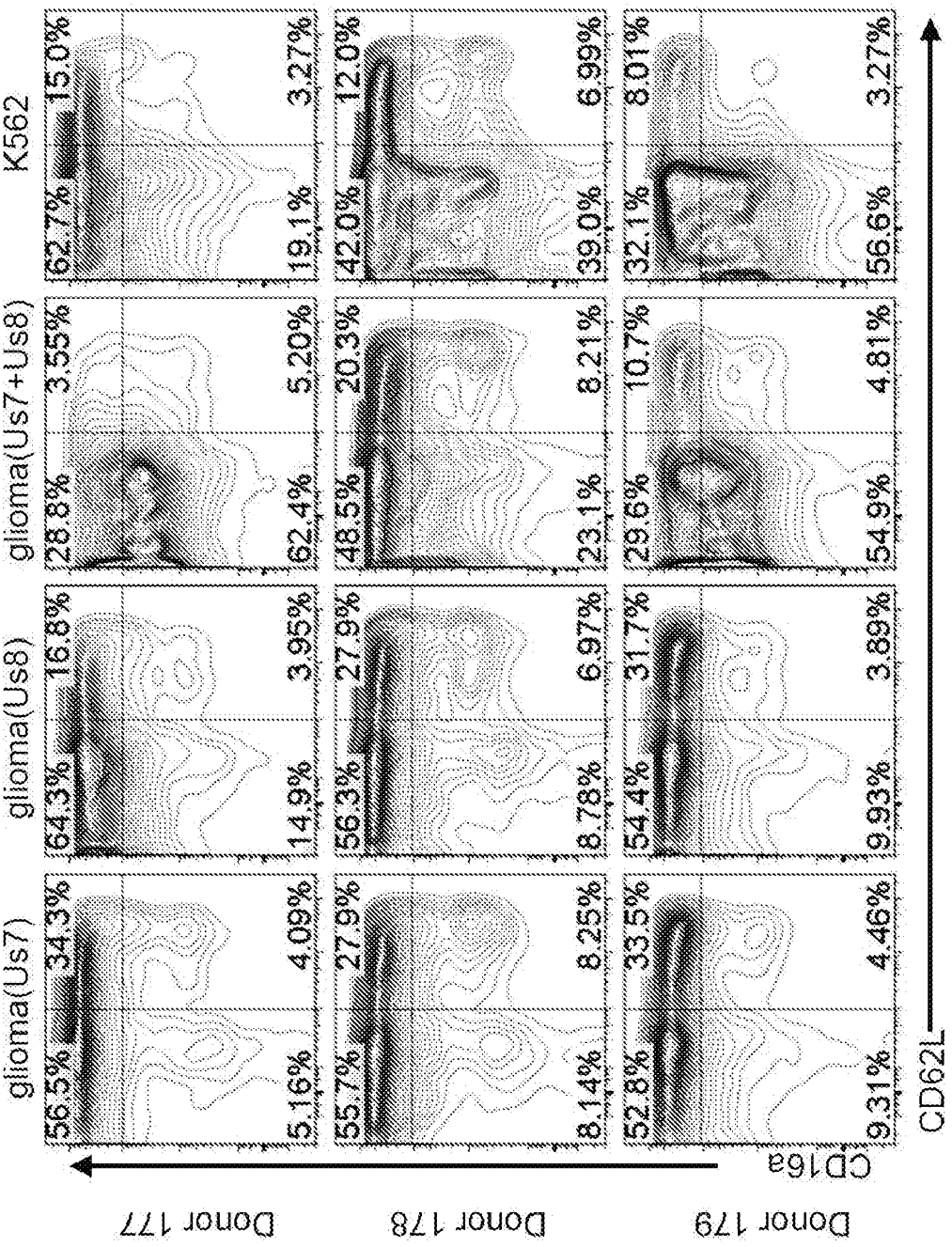

glioma(Us7)
glioma(Us8)
glioma(Us7+Us8)
K562
Donor 177
Donor 178
Donor 179
CD16a
CD62L
56.5%
34.3%
5.16%
4.09%
64.3%
16.8%
14.9%
3.95%
28.8%
3.55%
62.4%
5.20%
62.7%
15.0%
19.1%
3.27%
55.7%
27.9%
8.14%
8.25%
56.3%
27.9%
8.78%
6.97%
48.5%
20.3%
23.1%
8.21%
42.0%
12.0%
39.0%
6.99%
52.8%
33.5%
9.31%
4.46%
54.4%
31.7%
9.93%
3.89%
29.6%
10.7%
54.9%
4.81%
32.1%
8.01%
56.6%
3.27%

FIG. 8

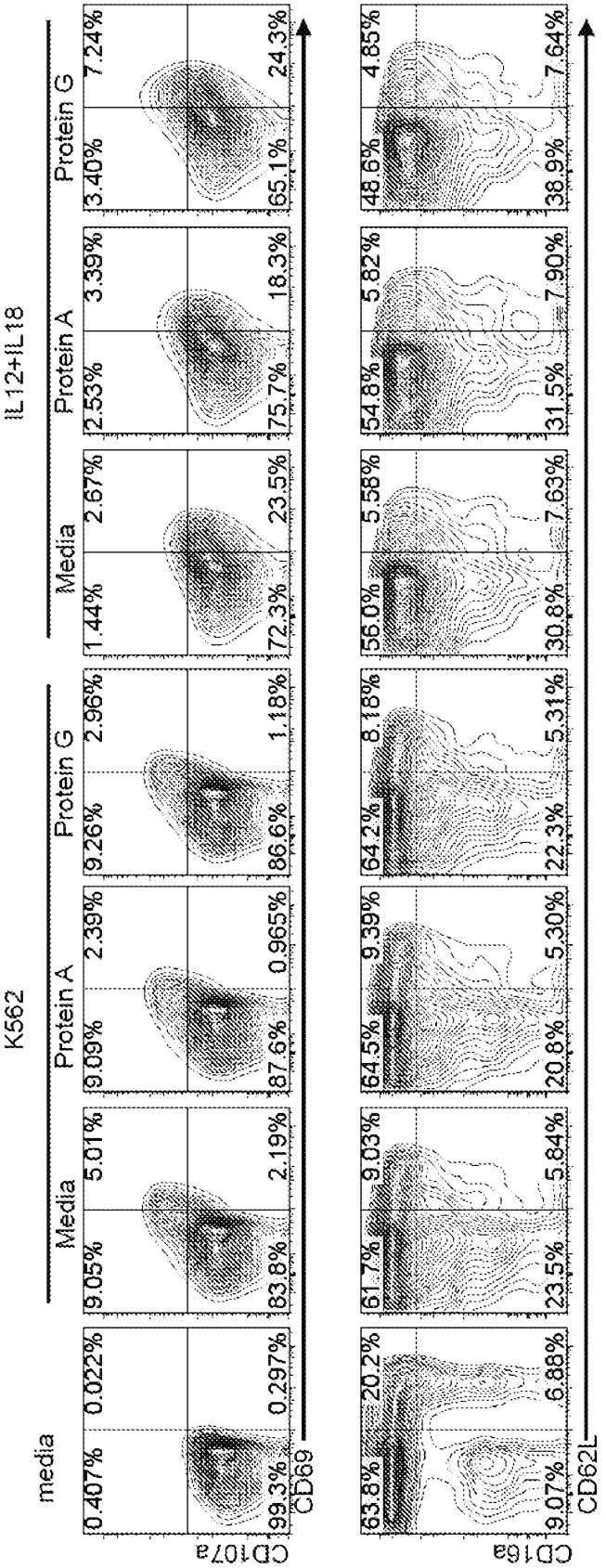

media
K562
IL12+IL18
Media
Protein A
Protein G
Media
Protein A
Protein G
0.407%
0.022%
9.05%
5.01%
9.09%
2.39%
9.26%
2.96%
1.44%
2.67%
2.53%
3.39%
3.40%
7.24%
99.3%
0.297%
83.8%
2.19%
87.6%
0.965%
86.6%
1.18%
72.3%
23.5%
75.7%
18.3%
65.1%
24.3%
CD107a
CD69
63.8%
20.2%
61.7%
9.03%
64.5%
9.39%
64.2%
8.18%
56.0%
5.58%
54.8%
5.82%
48.6%
4.85%
9.07%
6.88%
23.5%
5.84%
20.8%
5.30%
22.3%
5.31%
30.8%
7.63%
31.5%
7.90%
38.9%
7.64%
CD16a
CD62L

FIG. 9A

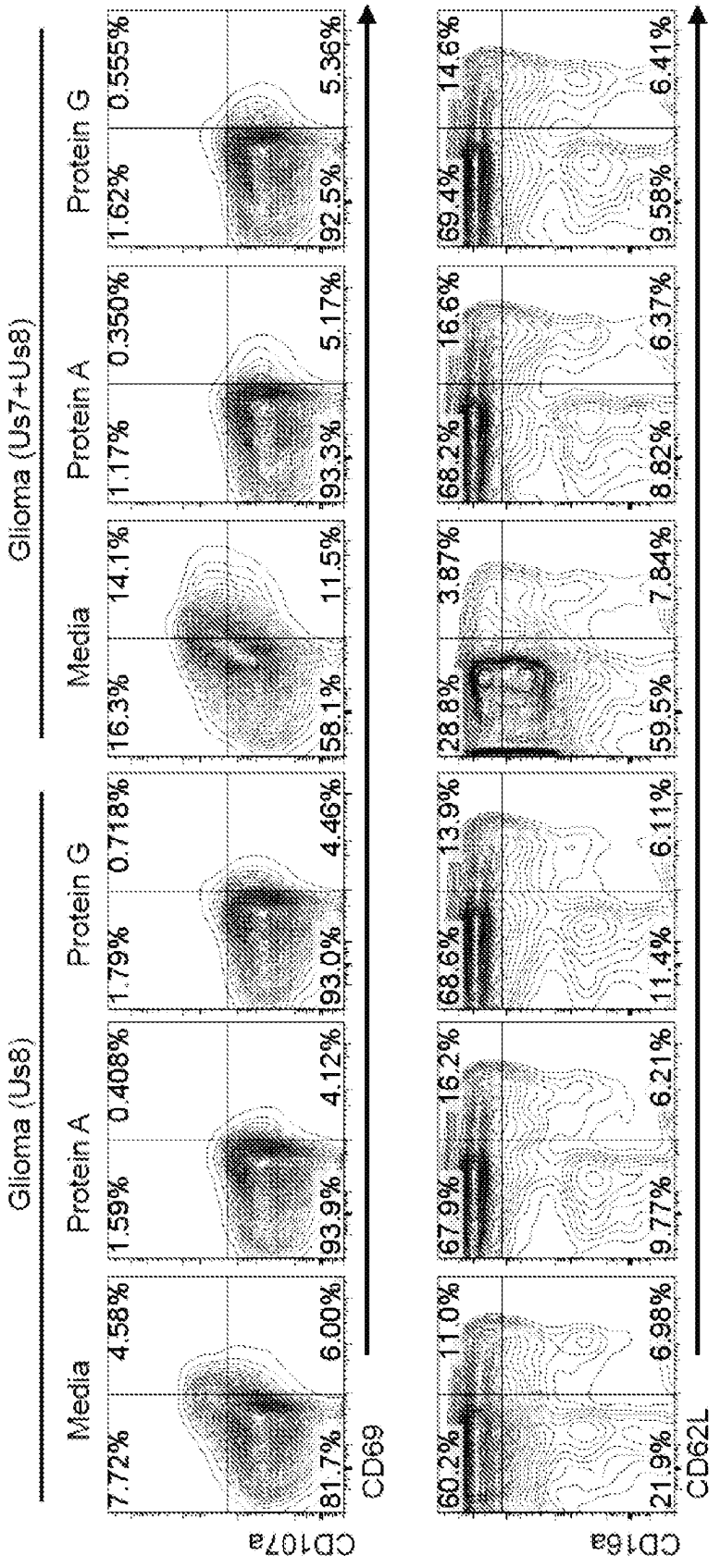

Glioma (Us8)
Glioma (Us7+Us8)
Media
Protein A
Protein G
Media
Protein A
Protein G
7.72%
4.58%
1.59%
0.408%
1.79%
0.718%
16.3%
14.1%
1.17%
0.350%
1.62%
0.555%
81.7%
6.00%
93.9%
4.12%
93.0%
4.46%
58.1%
11.5%
93.3%
5.17%
92.5%
5.36%
CD107a
CD69
60.2%
11.0%
67.9%
16.2%
68.6%
13.9%
28.8%
3.87%
68.2%
16.6%
69.4%
14.6%
21.9%
6.98%
9.77%
6.21%
11.4%
6.11%
59.5%
7.84%
8.82%
6.37%
9.58%
6.41%
CD16a
CD62L

FIG. 9B

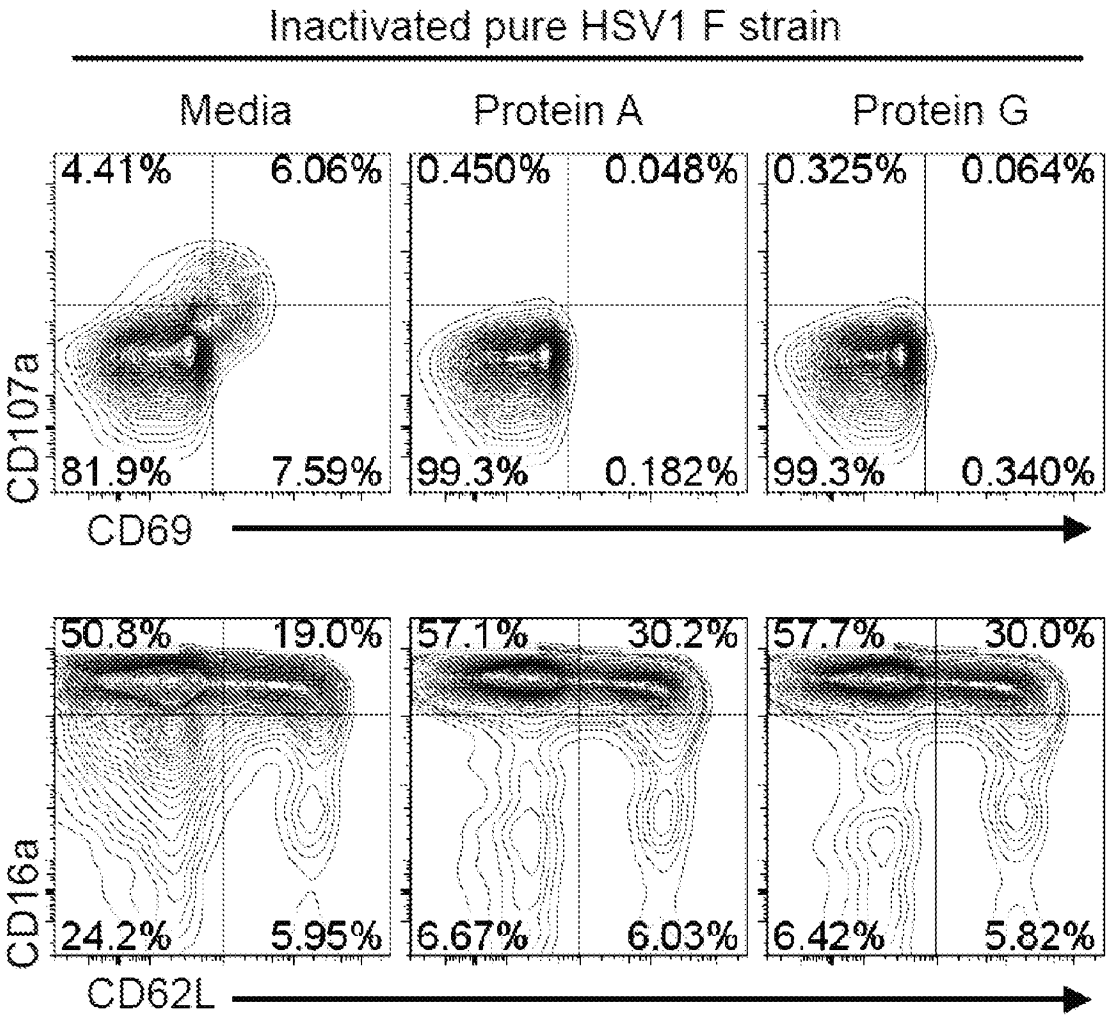

Inactivated pure HSV1 F strain
Media
Protein A
Protein G
4.41%
6.06%
81.9%
7.59%
0.450%
0.048%
99.3%
0.182%
0.325%
0.064%
99.3%
0.340%
CD107a
CD69
50.8%
19.0%
24.2%
5.95%
57.1%
30.2%
6.67%
6.03%
57.7%
30.0%
6.42%
5.82%
CD16a
CD62L

FIG. 10A

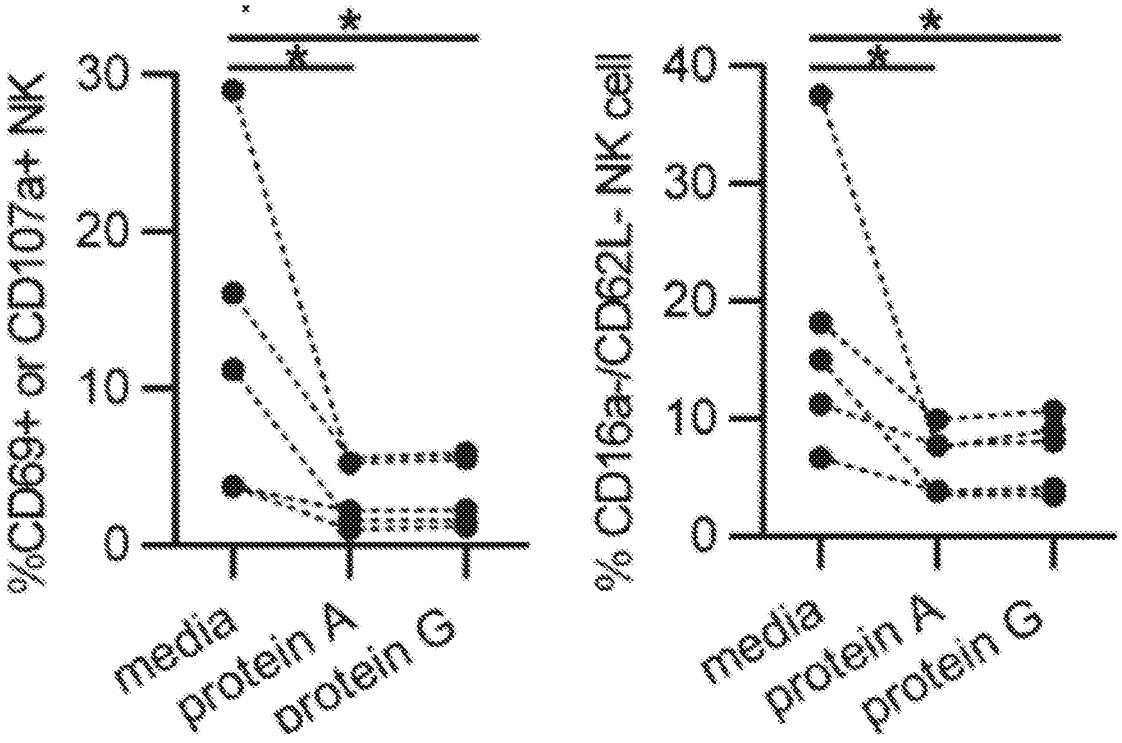

%CD69+ or CD107a+ NK
30
20
10
0
media
protein A
protein G
*
*
% CD16a-/CD62L- NK cell
40
30
20
10
0
media
protein A
protein G
*
*

FIG. 10B

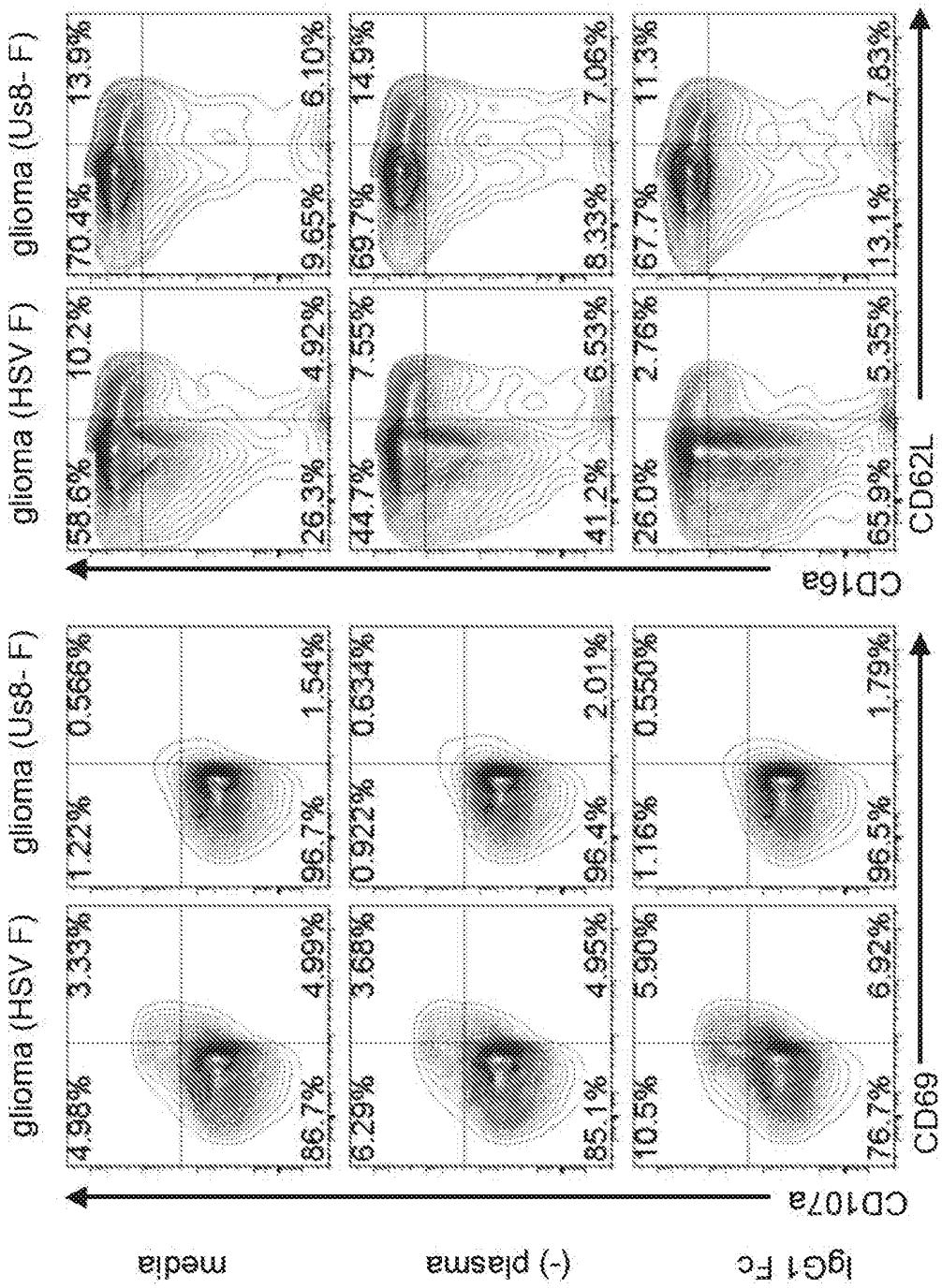

glioma (HSV F)
glioma (Us8- F)
glioma (HSV F)
glioma (Us8- F)
media
(-) plasma
IgG1 Fc
CD107a
CD69
CD16a
CD62L
4.98%
3.33%
86.7%
4.99%
1.22%
0.566%
96.7%
1.54%
6.29%
3.68%
85.1%
4.95%
0.922%
0.634%
96.4%
2.01%
10.5%
5.90%
76.7%
6.92%
1.16%
0.550%
96.5%
1.79%
58.6%
10.2%
26.3%
4.92%
70.4%
13.9%
9.65%
6.10%
44.7%
7.55%
41.2%
6.53%
69.7%
14.9%
8.33%
7.06%
26.0%
2.76%
65.9%
5.35%
67.7%
11.3%
13.1%
7.83%

FIG. 11A

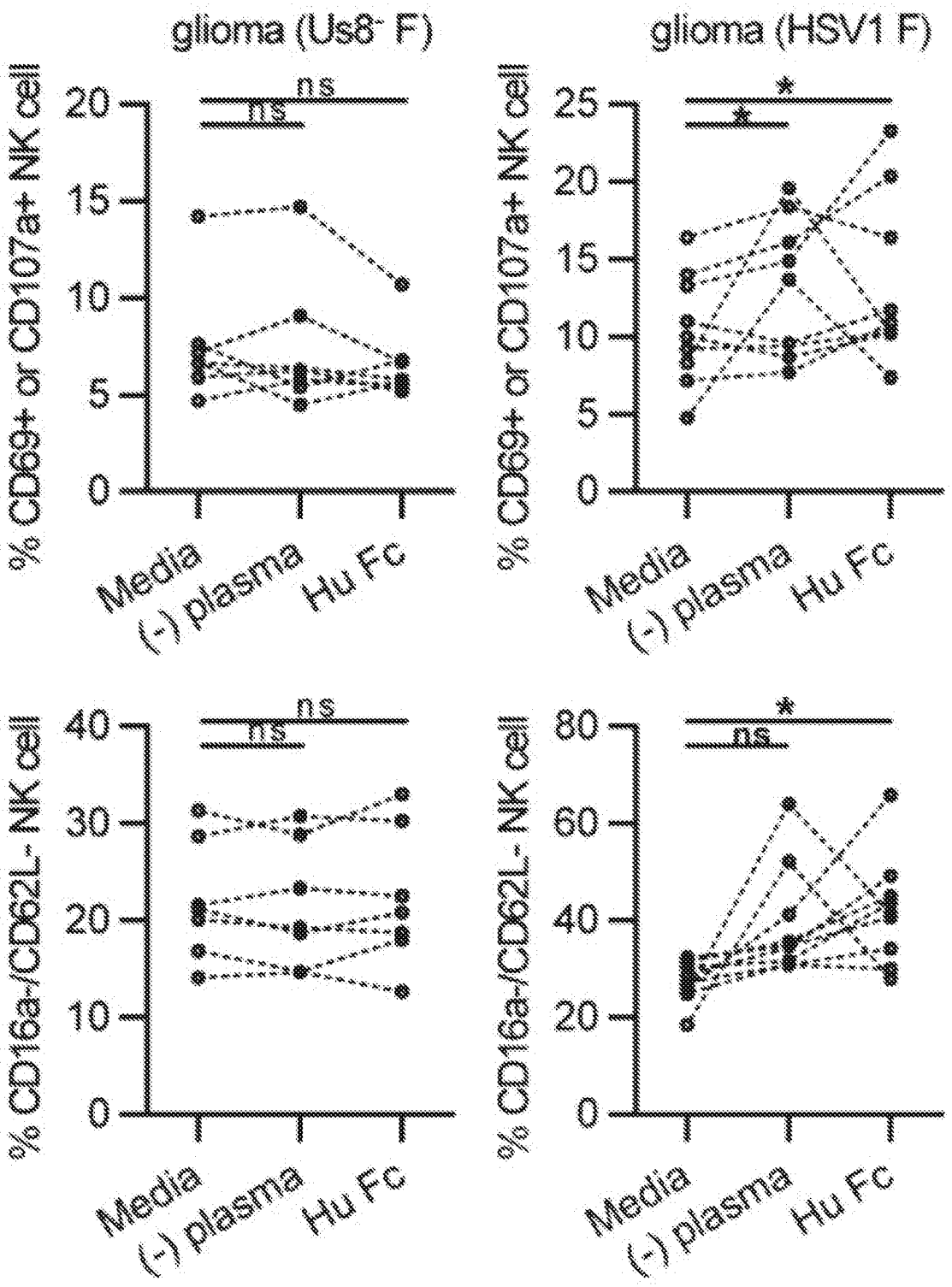

glioma (Us8- F)
glioma (HSV1 F)
% CD69+ or CD107a+ NK cell
% CD16a-/CD62L- NK cell
ns
*
Media
(-) plasma
Hu Fc

FIG. 11B

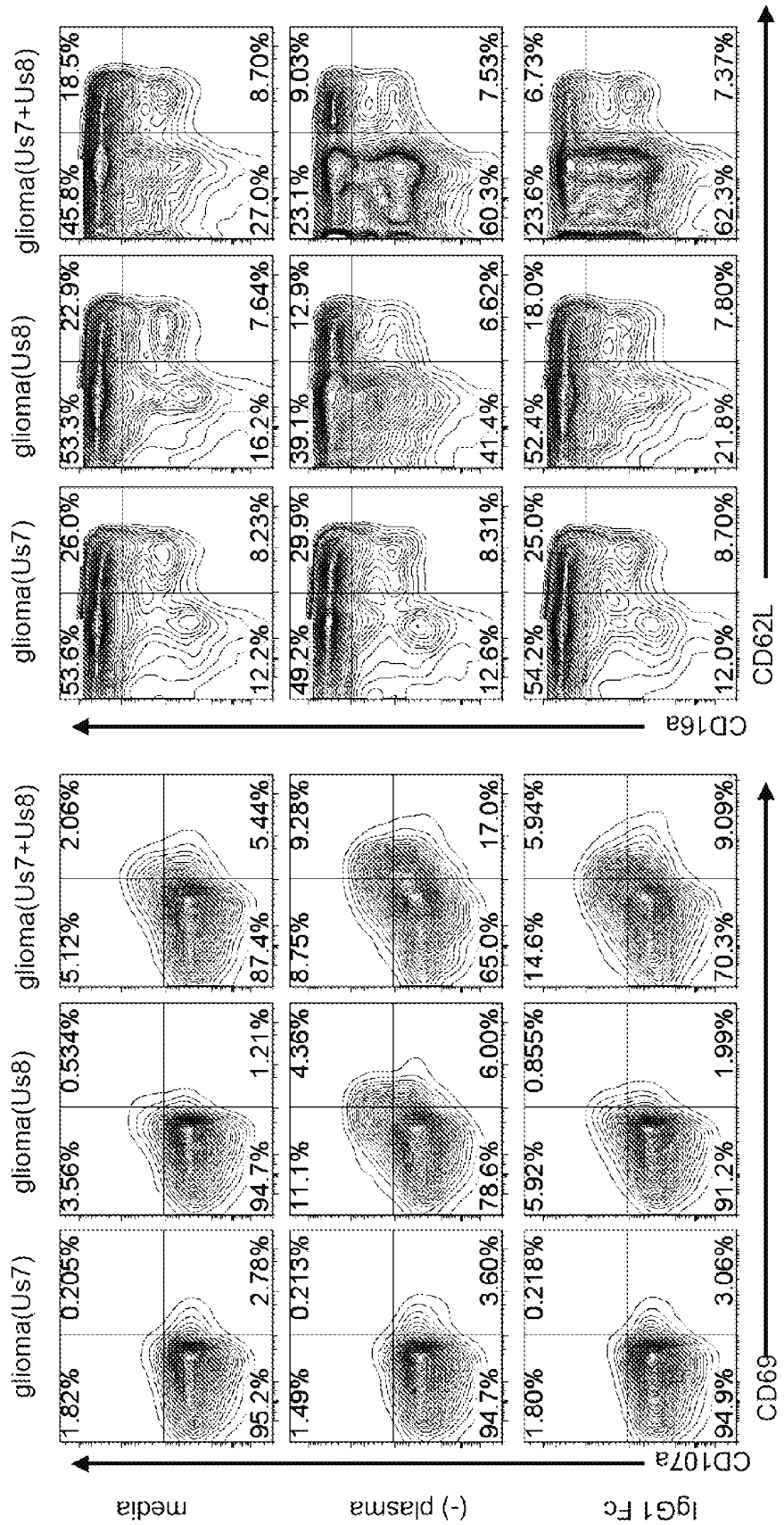

media
(-) plasma
IgG1 Fc
CD107a
CD69
glioma(Us7)
glioma(Us8)
glioma(Us7+Us8)
CD16a
CD62L
1.82%
0.205%
95.2%
2.78%
3.56%
0.534%
94.7%
1.21%
5.12%
2.06%
87.4%
5.44%
1.49%
0.213%
94.7%
3.60%
11.1%
4.36%
78.6%
6.00%
8.75%
9.28%
65.0%
17.0%
1.80%
0.218%
94.9%
3.06%
5.92%
0.855%
91.2%
1.99%
14.6%
5.94%
70.3%
9.09%
53.6%
26.0%
12.2%
8.23%
53.3%
22.9%
16.2%
7.64%
45.8%
18.5%
27.0%
8.70%
49.2%
29.9%
12.6%
8.31%
39.1%
12.9%
41.4%
6.62%
23.1%
9.03%
60.3%
7.53%
54.2%
25.0%
12.0%
8.70%
52.4%
18.0%
21.8%
7.80%
23.6%
6.73%
62.3%
7.37%

FIG. 11C

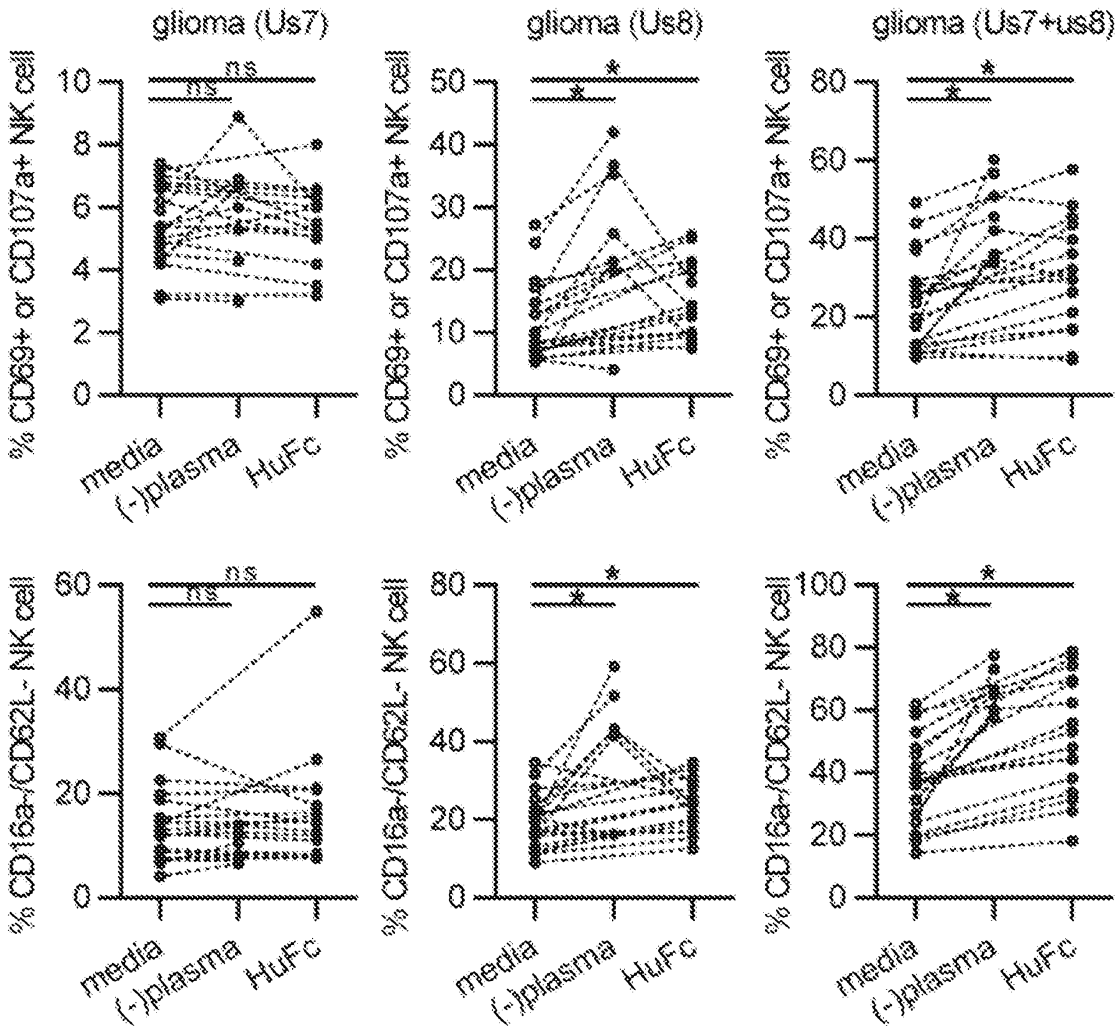

glioma (Us7)
glioma (Us8)
glioma (Us7+us8)
% CD69+ or CD107a+ NK cell
% CD16a-/CD62L- NK cell
ns
*
media
(-)plasma
HuFc

FIG. 11D

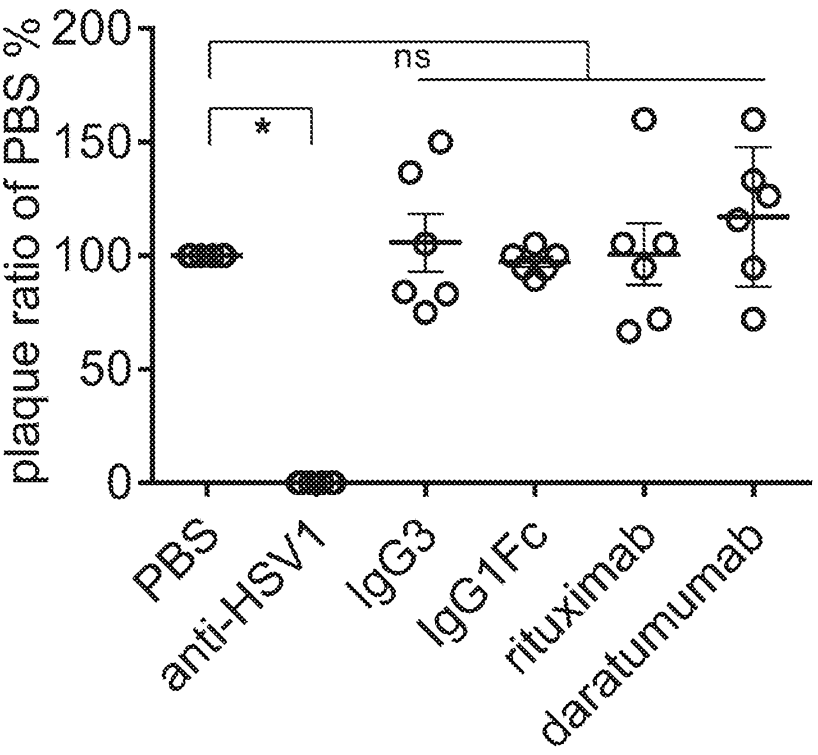

plaque ratio of PBS %
200
150
100
50
0
ns
*
PBS
anti-HSV1
IgG3
IgG1Fc
rituximab
daratumumab

FIG. 12A

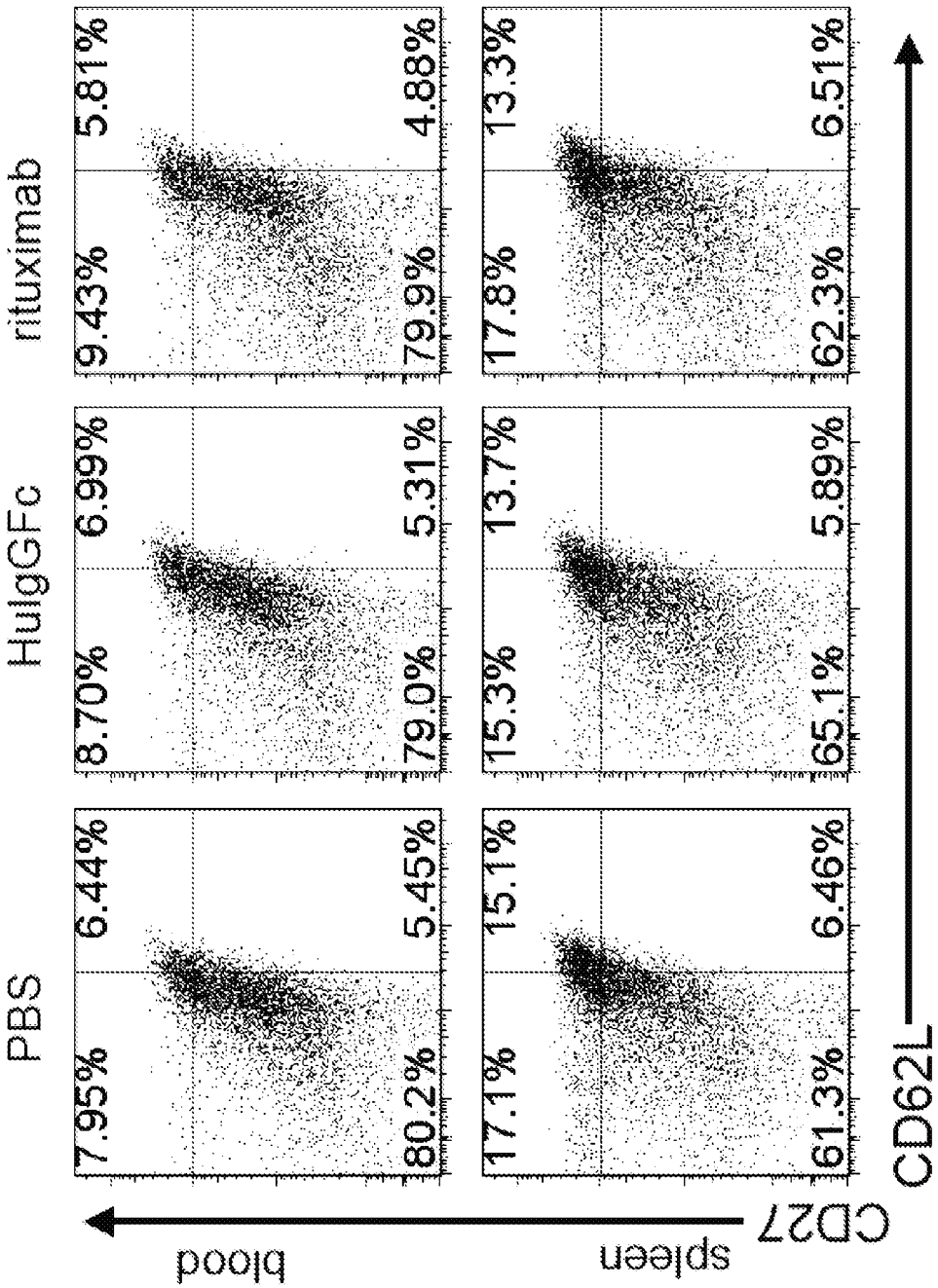

PBS
HuIgGFc
rituximab
blood
spleen
CD27
CD62L
7.95%
6.44%
80.2%
5.45%
8.70%
6.99%
79.0%
5.31%
9.43%
5.81%
79.9%
4.88%
17.1%
15.1%
61.3%
6.46%
15.3%
13.7%
65.1%
5.89%
17.8%
13.3%
62.3%
6.51%

FIG. 12B

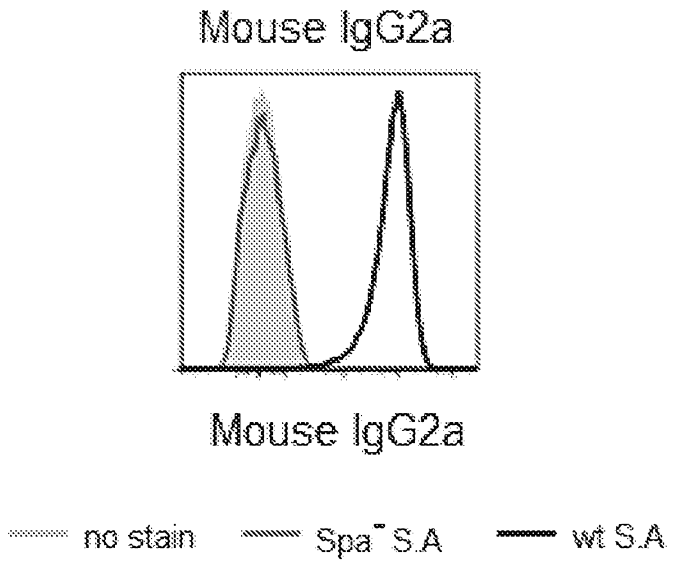


FIG. 13A

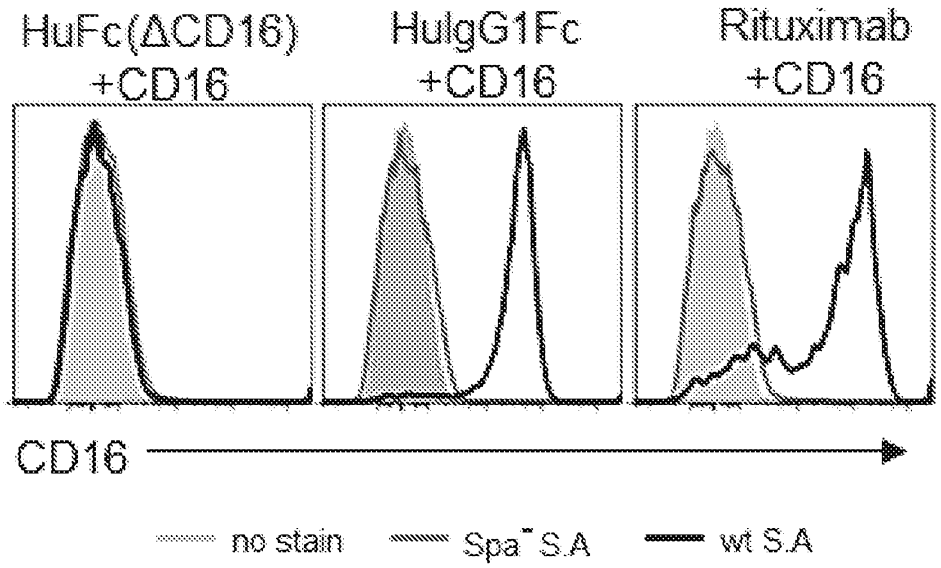


FIG. 13B

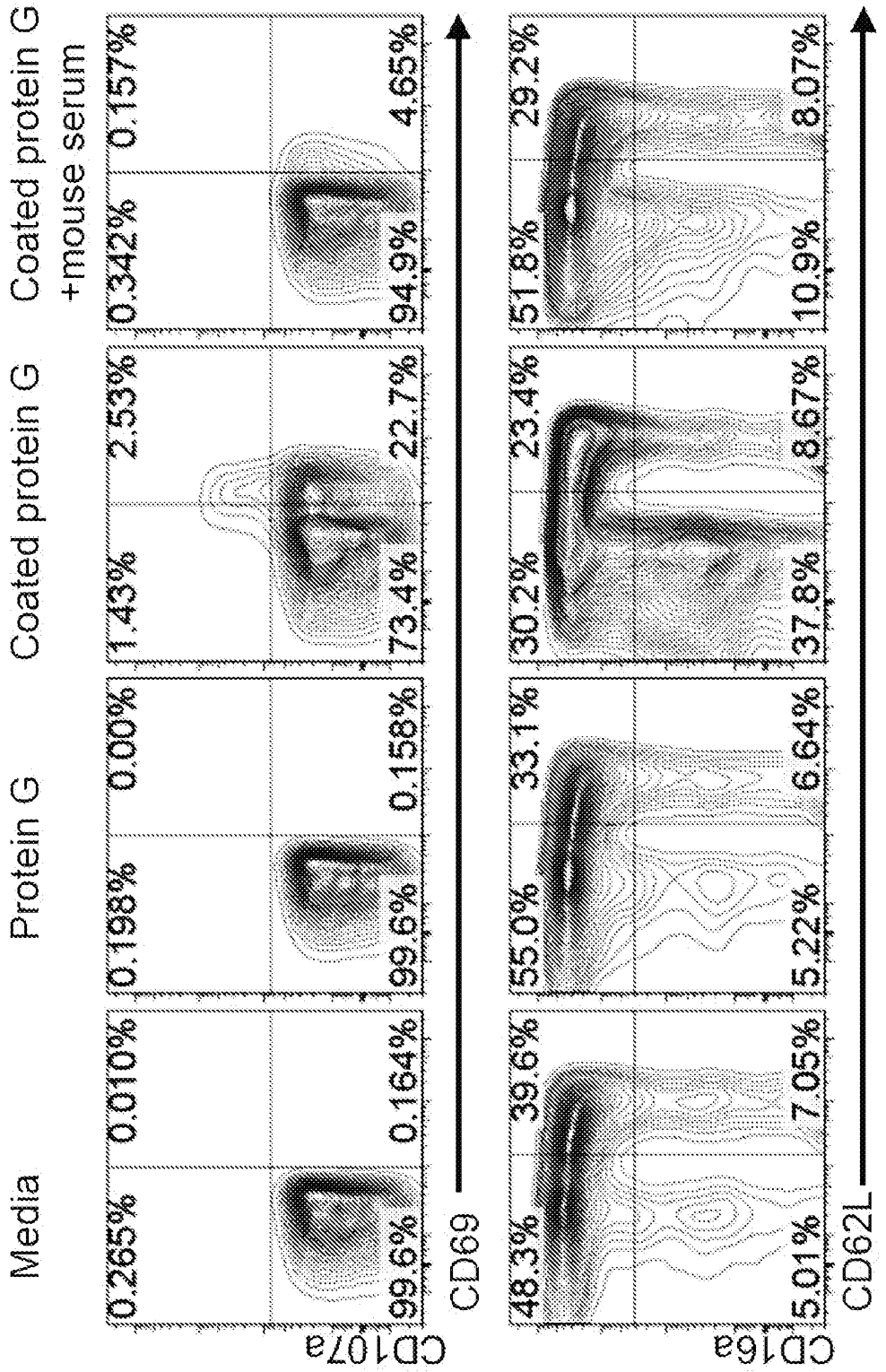

Media
Protein G
Coated protein G
Coated protein G
+mouse serum
0.265%
0.010%
0.198%
0.00%
1.43%
2.53%
0.342%
0.157%
99.6%
0.164%
99.6%
0.158%
73.4%
22.7%
94.9%
4.65%
CD107a
CD69
48.3%
39.6%
55.0%
33.1%
30.2%
23.4%
51.8%
29.2%
5.01%
7.05%
5.22%
6.64%
37.8%
8.67%
10.9%
8.07%
CD16a
CD62L

FIG. 14A

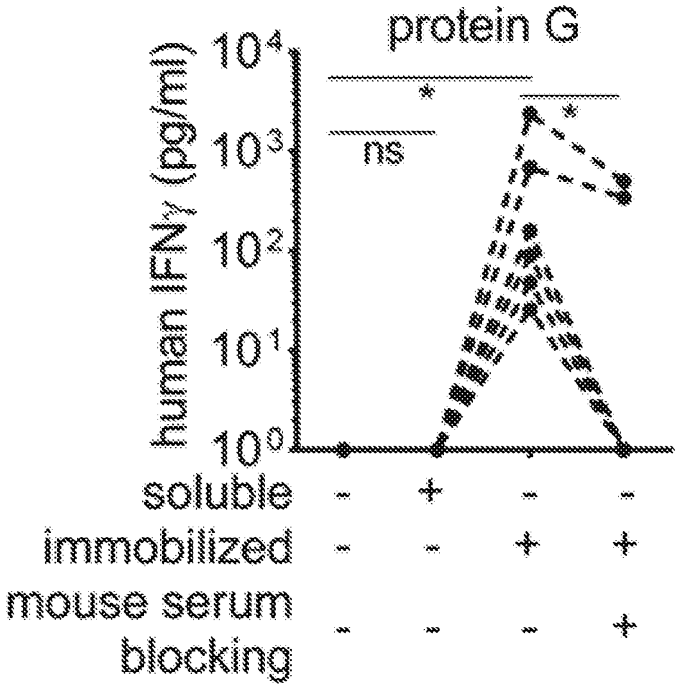


FIG. 14B

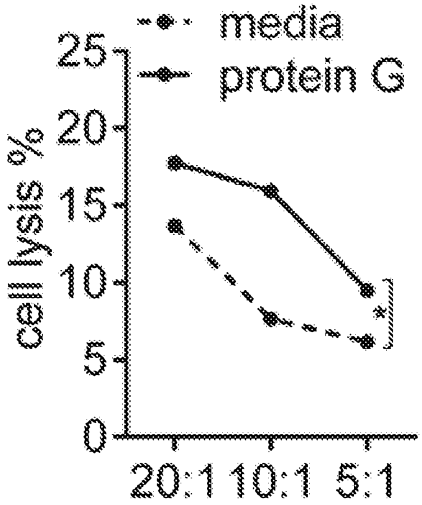


FIG. 14C

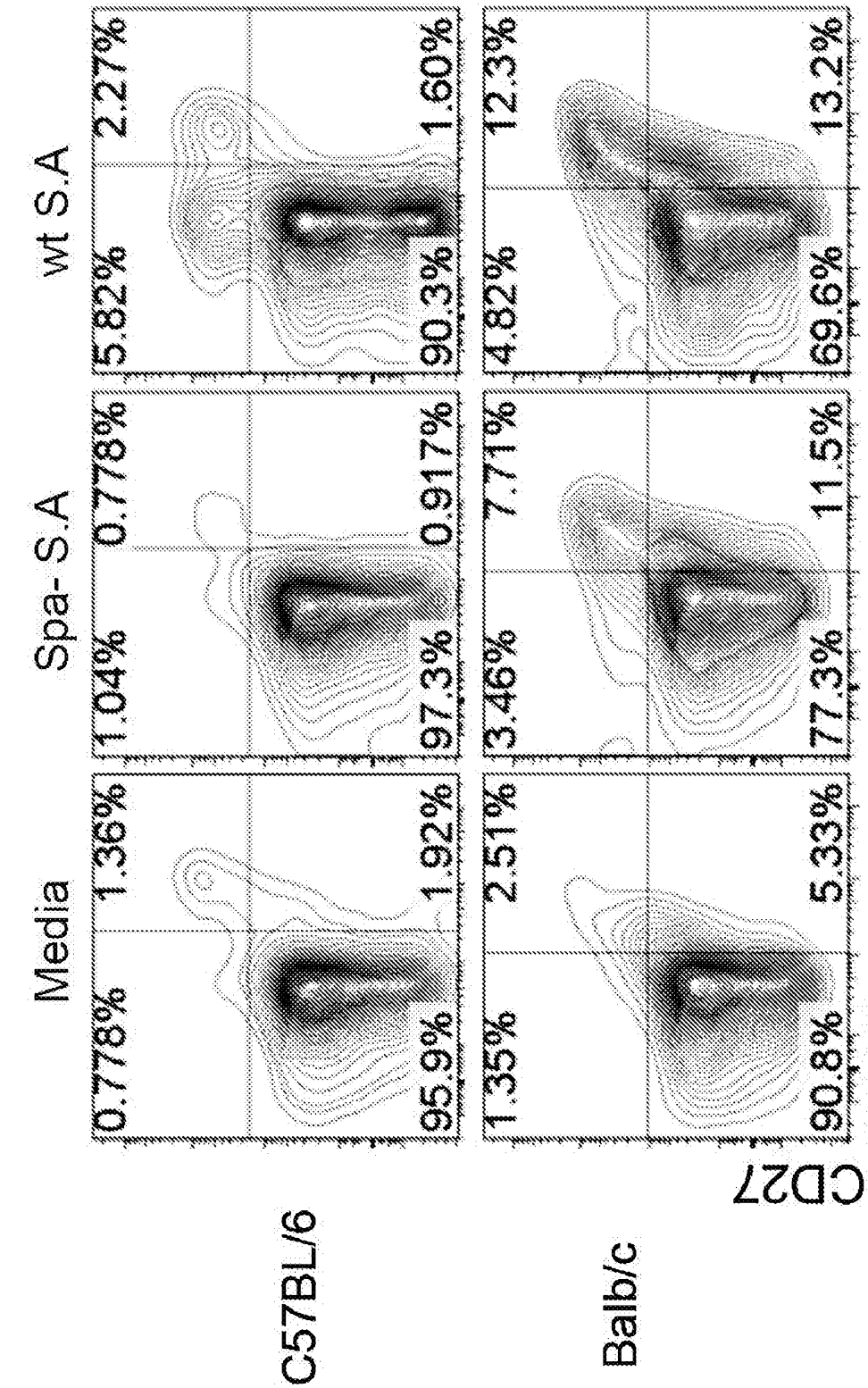

Media
Spa- S.A
wt S.A
C57BL/6
Balb/c
CD27
CD62L
0.778%
1.36%
95.9%
1.92%
1.04%
0.778%
97.3%
0.917%
5.82%
2.27%
90.3%
1.60%
1.35%
2.51%
90.8%
5.33%
3.46%
7.71%
77.3%
11.5%
4.82%
12.3%
69.6%
13.2%

FIG. 15A

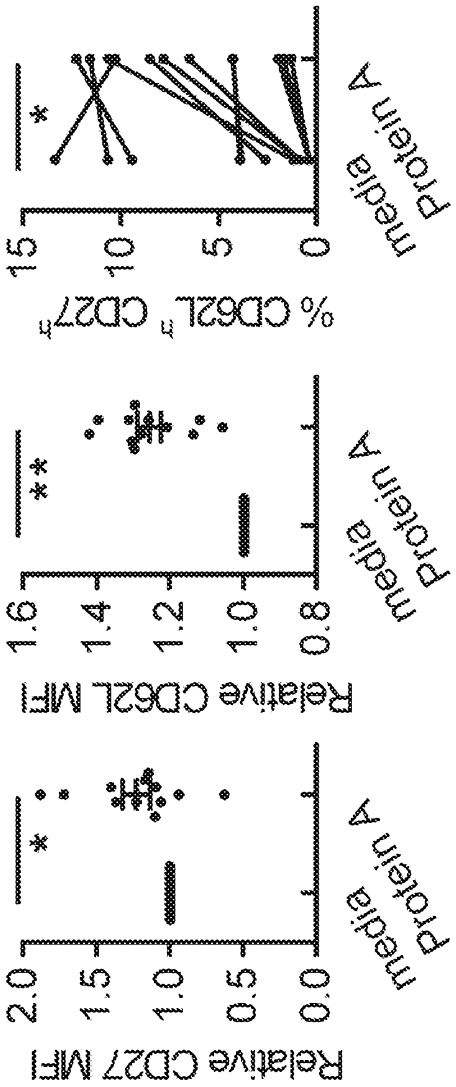

Relative CD27 MFI
2.0
1.5
1.0
0.5
0.0
*
Media
Protein A
Relative CD62L MFI
1.6
1.4
1.2
1.0
0.8
**
Media
Protein A
% CD62L^{h} CD27^{h}
15
10
5
0
*
Media
Protein A

FIG. 15B

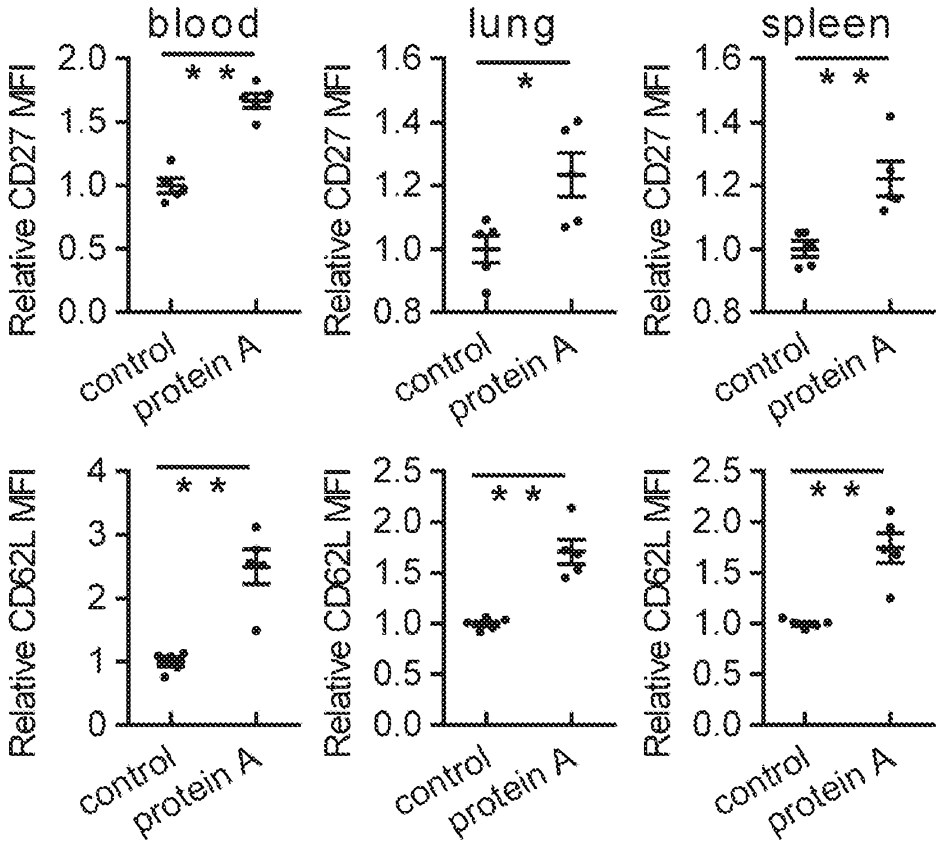

blood
lung
spleen
Relative CD27 MFI
Relative CD62L MFI
control
protein A
*
*

FIG. 15C

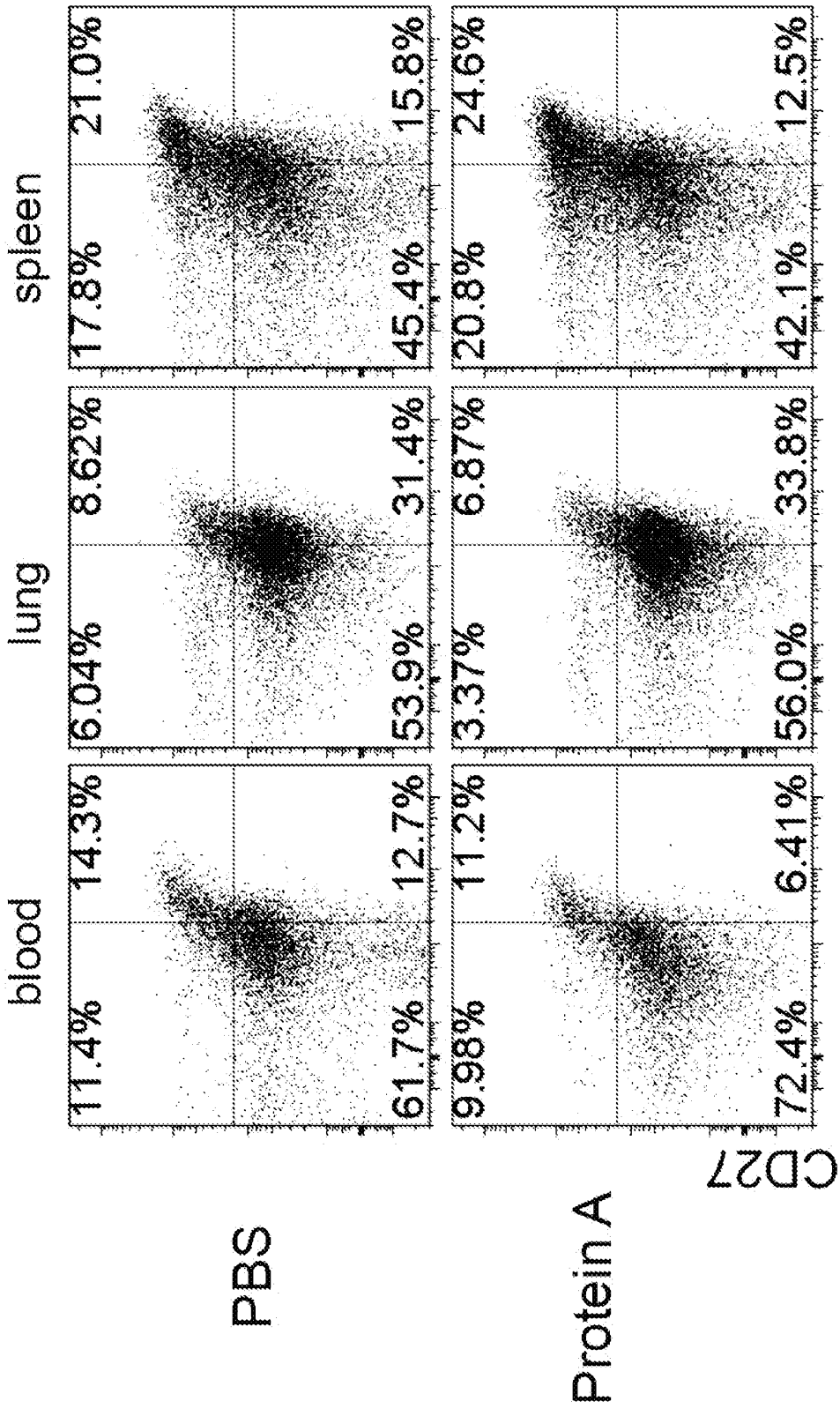

blood
lung
spleen
PBS
Protein A
CD27
CD62L
11.4%
14.3%
61.7%
12.7%
6.04%
8.62%
53.9%
31.4%
17.8%
21.0%
45.4%
15.8%
9.98%
11.2%
72.4%
6.41%
3.37%
6.87%
56.0%
33.8%
20.8%
24.6%
42.1%
12.5%

FIG. 15D

FIG. 16

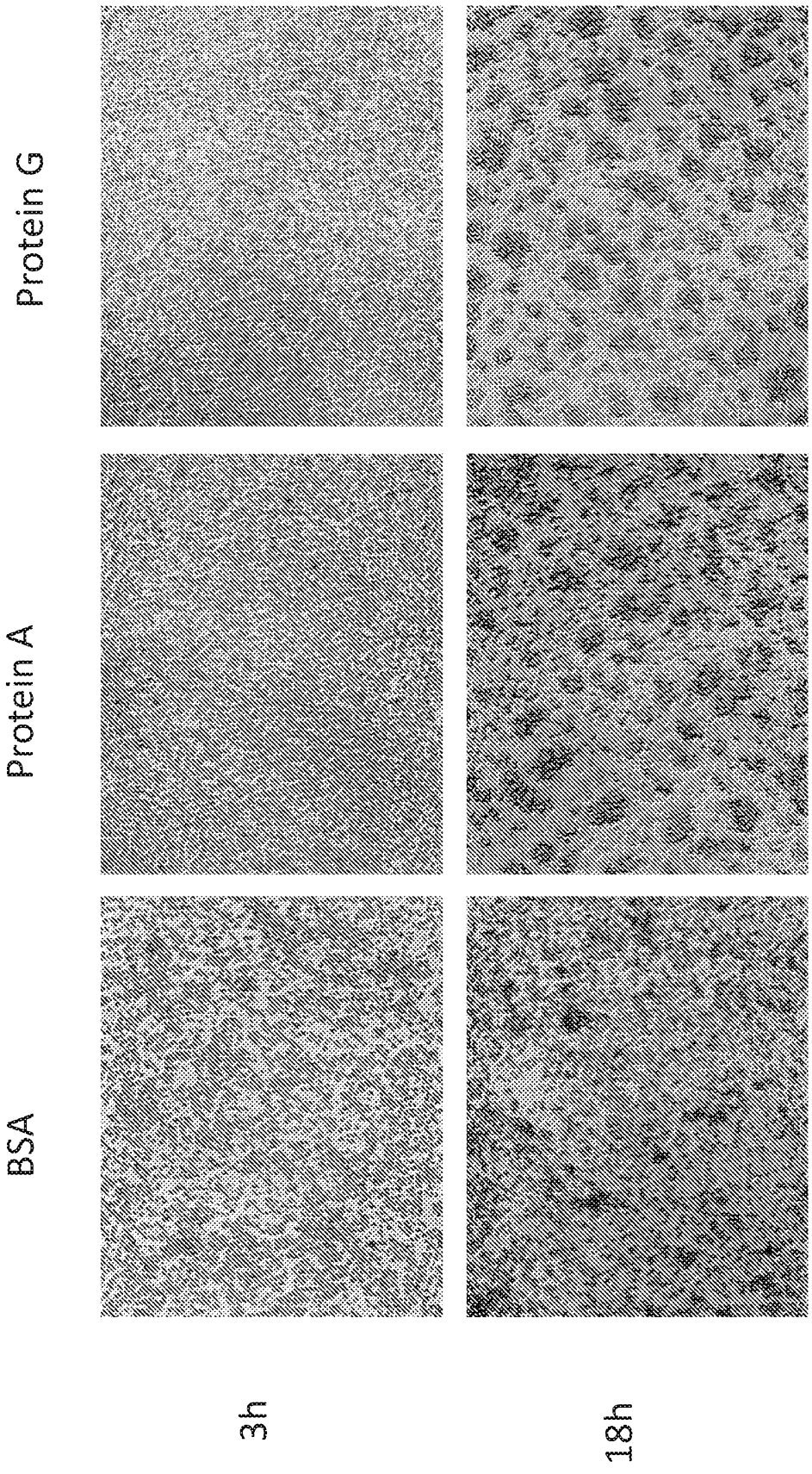


FIG. 17A

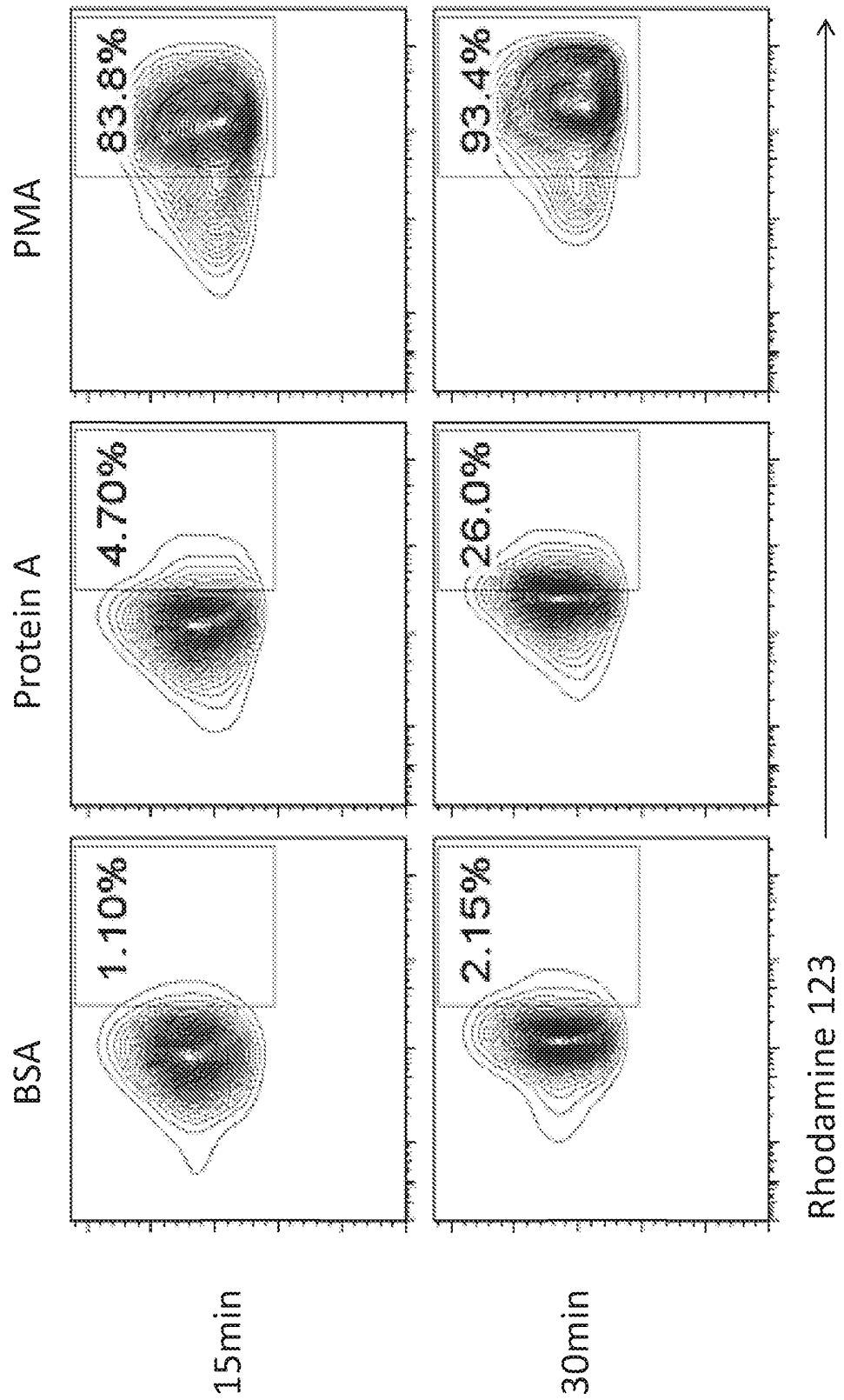

BSA
Protein A
PMA
1.10%
4.70%
83.8%
2.15%
26.0%
93.4%
15min
30min
Rhodamine 123

FIG. 17B

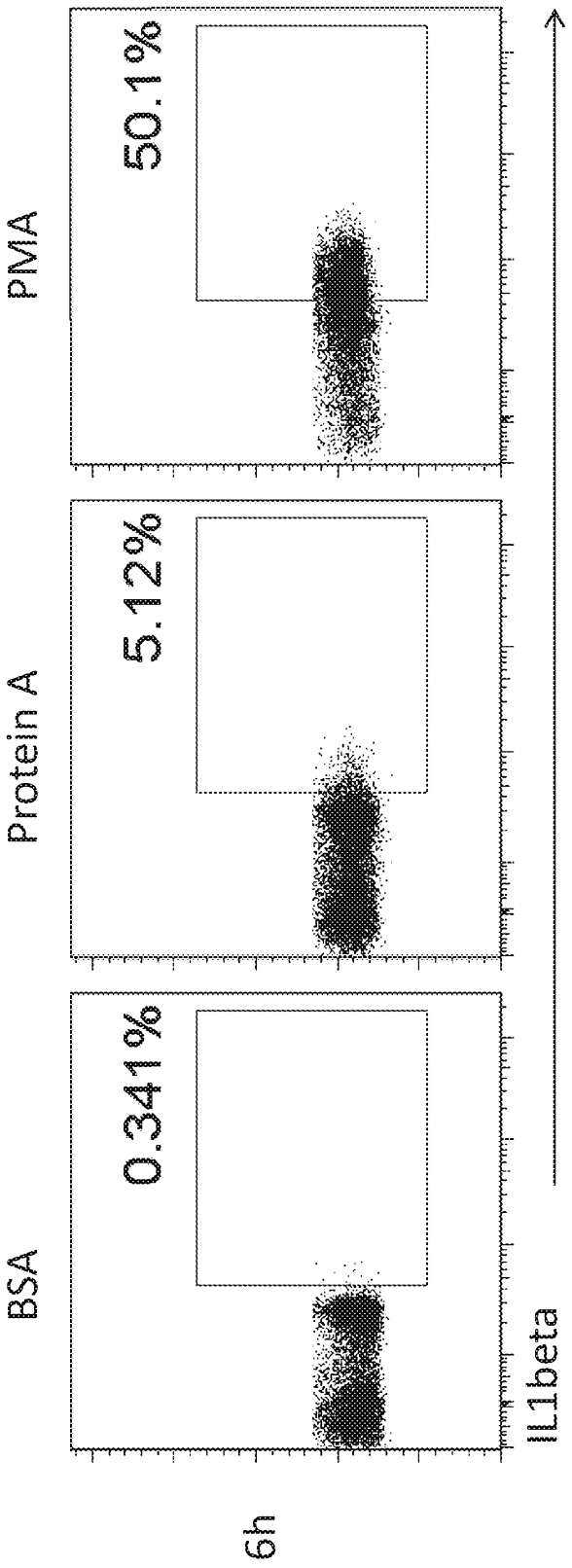


FIG. 17C

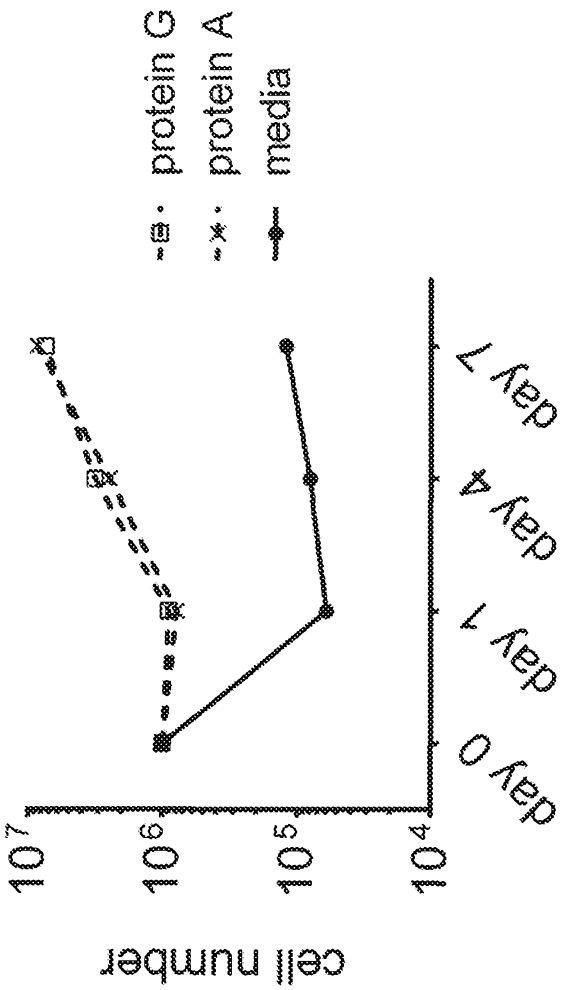

protein G
protein A
media
cell number
10^7
10^6
10^5
10^4
day 0
day 1
day 4
day 7

FIG. 18

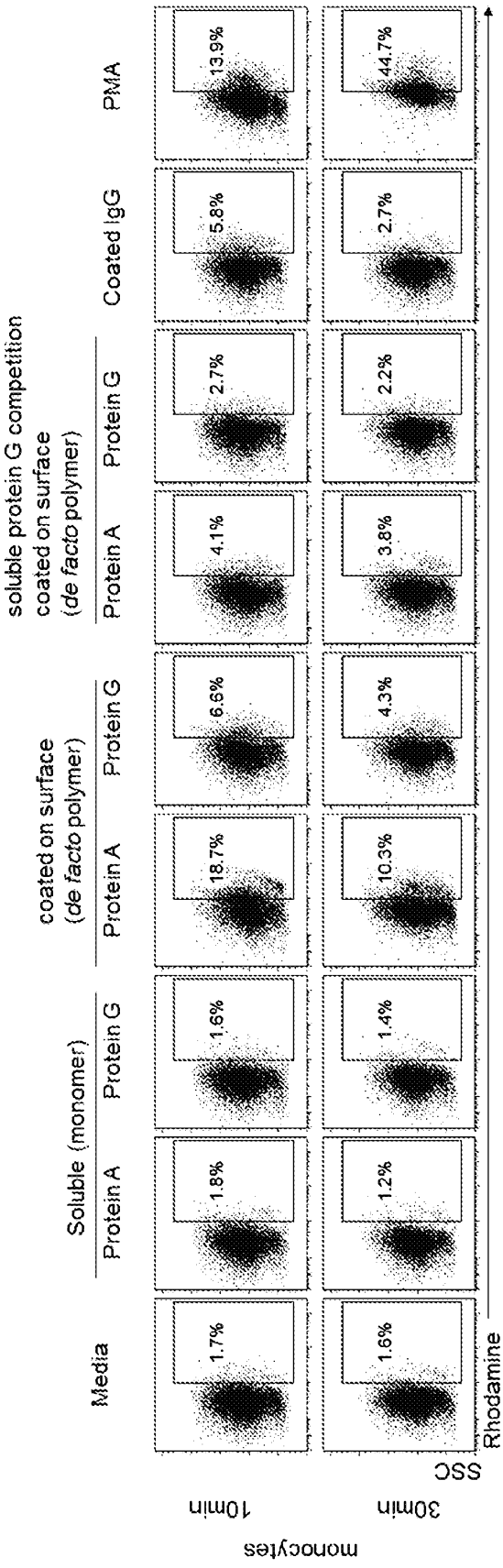

monocytes
10min
30min
SSC
Rhodamine
Media
Soluble (monomer)
Protein A
Protein G
coated on surface
(de facto polymer)
Protein A
Protein G
soluble protein G competition
coated on surface
(de facto polymer)
Protein A
Protein G
Coated IgG
PMA
1.7%
1.8%
1.6%
18.7%
6.6%
4.1%
2.7%
5.8%
13.9%
1.6%
1.2%
1.4%
10.3%
4.3%
3.8%
2.2%
2.7%
44.7%

FIG. 19

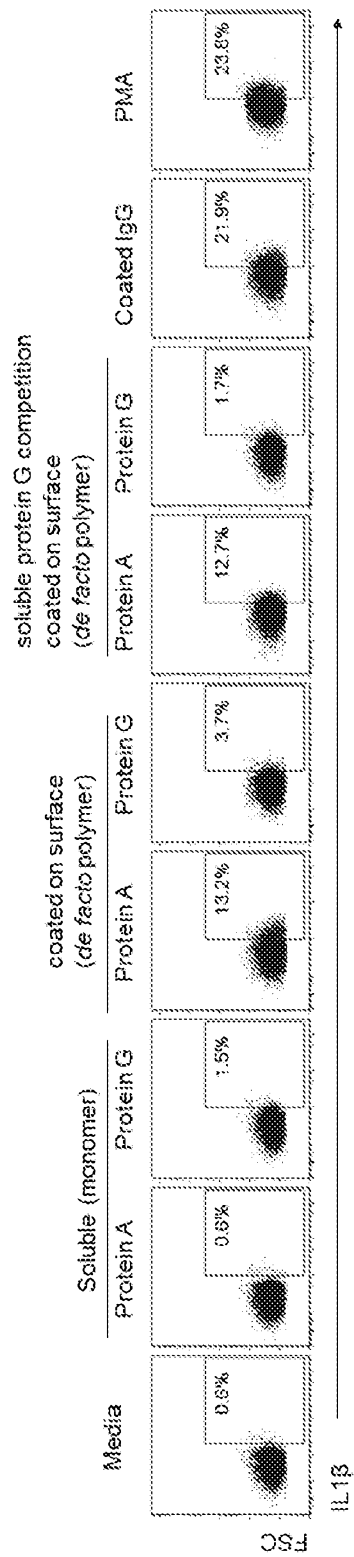

soluble protein G competition
coated on surface
(de facto polymer)
Media
Soluble (monomer)
Protein A
Protein G
coated on surface
(de facto polymer)
Protein A
Protein G
Protein A
Protein G
Coated IgG
PMA
0.6%
0.6%
1.5%
13.2%
3.7%
12.7%
1.7%
21.9%
23.8%
FSC
IL1β

FIG. 20

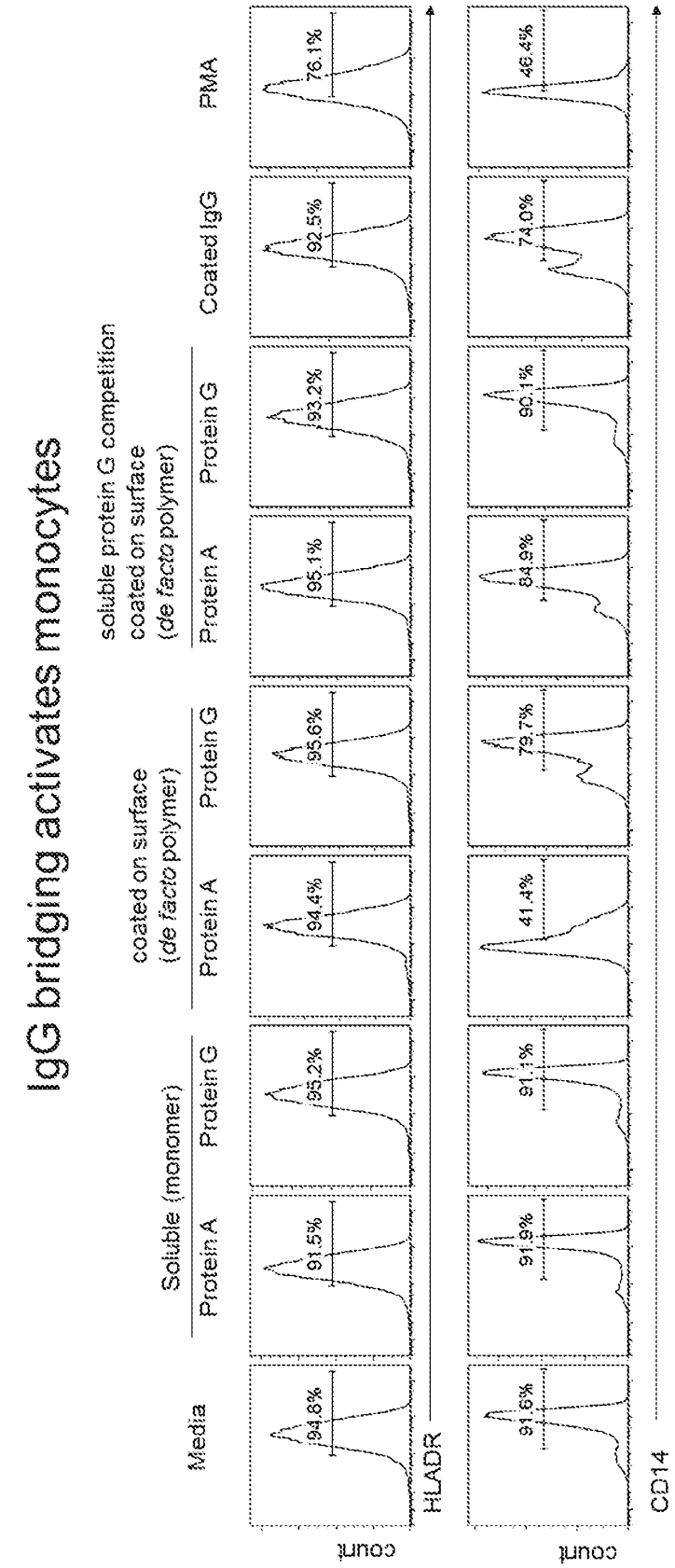

IgG bridging activates monocytes
soluble protein G competition
coated on surface
(de facto polymer)
Soluble (monomer)
coated on surface
(de facto polymer)
Media
Protein A
Protein G
Protein A
Protein G
Protein A
Protein G
Coated IgG
PMA
94.8%
91.5%
95.2%
94.4%
95.6%
95.1%
93.2%
92.5%
76.1%
91.6%
91.9%
91.1%
41.4%
79.7%
84.9%
90.1%
74.0%
46.4%
count
HLADR
CD14

FIG. 21

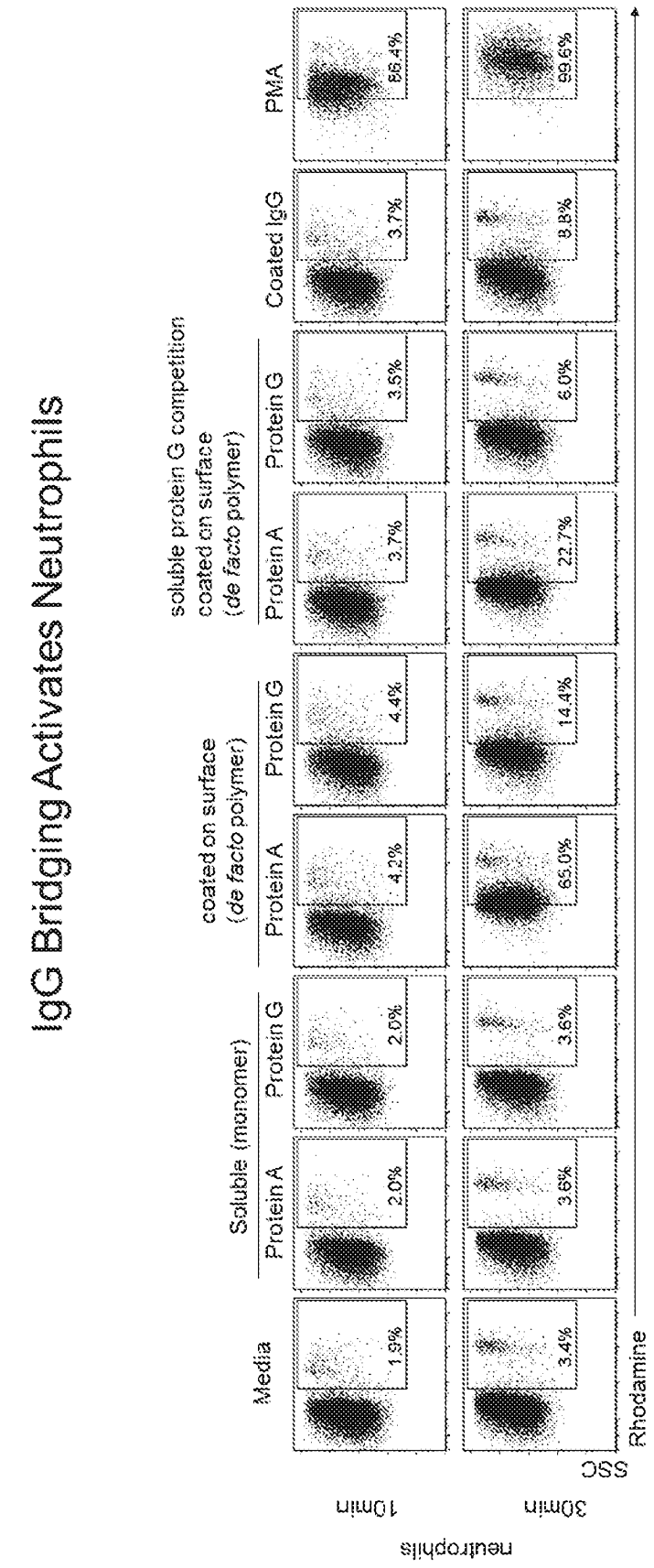

IgG Bridging Activates Neutrophils
neutrophils
10min
30min
SSC
Rhodamine
Media
Soluble (monomer)
coated on surface (de facto polymer)
soluble protein G competition coated on surface (de facto polymer)
Protein A
Protein G
Coated IgG
PMA
1.9%
2.0%
2.0%
4.2%
4.4%
3.7%
3.5%
3.7%
86.4%
3.4%
3.6%
3.6%
65.0%
14.4%
22.7%
6.0%
9.8%
89.6%

FIG. 22

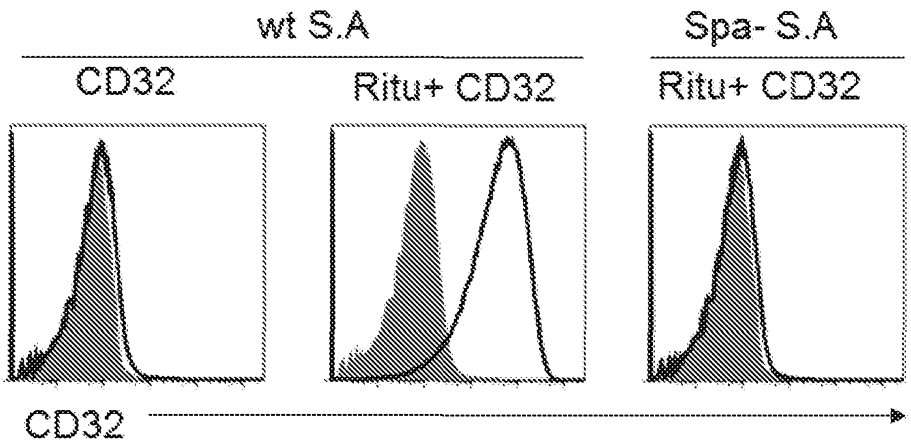


FIG 23A

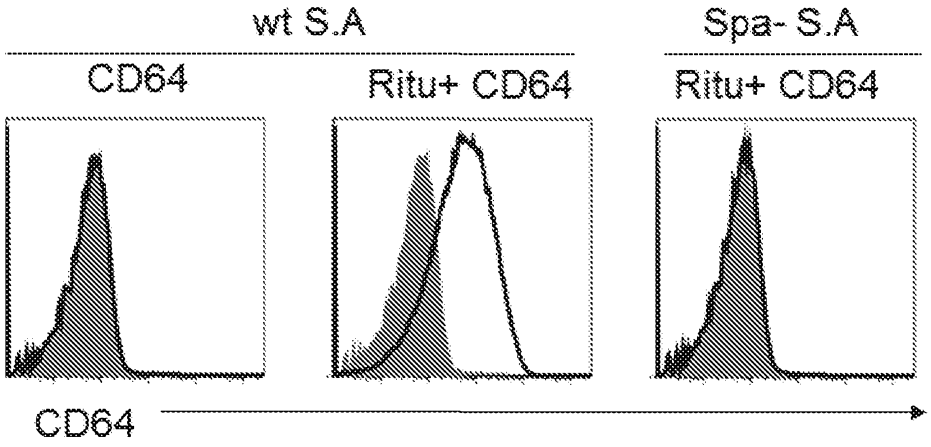


FIG 23B

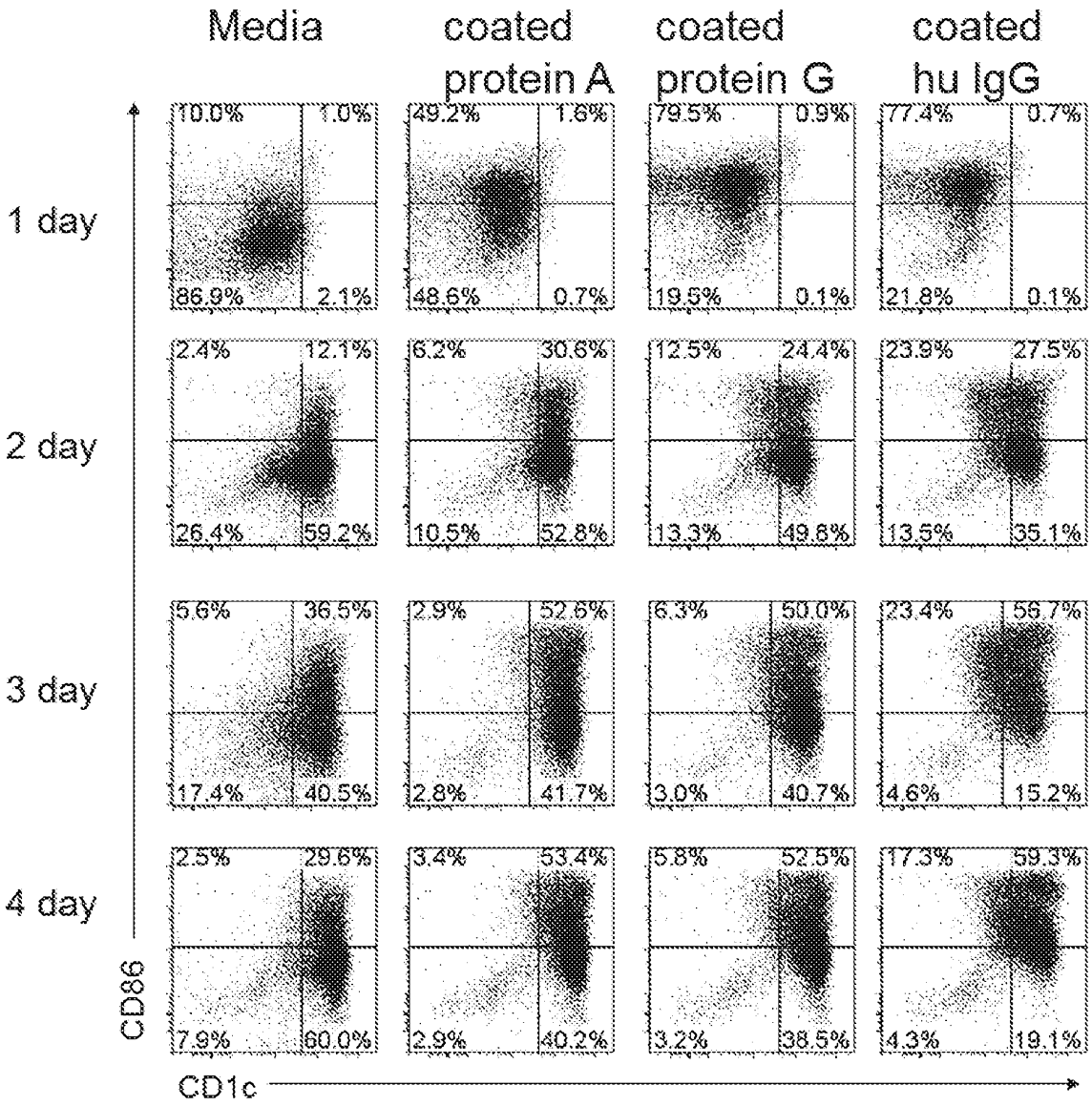

Media
coated protein A
coated protein G
coated hu IgG
1 day
2 day
3 day
4 day
CD86
CD1c
10.0% 1.0% 86.9% 2.1%
49.2% 1.6% 48.6% 0.7%
79.5% 0.9% 19.5% 0.1%
77.4% 0.7% 21.8% 0.1%
2.4% 12.1% 26.4% 59.2%
6.2% 30.6% 10.5% 52.8%
12.5% 24.4% 13.3% 49.8%
23.9% 27.5% 13.5% 35.1%
5.6% 36.5% 17.4% 40.5%
2.9% 52.6% 2.8% 41.7%
6.3% 50.0% 3.0% 40.7%
23.4% 56.7% 4.6% 15.2%
2.5% 29.6% 7.9% 60.0%
3.4% 53.4% 2.9% 40.2%
5.8% 52.5% 3.2% 38.5%
17.3% 59.3% 4.3% 19.1%

FIG. 24

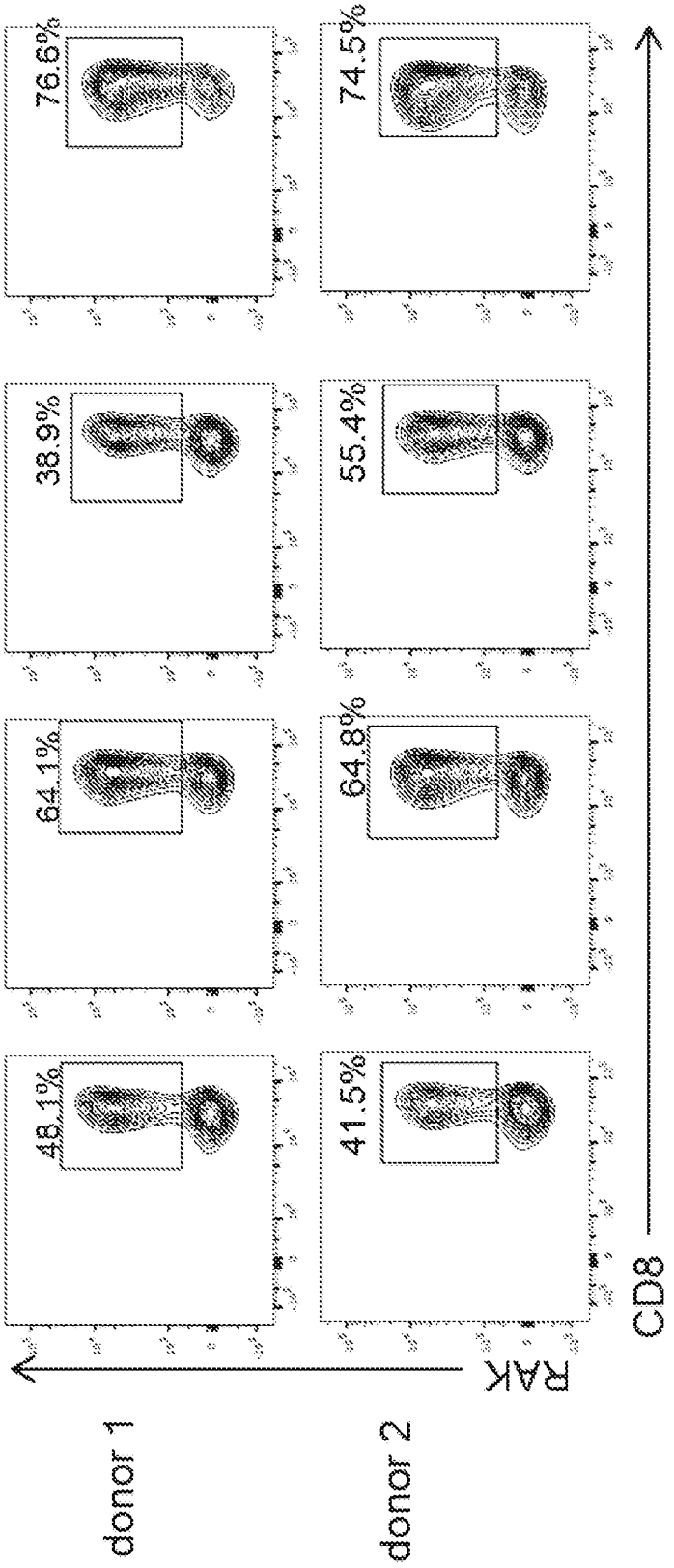


FIG. 25

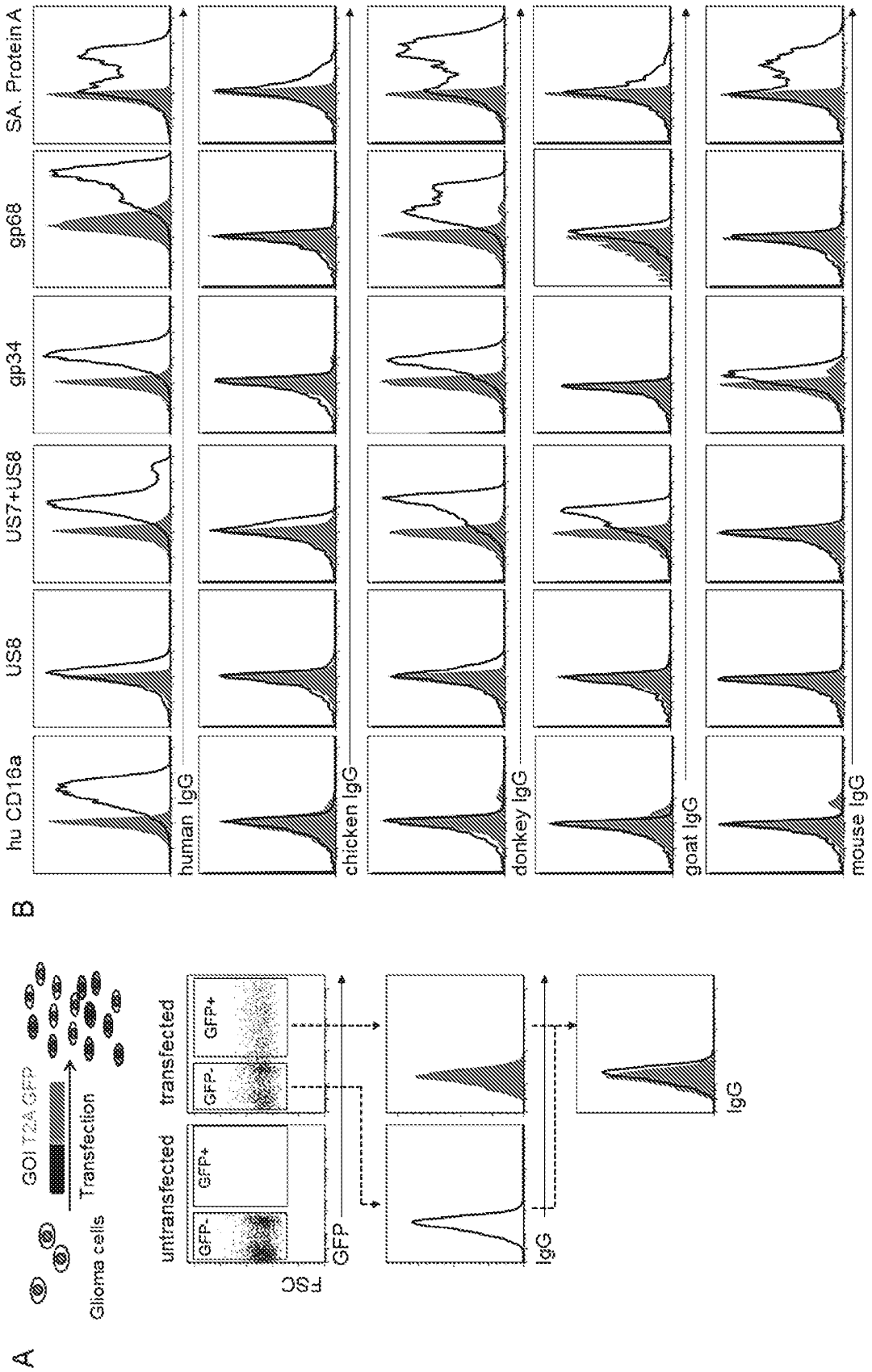

A
Glioma cells
GOI T2A GFP
Transfection
untransfected
transfected
GFP-
GFP+
GFP-
GFP+
FSC
GFP
IgG
IgG
B
hu CD16a
US8
US7+US8
gp34
gp68
SA. Protein A
human IgG
chicken IgG
donkey IgG
goat IgG
mouse IgG

FIG. 26

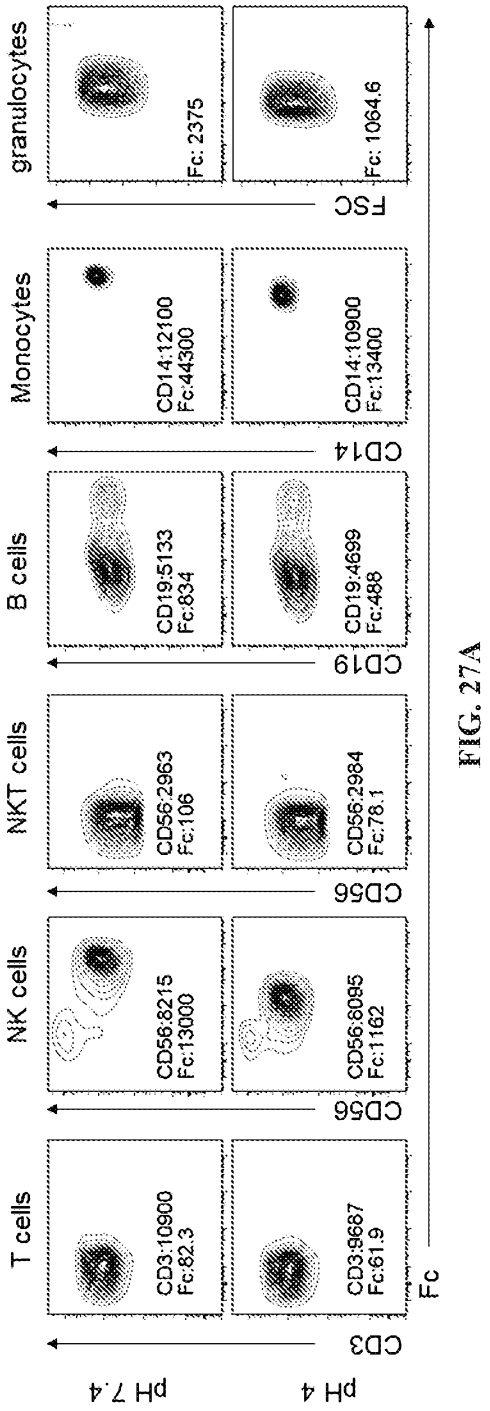


FIG. 27A

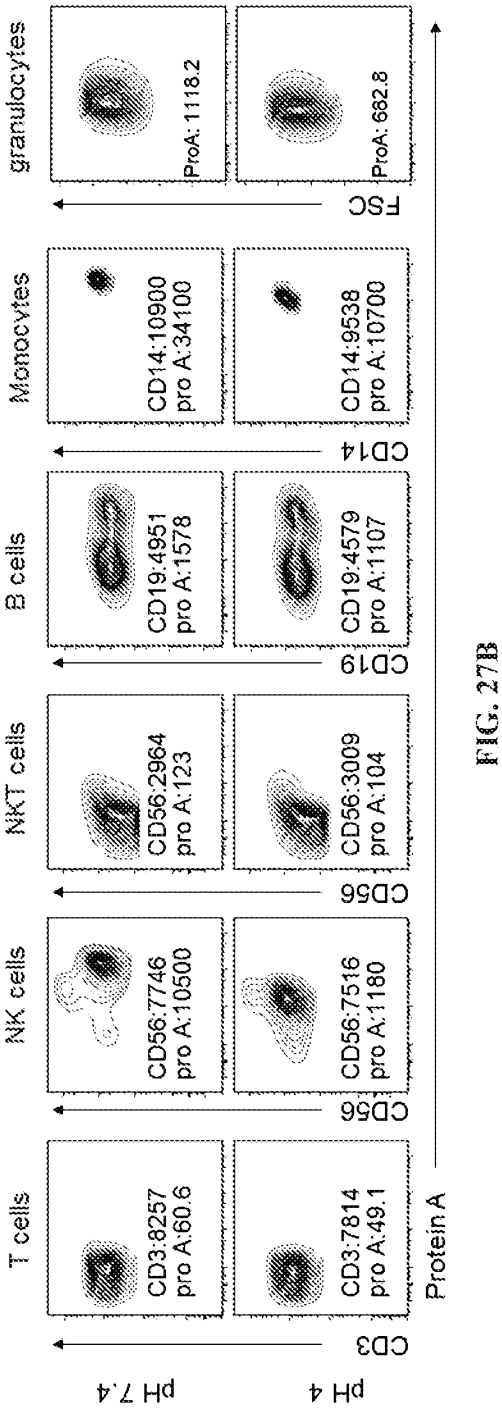


FIG. 27B

FIG 28A

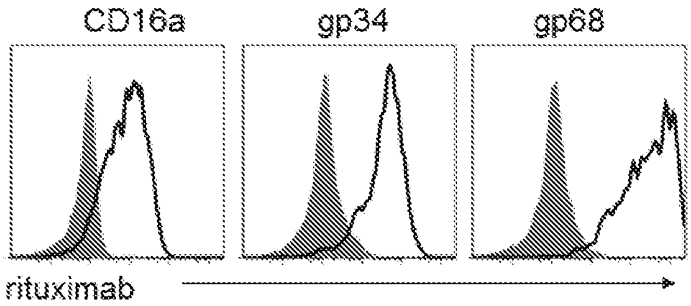


FIG 28B

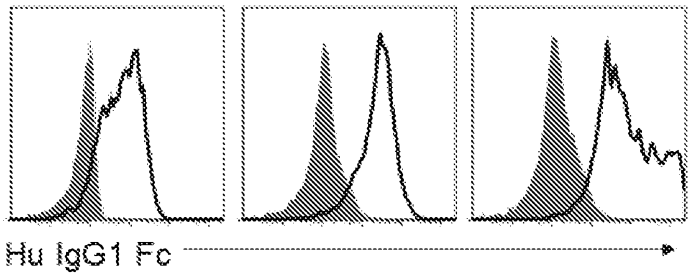


FIG 28C

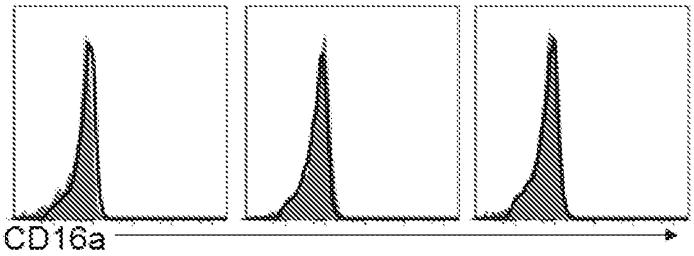


FIG 28D

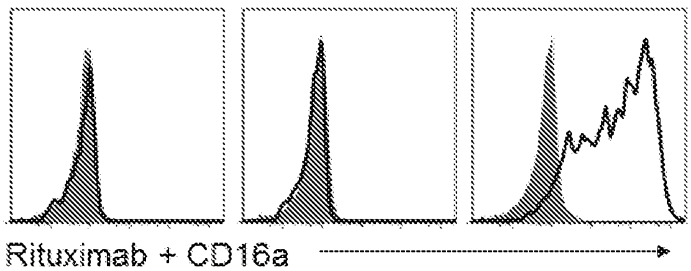
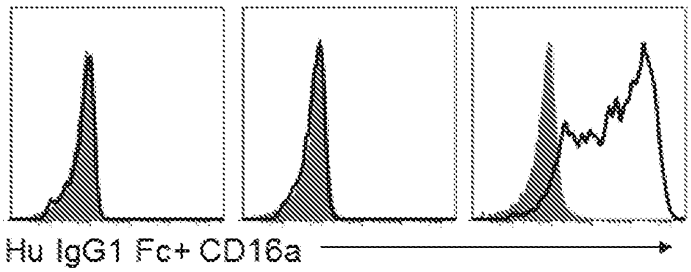


FIG 28E



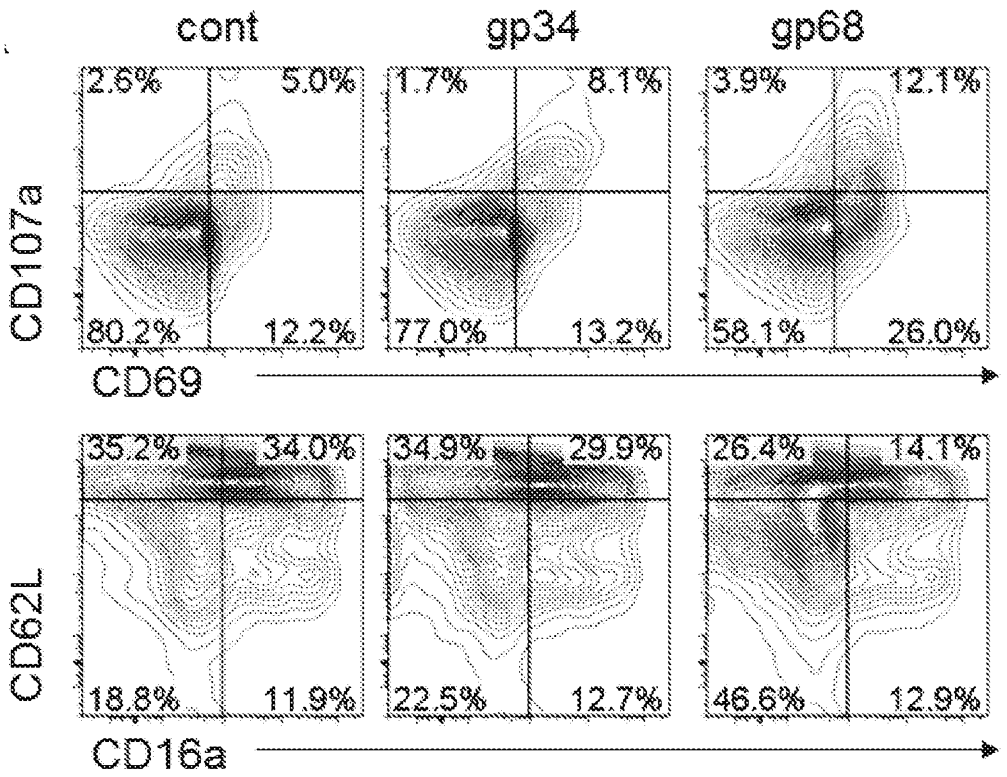


FIG. 29A

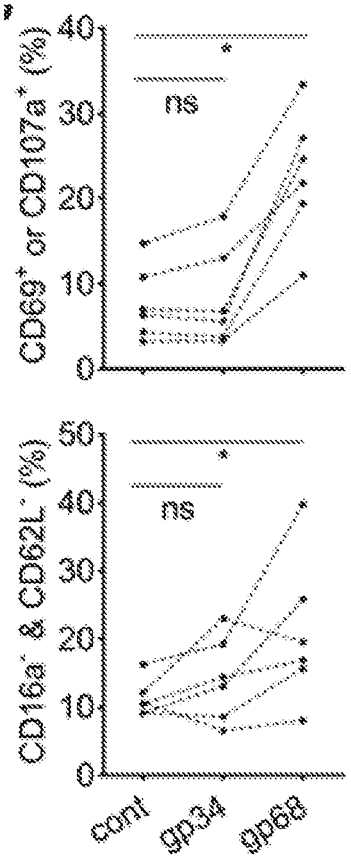


FIG. 29B

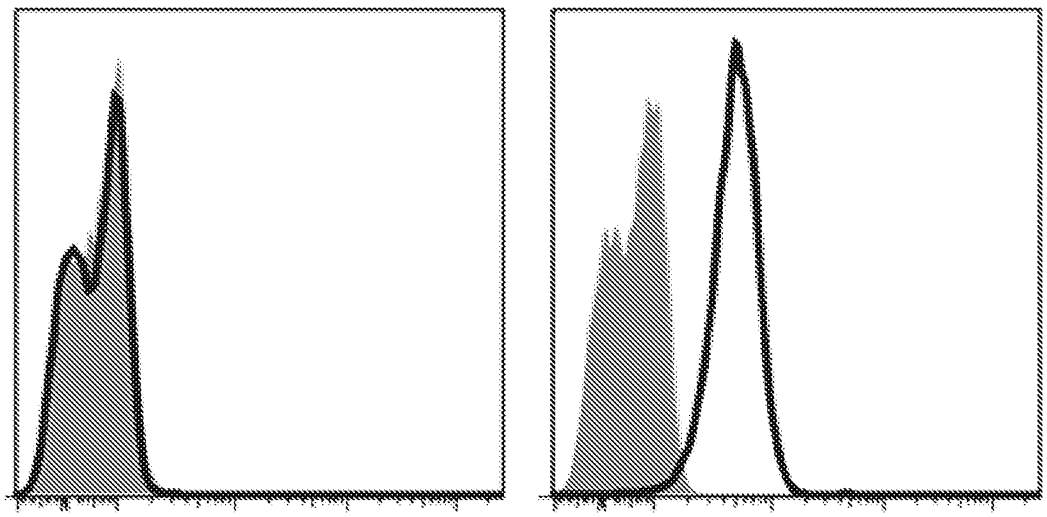


FIG. 30

SEQUENCE LISTING

<110> Ohio State Innovation Foundation

<120> Passive Antibody Dependent Cell-Mediated Activation

<130> 16-1848-WO

<160> 170

<170> PatentIn version 3.5

<210> 1
<211> 232
<212> PRT
<213> Homo sapiens

<400> 1

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1 5 10 15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro

```
        115                 120                 125


Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
    130                 135                 140


Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145                 150                 155                 160


Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175


Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190


Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205


Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220


Ser Leu Ser Leu Ser Pro Gly Lys
225                 230


<210>  2
<211>  205
<212>  PRT
<213>  Homo sapiens

<400>  2

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
1               5                   10                  15


Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
            20                  25                  30


Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
        35                  40                  45
```

```
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
    50                  55                  60

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
65                  70                  75                  80

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
                85                  90                  95

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
            100                 105                 110

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
        115                 120                 125

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
    130                 135                 140

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

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
                165                 170                 175

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
            180                 185                 190

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        195                 200                 205
```

<210> 3
<211> 450
<212> PRT
<213> Staphylococcus aureus

<400> 3

```
Met Lys Lys Lys Asn Ile Tyr Ser Ile Arg Lys Leu Gly Val Gly Ile
1               5                   10                  15


Ala Ser Val Thr Leu Gly Thr Leu Leu Ile Ser Gly Gly Val Thr Pro
            20                  25                  30


Ala Ala Asn Ala Ala Gln His Asp Glu Ala Gln Gln Asn Ala Phe Tyr
        35                  40                  45


Gln Val Leu Asn Met Pro Asn Leu Asn Ala Asp Gln Arg Asn Gly Phe
    50                  55                  60


Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Val Leu Gly
65                  70                  75                  80


Glu Ala Gln Lys Leu Asn Asp Ser Gln Ala Pro Lys Ala Asp Ala Gln
                85                  90                  95


Gln Asn Asn Phe Asn Lys Asp Gln Gln Ser Ala Phe Tyr Glu Ile Leu
            100                 105                 110


Asn Met Pro Asn Leu Asn Glu Ala Gln Arg Asn Gly Phe Ile Gln Ser
        115                 120                 125


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


Lys Leu Asn Glu Ser Gln Ala Pro Lys Ala Asp Asn Asn Phe Asn Lys
145                 150                 155                 160


Glu Gln Gln Asn Ala Phe Tyr Glu Ile Leu Asn Met Pro Asn Leu Asn
                165                 170                 175


Glu Glu Gln Arg Asn Gly Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser
            180                 185                 190
```

```
Gln Ser Ala Asn Leu Leu Ser Glu Ala Lys Lys Leu Asn Glu Ser Gln
        195                 200                 205

Ala Pro Lys Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe
    210                 215                 220

Tyr Glu Ile Leu His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Gly
225                 230                 235                 240

Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser Val Ser Lys Glu Ile Leu
                245                 250                 255

Ala Glu Ala Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys Glu Glu Asp
            260                 265                 270

Asn Lys Lys Pro Gly Lys Glu Asp Gly Asn Lys Pro Gly Lys Glu Asp
        275                 280                 285

Gly Asn Lys Pro Gly Lys Glu Asp Asn Lys Lys Pro Gly Lys Glu Asp
    290                 295                 300

Gly Asn Lys Pro Gly Lys Glu Asp Asn Asn Lys Pro Gly Lys Glu Asp
305                 310                 315                 320

Gly Asn Lys Pro Gly Lys Glu Asp Asn Asn Lys Pro Gly Lys Glu Asp
                325                 330                 335

Gly Asn Lys Pro Gly Lys Glu Asp Gly Asn Lys Pro Gly Lys Glu Asp
            340                 345                 350

Gly Asn Gly Val His Val Val Lys Pro Gly Asp Thr Val Asn Asp Ile
        355                 360                 365

Ala Lys Ala Asn Gly Thr Thr Ala Asp Lys Ile Ala Ala Asp Asn Lys
    370                 375                 380
```

```
Leu Ala Asp Lys Asn Met Ile Lys Pro Gly Gln Glu Leu Val Val Asp
385                 390                 395                 400


Lys Lys Gln Pro Ala Asn His Ala Asp Ala Asn Lys Ala Gln Ala Leu
                405                 410                 415


Pro Glu Thr Gly Glu Glu Asn Pro Phe Ile Gly Thr Thr Val Phe Gly
            420                 425                 430


Gly Leu Ser Leu Ala Leu Gly Ala Ala Leu Leu Ala Gly Arg Arg Arg
        435                 440                 445


Glu Leu
    450


<210>  4
<211>  448
<212>  PRT
<213>  Streptococcus

<400>  4

Met Glu Lys Glu Lys Lys Val Lys Tyr Phe Leu Arg Lys Ser Ala Phe
1               5                   10                  15


Gly Leu Ala Ser Val Ser Ala Ala Phe Leu Val Gly Ser Thr Val Phe
            20                  25                  30


Ala Val Asp Ser Pro Ile Glu Asp Thr Pro Ile Ile Arg Asn Gly Gly
        35                  40                  45


Glu Leu Thr Asn Leu Leu Gly Asn Ser Glu Thr Thr Leu Ala Leu Arg
    50                  55                  60


Asn Glu Glu Ser Ala Thr Ala Asp Leu Thr Ala Ala Ala Val Ala Asp
65                  70                  75                  80
```

```
Thr Val Ala Ala Ala Ala Ala Glu Asn Ala Gly Ala Ala Ala Trp Glu
                85                  90                  95

Ala Ala Ala Ala Ala Asp Ala Leu Ala Lys Ala Lys Ala Asp Ala Leu
            100                 105                 110

Lys Glu Phe Asn Lys Tyr Gly Val Ser Asp Tyr Tyr Lys Asn Leu Ile
        115                 120                 125

Asn Asn Ala Lys Thr Val Glu Gly Ile Lys Asp Leu Gln Ala Gln Val
    130                 135                 140

Val Glu Ser Ala Lys Lys Ala Arg Ile Ser Glu Ala Thr Asp Gly Leu
145                 150                 155                 160

Ser Asp Phe Leu Lys Ser Gln Thr Pro Ala Glu Asp Thr Val Lys Ser
                165                 170                 175

Ile Glu Leu Ala Glu Ala Lys Val Leu Ala Asn Arg Glu Leu Asp Lys
            180                 185                 190

Tyr Gly Val Ser Asp Tyr His Lys Asn Leu Ile Asn Asn Ala Lys Thr
        195                 200                 205

Val Glu Gly Val Lys Glu Leu Ile Asp Glu Ile Leu Ala Ala Leu Pro
    210                 215                 220

Lys Thr Asp Thr Tyr Lys Leu Ile Leu Asn Gly Lys Thr Leu Lys Gly
225                 230                 235                 240

Glu Thr Thr Thr Glu Ala Val Asp Ala Ala Thr Ala Glu Lys Val Phe
                245                 250                 255

Lys Gln Tyr Ala Asn Asp Asn Gly Val Asp Gly Glu Trp Thr Tyr Asp
            260                 265                 270
```

```
Asp Ala Thr Lys Thr Phe Thr Val Thr Glu Lys Pro Glu Val Ile Asp
        275                 280                 285


Ala Ser Glu Leu Thr Pro Ala Val Thr Thr Tyr Lys Leu Val Ile Asn
    290                 295                 300


Gly Lys Thr Leu Lys Gly Glu Thr Thr Thr Lys Ala Val Asp Ala Glu
305                 310                 315                 320


Thr Ala Glu Lys Ala Phe Lys Gln Tyr Ala Asn Asp Asn Gly Val Asp
                325                 330                 335


Gly Val Trp Thr Tyr Asp Asp Ala Thr Lys Thr Phe Thr Val Thr Glu
            340                 345                 350


Met Val Thr Glu Val Pro Gly Asp Ala Pro Thr Glu Pro Glu Lys Pro
        355                 360                 365


Glu Ala Ser Ile Pro Leu Val Pro Leu Thr Pro Ala Thr Pro Ile Ala
    370                 375                 380


Lys Asp Asp Ala Lys Lys Asp Asp Thr Lys Lys Glu Asp Ala Lys Lys
385                 390                 395                 400


Pro Glu Ala Lys Lys Asp Asp Ala Lys Lys Ala Glu Thr Leu Pro Thr
                405                 410                 415


Thr Gly Glu Gly Ser Asn Pro Phe Phe Thr Ala Ala Ala Leu Ala Val
            420                 425                 430


Met Ala Gly Ala Gly Ala Leu Ala Val Ala Ser Lys Arg Lys Glu Asp
        435                 440                 445


<210>  5
<211>  548
<212>  PRT
<213>  human herpesvirus 2
```

<400> 5

```
Met Ala Arg Gly Ala Gly Leu Val Phe Phe Val Gly Val Trp Val Val
1               5                  10                  15

Ser Cys Leu Ala Ala Ala Pro Arg Thr Ser Trp Lys Arg Val Thr Ser
            20                  25                  30

Gly Glu Asp Val Val Leu Leu Pro Ala Pro Ala Gly Pro Glu Glu Arg
        35                  40                  45

Thr Arg Ala His Lys Leu Leu Trp Ala Ala Glu Pro Leu Asp Ala Cys
    50                  55                  60

Gly Pro Leu Arg Pro Ser Trp Val Ala Leu Trp Pro Pro Arg Arg Val
65                  70                  75                  80

Leu Glu Thr Val Val Asp Ala Ala Cys Met Arg Ala Pro Glu Pro Leu
                85                  90                  95

Ala Ile Ala Tyr Ser Pro Pro Phe Pro Ala Gly Asp Glu Gly Leu Tyr
            100                 105                 110

Ser Glu Leu Ala Trp Arg Asp Arg Val Ala Val Val Asn Glu Ser Leu
        115                 120                 125

Val Ile Tyr Gly Ala Leu Glu Thr Asp Ser Gly Leu Tyr Thr Leu Ser
    130                 135                 140

Val Val Gly Leu Ser Asp Glu Ala Arg Gln Val Ala Ser Val Val Leu
145                 150                 155                 160

Val Val Glu Pro Ala Pro Val Pro Thr Pro Thr Pro Asp Asp Tyr Asp
                165                 170                 175

Glu Glu Asp Asp Ala Gly Val Ser Glu Arg Thr Pro Val Ser Val Pro
```

```
            180                 185                 190

Pro Pro Thr Pro Pro Arg Arg Pro Pro Val Ala Pro Pro Thr His Pro
        195                 200                 205

Arg Val Ile Pro Glu Val Ser His Val Arg Gly Val Thr Val His Met
    210                 215                 220

Glu Thr Pro Glu Ala Ile Leu Phe Ala Pro Gly Glu Thr Phe Gly Thr
225                 230                 235                 240

Asn Val Ser Ile His Ala Ile Ala His Asp Asp Gly Pro Tyr Ala Met
                245                 250                 255

Asp Val Val Trp Met Arg Phe Asp Val Pro Ser Ser Cys Ala Glu Met
            260                 265                 270

Arg Ile Tyr Glu Ala Cys Leu Tyr His Pro Gln Leu Pro Glu Cys Leu
        275                 280                 285

Ser Pro Ala Asp Ala Pro Cys Ala Val Ser Ser Trp Ala Tyr Arg Leu
    290                 295                 300

Ala Val Arg Ser Tyr Ala Gly Cys Ser Arg Thr Thr Pro Pro Pro Arg
305                 310                 315                 320

Cys Phe Ala Glu Ala Arg Met Glu Pro Val Pro Gly Leu Ala Trp Leu
                325                 330                 335

Ala Ser Thr Val Asn Leu Glu Phe Gln His Ala Ser Pro Gln His Ala
            340                 345                 350

Gly Leu Tyr Leu Cys Val Val Tyr Val Asp Asp His Ile His Ala Trp
        355                 360                 365

Gly His Met Thr Ile Ser Thr Ala Ala Gln Tyr Arg Asn Ala Val Val
```

```
    370                 375                 380

Glu Gln His Leu Pro Gln Arg Gln Pro Glu Pro Val Glu Pro Thr Arg
385                 390                 395                 400

Pro His Val Arg Ala Pro Pro Pro Ala Pro Ser Ala Arg Gly Pro Leu
                405                 410                 415

Arg Leu Gly Ala Val Leu Gly Ala Ala Leu Leu Leu Ala Ala Leu Gly
            420                 425                 430

Leu Ser Ala Trp Ala Cys Met Thr Cys Trp Arg Arg Arg Ser Trp Arg
        435                 440                 445

Ala Val Lys Ser Arg Ala Ser Ala Thr Gly Pro Thr Tyr Ile Arg Val
    450                 455                 460

Ala Asp Ser Glu Leu Tyr Ala Asp Trp Ser Ser Asp Ser Glu Gly Glu
465                 470                 475                 480

Arg Asp Gly Ser Leu Trp Gln Asp Pro Pro Glu Arg Pro Asp Ser Pro
                485                 490                 495

Ser Thr Asn Gly Ser Gly Phe Glu Ile Leu Ser Pro Thr Ala Pro Ser
            500                 505                 510

Val Tyr Pro His Ser Glu Gly Arg Lys Ser Arg Arg Pro Leu Thr Thr
        515                 520                 525

Phe Gly Ser Gly Ser Pro Gly Arg Arg His Ser Gln Ala Ser Tyr Ser
    530                 535                 540

Ser Val Leu Trp
545
```

<210> 6

<211> 330
<212> PRT
<213> Homo sapiens

<400> 6

```
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1               5                   10                  15


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


Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45


Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60


Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80


Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95


Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110


Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125


Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140


Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160


Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175
```

```
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190


His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205


Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220


Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225                 230                 235                 240


Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255


Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270


Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285


Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300


Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320


Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330
```

<210> 7
<211> 326
<212> PRT
<213> Homo sapiens

<400> 7

```
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1               5                  10                  15

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

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65                  70                  75                  80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
            100                 105                 110

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

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
    130                 135                 140

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
145                 150                 155                 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
                165                 170                 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
            180                 185                 190
```

```
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
        195                 200                 205


Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
    210                 215                 220


Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225                 230                 235                 240


Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
                245                 250                 255


Ser Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
            260                 265                 270


Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
        275                 280                 285


Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
    290                 295                 300


Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305                 310                 315                 320


Ser Leu Ser Pro Gly Lys
                325


<210>  8
<211>  377
<212>  PRT
<213>  Homo sapiens

<400>  8

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1               5                   10                  15
```

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

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80

Tyr Thr Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Arg Val Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro
            100                 105                 110

Arg Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg
        115                 120                 125

Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys
    130                 135                 140

Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro
145                 150                 155                 160

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
                165                 170                 175

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
            180                 185                 190

Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr
        195                 200                 205
```

```
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    210                 215                 220

Gln Tyr Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Leu His
225                 230                 235                 240

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                245                 250                 255

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln
            260                 265                 270

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
        275                 280                 285

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
    290                 295                 300

Ser Asp Ile Ala Val Glu Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn
305                 310                 315                 320

Tyr Asn Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu
                325                 330                 335

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile
            340                 345                 350

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln
        355                 360                 365

Lys Ser Leu Ser Leu Ser Pro Gly Lys
    370                 375
```

<210> 9
<211> 327
<212> PRT
<213> Homo sapiens

<400> 9

```
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1               5                  10                  15

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

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65                  70                  75                  80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro
            100                 105                 110

Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
        115                 120                 125

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
    130                 135                 140

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
145                 150                 155                 160

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
                165                 170                 175

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
```

180 185 190

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
195 200 205

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
210 215 220

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
225 230 235 240

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
245 250 255

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
260 265 270

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
275 280 285

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
290 295 300

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
305 310 315 320

Leu Ser Leu Ser Leu Gly Lys
325

<210> 10
<211> 466
<212> PRT
<213> Homo sapiens

<400> 10

Met Glu Phe Gly Leu Ser Trp Leu Phe Leu Val Ala Ile Leu Lys Gly
1 5 10 15

```
Val Gln Cys Glu Val Gln Leu Leu Glu Ser Gly Gly Asp Leu Val Gln
            20                  25                  30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
        35                  40                  45

Ser Thr Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
    50                  55                  60

Glu Trp Val Ser Gly Ile Gly Asp Ser Gly His Ser Ile Tyr Tyr Ala
65                  70                  75                  80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
                85                  90                  95

Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Thr Gly Ser Gln Trp Pro Gly Asp Tyr Trp Gly Gln
        115                 120                 125

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

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
145                 150                 155                 160

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
                165                 170                 175

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
            180                 185                 190

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
        195                 200                 205
```

```
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
    210                 215                 220

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
225                 230                 235                 240

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
                245                 250                 255

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
            260                 265                 270

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
        275                 280                 285

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
    290                 295                 300

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
305                 310                 315                 320

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
                325                 330                 335

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
            340                 345                 350

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
        355                 360                 365

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
    370                 375                 380

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
385                 390                 395                 400
```

```
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
                405                 410                 415


Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
            420                 425                 430


Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
        435                 440                 445


Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
    450                 455                 460


Gly Lys
465


<210>  11
<211>  6
<212>  PRT
<213>  Homo sapiens

<400>  11

Met Gly Lys His Arg Tyr
1               5


<210>  12
<211>  626
<212>  PRT
<213>  human herpesvirus 1

<400>  12

Met Glu Ser Thr Val Gly Pro Ala Cys Pro Pro Gly Arg Thr Val Thr
1               5                   10                  15


Lys Arg Pro Trp Ala Leu Ala Glu Asp Thr Pro Arg Gly Pro Asp Ser
            20                  25                  30
```

```
Pro Pro Lys Arg Pro Arg Pro Asn Ser Leu Pro Leu Thr Thr Thr Phe
        35                  40                  45

Arg Pro Leu Pro Pro Pro Pro Gln Thr Thr Ser Ala Val Asp Pro Ser
    50                  55                  60

Ser His Ser Pro Val Asn Pro Pro Arg Asp Gln His Ala Thr Asp Thr
65                  70                  75                  80

Ala Asp Glu Lys Pro Arg Ala Ala Ser Pro Ala Leu Ser Asp Ala Ser
                85                  90                  95

Gly Pro Pro Thr Pro Asp Ile Pro Leu Ser Pro Gly Gly Thr His Ala
            100                 105                 110

Arg Asp Pro Asp Ala Asp Pro Asp Ser Pro Asp Leu Asp Ser Met Trp
        115                 120                 125

Ser Ala Ser Val Ile Pro Asn Ala Leu Pro Ser His Ile Leu Ala Glu
    130                 135                 140

Thr Phe Glu Arg His Leu Arg Gly Leu Leu Arg Gly Val Arg Ala Pro
145                 150                 155                 160

Leu Ala Ile Gly Pro Leu Trp Ala Arg Leu Asp Tyr Leu Cys Ser Leu
                165                 170                 175

Ala Val Val Leu Glu Glu Ala Gly Met Val Asp Arg Gly Leu Gly Arg
            180                 185                 190

His Leu Trp Arg Leu Thr Arg Arg Gly Pro Pro Ala Ala Ala Asp Ala
        195                 200                 205

Val Ala Pro Arg Pro Leu Met Gly Phe Tyr Glu Ala Ala Thr Gln Asn
    210                 215                 220
```

```
Gln Ala Asp Cys Gln Leu Trp Ala Leu Leu Arg Arg Gly Leu Thr Thr
225                 230                 235                 240

Ala Ser Thr Leu Arg Trp Gly Pro Gln Gly Pro Cys Phe Ser Pro Gln
                245                 250                 255

Trp Leu Lys His Asn Ala Ser Leu Arg Pro Asp Val Gln Ser Ser Ala
            260                 265                 270

Val Met Phe Gly Arg Val Asn Glu Pro Thr Ala Arg Ser Leu Leu Phe
        275                 280                 285

Arg Tyr Cys Val Gly Arg Ala Asp Asp Gly Gly Glu Ala Gly Ala Asp
    290                 295                 300

Thr Arg Arg Phe Ile Phe His Glu Pro Ser Asp Leu Ala Glu Glu Asn
305                 310                 315                 320

Val His Thr Cys Gly Val Leu Met Asp Gly His Thr Gly Met Val Gly
                325                 330                 335

Ala Ser Leu Asp Ile Leu Val Cys Pro Arg Asp Ile His Gly Tyr Leu
            340                 345                 350

Ala Pro Val Pro Lys Thr Pro Leu Ala Phe Tyr Glu Val Lys Cys Arg
        355                 360                 365

Ala Lys Tyr Ala Phe Asp Pro Met Asp Pro Ser Asp Pro Thr Ala Ser
    370                 375                 380

Ala Tyr Glu Asp Leu Met Ala His Arg Ser Pro Glu Ala Phe Arg Ala
385                 390                 395                 400

Phe Ile Arg Ser Ile Pro Lys Pro Ser Val Arg Tyr Phe Ala Pro Gly
                405                 410                 415
```

```
Arg Val Pro Gly Pro Glu Glu Ala Leu Val Thr Gln Asp Gln Ala Trp
            420                 425                 430

Ser Glu Ala His Ala Ser Gly Glu Lys Arg Arg Cys Ser Ala Ala Asp
        435                 440                 445

Arg Ala Leu Val Glu Leu Asn Ser Gly Val Val Ser Glu Val Leu Leu
    450                 455                 460

Phe Gly Ala Pro Asp Leu Gly Arg His Thr Ile Ser Pro Val Ser Trp
465                 470                 475                 480

Ser Ser Gly Asp Leu Val Arg Arg Glu Pro Val Phe Ala Asn Pro Arg
                485                 490                 495

His Pro Asn Phe Lys Gln Ile Leu Val Gln Gly Tyr Val Leu Asp Ser
            500                 505                 510

His Phe Pro Asp Cys Pro Pro His Pro His Leu Val Thr Phe Ile Gly
        515                 520                 525

Arg His Arg Thr Ser Ala Glu Glu Gly Val Thr Phe Arg Leu Glu Asp
    530                 535                 540

Gly Ala Gly Ala Leu Gly Ala Ala Gly Pro Ser Lys Ala Ser Ile Leu
545                 550                 555                 560

Pro Asn Gln Ala Val Pro Ile Ala Leu Ile Ile Thr Pro Val Arg Ile
                565                 570                 575

Asp Pro Glu Ile Tyr Lys Ala Ile Gln Arg Ser Ser Arg Leu Ala Phe
            580                 585                 590

Asp Asp Thr Leu Ala Glu Leu Trp Ala Ser Arg Ser Pro Gly Pro Gly
        595                 600                 605
```

```
Pro Ala Ala Ala Glu Thr Thr Ser Ser Ser Pro Thr Thr Gly Arg Ser
    610                 615                 620


Ser Arg
625
```

<210> 13
<211> 1235
<212> PRT
<213> human herpesvirus 1

<400> 13

```
Met Phe Ser Gly Gly Gly Gly Pro Leu Ser Pro Gly Gly Lys Ser Ala
1               5                   10                  15


Ala Arg Ala Ala Ser Gly Phe Phe Ala Pro Ala Gly Pro Arg Gly Ala
            20                  25                  30


Ser Arg Gly Pro Pro Pro Cys Leu Arg Gln Asn Phe Tyr Asn Pro Tyr
        35                  40                  45


Leu Ala Pro Val Gly Thr Gln Gln Lys Pro Thr Gly Pro Thr Gln Arg
    50                  55                  60


His Thr Tyr Tyr Ser Glu Cys Asp Glu Phe Arg Phe Ile Ala Pro Arg
65                  70                  75                  80


Val Leu Asp Glu Asp Ala Pro Pro Glu Lys Arg Ala Gly Val His Asp
                85                  90                  95


Gly His Leu Lys Arg Ala Pro Lys Val Tyr Cys Gly Gly Asp Glu Arg
            100                 105                 110


Asp Val Leu Arg Val Gly Ser Gly Gly Phe Trp Pro Arg Arg Ser Arg
        115                 120                 125


Leu Trp Gly Gly Val Asp His Ala Pro Ala Gly Phe Asn Pro Thr Val
```

```
    130                 135                 140

Thr Val Phe His Val Tyr Asp Ile Leu Glu Asn Val Glu His Ala Tyr
145                 150                 155                 160

Gly Met Arg Ala Ala Gln Phe His Ala Arg Phe Met Asp Ala Ile Thr
                165                 170                 175

Pro Thr Gly Thr Val Ile Thr Leu Leu Gly Leu Thr Pro Glu Gly His
            180                 185                 190

Arg Val Ala Val His Val Tyr Gly Thr Arg Gln Tyr Phe Tyr Met Asn
        195                 200                 205

Lys Glu Glu Val Asp Arg His Leu Gln Cys Arg Ala Pro Arg Asp Leu
    210                 215                 220

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

Arg Gly Ile Ser Ala Asp His Phe Glu Ala Glu Val Val Glu Arg Thr
                245                 250                 255

Asp Val Tyr Tyr Tyr Glu Thr Arg Pro Ala Leu Phe Tyr Arg Val Tyr
            260                 265                 270

Val Arg Ser Gly Arg Val Leu Ser Tyr Leu Cys Asp Asn Phe Cys Pro
        275                 280                 285

Ala Ile Lys Lys Tyr Glu Gly Gly Val Asp Ala Thr Thr Arg Phe Ile
    290                 295                 300

Leu Asp Asn Pro Gly Phe Val Thr Phe Gly Trp Tyr Arg Leu Lys Pro
305                 310                 315                 320

Gly Arg Asn Asn Thr Leu Ala Gln Pro Ala Ala Pro Met Ala Phe Gly
```

325 330 335

Thr Ser Ser Asp Val Glu Phe Asn Cys Thr Ala Asp Asn Leu Ala Ile
340 345 350

Glu Gly Gly Met Ser Asp Leu Pro Ala Tyr Lys Leu Met Cys Phe Asp
355 360 365

Ile Glu Cys Lys Ala Gly Gly Glu Asp Glu Leu Ala Phe Pro Val Ala
370 375 380

Gly His Pro Glu Asp Leu Val Ile Gln Ile Ser Cys Leu Leu Tyr Asp
385 390 395 400

Leu Ser Thr Thr Ala Leu Glu His Val Leu Leu Phe Ser Leu Gly Ser
405 410 415

Cys Asp Leu Pro Glu Ser His Leu Asn Glu Leu Ala Ala Arg Gly Leu
420 425 430

Pro Thr Pro Val Val Leu Glu Phe Asp Ser Glu Phe Glu Met Leu Leu
435 440 445

Ala Phe Met Thr Leu Val Lys Gln Tyr Gly Pro Glu Phe Val Thr Gly
450 455 460

Tyr Asn Ile Ile Asn Phe Asp Trp Pro Phe Leu Leu Ala Lys Leu Thr
465 470 475 480

Asp Ile Tyr Lys Val Pro Leu Asp Gly Tyr Gly Arg Met Asn Gly Arg
485 490 495

Gly Val Phe Arg Val Trp Asp Ile Gly Gln Ser His Phe Gln Lys Arg
500 505 510

Ser Lys Ile Lys Val Asn Gly Met Val Asn Ile Asp Met Tyr Gly Ile

515 520 525

Ile Thr Asp Lys Ile Lys Leu Ser Ser Tyr Lys Leu Asn Ala Val Ala
530 535 540

Glu Ala Val Leu Lys Asp Lys Lys Lys Asp Leu Ser Tyr Arg Asp Ile
545 550 555 560

Pro Ala Tyr Tyr Ala Ala Gly Pro Ala Gln Arg Gly Val Ile Gly Glu
565 570 575

Tyr Cys Ile Gln Asp Ser Leu Leu Val Gly Gln Leu Phe Phe Lys Phe
580 585 590

Leu Pro His Leu Glu Leu Ser Ala Val Ala Arg Leu Ala Gly Ile Asn
595 600 605

Ile Thr Arg Thr Ile Tyr Asp Gly Gln Gln Ile Arg Val Phe Thr Cys
610 615 620

Leu Leu Arg Leu Ala Asp Gln Lys Gly Phe Ile Leu Pro Asp Thr Gln
625 630 635 640

Gly Arg Phe Arg Gly Ala Gly Gly Glu Ala Pro Lys Arg Pro Ala Ala
645 650 655

Ala Arg Glu Asp Glu Glu Arg Pro Glu Glu Glu Gly Glu Asp Glu Asp
660 665 670

Glu Arg Glu Glu Gly Gly Gly Glu Arg Glu Pro Glu Gly Ala Arg Glu
675 680 685

Thr Ala Gly Arg His Val Gly Tyr Gln Gly Ala Arg Val Leu Asp Pro
690 695 700

Thr Ser Gly Phe His Val Asn Pro Val Val Val Phe Asp Phe Ala Ser

```
705                 710                 715                 720

Leu Tyr Pro Ser Ile Ile Gln Ala His Asn Leu Cys Phe Ser Thr Leu
                725                 730                 735

Ser Leu Arg Ala Asp Ala Val Ala His Leu Glu Ala Gly Lys Asp Tyr
            740                 745                 750

Leu Glu Ile Glu Val Gly Gly Arg Arg Leu Phe Phe Val Lys Ala His
        755                 760                 765

Val Arg Glu Ser Leu Leu Ser Ile Leu Leu Arg Asp Trp Leu Ala Met
    770                 775                 780

Arg Lys Gln Ile Arg Ser Arg Ile Pro Gln Ser Ser Pro Glu Glu Ala
785                 790                 795                 800

Val Leu Leu Asp Lys Gln Gln Ala Ala Ile Lys Val Val Cys Asn Ser
                805                 810                 815

Val Tyr Gly Phe Thr Gly Val Gln His Gly Leu Leu Pro Cys Leu His
            820                 825                 830

Val Ala Ala Thr Val Thr Thr Ile Gly Arg Glu Met Leu Leu Ala Thr
        835                 840                 845

Arg Glu Tyr Val His Ala Arg Trp Ala Ala Phe Glu Gln Leu Leu Ala
    850                 855                 860

Asp Phe Pro Glu Ala Ala Asp Met Arg Ala Pro Gly Pro Tyr Ser Met
865                 870                 875                 880

Arg Ile Ile Tyr Gly Asp Thr Asp Ser Ile Phe Val Leu Cys Arg Gly
                885                 890                 895

Leu Thr Ala Ala Gly Leu Thr Ala Val Gly Asp Lys Met Ala Ser His
```

```
            900                 905                 910

Ile Ser Arg Ala Leu Phe Leu Pro Pro Ile Lys Leu Glu Cys Glu Lys
        915                 920                 925

Thr Phe Thr Lys Leu Leu Leu Ile Ala Lys Lys Lys Tyr Ile Gly Val
    930                 935                 940

Ile Tyr Gly Gly Lys Met Leu Ile Lys Gly Val Asp Leu Val Arg Lys
945                 950                 955                 960

Asn Asn Cys Ala Phe Ile Asn Arg Thr Ser Arg Ala Leu Val Asp Leu
                965                 970                 975

Leu Phe Tyr Asp Asp Thr Val Ser Gly Ala Ala Ala Ala Leu Ala Glu
            980                 985                 990

Arg Pro Ala Glu Glu Trp Leu Ala  Arg Pro Leu Pro Glu  Gly Leu Gln
        995                 1000                1005

Ala Phe  Gly Ala Val Leu Val  Asp Ala His Arg Arg  Ile Thr Asp
    1010                1015                1020

Pro Glu  Arg Asp Ile Gln Asp  Phe Val Leu Thr Ala  Glu Leu Ser
    1025                1030                1035

Arg His  Pro Arg Ala Tyr Thr  Asn Lys Arg Leu Ala  His Leu Thr
    1040                1045                1050

Val Tyr  Tyr Lys Leu Met Ala  Arg Arg Ala Gln Val  Pro Ser Ile
    1055                1060                1065

Lys Asp  Arg Ile Pro Tyr Val  Ile Val Ala Gln Thr  Arg Glu Val
    1070                1075                1080

Glu Glu  Thr Val Ala Arg Leu  Ala Ala Leu Arg Glu  Leu Asp Ala
```

```
    1085                1090                1095

Ala Ala  Pro Gly Asp Glu Pro  Ala Pro Pro Ala Ala  Leu Pro Ser
    1100                1105                1110

Pro Ala  Lys Arg Pro Arg Glu  Thr Pro Ser Pro Ala  Asp Pro Pro
    1115                1120                1125

Gly Gly  Ala Ser Lys Pro Arg  Lys Leu Leu Val Ser  Glu Leu Ala
    1130                1135                1140

Glu Asp  Pro Ala Tyr Ala Ile  Ala His Gly Val Ala  Leu Asn Thr
    1145                1150                1155

Asp Tyr  Tyr Phe Ser His Leu  Leu Gly Ala Ala Cys  Val Thr Phe
    1160                1165                1170

Lys Ala  Leu Phe Gly Asn Asn  Ala Lys Ile Thr Glu  Ser Leu Leu
    1175                1180                1185

Lys Arg  Phe Ile Pro Glu Val  Trp His Pro Pro Asp  Asp Val Ala
    1190                1195                1200

Ala Arg  Leu Arg Thr Ala Gly  Phe Gly Ala Val Gly  Ala Gly Ala
    1205                1210                1215

Thr Ala  Glu Glu Thr Arg Arg  Met Leu His Arg Ala  Phe Asp Thr
    1220                1225                1230

Leu Ala
    1235


<210>  14
<211>  92
<212>  PRT
<213>  human herpesvirus 1
```

<400> 14

Met Ser Leu Arg Ala Val Trp His Leu Gly Leu Leu Gly Ser Leu Val
1 5 10 15

Gly Ala Val Leu Ala Ala Thr His Arg Gly Pro Ala Ala Asn Thr Thr
20 25 30

Asp Pro Leu Thr His Ala Pro Val Ser Pro His Pro Ser Pro Leu Gly
35 40 45

Gly Phe Ala Val Pro Leu Val Val Gly Gly Leu Cys Ala Val Val Leu
50 55 60

Gly Ala Ala Cys Leu Leu Glu Leu Leu Arg Arg Thr Cys Arg Gly Trp
65 70 75 80

Gly Arg Tyr His Pro Tyr Met Asp Pro Val Val Val
85 90

<210> 15
<211> 186
<212> PRT
<213> human herpesvirus 5

<400> 15

Met Lys Pro Val Leu Val Leu Ala Ile Leu Ala Val Leu Phe Leu Arg
1 5 10 15

Leu Ala Asp Ser Val Pro Arg Pro Leu Asp Val Val Val Ser Glu Ile
20 25 30

Arg Ser Ala His Phe Arg Val Glu Glu Asn Gln Cys Trp Phe His Met
35 40 45

Gly Met Leu Tyr Phe Lys Gly Arg Met Ser Gly Asn Phe Thr Glu Lys
50 55 60

```
His Phe Val Asn Val Gly Ile Val Ser Gln Ser Tyr Met Asp Arg Leu
65                  70                  75                  80


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


Glu Trp Asn Ile Gly Gly His Pro Val Thr His Thr Val Asp Met Val
            100                 105                 110


Asp Ile Thr Leu Ser Thr Arg Trp Gly Asp Pro Lys Lys Tyr Ala Ala
        115                 120                 125


Cys Val Pro Gln Val Arg Met Asp Tyr Ser Ser Gln Thr Ile Asn Trp
    130                 135                 140


Tyr Leu Gln Arg Ser Met Arg Asp Asp Asn Trp Gly Leu Leu Phe Arg
145                 150                 155                 160


Thr Leu Leu Val Tyr Leu Phe Ser Leu Val Val Leu Val Leu Leu Thr
                165                 170                 175


Val Gly Val Ser Ala Arg Leu Arg Phe Ile
            180                 185


<210>  16
<211>  281
<212>  PRT
<213>  human herpesvirus 5

<400>  16

Met Val Gln Ile Gln Phe His Gln Gly Glu Pro Leu Gly His Lys Lys
1               5                   10                  15


Glu Lys Pro Pro Pro Val Ser Pro Pro Ser Pro Pro Pro Ile Arg Arg
            20                  25                  30
```

```
Val Thr Val Ile Thr Lys Asp Glu Asp Thr Leu Arg Ser Val Gln His
        35                  40                  45

Phe Leu Trp Met Val Arg Leu Tyr Gly Thr Val Val Phe Gln Thr Ser
    50                  55                  60

Ala Thr Ile Ala Thr Thr Ile Leu Phe Met Leu Ile Pro Trp Arg Val
65                  70                  75                  80

Thr Thr Pro Tyr Leu Arg Asp Thr Leu Pro Phe Trp Ser Thr Leu Leu
                85                  90                  95

Pro Cys Ala Leu Arg Cys His Ala Tyr Trp Leu Glu Arg Gln Arg Arg
            100                 105                 110

Pro Gly Thr Leu Met Leu Val Met Val Tyr Thr Thr Leu Thr Thr Ile
        115                 120                 125

Ser Val Ser Thr Ile Gly Leu Cys Phe Asp Arg Thr Val Val Ile Gln
    130                 135                 140

Ala Tyr Val Leu Ser Ser Met Leu Cys Val Trp Cys Thr Gly Leu Ala
145                 150                 155                 160

Trp Leu Met Ala Trp Asn Met Gln Arg Arg Leu Ala Ile Leu Cys Leu
                165                 170                 175

Leu Ser Phe Met Leu Pro Ile Leu Trp Leu Phe Ile Ala Val Gln Ser
            180                 185                 190

Trp Glu Pro Tyr Gln Arg Ile Ile Leu Ala Leu Thr Val Ser Phe Ile
        195                 200                 205

Tyr Gly Leu Lys Ile Val Leu Ile Arg Asp Thr Leu Thr Val Leu Tyr
    210                 215                 220
```

```
Arg Ser Pro Ser Asn Cys Tyr Thr Asp Gly Asp Leu Leu Arg Thr Ala
225                 230                 235                 240

Met Leu Leu Tyr Met Asp Gln Val Ile Met Phe Leu Leu Val Val Val
                245                 250                 255

Pro Leu Thr Ala Pro Ile Trp Tyr Pro Asn Tyr Ala Gly Ala Leu Gly
            260                 265                 270

Arg Thr Ala His Trp Leu Phe His Lys
        275                 280
```

<210> 17
<211> 225
<212> PRT
<213> human herpesvirus 5

<400> 17

```
Met Arg Ile Gln Leu Leu Leu Val Ser Thr Leu Val Ala Ser Ile Val
1               5                   10                  15

Ala Thr Arg Val Glu Asp Met Ala Thr Phe Arg Thr Glu Lys Gln Trp
            20                  25                  30

Gln Gln Asp Leu Gln Tyr Arg Arg Glu Phe Val Lys Arg Gln Leu Ala
        35                  40                  45

Pro Lys Pro Lys Ser Asn Ile Val Val Ser His Thr Val Ser Cys Val
    50                  55                  60

Ile Asp Gly Gly Asn Met Thr Ser Val Trp Arg Phe Glu Gly Gln Phe
65                  70                  75                  80

Asn Pro His Ile Ala Ser Glu Val Ile Leu His Asp Thr Ser Gly Leu
                85                  90                  95

Tyr Asn Val Pro His Glu Val Gln Asn Asp Gly Gln Val Leu Thr Val
```

```
            100                 105                 110

Thr Val Lys Arg Ser Ala Pro Ala Asp Ile Ala Lys Val Leu Ile Ser
        115                 120                 125

Leu Lys Pro Val Gln Leu Ser Ser Gly Gln Tyr Glu Cys Arg Pro Gln
    130                 135                 140

Leu Gln Leu Pro Trp Val Pro Arg Pro Ser Ser Phe Met Tyr Asp Ser
145                 150                 155                 160

Tyr Arg Leu Trp Tyr Glu Lys Arg Trp Leu Thr Ile Ile Leu Tyr Val
                165                 170                 175

Phe Met Trp Thr Tyr Leu Val Thr Leu Leu Gln Tyr Cys Ile Val Arg
            180                 185                 190

Phe Ile Gly Thr Arg Leu Phe Tyr Phe Leu Gln Arg Asn Ile Thr Ile
        195                 200                 205

Arg Phe Thr Gly Lys Pro Thr Tyr Asn Leu Leu Thr Tyr Pro Val Lys
    210                 215                 220

Gly
225


<210>  18
<211>  227
<212>  PRT
<213>  human herpesvirus 5

<400>  18

Met Arg Arg Trp Leu Arg Leu Leu Val Gly Leu Gly Cys Cys Trp Val
1               5                   10                  15

Thr Leu Ala His Ala Gly Asn Pro Tyr Glu Asp Asp Asp Tyr Tyr Tyr
            20                  25                  30
```

```
Tyr Arg Glu Asp Glu Pro Arg Gln His Gly Glu Pro Asn Tyr Val Ala
        35                  40                  45

Pro Pro Ala Arg Gln Phe Arg Phe Pro Pro Leu Asn Asn Val Ser Ser
    50                  55                  60

Tyr Gln Ala Ser Cys Val Val Lys Asp Gly Val Leu Asp Ala Val Trp
65                  70                  75                  80

Arg Val Gln Gly Thr Phe Tyr Pro Glu Lys Gly Ile Val Ala Arg Val
                85                  90                  95

Gly Trp Ser Gly Arg Arg Gly Arg Lys Trp Gly Arg Leu His Ala Pro
            100                 105                 110

Glu Cys Leu Val Glu Thr Thr Glu Ala Val Phe Arg Leu Arg Gln Trp
        115                 120                 125

Val Pro Thr Asp Leu Asp His Leu Thr Leu His Leu Val Pro Cys Thr
    130                 135                 140

Lys Cys Lys Pro Met Trp Cys Gln Pro Arg Tyr His Ile Arg Tyr Phe
145                 150                 155                 160

Ser Tyr Gly Asn Ser Val Asp Asn Leu Arg Arg Leu His Tyr Glu Tyr
                165                 170                 175

Arg His Leu Glu Leu Gly Val Val Ile Ala Ile Gln Met Ala Met Val
            180                 185                 190

Leu Leu Leu Gly Tyr Val Leu Ala Arg Thr Val Tyr Arg Val Ser Ser
        195                 200                 205

Ala Tyr Tyr Leu Arg Trp His Ala Cys Val Pro Gln Lys Cys Glu Lys
    210                 215                 220
```

Ser Leu Cys
225

<210> 19
<211> 183
<212> PRT
<213> human herpesvirus 5

<400> 19

Met Asp Leu Leu Ile Arg Leu Gly Phe Leu Leu Met Cys Ala Leu Pro
1 5 10 15

Thr Pro Gly Glu Arg Ser Ser Arg Asp Pro Lys Thr Leu Leu Ser Leu
20 25 30

Ser Pro Arg Gln Gln Ala Cys Val Pro Arg Thr Lys Ser His Arg Pro
35 40 45

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

Lys Gln Leu Gly Glu Asp Phe Ala His Gln Cys Leu Gln Ala Ala Lys
65 70 75 80

Lys Arg Pro Lys Thr His Lys Ser Arg Pro Asn Asp Arg Asn Leu Glu
85 90 95

Gly Arg Leu Thr Cys Gln Arg Val Arg Arg Leu Leu Pro Cys Asp Leu
100 105 110

Asp Ile His Pro Ser His Arg Leu Leu Thr Leu Met Asn Asn Cys Val
115 120 125

Cys Asp Gly Ala Val Trp Asn Ala Phe Arg Leu Ile Glu Arg His Gly
130 135 140

```
Phe Phe Ala Val Thr Leu Tyr Leu Cys Cys Gly Ile Thr Leu Leu Val
145                 150                 155                 160


Val Ile Leu Ala Leu Leu Cys Ser Ile Thr Tyr Glu Ser Thr Gly Arg
                165                 170                 175


Gly Ile Arg Arg Cys Gly Ser
            180


<210>  20
<211>  490
<212>  PRT
<213>  human herpesvirus 1

<400>  20

Met Asp Leu Leu Val Asp Glu Leu Phe Ala Asp Met Asn Ala Asp Gly
1               5                   10                  15


Ala Ser Pro Pro Pro Pro Arg Pro Ala Gly Gly Pro Lys Asn Thr Pro
            20                  25                  30


Ala Ala Pro Pro Leu Tyr Ala Thr Gly Arg Leu Ser Gln Ala Gln Leu
        35                  40                  45


Met Pro Ser Pro Pro Met Pro Val Pro Pro Ala Ala Leu Phe Asn Arg
    50                  55                  60


Leu Leu Asp Asp Leu Gly Phe Ser Ala Gly Pro Ala Leu Cys Thr Met
65                  70                  75                  80


Leu Asp Thr Trp Asn Glu Asp Leu Phe Ser Ala Leu Pro Thr Asn Ala
                85                  90                  95


Asp Leu Tyr Arg Glu Cys Lys Phe Leu Ser Thr Leu Pro Ser Asp Val
            100                 105                 110
```

```
Val Glu Trp Gly Asp Ala Tyr Val Pro Glu Arg Thr Gln Ile Asp Ile
        115                 120                 125


Arg Ala His Gly Asp Val Ala Phe Pro Thr Leu Pro Ala Thr Arg Asp
    130                 135                 140


Gly Leu Gly Leu Tyr Tyr Glu Ala Leu Ser Arg Phe Phe His Ala Glu
145                 150                 155                 160


Leu Arg Ala Arg Glu Glu Ser Tyr Arg Thr Val Leu Ala Asn Phe Cys
                165                 170                 175


Ser Ala Leu Tyr Arg Tyr Leu Arg Ala Ser Val Arg Gln Leu His Arg
            180                 185                 190


Gln Ala His Met Arg Gly Arg Asp Arg Asp Leu Gly Glu Met Leu Arg
        195                 200                 205


Ala Thr Ile Ala Asp Arg Tyr Tyr Arg Glu Thr Ala Arg Leu Ala Arg
    210                 215                 220


Val Leu Phe Leu His Leu Tyr Leu Phe Leu Thr Arg Glu Ile Leu Trp
225                 230                 235                 240


Ala Ala Tyr Ala Glu Gln Met Met Arg Pro Asp Leu Phe Asp Cys Leu
                245                 250                 255


Cys Cys Asp Leu Glu Ser Trp Arg Gln Leu Ala Gly Leu Phe Gln Pro
            260                 265                 270


Phe Met Phe Val Asn Gly Ala Leu Thr Val Arg Gly Val Pro Ile Glu
        275                 280                 285


Ala Arg Arg Leu Arg Glu Leu Asn His Ile Arg Glu His Leu Asn Leu
    290                 295                 300
```

```
Pro Leu Val Arg Ser Ala Ala Thr Glu Glu Pro Gly Ala Pro Leu Thr
305                 310                 315                 320

Thr Pro Pro Thr Leu His Gly Asn Gln Ala Arg Ala Ser Gly Tyr Phe
                325                 330                 335

Met Val Leu Ile Arg Ala Lys Leu Asp Ser Tyr Ser Ser Phe Thr Thr
            340                 345                 350

Ser Pro Ser Glu Ala Val Met Arg Glu His Ala Tyr Ser Arg Ala Arg
        355                 360                 365

Thr Lys Asn Asn Tyr Gly Ser Thr Ile Glu Gly Leu Leu Asp Leu Pro
    370                 375                 380

Asp Asp Asp Ala Pro Glu Glu Ala Gly Leu Ala Ala Pro Arg Leu Ser
385                 390                 395                 400

Phe Leu Pro Ala Gly His Thr Arg Arg Leu Ser Thr Ala Pro Pro Thr
                405                 410                 415

Asp Val Ser Leu Gly Asp Glu Leu His Leu Asp Gly Glu Asp Val Ala
            420                 425                 430

Met Ala His Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly
        435                 440                 445

Asp Gly Asp Ser Pro Gly Pro Gly Phe Thr Pro His Asp Ser Ala Pro
    450                 455                 460

Tyr Gly Ala Leu Asp Met Ala Asp Phe Glu Phe Glu Gln Met Phe Thr
465                 470                 475                 480

Asp Ala Leu Gly Ile Asp Glu Tyr Gly Gly
                485                 490
```

<210> 21
<211> 317
<212> PRT
<213> Homo sapiens

<400> 21

```
Met Thr Met Glu Thr Gln Met Ser Gln Asn Val Cys Pro Arg Asn Leu
1               5                  10                  15


Trp Leu Leu Gln Pro Leu Thr Val Leu Leu Leu Leu Ala Ser Ala Asp
            20                  25                  30


Ser Gln Ala Ala Ala Pro Pro Lys Ala Val Leu Lys Leu Glu Pro Pro
        35                  40                  45


Trp Ile Asn Val Leu Gln Glu Asp Ser Val Thr Leu Thr Cys Gln Gly
    50                  55                  60


Ala Arg Ser Pro Glu Ser Asp Ser Ile Gln Trp Phe His Asn Gly Asn
65                  70                  75                  80


Leu Ile Pro Thr His Thr Gln Pro Ser Tyr Arg Phe Lys Ala Asn Asn
                85                  90                  95


Asn Asp Ser Gly Glu Tyr Thr Cys Gln Thr Gly Gln Thr Ser Leu Ser
            100                 105                 110


Asp Pro Val His Leu Thr Val Leu Ser Glu Trp Leu Val Leu Gln Thr
        115                 120                 125


Pro His Leu Glu Phe Gln Glu Gly Glu Thr Ile Met Leu Arg Cys His
    130                 135                 140


Ser Trp Lys Asp Lys Pro Leu Val Lys Val Thr Phe Phe Gln Asn Gly
145                 150                 155                 160


Lys Ser Gln Lys Phe Ser His Leu Asp Pro Thr Phe Ser Ile Pro Gln
```

```
                165                 170                 175

Ala Asn His Ser His Ser Gly Asp Tyr His Cys Thr Gly Asn Ile Gly
            180                 185                 190

Tyr Thr Leu Phe Ser Ser Lys Pro Val Thr Ile Thr Val Gln Val Pro
        195                 200                 205

Ser Met Gly Ser Ser Ser Pro Met Gly Ile Ile Val Ala Val Val Ile
    210                 215                 220

Ala Thr Ala Val Ala Ala Ile Val Ala Ala Val Val Ala Leu Ile Tyr
225                 230                 235                 240

Cys Arg Lys Lys Arg Ile Ser Ala Asn Ser Thr Asp Pro Val Lys Ala
                245                 250                 255

Ala Gln Phe Glu Pro Pro Gly Arg Gln Met Ile Ala Ile Arg Lys Arg
            260                 265                 270

Gln Leu Glu Glu Thr Asn Asn Asp Tyr Glu Thr Ala Asp Gly Gly Tyr
        275                 280                 285

Met Thr Leu Asn Pro Arg Ala Pro Thr Asp Asp Asp Lys Asn Ile Tyr
    290                 295                 300

Leu Thr Leu Pro Pro Asn Asp His Val Asn Ser Asn Asn
305                 310                 315


<210>  22
<211>  310
<212>  PRT
<213>  Homo sapiens

<400>  22

Met Gly Ile Leu Ser Phe Leu Pro Val Leu Ala Thr Glu Ser Asp Trp
1               5                   10                  15
```

```
Ala Asp Cys Lys Ser Pro Gln Pro Trp Gly His Met Leu Leu Trp Thr
            20                  25                  30

Ala Val Leu Phe Leu Ala Pro Val Ala Gly Thr Pro Ala Ala Pro Pro
        35                  40                  45

Lys Ala Val Leu Lys Leu Glu Pro Gln Trp Ile Asn Val Leu Gln Glu
    50                  55                  60

Asp Ser Val Thr Leu Thr Cys Arg Gly Thr His Ser Pro Glu Ser Asp
65                  70                  75                  80

Ser Ile Gln Trp Phe His Asn Gly Asn Leu Ile Pro Thr His Thr Gln
                85                  90                  95

Pro Ser Tyr Arg Phe Lys Ala Asn Asn Asn Asp Ser Gly Glu Tyr Thr
            100                 105                 110

Cys Gln Thr Gly Gln Thr Ser Leu Ser Asp Pro Val His Leu Thr Val
        115                 120                 125

Leu Ser Glu Trp Leu Val Leu Gln Thr Pro His Leu Glu Phe Gln Glu
    130                 135                 140

Gly Glu Thr Ile Val Leu Arg Cys His Ser Trp Lys Asp Lys Pro Leu
145                 150                 155                 160

Val Lys Val Thr Phe Phe Gln Asn Gly Lys Ser Lys Lys Phe Ser Arg
                165                 170                 175

Ser Asp Pro Asn Phe Ser Ile Pro Gln Ala Asn His Ser His Ser Gly
            180                 185                 190

Asp Tyr His Cys Thr Gly Asn Ile Gly Tyr Thr Leu Tyr Ser Ser Lys
        195                 200                 205
```

```
Pro Val Thr Ile Thr Val Gln Ala Pro Ser Ser Ser Pro Met Gly Ile
    210                 215                 220

Ile Val Ala Val Val Thr Gly Ile Ala Val Ala Ala Ile Val Ala Ala
225                 230                 235                 240

Val Val Ala Leu Ile Tyr Cys Arg Lys Lys Arg Ile Ser Ala Leu Pro
                245                 250                 255

Gly Tyr Pro Glu Cys Arg Glu Met Gly Glu Thr Leu Pro Glu Lys Pro
            260                 265                 270

Ala Asn Pro Thr Asn Pro Asp Glu Ala Asp Lys Val Gly Ala Glu Asn
        275                 280                 285

Thr Ile Thr Tyr Ser Leu Leu Met His Pro Asp Ala Leu Glu Glu Pro
    290                 295                 300

Asp Asp Gln Asn Arg Ile
305                 310
```

<210> 23
<211> 390
<212> PRT
<213> human herpesvirus 1

<400> 23

```
Met Pro Cys Arg Pro Leu Gln Gly Leu Val Leu Val Gly Leu Trp Val
1               5                   10                  15

Cys Ala Thr Ser Leu Val Val Arg Gly Pro Thr Val Ser Leu Val Ser
            20                  25                  30

Asn Ser Phe Val Asp Ala Gly Ala Leu Gly Pro Asp Gly Val Val Glu
        35                  40                  45
```

```
Glu Asp Leu Leu Ile Leu Gly Glu Leu Arg Phe Val Gly Asp Gln Val
    50                  55                  60

Pro His Thr Thr Tyr Tyr Asp Gly Gly Val Glu Leu Trp His Tyr Pro
65                  70                  75                  80

Met Gly His Lys Cys Pro Arg Val Val His Val Val Thr Val Thr Ala
                85                  90                  95

Cys Pro Arg Arg Pro Ala Val Ala Phe Ala Leu Cys Arg Ala Thr Asp
            100                 105                 110

Ser Thr His Ser Pro Ala Tyr Pro Thr Leu Glu Leu Asn Leu Ala Gln
        115                 120                 125

Gln Pro Leu Leu Arg Val Gln Arg Ala Thr Arg Asp Tyr Ala Gly Val
    130                 135                 140

Tyr Val Leu Arg Val Trp Val Gly Asp Ala Pro Asn Ala Ser Leu Phe
145                 150                 155                 160

Val Leu Gly Met Ala Ile Ala Ala Glu Gly Thr Leu Ala Tyr Asn Gly
                165                 170                 175

Ser Ala Tyr Gly Ser Cys Asp Pro Lys Leu Leu Pro Ser Ser Ala Pro
            180                 185                 190

Arg Leu Ala Pro Ala Ser Val Tyr Gln Pro Ala Pro Asn Gln Ala Ser
        195                 200                 205

Thr Pro Ser Thr Thr Thr Ser Thr Pro Ser Thr Thr Ile Pro Ala Pro
    210                 215                 220

Ser Thr Thr Ile Pro Ala Pro Gln Ala Ser Thr Thr Pro Phe Pro Thr
225                 230                 235                 240
```

```
Gly Asp Pro Lys Pro Gln Pro Pro Gly Val Asn His Glu Pro Pro Ser
               245                 250                 255


Asn Ala Thr Arg Ala Thr Arg Asp Ser Arg Tyr Ala Leu Thr Val Thr
            260                 265                 270


Gln Ile Ile Gln Ile Ala Ile Pro Ala Ser Ile Ile Ala Leu Val Phe
        275                 280                 285


Leu Gly Ser Cys Ile Cys Phe Ile His Arg Cys Gln Arg Arg Tyr Arg
    290                 295                 300


Arg Ser Arg Arg Pro Ile Tyr Ser Pro Gln Met Pro Thr Gly Ile Ser
305                 310                 315                 320


Cys Ala Val Asn Glu Ala Ala Met Ala Arg Leu Gly Ala Glu Leu Lys
               325                 330                 335


Ser His Pro Ser Thr Pro Pro Lys Ser Arg Arg Arg Ser Ser Arg Thr
            340                 345                 350


Pro Met Pro Ser Leu Thr Ala Ile Ala Glu Glu Ser Glu Pro Ala Gly
        355                 360                 365


Ala Ala Gly Leu Pro Thr Pro Pro Val Asp Pro Thr Thr Pro Thr Pro
    370                 375                 380


Thr Pro Pro Leu Leu Val
385                 390


<210>  24
<211>  254
<212>  PRT
<213>  Homo sapiens

<400>  24
```

```
Met Trp Gln Leu Leu Leu Pro Thr Ala Leu Leu Leu Leu Val Ser Ala
1               5                  10                  15


Gly Met Arg Thr Glu Asp Leu Pro Lys Ala Val Val Phe Leu Glu Pro
            20                  25                  30


Gln Trp Tyr Arg Val Leu Glu Lys Asp Ser Val Thr Leu Lys Cys Gln
        35                  40                  45


Gly Ala Tyr Ser Pro Glu Asp Asn Ser Thr Gln Trp Phe His Asn Glu
    50                  55                  60


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


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


Ser Asp Pro Val Gln Leu Glu Val His Ile Gly Trp Leu Leu Leu Gln
            100                 105                 110


Ala Pro Arg Trp Val Phe Lys Glu Glu Asp Pro Ile His Leu Arg Cys
        115                 120                 125


His Ser Trp Lys Asn Thr Ala Leu His Lys Val Thr Tyr Leu Gln Asn
    130                 135                 140


Gly Lys Gly Arg Lys Tyr Phe His His Asn Ser Asp Phe Tyr Ile Pro
145                 150                 155                 160


Lys Ala Thr Leu Lys Asp Ser Gly Ser Tyr Phe Cys Arg Gly Leu Val
                165                 170                 175


Gly Ser Lys Asn Val Ser Ser Glu Thr Val Asn Ile Thr Ile Thr Gln
            180                 185                 190
```

```
Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln
        195                 200                 205


Val Ser Phe Cys Leu Val Met Val Leu Leu Phe Ala Val Asp Thr Gly
    210                 215                 220


Leu Tyr Phe Ser Val Lys Thr Asn Ile Arg Ser Ser Thr Arg Asp Trp
225                 230                 235                 240


Lys Asp His Lys Phe Lys Trp Arg Lys Asp Pro Gln Asp Lys
                245                 250


<210>  25
<211>  36
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  UL1 forward primer

<400>  25
gtctacacta gtatggggat tttgggttgg gtcggg                                    36


<210>  26
<211>  1137
<212>  PRT
<213>  human herpesvirus 1

<400>  26

Met Ala Ser Arg Pro Ala Ala Ser Ser Pro Val Glu Ala Arg Ala Pro
1               5                   10                  15


Val Gly Gly Gln Glu Ala Gly Gly Pro Ser Ala Ala Thr Gln Gly Glu
            20                  25                  30


Ala Ala Gly Ala Pro Leu Ala His Gly His His Val Tyr Cys Gln Arg
        35                  40                  45


Val Asn Gly Val Met Val Leu Ser Asp Lys Thr Pro Gly Ser Ala Ser
```

50 55 60

Tyr Arg Ile Ser Asp Ser Asn Phe Val Gln Cys Gly Ser Asn Cys Thr
65 70 75 80

Met Ile Ile Asp Gly Asp Val Val Arg Gly Arg Pro Gln Asp Pro Gly
85 90 95

Ala Ala Ala Ser Pro Ala Pro Phe Val Ala Val Thr Asn Ile Gly Ala
100 105 110

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

Arg Ser Ala Gly Thr Ser Thr Gly Thr Gln Thr Ala Asp Val Pro Ala
130 135 140

Glu Ala Leu Gly Gly Pro Pro Pro Pro Pro Arg Phe Thr Leu Gly Gly
145 150 155 160

Gly Cys Cys Ser Cys Arg Asp Thr Arg Arg Arg Ser Ala Val Phe Gly
165 170 175

Gly Glu Gly Asp Pro Val Gly Pro Ala Glu Phe Val Ser Asp Asp Arg
180 185 190

Ser Ser Asp Ser Asp Ser Asp Asp Ser Glu Asp Thr Asp Ser Glu Thr
195 200 205

Leu Ser His Ala Ser Ser Asp Val Ser Gly Gly Ala Thr Tyr Asp Asp
210 215 220

Ala Leu Asp Ser Asp Ser Ser Ser Asp Asp Ser Leu Gln Ile Asp Gly
225 230 235 240

Pro Val Cys Arg Pro Trp Ser Asn Asp Thr Ala Pro Leu Asp Val Cys

245 250 255

Pro Gly Thr Pro Gly Pro Gly Ala Asp Ala Gly Gly Pro Ser Ala Val
260 265 270

Asp Pro His Ala Pro Thr Thr Gly Ala Gly Ala Gly Leu Ala Ala Asp
275 280 285

Pro Ala Val Ala Arg Asp Asp Ala Glu Gly Leu Ser Asp Pro Arg Pro
290 295 300

Arg Leu Gly Thr Gly Thr Ala Tyr Pro Val Pro Leu Glu Leu Thr Pro
305 310 315 320

Glu Asn Ala Glu Ala Val Ala Arg Phe Leu Gly Asp Ala Val Asn Arg
325 330 335

Glu Pro Ala Leu Met Leu Glu Tyr Phe Cys Arg Cys Ala Arg Glu Glu
340 345 350

Thr Lys Arg Val Pro Pro Arg Thr Phe Cys Ser Pro Pro Arg Leu Thr
355 360 365

Glu Asp Asp Phe Gly Leu Leu Asn Tyr Ala Leu Val Glu Met Gln Arg
370 375 380

Leu Cys Leu Asp Val Pro Pro Val Pro Pro Asn Ala Tyr Met Pro Tyr
385 390 395 400

Tyr Leu Arg Glu Tyr Val Thr Arg Leu Val Asn Gly Phe Lys Pro Leu
405 410 415

Val Ser Arg Ser Val Arg Leu Tyr Arg Ile Leu Gly Val Leu Val His
420 425 430

Leu Arg Ile Arg Thr Arg Glu Ala Ser Phe Glu Glu Trp Leu Arg Ser

435 440 445

Lys Glu Val Ala Leu Asp Phe Gly Leu Thr Glu Arg Leu Arg Glu His
450 455 460

Glu Ala Gln Leu Val Ile Leu Ala Gln Ala Leu Asp His Tyr Asp Cys
465 470 475 480

Leu Ile His Ser Thr Pro His Thr Leu Val Glu Arg Gly Leu Gln Ser
485 490 495

Ala Leu Lys Tyr Glu Glu Phe Tyr Leu Lys Arg Phe Gly Gly His Tyr
500 505 510

Met Glu Ser Val Phe Gln Met Tyr Thr Arg Ile Ala Gly Phe Leu Ala
515 520 525

Cys Arg Ala Thr Arg Gly Met Arg His Ile Ala Leu Gly Arg Glu Gly
530 535 540

Ser Trp Trp Glu Met Phe Lys Phe Phe Phe His Arg Leu Tyr Asp His
545 550 555 560

Gln Ile Val Pro Ser Thr Pro Ala Met Leu Asn Leu Gly Thr Arg Asn
565 570 575

Tyr Tyr Thr Ser Ser Cys Tyr Leu Val Asn Pro Gln Ala Thr Thr Asn
580 585 590

Lys Ala Thr Leu Arg Ala Ile Thr Ser Asn Val Ser Ala Ile Leu Ala
595 600 605

Arg Asn Gly Gly Ile Gly Leu Cys Val Gln Ala Phe Asn Asp Ser Gly
610 615 620

Pro Gly Thr Ala Ser Val Met Pro Ala Leu Lys Val Leu Asp Ser Leu

625 630 635 640

Val Ala Ala His Asn Lys Glu Ser Ala Arg Pro Thr Gly Ala Cys Val
645 650 655

Tyr Leu Glu Pro Trp His Thr Asp Val Arg Ala Val Leu Arg Met Lys
660 665 670

Gly Val Leu Ala Gly Glu Glu Ala Gln Arg Cys Asp Asn Ile Phe Ser
675 680 685

Ala Leu Trp Met Pro Asp Leu Phe Phe Lys Arg Leu Ile Arg His Leu
690 695 700

Asp Gly Glu Lys Asn Val Thr Trp Thr Leu Phe Asp Arg Asp Thr Ser
705 710 715 720

Met Ser Leu Ala Asp Phe His Gly Glu Glu Phe Glu Lys Leu Tyr Gln
725 730 735

His Leu Glu Val Met Gly Phe Gly Glu Gln Ile Pro Ile Gln Glu Leu
740 745 750

Ala Tyr Gly Ile Val Arg Ser Ala Ala Thr Thr Gly Ser Pro Phe Val
755 760 765

Met Phe Lys Asp Ala Val Asn Arg His Tyr Ile Tyr Asp Thr Gln Gly
770 775 780

Ala Ala Ile Ala Gly Ser Asn Leu Cys Thr Glu Ile Val His Pro Ala
785 790 795 800

Ser Lys Arg Ser Ser Gly Val Cys Asn Leu Gly Ser Val Asn Leu Ala
805 810 815

Arg Cys Val Ser Arg Gln Thr Phe Asp Phe Gly Arg Leu Arg Asp Ala

820 825 830

Val Gln Ala Cys Val Leu Met Val Asn Ile Met Ile Asp Ser Thr Leu
835 840 845

Gln Pro Thr Pro Gln Cys Thr Arg Gly Asn Asp Asn Leu Arg Ser Met
850 855 860

Gly Ile Gly Met Gln Gly Leu His Thr Ala Cys Leu Lys Leu Gly Leu
865 870 875 880

Asp Leu Glu Ser Ala Glu Phe Gln Asp Leu Asn Lys His Ile Ala Glu
885 890 895

Val Met Leu Leu Ser Ala Met Lys Thr Ser Asn Ala Leu Cys Val Arg
900 905 910

Gly Ala Arg Pro Phe Asn His Phe Lys Arg Ser Met Tyr Arg Ala Gly
915 920 925

Arg Phe His Trp Glu Arg Phe Pro Asp Ala Arg Pro Arg Tyr Glu Gly
930 935 940

Glu Trp Glu Met Leu Arg Gln Ser Met Met Lys His Gly Leu Arg Asn
945 950 955 960

Ser Gln Phe Val Ala Leu Met Pro Thr Ala Ala Ser Ala Gln Ile Ser
965 970 975

Asp Val Ser Glu Gly Phe Ala Pro Leu Phe Thr Asn Leu Phe Ser Lys
980 985 990

Val Thr Arg Asp Gly Glu Thr Leu Arg Pro Asn Thr Leu Leu Leu Lys
995 1000 1005

Glu Leu Glu Arg Thr Phe Ser Gly Lys Arg Leu Leu Glu Val Met

1010 1015 1020

Asp Ser Leu Asp Ala Lys Gln Trp Ser Val Ala Gln Ala Leu Pro
1025 1030 1035

Cys Leu Glu Pro Thr His Pro Leu Arg Arg Phe Lys Thr Ala Phe
1040 1045 1050

Asp Tyr Asp Gln Lys Leu Leu Ile Asp Leu Cys Ala Asp Arg Ala
1055 1060 1065

Pro Tyr Val Asp His Ser Gln Ser Met Thr Leu Tyr Val Thr Glu
1070 1075 1080

Lys Ala Asp Gly Thr Leu Pro Ala Ser Thr Leu Val Arg Leu Leu
1085 1090 1095

Val His Ala Tyr Lys Arg Gly Leu Lys Thr Gly Met Tyr Tyr Cys
1100 1105 1110

Lys Val Arg Lys Ala Thr Asn Ser Gly Val Phe Gly Gly Asp Asp
1115 1120 1125

Asn Ile Val Cys Thr Ser Cys Ala Leu
1130 1135

<210> 27
<211> 36
<212> DNA
<213> Artificial Sequence

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