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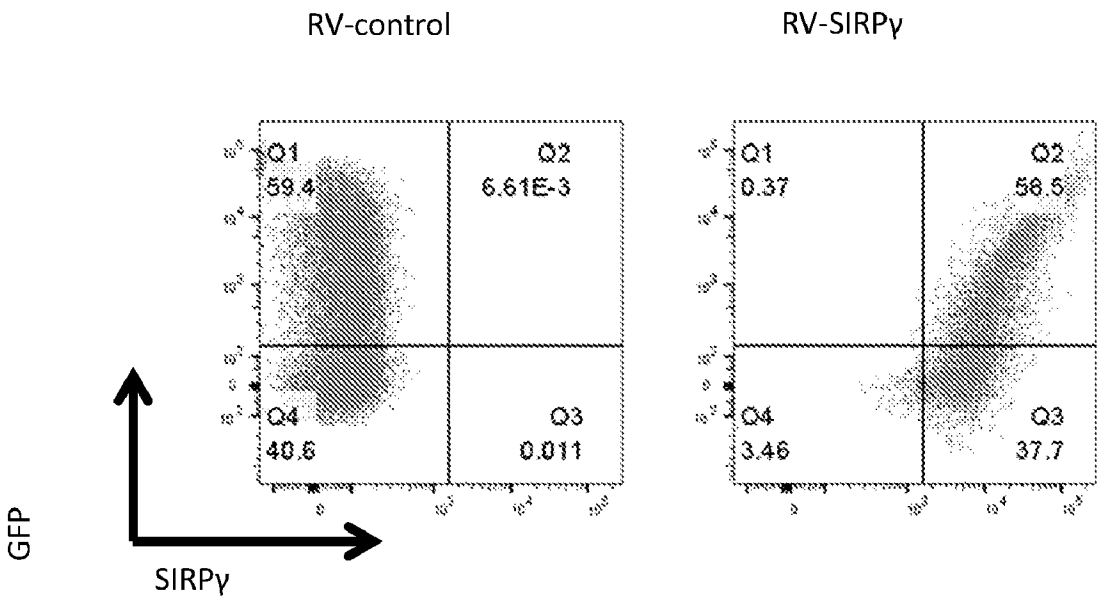
(54) Titre : INHIBITION DE LA SIRP-GAMMA POUR LE TRAITEMENT DU CANCER
(54) Title: INHIBITION OF SIRP-GAMMA FOR CANCER TREATMENT

FIGURE 1A



(57) Abrégé/Abstract:
Provided herein are methods of treating a subject with a tumor or cancer. In exemplary embodiments, the method comprises increasing an immune response against the tumor or cancer in the subject or increasing effector activity or reducing suppressive activity of T-cells in the subject. In exemplary embodiments, the method comprises administering to the subject a SIRPγ binder, e.g., SIRPγ inhibitor, in an amount effective to treat the tumor or cancer in the subject.

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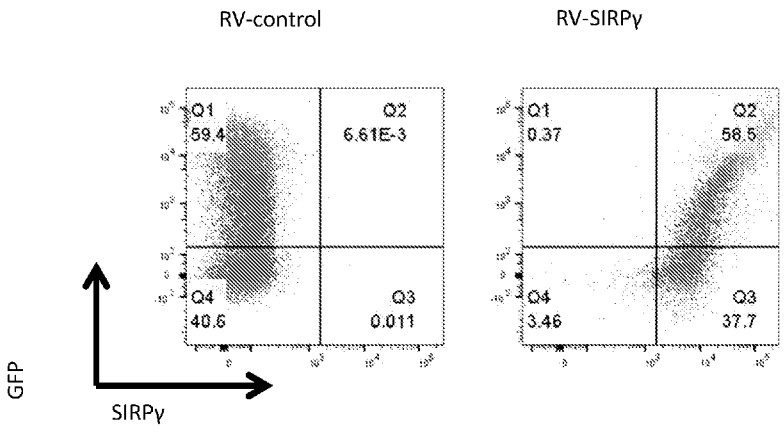
(54) Title: INHIBITION OF SIRP-GAMMA FOR CANCER TREATMENT

FIGURE 1A



(57) Abstract: Provided herein are methods of treating a subject with a tumor or cancer. In exemplary embodiments, the method comprises increasing an immune response against the tumor or cancer in the subject or increasing effector activity or reducing suppressive activity of T-cells in the subject. In exemplary embodiments, the method comprises administering to the subject a SIRPγ binder, *e.g.*, SIRPγ inhibitor, in an amount effective to treat the tumor or cancer in the subject.

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[Continued on next page]

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— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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INHIBITION OF SIRP-GAMMA FOR CANCER TREATMENT

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/865,537, filed on June 24, 2019, the entire contents of which are incorporated herein by reference in its entirety.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] Incorporated by reference in its entirety is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: 96,000 bytes ASCII (Text) file named "A-2396_Seqlisting.txt"; created on June 9, 2020.

BACKGROUND

[0003] Immune checkpoint blockade has shown to induce durable immune responses against various cancers. However, the patient responses to immunotherapies, such as anti-PD1/PDL1, are limited to only a small percentage of patients. PD1 is one of many co-inhibitory receptors expressed on T cells that are upregulated during T cell activation and its interaction with PD-L1 limits T cell activation. Although anti-PD-1 sufficiently blocks the inhibitory pathway and rejuvenates T cells inside various tumors, it is possible that many other inhibitory receptors are expressed on T cells. Therefore, exploration and further identification of novel inhibitory receptors on T cells will broaden the targets of immunotherapy and highly advance the efficacy to treat cancer.

SUMMARY

[0004] Provided herein for the first time are data demonstrating that SIRPγ is a potential novel inhibitory receptor in human T cells. These data were surprising given previous studies suggesting SIRPγ as a T cell co-stimulatory molecule (Piccio et al., Blood 105(6): 2421-2427 (2005); Leitner et al., Immunol Letters 128(2): 89-97 (2010)). The expression, regulation, and function of SIRPγ were evaluated and it was demonstrated that SIRPγ is mainly expressed on T cells and activated NK cells, and is highly expressed in memory CD8 T cells and tumor infiltrating exhausted T cells. It was also demonstrated herein that overexpression of SIRPγ inhibited CD8 T cell effector cytokine release, while knocked-out expression of SIRPγ (via CRISPR) enhanced the effector states of T cells as measured by T-cell proliferation and T-cell-mediated cytokine production. In addition, overexpression of SIRPγ in human Treg cells lead to enhanced Treg cell suppressive function. Furthermore, the data herein support that blocking the interaction between CD47 and SIRPγ is not a requirement for achieving enhanced T cell proliferation and IFNγ secretion, that the T-cell inhibitory function of SIRPγ may be mediated through a

unique epitope of SIRPγ, and that molecules that bind to the interface of D1 and D2 of SIRPγ may be useful in enhancing T cell function.

[0005] Without being bound to any particular theory, these data support the use of SIRPγ binders, e.g., SIRPγ inhibitors, for increasing effector activity or reducing suppressive activity of T-cells in a subject ultimately for the treatment of a tumor or cancer in the subject. Accordingly, in one embodiment, the present invention relates to a method of treating a tumor or cancer in a subject comprising administering to the subject an effective amount of a SIRPγ binder, e.g., SIRPγ inhibitor. The present disclosure also provides methods of increasing effector activity or reducing suppressive activity of T-cells in a subject with a tumor or cancer. In exemplary embodiments, the method comprises administering to the subject a SIRPγ binder, e.g., SIRPγ inhibitor, in an amount effective to increase the effector activity or reduce the suppressive activity in the subject. Methods of increasing an immune response against a tumor or cancer in a subject are additionally provided herein. In exemplary embodiments, the method comprises administering to the subject a SIRPγ binder, e.g., SIRPγ inhibitor, in an amount effective to increase an immune response against a tumor or cancer. In various aspects, the subject has hepatocellular carcinoma (HCC), colorectal cancer (CRC), lung cancer, or breast cancer, optionally, wherein the subject has non-small-cell lung cancer (NSCLC).

[0006] In various instances, the SIRPγ binder binds to Immunoglobulin (Ig) Domain 1 (D1) of SIRPγ. In various aspects, the SIRPγ binder binds to D1 and Ig Domain 2 (D2) of SIRPγ. In exemplary aspects, the SIRPγ binder binds to both D1 and D2, optionally at the interface between D1 and D2. In exemplary instances, the SIRPγ binder binds to the epitope to which SIRPγ monoclonal antibody OX117 binds, optionally, wherein the SIRPγ binder competes with a reference antibody known to bind to SIRPγ (e.g., OX117) for binding to SIRPγ. The SIRPγ binder in various instances binds to SIRPγ with the same or higher affinity as OX117, optionally, wherein the SIRPγ binder is OX117, or an antigen binding fragment thereof. The SIRPγ binder in various aspects forms hydrogen bonds with one or more of amino acid residues Q8, E10, G109, K11, L12, and D149 of SIRPγ. The SIRPγ binder causes a conformational change of SIRPγ, upon binding to SIRPγ in some aspects. In various instances, the SIRPγ binder simultaneously binds to two SIRPγ molecules or promotes SIRPγ dimerization. Optionally, the SIRPγ binder binds to an epitope which does not overlap with the CD47 binding site. In various aspects, the SIRPγ binder is an antigen-binding protein which binds SIRPγ. Optionally, the antigen-binding protein is an antibody, an antigen-binding antibody fragment, or an antibody protein product. In some aspects, the antigen-binding protein binds at an epitope within the CD47 binding site of SIRPγ.

[0007] In various instances of the presently disclosed methods, the SIRPγ binder is a SIRPγ inhibitor. In some aspects, the SIRPγ inhibitor reduces expression of SIRPγ in cells of the subject, optionally,

wherein the SIRPγ inhibitor reduces cell surface expression of SIRPγ on T-cells of the subject. Optionally, the T cells are effector T-cells of the subject. In various instances, the SIRPγ inhibitor reduces a binding interaction between SIRPγ and a SIRPγ binding partner, optionally, CD47.

[0008] In exemplary aspects of the presently disclosed methods of increasing effector activity or reducing suppressive activity of T-cells in a subject with a tumor or cancer, the T-cells are located within a tumor or a tumor microenvironment. In various instances, the T-cells are tumor-infiltrating T-cells. In some aspects, the T-cells are T regulatory cells (Tregs). In exemplary instances, the T-cells are exhausted T-cells, optionally, exhausted CD8+ T-cells. In exemplary instances, the T-cells are memory cells, optionally, CD8+ memory cells or CD4+ central memory cells.

[0009] In exemplary aspects of the presently disclosed methods of increasing an immune response against a tumor or cancer in a subject, the immune-response is mediated by T-cells. In various aspects, the T-cells are located within a tumor or a tumor microenvironment. In various instances, the T-cells are tumor-infiltrating T-cells. In some aspects, the T-cells are T regulatory cells (Tregs). In exemplary instances, the T-cells are exhausted T-cells, optionally, exhausted CD8+ T-cells. In exemplary instances, the T-cells are memory cells, optionally, CD8+ memory cells or CD4+ central memory cells.

[0010] The present disclosure additionally provides methods of treating a subject with a tumor or cancer. In exemplary embodiments, the method comprises increasing an immune response against the tumor or cancer in the subject in accordance with any one of the presently disclosed methods of increasing an immune response against a tumor or cancer in a subject. In exemplary embodiments, the method comprises increasing effector activity or reducing suppressive activity of T-cells in the subject in accordance with any one of the presently disclosed methods of increasing effector activity or reducing suppressive activity of T-cells in a subject with a tumor or cancer.

[0011] Additional embodiments and aspects of the presently disclosed pharmaceutical compositions and methods are provided below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Figures 1A – 1C demonstrate that SIRPγ is expressed on human T cells and NKT cells. Figure 1A is a series of FACS plots showing antibody against SIRPγ specifically detected SIRPγ overexpressed on 293T cells. Figure 1B is a series of FACS plots showing SIRPγ expression on different types of cells from human peripheral blood mononuclear cells (PBMCs). Figure 1C shows that SIRPγ expression level on T cells is not changed by TCR stimulation.

[0013] Figures 2A and 2B demonstrate that SIRPγ is highly expressed on memory T cells. Figure 2A is a series of FACS plots showing expression in different subsets of T cells. CD8+ memory T cells have

higher SIRPγ expression than CD8+ effector T cells in human PBMC samples. Figure 2B is a quantification of mean fluorescence intensity (MFI) of SIRPγ expression in different subsets of T cells from 4 different healthy donor PBMCs. Significance is denoted as ***$p \leq 0.0002$, **$p \leq 0.0021$, *$p \leq 0.0332$, and ns $p > 0.05$ using a paired t test. Error bars represent ± SEM.

[0014] Figures 3A-3C demonstrate that SIRPγ has heightened expression on tumor infiltrated exhaustion T cells. Figure 3A is a graph comparing the expression of SIRPγ on different subsets of tumor infiltrated T cells from HCC samples. Figure 3B is a graph comparing the expression of SIRPγ on different subsets of tumor infiltrated T cells from CRC samples. Figure 3C is a graph comparing the expression of SIRPγ on different subsets of tumor infiltrated T cells from lung cancer samples. SIRPγ exhibited a highly specific expression pattern in both tumor Tregs (CD4-CTLA4) and exhausted CD8 T cells (CD8-LAYN), marked with star.

[0015] Figure 4 demonstrates that SIRPγ has heightened expression on exhausted T cells derived from repeated TCR re-stimulation. Figure 4 is a series of FACS plots showing expression of SIRPγ on in vitro restimulated exhausted T cells and conventional T cells from three different healthy donors.

[0016] Figures 5A-5D demonstrate that SIRPγ overexpression on T cells inhibited IFNγ secretion. Figure 5A is a schematic diagram showing the experimental flow of SIRPγ overexpression and T cell restimulation. PanT cells were isolated from human PBMCs and activated by αCD3/CD28 Dynabeads for 3 days. Activated T cells were infected with retrovirus (RV) to overexpress SIRPγ (RV-SIRPγ) on T cells or infected with an RV vector without SIRPγ coding sequence as a control (RV-Vec). 5 days post spin-infection, GFP+ CD4 or CD8 T cells were FACS sorted and rested 2 days with human IL2. Rested T cells were then restimulated with plate bounded αCD3 and soluble CD28 antibodies for 24 hours. Cell supernatant was collected for ELISA analysis. Figure 5B is a series of flow cytometry plots showing human SIRPγ expression on both CD4+ T cells and CD8+ T cells. 3 days post spin-infection, cells were stained with human SIRPγ antibody. Figure 5C is an ELISA of human IFNγ in the cell supernatant. Significance is denoted as ****$p \leq 0.0001$, ***$p \leq 0.0002$, **$p \leq 0.0021$, *$p \leq 0.0332$, and ns $p > 0.05$ using two-way ANOVA test from Graphpad Prism. Error bars represent ± SEM. Data represent at least five independent experiments. Figure 5D is a paired T test of ELISA of human IFNγ in the cell supernatant from five independent experiments. * denotes a p value of <0.05 using a paired T test.

[0017] Figures 6A-6F demonstrate that SIRPγ knockdown on T cells enhanced IFNγ secretion. Figure 6A is a schematic diagram showing the experimental flow of SIRPγ knockdown and T cell restimulation. PanT cells were isolated from human PBMCs and activated by αCD3/CD28 Dynabeads for 2 days. Activated T cells were transfected with CRISPR gRNAs (guide RNAs) that targeted to SIRPγ genomic region to knockdown SIRPγ expression (SIRPγ KO) on T cells or transfected with a control. 3 days post

transfection, T cells were activated with αCD3/CD28 for 24 hours before FACS sort. SIRPγ- CD4 or CD8 T cells were FACS sorted and rested 2 days with human IL2. Rested T cells were then restimulated with plate bounded αCD3 and soluble CD28 antibodies for 24 hours. Cell supernatant was collected for ELISA analysis. Figure 6B are gel analyses of PCR product amplified from targeted genomic region. The arrow indicates the deletion on the gRNA targeted genomic region. Figure 6C are qPCR analyses of expression of SIRPγ after knockout. Significance is denoted as ****$p \leq 0.0001$, ***$p \leq 0.0002$, **$p \leq 0.0021$, *$p \leq 0.0332$, and ns $p > 0.05$ using unpaired t test from Graphpad Prism. Error bars represent ± SEM. Figure 6D is a series of flow cytometry plots showing human SIRPγ expression on both CD4+ T cells and CD8+ T cells after CRISPR knockdown. 4 days post spin-infection, cells were stained with human SIRPγ antibody. Figure 6E is a quantification of percentage of SIRPγ+ cells in total CD4 or CD8 T cells after CRISPR knockout. Figure 6F is an ELISA of human IFNγ in the cell supernatant from two independent experiments. Significance is denoted as ****$p \leq 0.0001$, ***$p \leq 0.0002$, **$p \leq 0.0021$, *$p \leq 0.0332$, and ns $p > 0.05$ using two-way ANOVA test from Graphpad Prism. Error bars represent ± SEM.

[0018] Figures 7A-7F demonstrate that SIRPγ overexpression on Treg cells enhanced suppressive function of Tregs. Figure 7A is a series of FACS plots and histogram showing expression of SIRPγ on tumor infiltrated lymphocytes from non-small-cell lung cancer tissues. Figure 7B is a schematic diagram showing the experimental flow of SIRPγ overexpression and Treg cells suppression assay. Tregs cells were FACS sorted from panT cells isolated from human PBMCs and activated by αCD3/CD28 Dynabeads for 2 days. Activated Treg cells were transfected with retrovirus to overexpress SIRPγ (RV-SIRPγ) on Treg cells or infected with an RV vector without SIRPγ coding sequence as a control (RV-Vec). 5 days post spin-infection, GFP+ Treg cells were FACS sorted and rested overnight with human IL2 (200U/ml) before adding to the suppression assay. On the day of setting up the suppression assay, responder CD4 T cells were isolated from a different healthy donor PBMCs and labelled with CellTrace Violet (CTV). Rested Treg cells were mixed with CTV labelled responder CD4 T cells at different ratios. Allogenic DCs were added and CD4 T cells proliferation was measured by CTV dilution. Figure 7C is a series of flow cytometry plots showing human SIRPγ expression on Treg cells after retrovirus spin infection. 5 days post spin-infection, cells were stained with human SIRPγ antibody. Figure 7D is a series of flow cytometry plots showing human FOXP3 expression on FACS sorted control or SIRPγ overexpression Treg cells. Figure 7E is a series of flow cytometry plots showing CTV dilution on T cells that mixed with either control and SIRPγ overexpression Tregs. Figure 7F is a graph showing the percentage of cell proliferation at different ratios of Treg vs responder cells. * denotes a p value of < 0.05 using a Student's t test. Error bars represent ± SEM.

[0019] Figures 8A-8F demonstrate that SIRPγ antibodies have non-specific inhibitory effects on T cell proliferation. Figure 8A is a series of FACS plots showing expression of SIRPγ (left plot) and CD47 (right plot) on Jurkat T cells after CRISPR knockout of SIRPγ (SIRPγ KO), CD47 (CD47 KO) or both SIRPγ and CD47 (DKO). The plot using an isotype matched control antibody (Isotype) or non-transfected control cells (NT control) are shown in each panel. Figure 8B shows binding analyses of SIRPγ-Fc and SIRPα-Fc proteins on Jurkat T cells. All Fc fusion proteins were added at 5 μg/ml. Binding of fusion proteins to cells was detected by flow cytometry using a PE conjugated anti-human IgG-Fc. Figure 8C shows antagonist activity study of anti-SIRPγ antibodies (LSB2.20 and OX119) on SIRPγ-Fc binding on Jurkat T cells. All IgG-Fc proteins were added at 5 μg/ml and all antibodies were added at 10 μg/ml. An IgG antibody (mIgG) was used as a control. Binding of fusion proteins to cells was detected by flow cytometry using a PE conjugated anti-human IgG-Fc. Figure 8D is a graph showing counts per minute (CPM) of human panT cells isolated from PBMCs from healthy donors stimulated with allogeneic dendritic cells (DCs) at a 10 T cell: 1 DC ratio for 7 days. Antibodies were added at day 0 of the culture at 10 μg/ml. T cell proliferation was measured by standard 3H-thymidine incorporation assay. Figure 8E is a graph showing CPM of control T cells, SIRPγ knockout or CD47 knockout panT cells stimulated with allogeneic dendritic cells (DCs) at a 10 T cell: 1 DC ratio for 7 days. Antibodies were added at day 0 of the culture at 10 μg/ml. T cell proliferation was measured by standard 3H-thymidine incorporation assay. Figure 8F is human panT cells or CD8 T cells isolated from PBMCs from healthy donors were stimulated with different concentrations of plate bounded anti-CD3 for 3 days. Antibodies against SIRPγ or CD47 were added at day 0 of the culture at 10 μg/ml. T cell proliferation was measured by standard 3H-thymidine incorporation assay.

[0020] Figures 9A-9E demonstrate that SIRPγ antibody clone OX117, which binds to a specific epitope of SIRPγ, enhances T cell proliferation and cytokine secretion. Figure 9A is a series of FACS plots showing binding of SIRPγ antibodies on parental Jurkat T cells and Jurkat T cells that overexpress SIRPγ. Figure 9B shows only antibody OX117 alters the binding of SIRPγ-Fc proteins on Jurkat T cells. Jurkat T cells were pre-treated with SIRPγ antibodies at 10 μg/ml. SIRPγ-Fc proteins were added at 10 μg/ml. Binding of fusion proteins to cells was detected by flow cytometry using a PE conjugated anti-human IgG-Fc. Figure 9C and 9D show specific anti-SIRPγ antibody clone OX117 has the strongest antagonist activity on human panT cell in promoting proliferation and cytokine production. Plates were coated with SIRPγ antibodies at 10 μg/ml. Human panT cells isolated from PBMCs from healthy donors were stimulated with ImmunoCult CD3/CD28 T cell activator along with plate bounded SIRPγ antibodies for 3 days. In Figure 9C, T cell proliferation was measured by standard 3H-thymidine incorporation assay. Figure 9D is an CBA analyses of human IFNγ in the cell supernatant from human panT cells stimulated for 48 hrs. Figure 9E is the overview of the binding epitopes between SIRPγ: FabOX117 and between

SIRPα: CD47 complexes, showing that CD47 and FabOX117 bind to different residues on SIRPγ (Nettleship et al., BMC Structural Biology 13: 13 (2013)).

[0021] Figure 10 provides a table summarizing the commercial SIRPγ antibodies properties and their functions on T cells.

DETAILED DESCRIPTION

[0022] *SIRPγ, SIRPγ Binders, and SIRPγ Inhibitors*

[0023] Signal regulatory protein gamma (SIRPγ or SIRPG) – also known as, CD172g, SIRPB2, SIRP-B2, and bA77C3.1 – is a member of the signal-regulatory protein (SIRP) family and also belongs to the immunoglobulin (Ig) superfamily. Like other members of the SIRP family, SIRPγ, has three Type I transmembrane glycoproteins, each comprising three Ig-like domains that make up an extracellular region, a single transmembrane domain, and a short cytoplasmic domain. Unlike other members of SIRP receptor family, SIRPγ lacks cytoplasmic immunoreceptor tyrosine based inhibitory motifs (ITIMs) to recruit the downstream signaling molecules to mediate cell signaling. SIRPγ functions in the negative regulation of receptor tyrosine kinase-coupled signaling processes and integrin-independent adhesion of lymphocytes to antigen-presenting cells. SIRPγ is highly expressed in human blood, thymus and splenic tissue. Within human PBMCs, SIRPγ is mainly expressed on T cells and activated natural killer (NK) cells. Additionally, several recent studies on RNAseq profiling of tumor and adjacent tissue showed that SIRPγ is highly expressed on T cells isolated from various tumors. Like SIRPα, SIRPγ binds to CD47, though with lower affinity than SIRPα (Brooke et al., J Immunol 173(4): 2562-2570 (2004)). SIRPγ and its role in the immune system is reviewed in van Beek et al., J Immunol 175(12): 7781-7787 (2005). The crystal structure of SIRPγ is described in Nettleship et al., BMC Structural Biology 13: 13 (2013).

[0024] The *SIRPγ* gene is a polymorphic gene and is found on human chromosome 20 (arm p13) and comprises 8 exons. Several SIRPγ variants have been described in human population and the protein sequences of such SIRPγ variants may be found at the National Center for Biotechnology Information (NCBI) website as Accession No. NP_001034597.1 (Isoform 3 precursor; SEQ ID NO: 1), NP_061026.2 (Isoform 1 precursor; SEQ ID NO: 3), and NP_543006.2 (Isoform 2 precursor; SEQ ID NO: 5). The messenger RNA (mRNA) sequences of SIRPγ may be found at the NCBI website as Accession No. NM_001039508.1 (Transcript variant 3; SEQ ID NO: 2); Accession No. NM_018556.4 (Transcript variant 1; SEQ ID NO: 4); and Accession No. NM_080816.2 (Transcript variant 2; SEQ ID NO: 6). Among the variants, a protective intron variant rs2281808 within *SIRPγ* intron has been identified to be associated with decreased risk in Type 1 diabetes (T1D) development through several genome-wide association studies. A recent study of the *rs2281808* intron variant indicated that the SNP variant resulted

in the reduction of SIRPγ expression on T cells. However, the biological activity of SIRPγ is still largely unknown, partially because of the lack of homolog gene in mouse.

[0025] Previous studies have shown that anti-SIRPγ or anti-CD47 antibody can inhibit T cell proliferation and T-cell secretion of IFNγ triggered by allogeneic immature DCs in mixed lymphocyte reactions (Piccio et al., Blood, 105:2421-2427, 2005). However, in these previous studies, the binding epitope of anti-SIRPγ antibody is unknown and the mechanisms of anti-SIRPγ antibody's inhibitory effect on T cell proliferation are unclear. In such studies, it is unknown whether the blocking of CD47 and SIRPγ interaction by the antibody is the cause of decreased T cell proliferation, and it is unclear whether there are additional biological functions of SIRPγ beyond those involving its interaction with CD47.

[0026] In exemplary embodiments of the presently disclosed methods, a SIRPγ binder is administered to a subject. As used herein, the term "SIRPγ binder" refers to any compound or molecule that binds to SIRPγ to form a binding interaction with SIRPγ. In exemplary aspects, the SIRPγ binder comprises or is a small molecular weight compound, an amino acid, a peptide, a polypeptide, a protein, a polymer, a carbohydrate, a lipid, a nucleic acid, an oligonucleotide, a DNA or RNA. Optionally, the SIRPγ binder is a protein, such as, for instance, an antigen binding protein described herein. In some embodiments, the SIRPγ binder is an antibody or antigen binding fragment thereof.

[0027] In various instances, the binding interaction formed between SIRPγ and the SIRPγ binder is a non-covalent binding interaction. For example, the SIRPγ binder may in various aspects form ionic bonds, van der Waals interactions, hydrophobic bonds, and/or hydrogen bonds with one or more amino acid residues of SIRPγ. Optionally, the non-covalent binding interaction is a reversible, non-covalent binding interaction. The binding interaction may be described in terms of K_D, the equilibrium dissociation constant, a ratio of k_{off}/k_{on}, between SIRPγ and the SIRPγ binder. The lower the K_D value of the SIRPγ binder the higher the affinity of the SIRPγ binder for SIRPγ. In exemplary aspects, the K_D value of the SIRPγ binder for SIRPγ is micromolar, nanomolar, picomolar or femtomolar. In exemplary aspects, the K_D of the antigen binding proteins provided herein is within a range of about 10^{-4} to 10^{-6} M, or 10^{-7} to 10^{-9} M, or 10^{-10} to 10^{-12} M, or 10^{-13} to 10^{-15} M. In exemplary aspects, the SIRPγ binder binds to SIRPγ with a K_D of about 0.01 nM to about 20 nM, 0.02 nM to 20 nM, 0.05 nM to 20 nM, 0.05 nM to 15 nM, 0.1 nM to 15 nM, 0.1 nM to 10 nM, 1 nM to 10 nM, or 5 nM to 10 nM.

[0028] In various instances, the SIRPγ binder binds to D1 and/or binds to the CD47 binding site of SIRPγ. In various aspects, the SIRPγ binder binds to D1 and Ig Domain 2 (D2) of SIRPγ. In exemplary aspects, the SIRPγ binder binds to both D1 and D2, optionally at the interface between D1 and D2. Optionally, the SIRPγ binder binds to the binding site of a SIRPγ binding partner other than CD47.

Figure 9E provides an illustration of SIRPγ, its Ig domains thereof and the CD47 binding site. In exemplary instances, the SIRPγ binder binds to the epitope to which SIRPγ monoclonal antibody OX117 binds. In some embodiments, the SIRPγ binder competes with a reference antibody known to bind to SIRPγ (e.g., OX117) for binding to SIRPγ. The SIRPγ binder in various instances binds to SIRPγ with the same or higher affinity as OX117. In some embodiments, the SIRPγ binder is OX117, or an antigen binding fragment thereof. Figure 9E provides an illustration of the binding interaction between SIRPγ and the fab of the OX117 antibody. The SIRPγ binder in various aspects forms hydrogen bonds with one or more of amino acid residues Q8, E10, G109, K11, L12, and D149 of SIRPγ. In some embodiments, the SIRPγ binder forms hydrogen bonds with each of amino acid residues Q8, E10, G109, K11, L12, and D149 of SIRPγ. In some embodiments, the SIRPγ binder binds to an epitope which does not overlap with the CD47 binding site.

[0029] In exemplary instances, upon binding to SIRPγ, the SIRPγ binder enhances T cell activation, T cell proliferation and cytokine secretion. In some instances, the methods of the disclosure increase the T cell activation, T cell proliferation and cytokine secretion to any degree or level relative to a control. For example, in some aspects, the increase provided by the methods of the disclosure is at least or about a 1% to about a 10% increase (e.g., at least or about a 1% increase, at least or about a 2% increase, at least or about a 3% increase, at least or about a 4% increase, at least or about a 5% increase, at least or about a 6% increase, at least or about a 7% increase, at least or about a 8% increase, at least or about a 9% increase, at least or about a 9.5% increase, at least or about a 9.8% increase, at least or about a 10% increase) relative to a control. In exemplary embodiments, the increase provided by the methods of the disclosure is over 100%, e.g., 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900% or even 1000% relative to a control. In exemplary embodiments, the T cell activation, T cell proliferation and cytokine secretion increases by at least or about 1.5-fold, at least or about 2.0 fold, at least or about 3.0 fold, at least or about 4.0 fold, at least or about 5.0 fold, at least or about 10.0 fold, at least or about 25 hold, at least or about 50 fold, at least or about 75 fold, or at least or about 100 fold or more, relative to a control. The control in various aspects is the T cell activation, T cell proliferation and cytokine secretion without the SIRPγ binder binding to SIRPγ.

[0030] In exemplary instances, the SIRPγ binder causes a conformational change of SIRPγ, upon binding to SIRPγ. The conformational change of SIRPγ may alter the accessibility of binding sites of binding partners. The conformational may also allow different binding partners to bind to SIRPγ. Additionally or alternatively, the conformational change may cause dimerization or multimerization of SIRPγ molecules. In exemplary aspects, the dimerization or multimerization of SIRPγ prevents one or more binding partners from binding to SIRPγ. In exemplary aspects, the dimerization or multimerization

of SIRPγ enhances the binding of one or more binding partners binding to SIRPγ. In various instances, the SIRPγ binder simultaneously binds to two SIRPγ molecules or promotes SIRPγ dimerization.

[0031] In some embodiments, the SIRPγ binder blocks the function of SIRPγ, e.g., the SIRPγ binder is a SIRPγ inhibitor. Accordingly, in exemplary embodiments of the presently disclosed methods, a SIRPγ inhibitor is administered to a subject. As used herein, the term "SIRPγ inhibitor" refers to any compound or molecule that reduces or inhibits the function of SIRPγ. In exemplary instances, the SIRPγ inhibitor reduces the signal transduction that ensues upon the binding of a SIRPγ binding partner to SIRPγ. In various instances, the SIRPγ inhibitor reduces a binding interaction between SIRPγ and a SIRPγ binding partner. In various aspects, the SIRPγ inhibitor reduces expression of SIRPγ in cells of the subject. In some embodiments, the SIRPγ inhibitor binds SIRPγ. In other embodiments, the SIRPγ inhibitor binds a SIRPγ binding partner.

[0032] As used herein, the terms "inhibit" and "reduce" and words stemming therefrom do not necessarily mean a 100% or complete inhibition or abrogation or reduction. Rather, there are varying degrees of inhibition and/or reduction of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the SIRPγ inhibitors of the present disclosure may reduce or inhibit the SIRPγ function to any amount or level. In exemplary embodiments, the reduction or inhibition provided by the SIRPγ inhibitor is at least or about a 10% reduction or inhibition (e.g., at least or about a 20% reduction or inhibition, at least or about a 30% reduction or inhibition, at least or about a 40% reduction or inhibition, at least or about a 50% reduction or inhibition, at least or about a 60% reduction or inhibition, at least or about a 70% reduction or inhibition, at least or about a 80% reduction or inhibition, at least or about a 90% reduction or inhibition, at least or about a 95% reduction or inhibition, at least or about a 98% reduction or inhibition, at least or about a 99% reduction or inhibition, or about a 100% reduction or inhibition).

[0033] In exemplary aspects, the SIRPγ inhibitor reduces expression of SIRPγ in cells of the subject. In certain instances, the SIRPγ inhibitor reduces cell surface expression of SIRPγ on T-cells. In exemplary aspects, the T cells are located within a tumor or a tumor microenvironment. In various instances, the T-cells are tumor-infiltrating T-cells. In exemplary aspects, the T-cells are T regulatory cells (Tregs). In various aspects, the T-cells are exhausted T-cells, optionally, exhausted CD8+ T-cells. Optionally, the T-cells are memory cells. In various aspects, the memory cells are CD8+ memory cells or CD4+ central memory cells. In exemplary instances, the SIRPγ inhibitor is a molecule that targets a nucleic acid encoding SIRPγ. In exemplary instances, the SIRPγ inhibitor is an antisense molecule which mediates RNA interference (RNAi). RNAi is a ubiquitous mechanism of gene regulation in plants and animals in which target mRNAs are degraded in a sequence-specific manner (Sharp, Genes Dev., 15, 485-

490 (2001); Hutvagner et al., Curr. Opin. Genet. Dev., 12, 225-232 (2002); Fire et al., Nature, 391, 806-811 (1998); Zamore et al., Cell, 101, 25-33 (2000)). The natural RNA degradation process is initiated by the dsRNA-specific endonuclease Dicer, which promotes cleavage of long dsRNA precursors into double-stranded fragments between 21 and 25 nucleotides long, termed small interfering RNA (siRNA; also known as short interfering RNA) (Zamore, et al., Cell. 101, 25-33 (2000); Elbashir et al., Genes Dev., 15, 188-200 (2001); Hammond et al., Nature, 404, 293-296 (2000); Bernstein et al., Nature, 409, 363-366 (2001)). siRNAs are incorporated into a large protein complex that recognizes and cleaves target mRNAs (Nykanen et al., Cell, 107, 309-321 (2001). The requirement for Dicer in maturation of siRNAs in cells can be bypassed by introducing synthetic 21-nucleotide siRNA duplexes, which inhibit expression of transfected and endogenous genes in a variety of mammalian cells (Elbashir et al., Nature, 411: 494-498 (2001)). In exemplary aspects, the SIRPγ inhibitor mediates RNAi and in various instances is a siRNA molecule specific for inhibiting the expression of the nucleic acid (e.g., the mRNA) encoding the SIRPγ protein. The term "siRNA" as used herein refers to an RNA (or RNA analog) comprising from about 10 to about 50 nucleotides (or nucleotide analogs) which is capable of directing or mediating RNAi. In exemplary embodiments, a siRNA molecule comprises about 15 to about 30 nucleotides (or nucleotide analogs) or about 20 to about 25 nucleotides (or nucleotide analogs), e.g., 21-23 nucleotides (or nucleotide analogs). The siRNA can be double or single stranded, preferably double-stranded.

[0034] In alternative aspects, the SIRPγ inhibitor is a short hairpin RNA (shRNA) molecule specific for inhibiting the expression of the nucleic acid (e.g., the mRNA) encoding the SIRPγ protein. The term "shRNA" as used herein refers to a molecule of about 20 or more base pairs in which a single-stranded RNA partially contains a palindromic base sequence and forms a double-strand structure therein (i.e., a hairpin structure). An shRNA can be a siRNA (or siRNA analog) which is folded into a hairpin structure. shRNAs typically comprise about 45 to about 60 nucleotides, including the approximately 21 nucleotide antisense and sense portions of the hairpin, optional overhangs on the non-loop side of about 2 to about 6 nucleotides long, and the loop portion that can be, e.g., about 3 to 10 nucleotides long. The shRNA can be chemically synthesized. Alternatively, the shRNA can be produced by linking sense and antisense strands of a DNA sequence in reverse directions and synthesizing RNA in vitro with T7 RNA polymerase using the DNA as a template. Though not wishing to be bound by any theory or mechanism, it is believed that after shRNA is introduced into a cell, the shRNA is degraded into a length of about 20 bases or more (e.g., representatively 21, 22, 23 bases), and causes RNAi, leading to an inhibitory effect. Thus, shRNA elicits RNAi and therefore can be used as an effective component of the disclosure. shRNA may preferably have a 3'-protruding end. The length of the double-stranded portion is not particularly limited, but is preferably about 10 or more nucleotides, and more preferably about 20 or more nucleotides. Here,

the 3'-protruding end may be preferably DNA, more preferably DNA of at least 2 nucleotides in length, and even more preferably DNA of 2-4 nucleotides in length.

[0035] In exemplary aspects, the SIRPγ inhibitor is a microRNA (miRNA). As used herein the term "microRNA" refers to a small (e.g., 15-22 nucleotides), non-coding RNA molecule which base pairs with mRNA molecules to silence gene expression via translational repression or target degradation. microRNA and the therapeutic potential thereof are described in the art. See, e.g., Mulligan, *MicroRNA: Expression, Detection, and Therapeutic Strategies*, Nova Science Publishers, Inc., Hauppauge, NY, 2011; Bader and Lammers, "The Therapeutic Potential of microRNAs" *Innovations in Pharmaceutical Technology*, pages 52-55 (March 2011).

[0036] In exemplary instances, the SIRPγ inhibitor reduces the signal transduction that ensues upon the binding of a SIRPγ binding partner to SIRPγ. In various aspects, the SIRPγ inhibitor reduces the signal transduction that ensues upon the binding of CD47 to SIRPγ, e.g., the signaling in endothelial cells induced by CD47-SIRPγ binding interactions that lead to T-cell transendothelial migration (Stefanidakis et al., Blood 112: 1280-1289 (2008)). In various aspects, the SIRPγ inhibitor reduces the signal transduction that ensues upon the binding of a SIRPγ binding partner to SIRPγ immunoglobulin domain D1 (D1) and/or immunoglobulin domain D2 (D2). In one instance, the SIRPγ inhibitor reduces the signal transduction that ensues upon the binding of a SIRPγ binding partner to the interface between SIRPγ immunoglobulin domain D1 and immunoglobulin domain D2. In various aspects, the SIRPγ inhibitor enhances secretion of IFNγ by activated T cells.

[0037] In various instances, the SIRPγ inhibitor reduces a binding interaction between SIRPγ and a SIRPγ binding partner. In exemplary aspects, the SIRPγ inhibitor inhibits at least or about 10% of the binding interactions between SIRPγ and the SIRPγ binding partner (e.g., at least or about 20% of the binding interactions, at least or about 30% of the binding interactions, at least or about 40% of the binding interactions, at least or about 50% of the binding interactions, at least or about 60% of the binding interactions, at least or about 70% of the binding interactions, at least or about 80% of the binding interactions, at least or about 90% of the binding interactions, at least or about 95% of the binding interactions, at least or about 98% of the binding interactions, at least or about 99% of the binding interactions, or about 100% of the binding interactions). In other instances, the SIRPγ binding partner binds to the interface between SIRPγ immunoglobulin domain D1 and immunoglobulin domain D2. In various instances, the SIRPγ binding partner is CD47. In various aspects, the SIRPγ binding partner binds to D1 and/or D2.

[0038] In exemplary aspects, the SIRPγ inhibitor is a soluble portion of SIRPγ which binds to CD47 or another SIRPγ binding partner. In various aspects, the soluble portion of SIRPγ is a decoy which, upon

binding to CD47 or other SIRPγ binding partner, leads to a null response, e.g., a lack of SIRPγ-CD47-mediated signaling. In various aspects, the soluble portion of SIRPγ comprises at least amino acids 29-360 of the SIRPγ amino acid sequence. In exemplary aspects, the soluble portion of SIRPγ comprises at least amino acids 29-360 of SEQ ID NO: NP_061026.2, the human SIRPγ amino acid sequence.

[0039] In some embodiments, the SIRPγ inhibitor is a SIRPγ-Fc which binds to CD47 or other SIRPγ binding partners. In various aspects, the SIRPγ-Fc is a decoy which, upon binding to CD47 or other SIRPγ binding partners, leads to a null response, e.g., a lack of SIRPγ-CD47-mediated signaling. In various aspects, the SIRPγ-Fc comprises at least amino acids 29-360 of the SIRPγ amino acid sequence.

[0040] In some embodiments, the SIRPγ inhibitor is a CRISPR gRNA. CRISPR knockout systems contain a guide RNA (gRNA) and a CRISPR-associated endonuclease (Cas protein). The term "gRNA" as used herein refers to a short RNA molecule of about 100 or more base pairs. The gRNA contains ~20 base pairs of nucleotide spacer and a scaffold sequence required for Cas protein binding. By altering the 20 base pairs towards the 5' end of the gRNA, the gRNA can be targeted towards any genomic region complementary to that sequence. The 20 base pairs long nucleotide spacer can be chemically synthesized and annealed to scaffold RNA to form gRNA *in vitro*. Full length of gRNA can be chemically synthesized *in vitro*. Alternatively, the gRNA can be produced by viral vectors that driven by U6 RNA polymerase III promoter. The desired 20 base pair target sequence immediately precedes a protospacer adjacent motif (PAM). The gRNA guides the Cas nuclease to the target sequence by complementary base pairing and Cas nuclease mediates a double strand break a few nucleotides upstream of the PAM sequence. Targeted cells will use non-homologous end joining (NHEJ) or homology directed repair (HDR) to repair the double strand break. In many cases, NHEJ causes deletions, insertions, or frameshift mutations in the targeted DNA region and results a loss-of function mutation of the targeted gene.

[0041] In exemplary aspects, the SIRPγ inhibitor is a soluble portion of CD47 which binds to SIRPγ. In various aspects, the soluble portion of CD47 is a decoy which, upon binding to SIRPγ, leads to a null response, e.g., a lack of SIRPγ-CD47-mediated signaling. In various aspects, the soluble portion of CD47 comprises at least amino acids 26-133 of the CD47 amino acid sequence. In exemplary aspects, the soluble portion of CD47 comprises at least amino acids 26-133 of SEQ ID NO: NP_001768.1, the human CD47 amino acid sequence.

[0042] *Antigen-Binding Proteins*

[0043] In exemplary instances, the SIRPγ binder, e.g., SIRPγ inhibitor, is an antigen-binding protein that binds to SIRPγ. In exemplary aspects, the SIRPγ inhibitor, is an antigen-binding protein that binds to SIRPγ or the SIRPγ binding partner (e.g., CD47). The antigen-binding protein in various aspects is an

antibody, an antigen-binding antibody fragment, or an antibody protein product. As used herein, the term "antibody" refers to a protein having a known immunoglobulin format, comprising heavy and light chains, and comprising variable and constant regions. For example, an antibody can be an IgG which is a "Y-shaped" structure of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). An antibody has variable regions and constant regions. In IgG formats, the variable region is generally about 100-110 or more amino acids, comprises three complementarity determining regions (CDRs), is primarily responsible for antigen recognition, and substantially varies among other antibodies that bind to different antigens. The constant region allows the antibody to recruit cells and molecules of the immune system. The variable region is made of the N-terminal regions of each light chain and heavy chain, while the constant region is made of the C-terminal portions of each of the heavy and light chains. (Janeway *et al.*, "Structure of the Antibody Molecule and the Immunoglobulin Genes", *Immunobiology: The Immune System in Health and Disease*, 4th ed. Elsevier Science Ltd./Garland Publishing, (1999)).

[0044] The general structure and properties of CDRs of antibodies have been described in the art. Briefly, in an antibody scaffold, the CDRs are embedded within the heavy and light chain variable regions where they constitute the regions largely responsible for antigen binding and recognition. A variable region typically comprises at least three heavy or light chain CDRs (Kabat *et al.*, 1991, *Sequences of Proteins of Immunological Interest*, Public Health Service N.I.H., Bethesda, Md.; see also Chothia and Lesk, 1987, *J. Mol. Biol.* 196:901-917; Chothia *et al.*, 1989, *Nature* 342: 877-883), within a framework region (designated framework regions 1-4, FR1, FR2, FR3, and FR4, by Kabat *et al.*, 1991; see also Chothia and Lesk, 1987, *supra*).

[0045] Antibodies can comprise any constant region known in the art. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. IgM has subclasses, including, but not limited to, IgM1 and IgM2. Embodiments of the present disclosure include all such classes or isotypes of antibodies. The light chain constant region can be, for example, a kappa- or lambda-type light chain constant region, *e.g.*, a human kappa- or lambda-type light chain constant region. The heavy chain constant region can be, for example, an alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant regions, *e.g.*, a human alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant region. Accordingly, in exemplary embodiments, the antibody is an antibody of isotype IgA, IgD, IgE, IgG, or

IgM, including any one of IgG1, IgG2, IgG3 or IgG4. In some embodiments, the antibody is an IgG1, IgG2, IgG3, or IgG4 antibody.

[0046] The antibody can be a monoclonal antibody or a polyclonal antibody. In some embodiments, the antibody comprises a sequence that is substantially similar to a naturally-occurring antibody produced by a mammal, *e.g.*, mouse, rabbit, goat, horse, chicken, hamster, human, and the like. In this regard, the antibody can be considered as a mammalian antibody, *e.g.*, a mouse antibody, rabbit antibody, goat antibody, horse antibody, chicken antibody, hamster antibody, human antibody, and the like. In certain aspects, the antibody is a human antibody. In certain aspects, the antibody is a chimeric antibody or a humanized antibody. The term "chimeric antibody" refers to an antibody containing domains from two or more different antibodies. A chimeric antibody can, for example, contain the constant domains from one species and the variable domains from a second, or more generally, can contain stretches of amino acid sequence from at least two species. A chimeric antibody also can contain domains of two or more different antibodies within the same species. The term "humanized" when used in relation to antibodies refers to antibodies having at least CDR regions from a non-human source which are engineered to have a structure and immunological function more similar to true human antibodies than the original source antibodies. For example, humanizing can involve grafting a CDR from a non-human antibody, such as a mouse antibody, into a human antibody. Humanizing also can involve select amino acid substitutions to make a non-human sequence more similar to a human sequence.

[0047] An antibody can be cleaved into fragments by enzymes, such as, *e.g.*, papain and pepsin. Papain cleaves an antibody to produce two Fab fragments and a single Fc fragment. Pepsin cleaves an antibody to produce a $F(ab')_2$ fragment and a pFc' fragment. In exemplary aspects of the present disclosure, the antigen-binding protein is an antigen binding fragment of an antibody. As used herein, the term "antigen binding antibody fragment" refers to a portion of an antibody that is capable of binding to the antigen of the antibody and is also known as "antigen-binding fragment" or "antigen-binding portion". In exemplary instances, the antigen binding antibody fragment is a Fab fragment or a $F(ab')_2$ fragment.

[0048] In various aspects, the antigen-binding protein is an antibody protein product. As used herein, the term "antibody protein product" refers to any one of several antibody alternatives which in various instances is based on the architecture of an antibody but is not found in nature. In some aspects, the antibody protein product has a molecular-weight within the range of at least about 12–150 kDa. In certain aspects, the antibody protein product has a valency (n) range from monomeric (n = 1), to dimeric (n = 2), to trimeric (n = 3), to tetrameric (n = 4), if not higher order valency. Antibody protein products in some aspects are those based on the full antibody structure and/or those that mimic antibody fragments which retain full antigen-binding capacity, *e.g.*, scFvs, Fabs and VHH/VH (discussed below).

[0049] The smallest antigen binding antibody fragment that retains its complete antigen binding site is the Fv fragment, which consists entirely of variable (V) regions. A soluble, flexible amino acid peptide linker is used to connect the V regions to a scFv (single chain fragment variable) fragment for stabilization of the molecule, or the constant (C) domains are added to the V regions to generate a Fab fragment [fragment, antigen-binding]. Both scFv and Fab fragments can be easily produced in host cells, *e.g.*, prokaryotic host cells. Other antibody protein products include disulfide-bond stabilized scFv (ds-scFv), single chain Fab (scFab), as well as di- and multimeric antibody formats like dia-, tria- and tetra-bodies, or minibodies (miniAbs) that comprise different formats consisting of scFvs linked to oligomerization domains. The smallest fragments are VHH/VH of camelid heavy chain Abs as well as single domain Abs (sdAb). The building block that is most frequently used to create novel antibody formats is the single-chain variable (V)-domain antibody fragment (scFv), which comprises V domains from the heavy and light chain (VH and VL domain) linked by a peptide linker of ~15 amino acid residues. A peptibody or peptide-Fc fusion is yet another antibody protein product. The structure of a peptibody consists of a biologically active peptide grafted onto an Fc domain. Peptibodies are well-described in the art. See, *e.g.*, Shimamoto *et al.*, *mAbs* 4(5): 586-591 (2012).

[0050] Other antibody protein products include a single chain antibody (SCA); a diabody; a triabody; a tetrabody; bispecific or trispecific antibodies, and the like. Bispecific antibodies can be divided into five major classes: BsIgG, appended IgG, BsAb fragments, bispecific fusion proteins and BsAb conjugates. See, *e.g.*, Spiess *et al.*, *Molecular Immunology* 67(2) Part A: 97-106 (2015).

[0051] In exemplary aspects, the antigen-binding protein is a bispecific T cell engager (BiTE®) molecule. BiTE® molecules are fusion proteins comprising two scFvs of different antibodies. One binds to CD3 and the other binds to a target antigen. BiTE® molecules are known in the art. See, *e.g.*, Huehls *et al.*, *Immuno Cell Biol* 93(3): 290-296 (2015); Rossi *et al.*, *MAbs* 6(2): 381-91 (2014); Ross *et al.*, *PLoS One* 12(8): e0183390.

[0052] In various aspects, the antigen-binding protein (e.g., an antibody or antigen binding fragment thereof) binds to a SIRPγ. The antigen-binding protein in some aspects binds to SIRPγ in a non-covalent and reversible manner. In exemplary embodiments, the binding strength of the antigen-binding proteins may be described in terms of its affinity, a measure of the strength of interaction between the binding site of SIRPγ and the SIRPγ binding partner. In exemplary aspects, the antigen-binding proteins have high-affinity for SIRPγ and thus will bind a greater amount of SIRPγ in a shorter period of time than low-affinity antigen-binding proteins. In exemplary aspects, the antigen-binding proteins have low-affinity for SIRPγ and thus will bind a lesser amount of SIRPγ in a longer period of time than high-affinity antigen-binding proteins. In exemplary aspects, the antigen-binding proteins have an equilibrium association

constant, KA, which is at least 10^5 M^{-1}, at least 10^6 M^{-1}, at least 10^7 M^{-1}, at least 10^8 M^{-1}, at least 10^9 M^{-1}, or at least 10^{10} M^{-1}. As understood by the artisan of ordinary skill, KA can be influenced by factors including pH, temperature and buffer composition.

[0053] In exemplary embodiments, the binding strength of the antigen-binding protein (e.g., an antibody or antigen binding fragment thereof) to SIRPγ may be described in terms of its sensitivity. K_D is the equilibrium dissociation constant, a ratio of k_{off}/k_{on}, between the antigen-binding protein and SIRPγ. K_D and KA are inversely related. The K_D value relates to the concentration of the antigen-binding protein (the amount of antigen-binding protein needed for a particular experiment); the lower the K_D value (lower concentration needed) the higher the affinity of the antigen-binding protein. In exemplary aspects, the binding strength of the antigen-binding protein to SIRPγ may be described in terms of K_D. In exemplary aspects, the K_D of the antigen-binding proteins is about 10^{-1} M, about 10^{-2} M, about 10^{-3} M, about 10^{-4} M, about 10^{-5} M, about 10^{-6} M, or less. In exemplary aspects, the K_D of the antigen-binding protein is micromolar, nanomolar, picomolar or femtomolar. In exemplary aspects, the K_D of the antigen-binding proteins is within a range of about 10^{-4} to 10^{-6} M, or 10^{-7} to 10^{-9} M, or 10^{-10} to 10^{-12} M, or 10^{-13} to 10^{-15} M. In exemplary aspects, the antigen-binding protein binds to the human SIRPγ with a K_D that is greater than or is about 0.04 nM. In exemplary aspects, the antigen-binding protein binds to the human SIRPγ with a K_D of about 0.01 nM to about 20 nM, 0.02 nM to 20 nM, 0.05 nM to 20 nM, 0.05 nM to 15 nM, 0.1 nM to 15 nM, 0.1 nM to 10 nM, 1 nM to 10 nM, or 5 nM to 10 nM. In various aspects, the KD is less than the KD that SIRPγ has for CD47, optionally, less than about 23 μM.

[0054] Optionally, the antigen-binding protein comprises a fully human antibody or antigen binding fragment thereof, a humanized antibody or antigen binding fragment thereof, a chimeric antibody or antigen binding fragment thereof. The antigen-binding protein may also comprise a Fab, Fab', F(ab')2, or a single chain Fv. In various aspects, the SIRPγ inhibitor comprises one, two, three, four, five or more of the heavy and light chain complementarity determining region (CDR) of an anti-SIRPγ antibody.

[0055] In certain aspects, the antigen-binding protein binds to an epitope on SIRPγ, optionally, wherein the epitope is located within or near or different from the CD47 binding site of SIRPγ. In various aspects, the antigen-binding protein binds to an epitope comprising the amino acid sequence of SLLPVGP (SEQ ID NO: 21; amino acids 29-35 of the SIRPγ amino acid sequence, LTKRNNMDF (SEQ ID NO: 22), and KFRKGS (SEQ ID NO: 23).

[0056] In exemplary aspects, the antigen-binding protein comprises a fully human antibody or antigen binding fragment thereof, a humanized antibody or antigen binding fragment thereof, a chimeric antibody or antigen binding fragment thereof, or a Fab, Fab', F(ab')2, or a single chain Fv, that competes with a reference antibody, which reference antibody is known to bind to SIRPγ (e.g., OX117), for binding to

SIRPγ. In exemplary aspects, the antigen-binding protein binds to an epitope to which the reference antibody (e.g., OX117) binds. In exemplary aspects, the antigen-binding protein exhibits a K_D for SIRPγ that is similar to, or the same as, the K_D of the reference antibody (e.g., OX117). In exemplary aspects, the antigen-binding protein exhibits a K_D for SIRPγ that is lower than the K_D of the reference antibody (e.g., OX117) and thus exhibits higher affinity for SIRPγ relative to the reference antibody. Suitable techniques for determining binding affinity for a ligand or target of an antigen binding protein are known in the art and include, e.g., surface plasmon resonance (SPR)-based methods, flow cytometry- or fluorescence microscopy-based methods, KinExA® method (see, e.g., International Patent Application Publication No. WO2019140196, Azimzadeh and Regenmortel, J Mol Recognit 3(3): 108-116 (1990); Schuck et al., Curr Protoc Cell Biol Chapter 17: Unit 17.6 (2004); Tseng et al., Electrophoresis 23(6): 836-846 (2002); Van Regenmortel et al., Immunol Invest 26(1-2): 67-82 (1997)).

[0057] In exemplary instances, the antigen-binding protein that competes with a reference antibody (e.g., OX117) for binding to SIRPγ reduces the amount of the anti-SIRPγ antibody (e.g., OX117) bound to SIRPγ in an *in vitro* competitive binding assay. In exemplary aspects, the amount of the reference antibody (e.g., OX117) bound to SIRPγ in the presence of the antigen binding protein of the present disclosure is reduced by at least or about 25%, at least or about 30%, at least or about 35%, at least or about 40%, at least or about 45%, at least or about 50%, at least or about 55%, at least or about 60%, at least or about 65%, at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90% or more (e.g., at least or about 95%, at least or about 98%). In various aspects, the antigen-binding proteins of the present disclosure inhibit the binding interaction between SIRPγ and the reference antibody and the inhibition are characterized by an IC_{50}. In various aspects, the antigen-binding proteins exhibit an IC_{50} of less than about 250 nM for inhibiting the binding interaction between SIRPγ and the reference antibody. In various aspects, the antigen-binding proteins exhibit an IC_{50} of less than about 200 nM, less than about 150 nM, less than about 100 nM, less than about 50 nm, less than about 25 nm, less than about 10 nm, less than about 5 nM, less than about 1 nM, less than 0.5 nM or less than 0.1 nM.

[0058] A suitable competitive binding assay that can be used to determine the reduced amount of the reference antibody (e.g., OX117) bound to SIRPγ comprises the steps of incubating the reference antibody (e.g., OX117) with SIRPγ or cells expressing SIRPγ in the presence of a presently disclosed antigen-binding protein (e.g., an antibody or antigen binding fragment thereof) that competes with the reference antibody (e.g., OX117) for binding to SIRPγ. The amount of the reference antibody (e.g., OX117) bound to SIRPγ is measured with and without the antigen-binding protein (e.g., an antibody or antigen binding fragment thereof) of the present disclosure that competes for binding to SIRPγ.

[0059] In various instances, the antigen-binding proteins of the present disclosure compete with the reference antibody for binding to SIRPγ and thereby reduce the amount of SIRPγ bound to the reference antibody as determined by a FACS-based assay in which the fluorescence of a fluorophore-conjugated secondary antibody which binds to the Fc of the reference antibody is measured in the absence or presence of a particular amount of the antigen-binding protein of the present disclosure. In various aspects, the FACS-based assay is carried out with the reference antibody, fluorophore-conjugated secondary antibody and cells which express SIRPγ. In various aspects, the cells are genetically-engineered to overexpress SIRPγ. In some aspects, the cells are HEK293T cells transduced with a viral vector to express SIRPγ. In alternative aspects, the cells endogenously express SIRPγ. Before the FACS-based assay is carried out, in some aspects, the cells which endogenously express SIRPγ are pre-determined as low SIRPγ -expressing cells or high SIRPγ -expressing cells.

[0060] Other binding assays, e.g., competitive binding assays or competition assays, which test the ability of an antibody to compete with another antigen-binding protein for binding to an antigen, or to an epitope thereof, are known in the art. For example, suitable receptor-ligand competition assays are described in International Patent Application Publication No. WO2019140196, incorporated herein by reference. See, e.g., Trikha et al., Int J Cancer 110: 326-335 (2004); Tam et al., Circulation 98(11): 1085-1091 (1998); U.S. Patent Application Publication No. US20140178905, Chand et al., Biologicals 46: 168-171 (2017); Liu et al., Anal Biochem 525: 89-91 (2017); Goolia et al., J Vet Diagn Invest 29(2): 250-253 (2017); Hunter and Cochran, Methods Enzymol 250: 21-44 (2016); Cox et al., Immunoassay Methods, Immunoassay Methods. 2012 May 1 [Updated 2019 Jul 8]. In: Sittampalam GS, Grossman A, Brimacombe K, et al., editors. Assay Guidance Manual [Internet]. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK92434/; Clarke, William, "Immunoassays for Therapeutic Drug Monitoring and Clinical Toxicology", *Handbook of Analytical Separations*, Volume 5, pages 95-112 (2004), and Goolia et al., J Vet Diagn Invest 29(2): 250-253 (2017). In exemplary aspects, the SIRPγ binder competes with OX117 for binding to SIRPγ as determined by any of the assays described in these references.

[0061] *Treatment of Cancer*

[0062] The present disclosure provides methods of treating a subject with a tumor or cancer. In exemplary embodiments, the method of treating cancer comprises administering to the subject a SIRPγ binder (e.g., an antibody or antigen binding fragment thereof) in an amount effective to treat the tumor or cancer in the subject. In some embodiments, the SIRPγ binder is a SIRPγ inhibitor. In some

embodiments, the method of treating cancer comprises administering to the subject an antigen-binding protein (e.g., an antibody or antigen binding fragment thereof) that binds to an epitope on SIRPγ in an amount effective to treat the tumor or cancer in the subject.

[0063] Any of the antigen-binding proteins that bind to an epitope on SIRPγ (e.g., SIRPγ binders and SIRPγ inhibitors) discussed herein may be used in such methods. In certain aspects, the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) binds to an epitope on SIRPγ. In some embodiments, the epitope on SIRPγ is located within or near or different from the CD47 binding site of SIRPγ. In various aspects, the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) binds to an epitope comprising the amino acid sequence of SLLPVGP (SEQ ID NO: 21; amino acids 29-35 of the SIRPγ amino acid sequence, LTKRNNMDF (SEQ ID NO: 22), and KFRKGS (SEQ ID NO: 23).

[0064] In various instances, the SIRPγ binder binds to D1 and/or binds to the CD47 binding site of SIRPγ. In various aspects, the SIRPγ binder binds to D1 and Ig Domain 2 (D2) of SIRPγ. In exemplary aspects, the SIRPγ binder binds to both D1 and D2, optionally at the interface between D1 and D2. Optionally, the SIRPγ binder binds to the binding site of a SIRPγ binding partner other than CD47. Figure 9E provides an illustration of SIRPγ, its Ig domains thereof and the CD47 binding site. In exemplary instances, the SIRPγ binder binds to the epitope to which SIRPγ monoclonal antibody OX117 binds, optionally, wherein the SIRPγ binder competes with a reference antibody known to bind to SIRPγ (e.g., OX117) for binding to SIRPγ. In some embodiments, the SIRPγ binder competes with OX117 for binding to SIRPγ. The SIRPγ binder in various instances binds to SIRPγ with the same or higher affinity as OX117. In some embodiments, the SIRPγ binder is OX117 or an antigen binding fragment thereof. Figure 9E provides an illustration of the binding interaction between SIRPγ and the fab of the OX117 antibody. The SIRPγ binder in various aspects forms hydrogen bonds with one or more of amino acid residues Q8, E10, G109, K11, L12, and D149 of SIRPγ. The SIRPγ binder in various aspects forms hydrogen bonds with each of amino acid residues Q8, E10, G109, K11, L12, and D149 of SIRPγ. Optionally, the SIRPγ binder binds to an epitope which does not overlap with the CD47 binding site.

[0065] In exemplary instances, upon binding to SIRPγ, the SIRPγ binder enhances T cell activation, T cell proliferation and cytokine secretion. In some instances, the methods of the disclosure increase the T cell activation, T cell proliferation and cytokine secretion to any degree or level relative a control. For example, in some aspects, the increase provided by the methods of the disclosure is at least or about a 1 % to about a 10% increase (e.g., at least or about a 1 % increase, at least or about a 2% increase, at least or about a 3% increase, at least or about a 4% increase, at least or about a 5% increase, at least or about a 6% increase, at least or about a 7% increase, at least or about a 8% increase, at least or about a 9% increase, at

least or about a 9.5% increase, at least or about a 9.8% increase, at least or about a 10% increase) relative a control. In exemplary embodiments, the increase provided by the methods of the disclosure is over 100%, e.g., 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900% or even 1000% relative a control. In exemplary embodiments, the T cell activation, T cell proliferation and cytokine secretion increases by at least or about 1.5-fold, at least or about 2.0 fold, at least or about 3.0 fold, at least or about 4.0 fold, at least or about 5.0 fold, at least or about 10.0 fold, at least or about 25 hold, at least or about 50 fold, at least or about 75 fold, at least or about 100 fold or more, relative to a control. The control in various aspects is the T cell activation, T cell proliferation and cytokine secretion without the SIRPγ binder binding to SIRPγ.

[0066] In exemplary instances, the SIRPγ binder causes a conformational change of SIRPγ, upon binding to SIRPγ. The conformational change of SIRPγ in various instances may alter the accessibility of binding sites of binding partners. Optionally, the conformational change in various aspects allows different binding partners to bind to SIRPγ. Additionally or alternatively, the conformational change may cause dimerization or multimerization of SIRPγ molecules. In exemplary aspects, the dimerization or multimerization of SIRPγ prevents one or more binding partners from binding to SIRPγ. In exemplary aspects, the dimerization or multimerization of SIRPγ enhances the binding of one or more binding partners binding to SIRPγ. In various instances, the SIRPγ binder simultaneously binds to two SIRPγ molecules or promotes SIRPγ dimerization.

[0067] As used herein, the term "treat," as well as words related thereto, do not necessarily imply 100% or complete treatment. Rather, there are varying degrees of treatment of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the methods of treating cancer of the present disclosure can provide any amount or any level of treatment. Furthermore, the treatment provided by the methods of the present disclosure can include treatment of one or more conditions or symptoms or signs of the cancer being treated. Also, the treatment provided by the methods of the present disclosure can encompass slowing the progression of the cancer. For example, the methods can treat cancer by virtue of increasing T cell activity (e.g., T cell effector activity) or increasing an immune response against the tumor or cancer, reducing tumor or cancer growth or tumor burden, reducing metastasis of tumor cells, increasing cell death of tumor or cancer cells or increasing tumor regression, reducing T cell suppressive activity, and the like. In accordance with the foregoing, provided herein are methods of increasing effector activity or reducing suppressive activity of T-cells in a subject with a tumor or cancer. In exemplary embodiments, the method comprises administering to the subject a SIRPγ inhibitor in an amount effective to increase the effector activity or reduce the suppressive activity in the subject. Also, in accordance with the foregoing, provided herein are methods of increasing an

immune response against a tumor or cancer in a subject. In exemplary embodiments, the method comprises administering to the subject a SIRPγ inhibitor in an amount effective to increase an immune response against a tumor or cancer.

[0068] In various aspects, the methods treat by way of delaying the onset or recurrence of the cancer by at least 1 day, 2 days, 4 days, 6 days, 8 days, 10 days, 15 days, 30 days, two months, 3 months, 4 months, 6 months, 1 year, 2 years, 3 years, 4 years, or more. In various aspects, the methods treat by way increasing the survival of the subject. In exemplary aspects, the methods of the present disclosure provide treatment by way of delaying the occurrence or onset of metastasis. In various instances, the methods provide treatment by way of delaying the occurrence or onset of a new metastasis.

[0069] *SIRPγ Binder Pharmaceutical Compositions, Routes and Timing of Administration*

[0070] The following embodiments disclose pharmaceutical compositions, routes and timing of administration of the antigen-binding proteins (e.g., an antibody or antigen binding fragment thereof) of the present invention that bind to SIRPγ. In some embodiments the antigen-binding protein is a SIRPγ binder or a SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof).

[0071] In some embodiments, the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ is administered to a subject as part of a pharmaceutical composition. In other embodiments, the pharmaceutical composition comprises a SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) or a pharmaceutically-acceptable salt thereof that binds to SIRPγ. In various aspects, the pharmaceutically-acceptable salt of the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ is prepared *in situ* during the final isolation and purification of the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ, or separately prepared by reacting a free base function with a suitable acid. Examples of acids which can be employed to form pharmaceutically acceptable acid addition salts include, for example, an inorganic acid, e.g., hydrochloric acid, hydrobromic acid, sulphuric acid, and phosphoric acid, and an organic acid, e.g., oxalic acid, maleic acid, succinic acid, and citric acid. The acid addition salts in various aspects is acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphor sulfonate, digluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethansulfonate (isothionate), lactate, maleate, methane sulfonate, nicotinate, 2-naphthalene sulfonate, oxalate, palmitoate, pectinate, persulfate, 3-phenylpropionate, picrate,

pivalate, propionate, succinate, tartrate, thiocyanate, phosphate, glutamate, bicarbonate, p-toluenesulfonate, and undecanoate.

[0072] In various aspects, the pharmaceutically-acceptable salt of the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ is a basic addition salt. Basic addition salts also can be prepared *in situ* during the final isolation and purification of the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ, or by reacting a carboxylic acid-containing moiety with a suitable base such as the hydroxide, carbonate, or bicarbonate of a pharmaceutically acceptable metal cation or with ammonia or an organic primary, secondary, or tertiary amine. In various instances, the pharmaceutically acceptable salt of the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ is a cation based on alkali metals or alkaline earth metals such as lithium, sodium, potassium, calcium, magnesium, and aluminum salts, and the like, and nontoxic quaternary ammonia and amine cations including ammonium, tetramethylammonium, tetraethylammonium, methylammonium, dimethylammonium, trimethylammonium, triethylammonium, diethylammonium, and ethylammonium, amongst others. Other representative organic amines useful for the formation of base addition salts include, for example, ethylenediamine, ethanolamine, diethanolamine, piperidine, piperazine, and the like. Further, basic nitrogen-containing groups can be quaternized with such SIRPγ inhibitors as lower alkyl halides such as methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides; long chain halides such as decyl, lauryl, myristyl, and stearyl chlorides, bromides, and iodides; arylalkyl halides like benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

[0073] In various aspects, the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ of the presently disclosed methods is formulated with a pharmaceutically acceptable carrier, diluent, or excipient prior to administration to the subject. Depending on the route of administration and other factors, the particular SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ, may be admixed with one or more additional pharmaceutically acceptable ingredients, including, for example, acidifying agents, additives, adsorbents, aerosol propellants, air displacement agents, alkalizing agents, anticaking agents, anticoagulants, antimicrobial preservatives, antioxidants, antiseptics, bases, binders, buffering agents, chelating agents, coating agents, coloring agents, desiccants, detergents, diluents, disinfectants, disintegrants, dispersing agents, dissolution enhancing agents, dyes, emollients, emulsifying agents, emulsion stabilizers, fillers, film forming agents, flavor enhancers, flavoring agents, flow enhancers, gelling agents, granulating agents, humectants, lubricants, mucoadhesives, ointment bases, ointments, oleaginous vehicles, organic bases, pastille bases, pigments, plasticizers, polishing agents, preservatives,

sequestering agents, skin penetrants, solubilizing agents, solvents, stabilizing agents, suppository bases, surface active agents, surfactants, suspending agents, sweetening agents, therapeutic agents, thickening agents, tonicity agents, toxicity agents, viscosity-increasing agents, water-absorbing agents, water-miscible cosolvents, water softeners, or wetting agents.

[0074] The SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ of the presently disclosed methods can be administered to the subject via any suitable route of administration. For example, the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ can be administered to a subject via parenteral, nasal, oral, pulmonary, topical, vaginal, or rectal administration. The following discussion on routes of administration is merely provided to illustrate exemplary embodiments and should not be construed as limiting the scope in any way.

[0075] In exemplary aspects, the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ of the presently disclosed methods is formulated for parenteral administration. The term, "parenteral" means not through the alimentary canal but by some other route such as subcutaneous, intramuscular, intraspinal, or intravenous. Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ can be administered with a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol or hexadecyl alcohol, a glycol, such as propylene glycol or polyethylene glycol, dimethylsulfoxide, glycerol, ketals such as 2,2- dimethyl-l53-dioxolane-4-methanol, ethers, poly(ethyleneglycol) 400, oils, fatty acids, fatty acid esters or glycerides, or acetylated fatty acid glycerides with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants. Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters. In exemplary aspects, the formulation for parenteral administration includes a soap. Suitable soaps for use in parenteral formulations include fatty alkali

metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-β-aminopropionates, and 2-alkyl -imidazoline quaternary ammonium salts, and (e) mixtures thereof. In exemplary instances, preservatives and buffers are present in the parenteral formulation. In order to minimize or eliminate irritation at the site of injection, such compositions can contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations typically ranges from about 5% to about 15% by weight. Suitable surfactants include polyethylene glycol sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations in some aspects are presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, syringes, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions in some aspects are prepared from sterile powders, granules, and tablets of the kind previously described.

[0076] In exemplary aspects, the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ is formulated for injection. Injectable formulations are in accordance with the present disclosure. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J. B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., pages 622-630 (1986)).

[0077] Optionally, the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ is administered to the subject via subcutaneous injection.

[0078] In various instances the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ is administered orally to the subject. Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the analog of the present disclosure dissolved in diluents, such as water, saline, or orange juice; (b) capsules, sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations may include diluents, such as water and alcohols, for example, ethanol, benzyl alcohol, and

the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant. Capsule forms can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and other pharmacologically compatible excipients. Lozenge forms can comprise the analog of the present disclosure in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the analog of the present disclosure in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to, such excipients as are known in the art.

[0079] The SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ may be administered according to any regimen including, for example, daily (1 time per day, 2 times per day, 3 times per day, 4 times per day, 5 times per day, 6 times per day), three times a week, twice a week, every two days, every three days, every four days, every five days, every six days, weekly, bi-weekly, every three weeks, monthly, or bi-monthly.

[0080] *Dosages*

[0081] The following embodiments disclose dosages of the antigen-binding proteins (e.g., an antibody or antigen binding fragment thereof) of the present invention that bind to SIRPγ. In some embodiments the antigen-binding protein is a SIRPγ binder or a SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof).

[0082] The SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ is believed to be useful in methods of increasing effector activity or reducing suppressive activity of T-cells or increasing an immune response against a tumor or cancer in a subject, as described herein, and are thus believed to be useful in methods of treating or preventing one or more diseases, e.g., cancer. The amount or dose of the SIRPγ binder or SIRPγ inhibitor, or antigen-binding protein (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ administered should be sufficient to effect, e.g., a therapeutic or prophylactic response, in the subject or animal over a reasonable time frame. For example, the dose of the SIRPγ binder or SIRPγ inhibitor, or antigen-binding protein (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ (e.g., a SIRPγ inhibitor) should be sufficient to treat cancer in a period of from about 1 to 4 about days or about 1 to about 4 weeks or longer, e.g., about 5 to about 20 or more weeks, from the time of administration. In certain embodiments, the

time period could be even longer. The dose will be determined by the efficacy of the particular active agent and the condition of the animal (e.g., human), as well as the body weight of the animal (e.g., human) to be treated.

[0083] Many assays for determining an administered dose are known in the art. For purposes herein, an assay, which comprises comparing secretion of IFNγ by activated T cells, upon administration of a given dose of the SIRPγ binder or SIRPγ inhibitor, or antigen-binding protein (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ to a mammal among a set of mammals, each set of which is given a different dose, could be used to determine a starting dose to be administered to a mammal in a clinical trial. Methods of measuring secretion of IFNγ by activated T cells are known in the art and described herein.

[0084] The dose of the SIRPγ binder or SIRPγ inhibitor, or antigen-binding protein (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular active agent. Typically, the attending physician will decide the dosage of the SIRPγ binder, SIRPγ inhibitor, or antigen-binding protein (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ with which to treat each individual patient, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, SIRPγ binder, SIRPγ inhibitor, or antigen-binding protein (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ to be administered, route of administration, and the severity of the condition being treated. By way of example and not intending to limit the present disclosure, the dose of the SIRPγ binder, SIRPγ inhibitor, or antigen-binding protein (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ of the presently disclosed methods can be about 0.0001 to about 1 g/kg body weight of the subject being treated/day, from about 0.0001 to about 0.001 g/kg body weight/day, or about 0.01 mg to about 1 g/kg body weight/day.

[0085] *Controlled Release Formulations*

[0086] The following embodiments disclose controlled release formulations of the antigen-binding proteins (e.g., an antibody or antigen binding fragment thereof) of the present invention that bind to SIRPγ. In some embodiments the antigen-binding protein is a SIRPγ binder or a SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof).

[0087] In some embodiments, the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ described herein can be modified into a depot form, such that the manner in which the active agent is released into the body to which it is administered is controlled with

respect to time and location within the body (see, for example, U.S. Patent No. 4,450,150). Depot forms of the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ can be, for example, an implantable composition comprising the SIRPγ binder or SIRPγ inhibitor(e.g., an antibody or antigen binding fragment thereof) and a porous or non-porous material, such as a polymer, wherein the SIRPγ binder or SIRPγ inhibitor(e.g., an antibody or antigen binding fragment thereof) is encapsulated by or diffused throughout the material and/or degradation of the non-porous material. The depot is then implanted into the desired location within the body of the subject and the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ is released from the implant at a predetermined rate.

[0088] In various aspects, the pharmaceutical composition comprising the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ may be modified to have any type of *in vivo* release profile. In some aspects, the pharmaceutical composition is an immediate release, controlled release, sustained release, extended release, delayed release, or bi-phasic release formulation. Methods of formulating peptides for controlled release are known in the art. See, for example, Qian et al., *J Pharm* 374: 46-52 (2009) and International Patent Application Publication Nos. WO 2008/130158, WO2004/033036; WO2000/032218; and WO 1999/040942.

[0089] In various instances, the pharmaceutical composition comprising the SIRPγ binder or SIRPγ inhibitor(e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ can further comprise, for example, micelles or liposomes, or some other encapsulated form, for prolonged storage and/or delivery effect.

[0090] *Combinations*

[0091] The following embodiments disclose combinations of the antigen-binding proteins (e.g., an antibody or antigen binding fragment thereof) of the present invention that bind to SIRPγ. In some embodiments the antigen-binding protein is a SIRPγ binder or a SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof).

[0092] In various instances, the SIRPγ binder or SIRPγ inhibitor that binds to SIRPγ is administered to the subject alone, e.g., without any additional pharmaceutical actives. In various aspects, the SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ is administered to the subject in combination with a chemotherapeutic agent. Chemotherapeutic agents suitable for use in the presently disclosed methods are known in the art, and include, but not limited to,

platinum coordination compounds, topoisomerase inhibitors, antibiotics, antimitotic alkaloids and difluoronucleosides, as described in U.S. Pat. No. 6,630,124.

[0093] In some embodiments, the chemotherapeutic agent is a platinum coordination compound. The term "platinum coordination compound" refers to any tumor cell growth inhibiting compound that provides a platinum in the form of an ion. In some embodiments, the platinum coordination compound is cis-diamminediaquoplatinum (II)-ion; chloro(diethylenetriamine)-platinum(II)chloride; dichloro(ethylenediamine)-platinum(II), diammine(1,1-cyclobutanedicarboxylato) platinum(II) (carboplatin); spiroplatin; iproplatin; diammine(2-ethylmalonato)-platinum(II); ethylenediaminemalonatoplatinum(II); aqua(1,2-diaminodyclohexane)-sulfatoplatinum(II); (1,2-diaminocyclohexane)malonatoplatinum(II); (4-caroxyphthalato)(1,2-diaminocyclohexane)platinum(II); (1,2-diaminocyclohexane)-(isocitrato)platinum(II); (1,2-diaminocyclohexane)cis(pyruvato)platinum(II); (1,2-diaminocyclohexane)oxalatoplatinum(II); ormaplatin; and tetraplatin.

[0094] In some embodiments, cisplatin is the platinum coordination compound employed in the compositions and methods of the present invention. Cisplatin is commercially available under the name PLATINOL™ from Bristol Myers-Squibb Corporation and is available as a powder for constitution with water, sterile saline or other suitable vehicle. Other platinum coordination compounds suitable for use in the present invention are known and are available commercially and/or can be prepared by known techniques. Cisplatin, or cis-dichlorodiammineplatinum II, has been used successfully for many years as a chemotherapeutic agent in the treatment of various human solid malignant tumors. More recently, other diamino-platinum complexes have also shown efficacy as chemotherapeutic agents in the treatment of various human solid malignant tumors. Such diamino-platinum complexes include, but are not limited to, spiroplatinum and carboplatinum. Although cisplatin and other diamino-platinum complexes have been widely used as chemotherapeutic agents in humans, they have had to be delivered at high dosage levels that can lead to toxicity problems such as kidney damage.

[0095] In some embodiments, the chemotherapeutic agent is a topoisomerase inhibitor. Topoisomerases are enzymes that are capable of altering DNA topology in eukaryotic cells. Topoisomerases are critical for cellular functions and cell proliferation. Generally, there are two classes of topoisomerases in eukaryotic cells, type I and type II. Topoisomerase I is a monomeric enzyme of approximately 100,000 molecular weight. The enzyme binds to DNA and introduces a transient single-strand break, unwinds the double helix (or allows it to unwind), and subsequently reseals the break before dissociating from the DNA strand. Various topoisomerase inhibitors have been shown clinical efficacy in the treatment of humans afflicted with ovarian cancer, breast cancer, esophageal cancer or non-small cell lung carcinoma.

[0096] In some aspects, the topoisomerase inhibitor is camptothecin or a camptothecin analog. Camptothecin is a water-insoluble, cytotoxic alkaloid produced by Camptotheca accuminata trees indigenous to China and Nothapodytes foetida trees indigenous to India. Camptothecin inhibits growth of a number of tumor cells. Compounds of the camptothecin analog class are typically specific inhibitors of DNA topoisomerase I. Compounds of the camptothecin analog class include, but are not limited to; topotecan, irinotecan and 9-amino-camptothecin.

[0097] In additional embodiments, the chemotherapeutic agent is any tumor cell growth inhibiting camptothecin analog claimed or described in: U.S. Pat. No. 5,004,758, issued on Apr. 2, 1991 and European Patent Application Number 88311366.4, published on Jun. 21, 1989 as 20' Publication Number EP 0 321 122; U.S. Pat. No. 4,604,463, issued on Aug. 5, 1986 and European Patent Application Publication Number EP 0 137 145, published on Apr. 17, 1985; U.S. Pat. No. 4,473,692, issued on Sep. 25, 1984 and European Patent Application Publication Number EP 0 074 256, published on Mar. 16, 1983; U.S. Pat. No. 4,545,880, issued on Oct. 8, 1985 and European Patent Application Publication Number EP 0 074 256, published on Mar. 16, 1983; European Patent Application Publication Number EP 0 088 642, published on Sep. 14, 1983; Wani et al., J. Med. Chem., 29, 2358-2363 (1986); Nitta et al., Proc. 14th International Congr. Chemotherapy, Kyoto, 1985, Tokyo Press, Anticancer Section 1, p. 28-30, especially a compound called CPT-11. CPT-11 is a camptothecin analog with a 4-(piperidino)-piperidine side chain joined through a carbamate linkage at C-10 of 10-hydroxy-7-ethyl camptothecin. CPT-11 is currently undergoing human clinical trials and is also referred to as irinotecan; Wani et al, J. Med. Chem., 23, 554 (1980); Wani et. al., J. Med. Chem., 30, 1774 (1987); U.S. Pat. No. 4,342,776, issued on Aug. 3, 1982; U.S. patent application Ser. No. 581,916, filed on Sep. 13, 1990 and European Patent Application Publication Number EP 418 099, published on Mar. 20, 1991; U.S. Pat. No. 4,513,138, issued on Apr. 23, 1985 and European Patent Application Publication Number EP 0 074 770, published on Mar. 23, 1983; U.S. Pat. No. 4,399,276, issued on Aug. 16, 1983 and European Patent Application Publication Number 0 056 692, published on Jul. 28, 1982; the entire disclosure of each of which is hereby incorporated by reference. All of the above-listed compounds of the camptothecin analog class are available commercially and/or can be prepared by known techniques including those described in the above-listed references. The topoisomerase inhibitor may be selected from the group consisting of topotecan, irinotecan and 9-aminocamptothecin.

[0098] The preparation of numerous compounds of the camptothecin analog class (including pharmaceutically acceptable salts, hydrates and solvates thereof) as well as the preparation of oral and parenteral pharmaceutical compositions comprising such a compounds of the camptothecin analog class and an inert, pharmaceutically acceptable carrier or diluent, is extensively described in U.S. Pat. No.

5,004,758, issued on Apr. 2, 1991 and European Patent Application Number 88311366.4, published on Jun. 21, 1989 as Publication Number EP 0 321 122, the teachings of which are incorporated herein by reference.

[0099] In still yet other embodiments, the chemotherapeutic agent is an antibiotic compound. Suitable antibiotics include, but are not limited to, doxorubicin, mitomycin, bleomycin, daunorubicin and streptozocin.

[00100] In some embodiments, the chemotherapeutic agent is an antimitotic alkaloid. In general, antimitotic alkaloids can be extracted from Cantharanthus roseus, and have been shown to be efficacious as anticancer chemotherapy agents. A great number of semi-synthetic derivatives have been studied both chemically and pharmacologically (see, O. Van Tellingen et al, Anticancer Research, 12, 1699-1716 (1992)). The antimitotic alkaloids of the present invention include, but are not limited to, vinblastine, vincristine, vindesine, paclitaxel (PTX; Taxol®) and vinorelbine. The latter two antimitotic alkaloids are commercially available from Eli Lilly and Company, and Pierre Fabre Laboratories, respectively (see, U.S. Pat. No. 5,620,985). In an exemplary aspect of the present invention, the antimitotic alkaloid is vinorelbine.

[00101] In other embodiments of the invention, the chemotherapeutic agent is a difluoronucleoside. 2'-deoxy-2',2'-difluoronucleosides are known in the art as having antiviral activity. Such compounds are disclosed and taught in U.S. Pat. Nos. 4,526,988 and 4,808614. European Patent Application Publication 184,365 discloses that these same difluoronucleosides have oncolytic activity. In certain specific aspects, the 2'-deoxy-2',2'-difluoronucleoside used in the compositions and methods of the present invention is 2'-deoxy-2',2'-difluorocytidine hydrochloride, also known as gemcitabine hydrochloride. Gemcitabine is commercially available or can be synthesized in a multi-step process as disclosed and taught in U.S. Pat. Nos. 4,526,988, 4,808,614 and 5,223,608, the teachings of which are incorporated herein by reference.

[00102] In exemplary aspects, the chemotherapeutic agent is a hormone therapy agent. In exemplary instances, the hormone therapy agent is, for instance, letrozole, tamoxifen, bazedoxifene, exemestane, leuprolide, goserelin, fulvestrant, anastrozole, or toremifene. In exemplary aspects, the hormone therapy agent is a luteinizing hormone (LH) blocker, e.g., gosarelin, or an LH releasing hormone (RH) agonist. In exemplary aspects, the hormone therapy agent is an ER-targeted agent (e.g., fulvestrant or tamoxifen), rapamycin, a rapamycin analog (e.g., everolimus, temsirolimus, ridaforolimus, zotarolimus, and 32-deoxo-rapamycin), an anti-HER2 drug (e.g., trastuzumab, pertuzumab, lapatinib, T-DM1, or neratinib) or a PI3K inhibitor (e.g., taselisib, alpelisib or buparlisib).

[00103] In exemplary aspects, the chemotherapeutic agent is a CDK4/6 inhibitor such as palbociclib, ribociclib, or abemaciclib (see, e.g., Knudsen and Witkiewicz, Trends Cancer 3(1): 39-55 (2017)).

[00104] *Subjects*

[00105] In exemplary embodiments of the present disclosure, the subject is a mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits, mammals from the order Carnivora, including Felines (cats) and Canines (dogs), mammals from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). In some aspects, the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). In some aspects, the mammal is a human.

[00106] In exemplary aspects, the subject has cancer or a tumor. The cancer in some aspects is one selected from the group consisting of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer, esophageal cancer, cervical cancer, gastrointestinal carcinoid tumor, Hodgkin lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, liver cancer, lung cancer, malignant mesothelioma, melanoma, multiple myeloma, nasopharynx cancer, non-Hodgkin lymphoma, ovarian cancer, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer (e.g., renal cell carcinoma (RCC)), small intestine cancer, soft tissue cancer, stomach cancer, testicular cancer, thyroid cancer, ureter cancer, and urinary bladder cancer. In particular aspects, the cancer is selected from the group consisting of: head and neck, ovarian, cervical, bladder and oesophageal cancers, pancreatic, gastrointestinal cancer, gastric, breast, endometrial and colorectal cancers, hepatocellular carcinoma, glioblastoma, bladder, lung cancer, e.g., non-small cell lung cancer (NSCLC), bronchioloalveolar carcinoma. In particular embodiments, the tumor is non-small cell lung cancer (NSCLC), head and neck cancer, renal cancer, triple negative breast cancer, or gastric cancer. In exemplary aspects, the subject has a tumor (e.g., a solid tumor, a hematological malignancy, or a lymphoid malignancy) and the pharmaceutical composition is administered to the subject in an amount effective to treat the tumor in the subject. In other exemplary aspects, the tumor is non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), head and neck cancer, renal cancer, breast cancer, melanoma, ovarian cancer, liver cancer, pancreatic cancer, colon cancer, prostate cancer, gastric cancer, lymphoma or leukemia, and the

pharmaceutical composition is administered to the subject in an amount effective to treat the tumor in the subject.

[00107] Optionally, the subject has hepatocellular carcinoma (HCC), colorectal cancer (CRC), lung cancer, optionally, non-small-cell lung cancer (NSCLC).

[00108] *Relief of Immune Suppression and Enhancement of Immune Response*

[00109] Without being bound to any particular theory, the antigen-binding protein (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ described herein is useful for increasing effector activity or reducing suppressive activity of T-cells. In some embodiments, the antigen-binding protein is a SIRPγ binder or SIRPγ inhibitor. Also, it is postulated that the SIRPγ binders or SIRPγ inhibitors (e.g., an antibody or antigen binding fragment thereof) that bind to SIRPγ described herein are useful for increasing an immune response against a tumor or cancer. Accordingly, the present disclosure provides methods of increasing effector activity or reducing suppressive activity of T-cells in a subject with a tumor or cancer. In exemplary embodiments, the method comprises administering to the subject an antigen-binding protein (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ in an amount effective to increase the effector activity or reduce the suppressive activity in the subject. In some embodiments, the antigen-binding protein is a SIRPγ binder or SIRPγ inhibitor. Also, the present disclosure accordingly provides methods of increasing an immune response against a tumor or cancer in a subject. In exemplary embodiments, the method comprises administering to the subject a SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ in an amount effective to increase an immune response against a tumor or cancer.

[00110] The increase in effector activity of T-cells provided by the methods of the present disclosure may be at least or about a 1% to about a 10% increase (e.g., at least or about a 1% increase, at least or about a 2% increase, at least or about a 3% increase, at least or about a 4% increase, at least or about a 5% increase, at least or about a 6% increase, at least or about a 7% increase, at least or about a 8% increase, at least or about a 9% increase, at least or about a 9.5% increase, at least or about a 9.8% increase, at least or about a 10% increase) relative to a control. The increase in effector activity of T-cells provided by the methods of the present disclosure may be at least or about a 10% to greater than about a 95% increase (e.g., at least or about a 10% increase, at least or about a 20% increase, at least or about a 30% increase, at least or about a 40% increase, at least or about a 50% increase, at least or about a 60% increase, at least or about a 70% increase, at least or about a 80% increase, at least or about a 90% increase, at least or about a 95% increase, at least or about a 98% increase, at least or about a 99% increase, or about a 100%

increase) relative to a control. In exemplary aspects, the control is cancer or tumor or a subject or a population of subjects that was not treated with the presently disclosed SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ or wherein the subject or population of subjects was treated with a placebo.

[00111] The increase in an immune response against a tumor or cancer provided by the methods of the present disclosure may be at least or about a 1% to about a 10% increase (e.g., at least or about a 1% increase, at least or about a 2% increase, at least or about a 3% increase, at least or about a 4% increase, at least or about a 5% increase, at least or about a 6% increase, at least or about a 7% increase, at least or about a 8% increase, at least or about a 9% increase, at least or about a 9.5% increase, at least or about a 9.8% increase, at least or about a 10% increase) relative to a control. The increase in an immune response against a tumor or cancer provided by the methods of the present disclosure may be at least or about a 10% to greater than about a 95% increase (e.g., at least or about a 10% increase, at least or about a 20% increase, at least or about a 30% increase, at least or about a 40% increase, at least or about a 50% increase, at least or about a 60% increase, at least or about a 70% increase, at least or about a 80% increase, at least or about a 90% increase, at least or about a 95% increase, at least or about a 98% increase, at least or about a 99% increase, or about a 100% increase) relative to a control. In exemplary aspects, the control is cancer or tumor or a subject or a population of subjects that was not treated with the presently disclosed SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ or wherein the subject or population of subjects was treated with a placebo.

[00112] The reduction in suppressive activity of T-cells provided by the methods of the present disclosure may be at least or about a 1% to about a 10% reduction (e.g., at least or about a 1% reduction, at least or about a 2% reduction, at least or about a 3% reduction, at least or about a 4% reduction, at least or about a 5% reduction, at least or about a 6% reduction, at least or about a 7% reduction, at least or about a 8% reduction, at least or about a 9% reduction, at least or about a 9.5% reduction, at least or about a 9.8% reduction, at least or about a 10% reduction) relative to a control. The reduction in suppressive activity of T-cells provided by the methods of the present disclosure may be at least or about a 10% to greater than a 95% reduction (e.g., at least or about a 10% reduction, at least or about a 20% reduction, at least or about a 30% reduction, at least or about a 40% reduction, at least or about a 50% reduction, at least or about a 60% reduction, at least or about a 70% reduction, at least or about a 80% reduction, at least or about a 90% reduction, at least or about a 95% reduction, at least or about a 98% reduction, at least or about a 99% reduction, or about a 100% reduction) relative to a control. In exemplary aspects, the control is cancer or tumor or a subject or a population of subjects that was not treated with the

presently disclosed SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ or wherein the subject or population of subjects was treated with a placebo.

[00113] With regard to the methods of increasing effector activity or reducing suppressive activity of T-cells, the T-cells in various aspects are located within a tumor or a tumor microenvironment, optionally, the T-cells are tumor-infiltrating T-cells. In some aspects, the T-cells are T regulatory cells (Tregs). In various aspects, the T-cells are exhausted T-cells, optionally, exhausted CD8+ T-cells. In certain instances, the T-cells are memory cells, optionally, the memory cells are CD8+ memory cells or CD4+ central memory cells.

[00114] With regard to the methods of increasing an immune response against a tumor or cancer, the immune-response is mediated by T-cells in various aspects. Optionally, the T-cells are located within a tumor or a tumor microenvironment. In various instances, the T-cells are tumor-infiltrating T-cells. In some aspects, the T-cells are T regulatory cells (Tregs). In various aspects, the T-cells are exhausted T-cells, optionally, exhausted CD8+ T-cells. In certain instances, the T-cells are memory cells, optionally, the memory cells are CD8+ memory cells or CD4+ central memory cells.

[00115] In exemplary aspects, the subject of the presently disclosed method of increasing effector activity or reducing suppressive activity of T-cells or the presently disclosed method of increasing an immune response against a tumor or cancer the immune-response is a subject as described herein. In various aspects, the subject has a tumor or a cancer. Optionally, the subject has hepatocellular carcinoma (HCC), colorectal cancer (CRC), lung cancer, optionally, non-small-cell lung cancer (NSCLC), or breast cancer.

[00116] Without being bound to any particular theory, increased effector activity or reduced suppressive activity of T-cells and/or increased immune responses against a tumor or cancer in a subject lead to treatment of a tumor or cancer in the subject. Accordingly, the present disclosure additionally provides methods of treating a subject with a tumor or cancer. In exemplary embodiments, the method comprises increasing effector activity or reducing suppressive activity of T-cells in the subject and/or increasing an immune response against the tumor or cancer in the subject. The considerations and details of the above-described methods of treatment comprising administering to the subject a SIRPγ binder or SIRPγ inhibitor (e.g., an antibody or antigen binding fragment thereof) that binds to SIRPγ apply to the instantly-described methods of treatment (comprising increasing effector activity or reducing suppressive activity of T-cells in the subject and/or increasing an immune response against the tumor or cancer in the subject).

[00117] The following examples are given merely to illustrate the present invention and not in any way to limit its scope.

EXAMPLES

[00118] The studies described below confirm the T cell specific expression pattern of SIRPγ in humans. Although most of T cells express SIRPγ highly, interestingly, memory CD8 T cells and tumor infiltrated CD8 exhaustion cells showed a heightened expression of SIRPγ compared to other T cells. Previous studies on the characterization of CD8 TILs from breast tumor and melanoma tumor tissues suggest that tumor CD8 TILs are predominantly effector memory cells. The high SIRPγ expression pattern on both memory and exhaustion T cells suggest that SIRPγ may negatively impact T cell effector function within tumor environment. Interestingly, the in vitro functional data herein is consistent with this hypothesis and confirms that SIRPγ is a negative regulator of T cell effector function. Furthermore, SIRPγ enhanced Treg suppressive function. These data provide insight into potential therapeutic intervention by targeting SIRPγ on both T cells and Tregs to improve immune response against tumors.

EXAMPLE 1

[00119] The following example describes the materials and methods used in the Examples.

[00120] *Cell preparation and MACS bead sorting*: PBMC were isolated from blood samples from healthy donors using Ficoll Hypaque (GE Healthcare Biosciences, Pittsburg, PA) density gradient. PanT and CD8 T cells were isolated from PBMCs using Miltenyi microbead negative selection kits (#130-096-535 and 130-096-495, Miltenyi) according to manufacturer instructions. To isolate human regulatory T cells, CD4+ T cells were isolated from PBMCs using the Human CD4+ T Cell Isolation kit (130-096-533, Miltenyi) followed by enrichment of CD25+ T cells with CD25 microbeads (130-092-983, Miltenyi) according to the manufacturer's protocol. Finally, CD4+CD25+CD127- human regulatory T cells were FACS sorted. Sorted panT, CD8 and Treg cells were subjected to downstream function assays.

[00121] *In vitro induction of CD8 exhaustion*: Purified human CD8 T cells were seeded at 1-2 x10^6 cells/ml and subjected to anti-CD3 (UCHT1, 0.2 μg/ml, BD Biosciences) and anti-CD28 (CD28.2, 2 μg/ml, BD Biosciences) stimulation for 3-4 days. CD8 T cells were re-stimulated every 3-4 days with anti-CD3 (UCHT1, 1 μg/ml, BD Biosciences) and anti-CD28 (CD28.2, 2 μg/ml, BD Biosciences). Cells were subjected to the re-stimulation at least two rounds as described above.

[00122] *Flow cytometric antibody staining*: Anti-human antibodies used for multi-color flow cytometric analysis included: CD3 (Biolegend, 344804), CD4 (Biolegend,300520), CD8 (Biolegend, 301040), SIRPγ (Biolegend, 336606), mouse IgG1 K isotype control (Biolegend, 400112), CD14 (BD Biosciences, 558121), CD56 (Biolegend, 318321), CD45RA (Biolegend, 304112), CCR7 (Biolegend, 353232), CD127 (Biolegend, 351318), Foxp3 (Biolegend, 320214). PBMC samples were washed with MACS buffer and stained with fluorescently labeled anti-human antibodies. For Foxp3 intracellular staining, cells were fixed with intracellular fixation and permeabilization buffer set (eBioscience, 00-5523-00) and stained with Foxp3 antibody. Flow cytometric data were acquired on LSRII using FACSDiva software (Becton Dickinson). Data were analyzed using Flow Jo (TreeStar, Ashland, OR).

[00123] *SIRPγ overexpression, T cell re-stimulation and cytokine detection*: To overexpress human SIRPγ in human T cells, human SIRPγ was cloned into MSCV-IRES-EGFP retroviral vector. Retrovirus was generated by using pAmpho packaging system (Clontech, #631530) before infecting T cells. For T cell infection, pan T cells were isolated from human PBMCs by using human PanT cell isolation kit from Miltenyi and activated with Dynabeads Human T-Activator CD3/CD28 (ThermoFisher Scientific, #11131D) at 1:1 ratio for 72 hours. 72 hours later, Dynabeads were removed and activated T cells were spin-infected with retrovirus at 2000 rpm for 1 hour under 32 degrees.

[00124] D5 post retrovirus spin-infection, GFP+ human SIRPγ overexpressing T cells were FACS sorted and rested with human IL2 for 2 days. Equal numbers of control or human SIRPγ overexpressing CD8 or CD4 T cells were seeded into 96 round-bottom wells and re-stimulated with plate-bound anti-CD3 (0.5 μg/ml) and anti-CD28 (1 μg/ml) (eBioscience 16-0037-85 and 16-0289-85). Cell supernatant was collected 24 hours after re-stimulation and human IFNγ was measured by ELISA (eBioscience, 88-7316-88).

[00125] *CRISPR knockout in naïve T cells*: For CRISPR knockout in naïve T cells, crRNA–tracrRNA duplex was prepared by mixing equimolar concentrations of Alt-R crRNA and Alt-R tracrRNA (IDT) oligos. Mixed oligos were annealed by heating at 95°C for 5 min in PCR thermocycler and the mix was slowly cooled to room temperature. Three crRNA–tracrRNA duplexes (3 μl equal to 150 pmol each, total of 9 μl) and 6 μl (equal to180 pmol) TrueCut Cas9 Protein v2 (catalog number A36499; Thermo Fisher Scientific, 5μg/ml) were gently mix by pipetting up and down and incubated at room temperature for10-20 min. 200 μl complete T cell media per well of a 96-well plate was prewarmed. 1–2 million T cells were resuspended in 20 μl primary cell nucleofection solution (P2 Primary Cell 4D-Nucleofector X kit S [32 RCT, V4XP-2032; Lonza]). T cells were mixed and incubated with 15 μl RNP at room temperature for 2 min in round bottom 96-well plate. The cell/RNP mix was transferred to Nucleofection cuvette strips (4D-Nucleofector X kit S; Lonza). Cells were electroporated using a 4D nucleofector. Pulse for

human naive T cell populations is EH100. After nucleofection, prewarmed T cell media was used to transfer transfected cells in 96-well plates. Resting human T cells were cultured at 1 × 106 per well in 200 µl complete T cell media for 3–5 d (with IL2 and IL7). Knockdown were checked by FACS at D5 post electroporation. The following crRNA targeting sequences were used in the study: SIRPγ-crRNA1: 5′-GGGACCCGTCCTGTGGTTCAG-3′ (SEQ ID NO: 7), SIRPγ-crRNA2: 5′-AAAAGGGAGCCCTGAGAACG-3′ (SEQ ID NO: 8), SIRPγ-crRNA3: 5′-GTATGTGCCGACATCTGCTG-3′ (SEQ ID NO: 9), CD47-crRNA1: 5′-TACGTAAAGTGGAAATTTAA-3′ (SEQ ID NO: 10), CD47-crRNA2: 5′-TTTGCACTACTAAAGTCAGT-3′ (SEQ ID NO: 11), CD47-crRNA3: 5′-TCCATATTAGTAACAAAGCA-3′ (SEQ ID NO: 12), PD1-crRNA1: 5′-GCAGTTGTGTGACACGGAAG-3′ (SEQ ID NO: 13), PD1-crRNA2: 5′-GGGCCCTGACCACGCTCATG-3′ (SEQ ID NO: 14), PD1-crRNA3: 5′-GATCTGCGCCTTGGGGGCCA-3′ (SEQ ID NO: 15).

[00126] *CRISPR knockout in activated T cells and Jurkat T cells*: Human panT cells were activated with Dynabeads Human T-Activator CD3/CD28 (ThermoFisher Scientific, #11131D) at 1:1 ratio for 48 hours. 48 hours later, Dynabeads were removed and 100,000-200,000 activated T cells were resuspended in 20 µl primary cell nucleofection solution (P2 Primary Cell 4D-Nucleofector X kit S [32 RCT, V4XP-2032; Lonza]) and mixed with RNP complex. The cell/RNP mix was transferred to Nucleofection cuvette strips (4D-Nucleofector X kit S; Lonza). Cells were electroporated using a 4D nucleofector. Pulse for human activated T cell populations is CM138. After nucleofection, prewarmed T cell media was used to transfer transfected cells in 96-well plates. Human T cells were cultured at 1 × 105 per well in 200 µl complete T cell media (with IL2). Knockdown were checked by FACS at D2 post electroporation.

[00127] For Jurkat T cell knockout, 200,000 Jurkat T cells were resuspended in 20 µl primary cell nucleofection solution (P4 Primary Cell 4D-Nucleofector X kit S [32 RCT, V4XP-4032; Lonza]) and mixed with RNP complex. The cell/RNP mix was transferred to Nucleofection cuvette strips (4D-Nucleofector X kit S; Lonza). Cells were electroporated using a 4D nucleofector with CM138 program. D3 post electroporation, knockout T cells were FACS sorted based on the protein expression on cell surface and further expanded for future experiments.

[00128] *Re-stimulation of knockout T cells*: D3 post CRISPR knockout, T cells were restimulated and SIRPγ- CD4 or CD8 T cells were FACS sorted D4 post CRISPR knockout. Sorted SIRPγ knockout T cells were rested with human IL2 for 2 days. Equal numbers of control or human SIRPγ knockout CD8 or CD4 T cells were seeded into 96 round-bottom wells and re-stimulated with plate-bound anti-CD3 (0.5

μg/ml) and anti-CD28 (1 μg/ml). Cell supernatant was collected 24 hours after re-stimulation and human IFNγ was measured by ELISA.

[00129] *Real time PCR*: For qRT-PCR, total RNA was isolated from sorted and restimulated control or SIRPγ- T cells 9 days after CRISPR/Cas9 delivery using RNeasy Mini kit (Qiagen) following the manufacturer's instruction. cDNA was reverse transcribed from these RNAs using SuperScript IV First-Strand Synthesis System (#18091050, Invitrogen) and qRT-PCR was done with QuantStudio3 (Applied Biosystems) using TaqMan gene expression assay kit/probe sets (Thermo Scientific). The primers used in this study were as follows: GAPDH: Hs03929097_g1, SIRPγ F:5′- AGGTGAGGAGGAGCTACAGA-3′ (SEQ ID NO: 16), SIRPγ R:5′- GGTCCAACTCCTCTGAACCA-3′ (SEQ ID NO: 17), SIRPγ probe:5′- CCCTGCTTCCCGTGGGACCCG-3′ (SEQ ID NO: 18). SIRPγ expressions between samples were normalized to GAPDH.

[00130] *PCR amplification and analysis of target region*: Genomic DNAs were isolated from sorted and restimulated control or SIRPγ- T cells 9 days after CRISPR/Cas9 delivery using using Qiagen DNeasy Blood & Tissue kit (Qiagen) following the manufacturer's instruction. Genomic regions, containing the SIRPγ target site, were PCR-amplified using the following primers: SIRPγ F:5′- CCAGATTGGGAAGGACAAGAGCTGT-3′ (SEQ ID NO: 19), SIRPγ R:5′- GGCATGTTGTGAGGGTTAAATGAGA-3′ (SEQ ID NO: 20). PCR products were analyzed through gel electrophoresis or purified on 2% (wt/vol) agarose gel containing SYBR Safe (Life Technologies) by using Qiagen Gel Extraction kit and subjected to sanger sequencing.

[00131] *SIRPγ expression on Treg cells and Treg suppression assay*: To overexpress human SIRPγ in human T regulatory cells, FACS sorted CD4+CD25+CD127- Treg cells were activated with Dynabeads Human T-Activator CD3/CD28 (ThermoFisher Scientific, #11131D) at 1:1 ratio for 48 hours in the presence of 200U/ml human IL2 (202-IL-010/CF, R&D). 48 hours later, Dynabeads were removed and activated Treg cells were spin-infected with retrovirus at 2000 rpm for 1 hour under 32 degree. D5 post retrovirus spin-infection, GFP+ human SIRPγ overexpressing Treg cells were FACS sorted and rested with human IL2 for overnight before setting up the suppression assay.

[00132] To set up the suppression assay, responder CD4 T cells were isolated from a different healthy donor PBMCs by using naïve CD4 T cell isolation kit (130-094-131, Miltenyi) and isolated CD4 T cells were labelled by cell trace violet proliferation kit (C34557, Thermo Fisher). Rested Treg cells were mixed with CTV labelled responder CD4 T cells at different ratio. Allogenic DCs were added to the reaction and CD4 T cells proliferation was measured by CTV dilution.

[00133] *Mixed lymphocyte reaction and T cell proliferation assay*: D6 post CRISPR knockdown, T cells were subjected to mixed lymphocyte reactions (MLRs) or TCR stimulated proliferation. MLRs were performed by incubating 100,000 pan T cells from a healthy donor (responder) with 10,000 allogeneic DCs (stimulators). T-cell proliferation was measured by standard 3H thymidine incorporation assay on D7.

[00134] For TCR stimulation, isolated T cells or CRISPR knockout T cells were plated on serial dilution of mAb anti-CD3 (OKT3, eBioscience) precoated 96 well round bottom plate. Anti-CD47 mAb (B6H12), anti- SIRPγ (LSB2.20), or control mouse IgG was added to T-cell cultures as indicated. After 72 hours, T-cell proliferation was measured by standard 3H-thymidine incorporation assay.

[00135] *SIRP-IgG fusion proteins*: Extracellular domain of SIRPγ and SIRPα were amplified by PCR and cloned into pTT5.2-CMV vector in frame with a cDNA fragment encoding the Fc portion of human IgG fusion proteins. SIRPγ and SIRPα chimeric cDNAs were transiently expressed in 293 cells and secreted SIRP-IgG fusion proteins were purified from culture supernatant on proteinA.

[00136] *Binding assay*: SIRPγ and SIRPα-IgG FC proteins (5μg/mL) were incubated with various cells for 1 hour at 4°C in the absence of presence of antibodies against SIRPγ and CD47. Then cells were washed with FACS staining buffer twice and stained with anti-IgG-FC (PE) antibody (1:50) for 15 minutes at 4 degree. After two washes, binding of fusion proteins to cells was detected by flow cytometry using a PE conjugated anti-human IgG-Fc (#409304, Biolegend) followed by FACS analyses.

[00137] *In vitro antibody interfering assay:* SIRPγ antibodies (10μg/mL) were incubated with Jurkat cells for 30 minutes at room temperature. Cells were washed with FACS staining buffer and incubated with SIRPγ-Fc fusion proteins (10μg/mL) for 30 minutes at 4°C. Then cells were washed with FACS staining buffer twice and stained with anti-IgG-Fc (PE) antibody (#409304, Biolegend, 1:50) for 15 minutes at 4 degree. After two washes, binding of fusion proteins to cells was detected by flow cytometry followed by FACS analyses.

[00138] *Antibody crosslinking and T cell proliferation assay:* 96 well plates were coated with 10μg/mL SIRPγ antibodies (50μl/well) overnight at 4°. The next day, pan T cells from healthy donors were plated in the wells and T cells were stimulated with ImmunoCult™ Human CD3/CD28 T Cell Activator (#10971, Stemcell Technologies). T-cell proliferation was measured by standard 3H thymidine incorporation assay on D3. Cell culture supernatant was collected and subjected to Cytometric Bead Array (CBA) (#558269, BD Biosciences) analyses.

[00139] *Statistical analysis*: Statistical significance was determined by performing t test with Graphpad Prism. Significance is denoted as ***p ≤ 0.0002, **p ≤ 0.0021, *p ≤ 0.0332, and ns p > 0.05.

EXAMPLE 2

[00140] This example demonstrates that SIRPγ is highly expressed on T cells.

[00141] To study the function of SIRPγ in T cells, the expression profile of SIRPγ was first examined in immune cell populations. The specificity of SIRPγ antibody was confirmed by specific recognition of overexpressed SIRPγ protein on cell surface of 293T cells (Figure 1A). Cell surface staining of SIRPγ on human PBMC cells showed that SIRPγ is mainly expressed on $CD4^+$ and $CD8^+$ T cells, but not on $CD14^+$ monocytes (Figure 1B). SIRPγ showed high level of expression in both human CD4 and CD8 T cells at the resting stage. Natural Killer T (NKT) cells also showed positive SIRPγ expression on cell surface. This data is consistent with previous studies (Piccio et al., Blood, 105:2421-2427(2005)), indicating that the expression of SIRPγ is T cell specific.

[00142] Previous studies (Piccio et al., Blood, 105:2421-2427(2005)) have shown that SIRPγ receptor ligation by anti-SIRPγ antibody functions as a co-stimulatory factor for T cell proliferation, suggesting a potential interplay between SIRPγ and the T cell receptor (TCR). To test if SIRPγ expression is regulated by TCR signaling, T cells were stimulated with anti-CD3 and anti-CD28 antibodies and the expression level of SIRPγ by the stimulated T cells was examined. SIRPγ maintained a high expression level on T cells and its expression level was not changed during TCR stimulation (Figure 1C).

EXAMPLE 3

[00143] This example demonstrates that SIRPγ has heightened expression on memory T cells and tumor infiltrated exhausted T cells.

[00144] To further understand the expression profiling of SIRPγ in subsets of memory and effector T cells, T cells from multiple healthy donor PBMCs were stained using antibodies. Naïve T cells, central memory, effector memory and effector T cells were identified by CD45RA and CCR7 staining. CD8+ memory T cells demonstrated a significantly heightened expression of SIRPγ compared to effector T cells and naïve T cells (Figures 2A and 2B). Within CD4 T cell population, central memory CD4 T cells demonstrated a higher expression of SIRPγ, than effector T cells (Figures 2A and 2B). These data suggest that SIRPγ may contribute to the memory function of T cells during immune regulation.

[00145] Interestingly, single-cell RNA sequencing profiling of tumor infiltrated T cells showed that SIRPγ is highly expressed in exhausted CD8 T cells and tumor Tregs isolated from different type of

human cancers including HCC, CRC and lung cancer (Figures 3A-3C). Because the association of SIRPγ with tumor-infiltrating exhausted $CD8^+$ T cells in tumors was not previously reported, its expression and regulation on *in vitro* derived exhausted $CD8^+$ T cells was further characterized. The SIRPγ protein expression was increased on $CD8^+$ T cells that were repeatedly *in vitro* stimulated with low dose of αCD3 TCR activation, a condition which mimics T cell exhaustion (Figure 4). The heightened expression pattern of SIRPγ in exhausted T cells is correlated with decreased effector cytokine production and increased expression of other T cell exhaustion markers such as Tim3 and PD1, suggesting that SIRPγ might serve as a marker for T cell exhaustion within tumor microenvironment.

EXAMPLE 4

[00146] This example demonstrates that SIRPγ inhibits T cell effector cytokine release.

[00147] To study the function of SIRPγ in T cells, retrovirus-mediated overexpression of SIRPγ in T cells was carried out to mimic the high expression of SIRPγ on tumor-infiltrating CD8 T cells. Increased SIRPγ expression on CD8 T cells was observed on day 3 after retroviral infection (Figure 5B). Interestingly, the SIRPγ-overexpressed CD8 T cells produced significantly less IFNγ than that by control virus-infected cells (Figure 5C-5D), supporting an inhibitory role of SIRPγ in CD8 T cells.

[00148] To further evaluate whether SIRPγ is a negative regulator of T cell effector function, the level of SIRPγ on human T cells was knocked down using CRISPR. The success of CRISPR knockdown of SIRPγ was evaluated at genomic, mRNA, and protein levels. CRISPR delivery of SIRPγ guide RNAs (gRNAs) led to the deletion of targeted SIRPγ genomic coding region, significantly reduced SIRPγ mRNA expression, and SIRPγ protein expression on the T cell surface (Figure 6B-6E). Notably, reduced expression of SIRPγ significantly enhanced secretion of IFNγ in CD8 T cells when stimulated with TCR signal *in vitro* (Figure 6F), indicating that SIRPγ functions as a negative regulator of T cells. As noted herein, these results were surprising given previous studies suggesting SIRPγ as a T cell co-stimulatory molecule (Piccio et al., Blood 105(6): 2421-2427 (2005); Leitner et al., Immunol Letters 128(2): 89-97 (2010)).

EXAMPLE 5

[00149] This example demonstrates that SIRPγ enhances regulatory T cell suppressive function and that monoclonal antibodies to SIRPγ have an inhibitory effect on T cell proliferation.

[00150] Previous RNA sequencing profiling on tumor infiltrating T cells also suggest that SIRPγ is upregulated in Tregs within HCC, CRC and lung cancer (Figures 3A-3C). Increased expression of SIRPγ in breast cancer additionally has been demonstrated (Ascension:GSE89225 dataset; Pitas et al. Immunity. 2016 Nov 15;45(5):1122-1134). A higher expression of SIRPγ in tumor infiltrated Tregs, compared to

expression in non-Tregs, was confirmed in NSCLC tumor samples (Figure 7A). Overexpression of SIRPγ in human Treg cells by retroviral transduction did not change FOXP3 expression levels on Tregs (Figure 7C-7D). However, SIRPγ enhanced Treg suppression activity on T cell proliferation in the *in vitro* suppression assay (Figure 7E-7F). These data suggest that heightened expression of SIRPγ in Tregs within tumor environment contributes to the suppression of effector T cell function. These data also suggest that inhibition of SIRPγ may lead to enhanced levels of T cell proliferation within the tumor environment.

[00151] Previous studies (Piccio et al., Blood, 105:2421-2427(2005)) indicated that CD47 is a ligand for SIRPγ. Piccio et al. also showed that anti-SIRPγ mAbs and anti-CD47 mAbs inhibited T-cell proliferation and ligation of SIRPγ co-stimulated T cell proliferation, which results supported SIRPγ as a positive regulator of T cell function. However, by using SIRPγ overexpression and SIRPγ knock-out system, the studies described herein suggest that SIRPγ is a negative regulator of T cell function. To further examine the discrepancy between previous results and the results described herein, the function of mAbs against SIRPγ during in vitro T cell proliferations was tested. To screen the functional blocking mAbs that can block the interaction between SIRPγ and CD47, SIRPγ and CD47 in Jurkat T cells was depleted through CRISPR (Figure 8A). By using CD47 knockout cell lines, it was confirmed that SIRPγ binds to the T cell surface in a CD47-dependent manner (Figure 8B). Furthermore, the mAbs clone LSB2.20 and OX119, which functionally block the interaction of SIRPγ-CD47 on Jurkat T cells, were identified through the *in vitro* binding assay (Figure 8C).

[00152] During mixed lymphocyte reactions (MLRs), some anti-SIRPγ mAbs inhibited T cell proliferation (Figure 8D). In addition, treatment with anti-SIRPγ antibody inhibited the activation of T cells in the presence of serial dilution of a TCR ligand (8E). Interestingly, the inhibitory effect of the anti-SIRPγ antibody was independent of the presence of SIRPγ (Figure 8F), since the inhibitory effect was still observed when SIRPG gene was knocked down by CRISPR mediated method as mentioned above. It is possible that the activities of these particular antibodies may be due to off-target effects or that the residues on SIRPγ that interact with CD47 are also required for interaction with other proteins to mediate its activity.

EXAMPLE 6

[00153] This example demonstrates that anti-SIRPγ monoclonal antibodies which bind to a particular epitope of SIRPγ can enhance T cell proliferation and function.

[00154] To explore the inhibitory effect of SIRPγ on T cell function, a panel of commercially-available SIRPγ antibodies were screened for their ability to bind to SIRPγ and perform as blockers of the CD47-

SIRPγ interaction. T cells were treated with anti-SIRPγ monoclonal antibody clone LSB2.20, clone OX117, clone OX119, LS-C484765, clone 4F8C10 (MAB21425), or with polyclonal anti-SIRPγ antibodies (AF4486), and the binding of the antibodies to the T cells were assayed by FACS As shown in Figure 9A, several anti-SIRPγ antibodies were capable of binding to SIRPγ endogenously expressed or exogenously overexpressed on Jurkat cells (Figure 9A).

[00155] The ability of the anti-SIRPγ antibodies to alter the binding interactions between SIRPγ and its binding partners on T cells was evaluated by carrying out an *in vitro* antibody blocking assay. In this assay, SIRPγ-Fc binds to T cells, possibly through its interaction with SIRPγ binding patterns, such as CD47 or other unidentified receptors, expressed by T cells. At this moment, CD47 is the only known high affinity receptor for SIRPγ (Figure 8C). However, in the absence of any SIRPγ antibodies, since SIRPγ is also expressed on T cells, it can potentially interact with CD47 and/or other putative binding partners in-cis on T cell surface. As a result, these potential in-cis interactions may inhibit the binding of SIRPγ-Fc with its binding partners on T cells in this assay. However, if preincubation of an anti-SIRPγ antibody, which disrupts the interaction between cell surface in-cis interaction between SIRPγ and its binding patterns, it could release these binding patterns on cell surface to subsequently interact with SIRPγ-Fc protein in this assay. Binding of SIRPγ-Fc to T cells was measured in the presence of the various anti-SIRPγ antibodies and compared to the binding in the absence of the anti-SIRPγ antibodies. As shown in Figure 9B, cells pretreated with anti SIRPγ antibody clone LSB2.20 or clone OX119 didn't have enhanced binding of SIRPγ-Fc, suggesting that disruption of SIRPγ-CD47 interaction with these antibody clones does not result in the release of more CD47 or other binding partners for further interaction with SIRPγ-Fc fusion proteins, although these antibodies have been previously shown to block the interaction between SIRPγ and CD47. These data suggested that either the affinity of these antibodies might not be strong enough to release the cis-interaction between on the T cell surface, or CD47 and SIRPγ do not interact in-cis on the cell surface. In addition, these antibodies also do not block the potential interactions between SIRPγ and other putative binding partners. Interestingly, unlike clone LSB2.20 and OX119, clone OX117 treatment showed enhanced binding of SIRPγ-Fc fusion proteins. Since OX119 is known to bind epitopes on SIRPγ different from the epitopes interact with CD47 (Figure 9E), these results suggesting that OX117 may be able to block interactions between SIRPγ and other putative binding proteins on Jurkat cell surface. After treatment with clone OX117, Jurkat cells released the putative binding partners for their binding by SIRPγ-Fc fusion protein (Figure 9B).

[00156] The effect anti-SIRPγ antibodies have on T cell proliferation and IFNγ secretion was also tested. In this assay, T cells were treated with anti-SIRPγ monoclonal antibody clone LSB2.20, clone OX117, clone OX119, LS-C484765, clone 4F8C10 (MAB21425), polyclonal anti-SIRPγ antibodies

(AF4486), one of three non-specific IgG controls, or with no antibody control. The level of T cell proliferation and IFNγ production were measured (Figure 9C and 9D). Interestingly, treatment of T cells with SIRPγ antibody clone OX117 demonstrated statistically significant and the highest level of T cell proliferation and IFNγ secretion in vitro (Figure 9C-9D). The level of IFNγ secreted by T cells treated with OX117 was twice the level of IFNγ secreted by cells treated with LSB2.20, and more than 6 times the level of IFNγ secreted by cells OX119-treated. Reportedly, the fab fragment of anti-SIRPγ monoclonal antibody clone OX117 binds SIRPγ at the interface of the first and second immunoglobulin domains (D1 and D2) of SIRPγ (Nettleship et al., BMC Struct Biol 13: 13 (2013)) which region is distinct from D1 (which interacts with CD47). In addition, the epitope to which OX117 binds is distinct from the epitope to which SIRPγ monoclonal antibody clones OX119 and LSB2.20 bind. This suggests that the T cell proliferation and IFNγ secretion observed with OX117 is likely not caused by a direct blocking of CD47 binding to SIRPγ.

[00157] A summary of results from the above assays carried out with the panel of commercially-available anti-SIRPγ antibodies is provided in Figure 10. Taken together, these data do not support a specific co-stimulatory function of SIRPγ, especially through its interaction with CD47. Instead, we identified a novel inhibitory function of SIRPγ in T cell proliferation, activation and cytokine production. Certain antibodies may interfere with this inhibitory function of SIRPγ and enhance T cell activities, which might be achieved through the blocking the interaction between CD47 and SIRPγ. These data also suggest that the T-cell inhibitory function of SIRPγ may be mediated through unique epitopes of SIRPγ and that molecules that bind to the interface of D1 and D2 of SIRPγ, similar to what is achieved by OX117, but is not limited by, may be useful in enhancing T cell function.

EXAMPLE 7

[00158] This example demonstrates that SIRPγ inhibitors increase an immune response against a tumor or cancer in a subject.

[00159] T cells can kill cancer cells when they recognize tumor specific antigens. To amplify the T cell specific killing effect, T cells are engineered to express tumor antigen-specific T cell receptors (TCR) or chimeric antigen receptors (CAR) and thereby kill cancer cells. CRISPR knockout of SIRPγ is performed on antigen specific TCR-T or CAR-T cells. Human cancer cells that express tumor specific antigen are co-cultured with control or SIRPγ knockout antigen specific T cells *in vitro* to measure the anti-tumor immune response. The killing activity of SIRPγ knockout antigen specific T cells is measured through the quantification of surviving cancer cells. Secreted IFNγ is detected with standard ELISA assay. SIRPγ knockout antigen specific T cells exhibit increased IFNγ secretion and enhanced cancer cell killing. The tumor specific antigens include, but are not limited to, NY-ESO-1 and MART1/Melan-A.

EXAMPLE 8

[00160] This example demonstrates that SIRPγ inhibitors lead to reduced tumor size and/or reduced tumor growth in a subject.

[00161] SIRPγ is not expressed on mouse cells. To establish a mouse tumor model for testing SIRPγ's effect on tumor growth, NOD scid gamma (NSG) immunodeficient mice are implanted with human cancer cells or finely minced patient-derived tumors that express specific tumor antigens. Control or SIRPγ knockout antigen specific T cells are engineered and expanded *in vitro*. After a tumor reaches a certain size (e.g., 50–120 mm^3 in volume), control or SIRPγ knockout antigen specific T cells are adoptively transferred into NSG mice and tumor size is measured. The tumor specific antigens include, but are not limited to, NY-ESO-1 and MART1/Melan-A. Mice with SIRPγ knockout antigen specific T cells transfer exhibit reduced tumor size and tumor growth.

[00162] To establish an in vivo tumor model to test the effect of SIRPγ inhibitors on tumor immune response, humanized NSG (HuNSG) mice are generated through human pluripotent stem cell (HPSC) transplantation. 12 weeks post-human CD34+ HPSC transplantation, human cancer cells are injected into mice to develop tumor. Alternatively, for patient derived xenograft (PDX) tumor model, finely minced patient derived tumors are injected subcutaneously into HuNSG mice. Treatment is started when the tumors reached a certain size (e.g., 50–120 mm^3 in volume). SIRPγ inhibitors are injected into HuNSG mice intravenously and tumor size and tumor volume is measured. Mice with SIRPγ inhibitors treatment exhibit reduced tumor size and tumor growth.

[00163] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[00164] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the disclosure (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted.

[00165] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range and each endpoint, unless otherwise indicated herein, and each separate value and endpoint is incorporated into the specification as if it were individually recited herein.

[00166] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the disclosure and does not pose a limitation on the scope of the disclosure unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the disclosure.

[00167] Preferred embodiments of this disclosure are described herein, including the best mode known to the inventors for carrying out the disclosure. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the disclosure to be practiced otherwise than as specifically described herein. Accordingly, this disclosure includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the disclosure unless otherwise indicated herein or otherwise clearly contradicted by context.

WHAT IS CLAIMED:

1. A method of treating a subject with a tumor or cancer, comprising administering to the subject a SIRPγ binder in an amount effective to treat the tumor or cancer in the subject.
2. A method of increasing effector activity or reducing suppressive activity of T-cells in a subject with a tumor or cancer, comprising administering to the subject a SIRPγ binder in an amount effective to increase the effector activity or reduce the suppressive activity in the subject.
3. A method of increasing an immune response against a tumor or cancer in a subject comprising administering to the subject a SIRPγ binder in an amount effective to increase an immune response against a tumor or cancer.
4. The method of claim 3, wherein the immune-response is mediated by T-cells.
5. The method of any one of claims 2-4, wherein the T-cells are located within a tumor or a tumor microenvironment.
6. The method of any one of claims 2-5, wherein the T-cells are tumor-infiltrating T-cells.
7. The method of claim 2-5, wherein the T-cells are T regulatory cells (Tregs).
8. The method of any one of claims 2-5, wherein the T-cells are exhausted T-cells, optionally, exhausted CD8+ T-cells.
9. The method of any one of claims 2-5, wherein the T-cells are memory cells.
10. The method of claim 9, wherein the memory cells are CD8+ memory cells or CD4+ central memory cells.
11. The method of any one of the preceding claims, wherein the SIRPγ binder is a SIRPγ inhibitor.
12. The method of claim 11, wherein the SIRPγ inhibitor reduces expression of SIRPγ in cells of the subject, optionally, wherein the SIRPγ inhibitor reduces cell surface expression of SIRPγ on T-cells of the subject.
13. The method of claim 12, wherein the T cells are effector T-cells of the subject.
14. The method of any one of claims 11-13, wherein the SIRPγ inhibitor reduces a binding interaction between SIRPγ and a SIRPγ binding partner.
15. The method of claim 14, wherein the SIRPγ binding partner is CD47.
16. The method of any one of the preceding claims, wherein the SIRPγ binder binds to Immunoglobulin (Ig) Domain 1 (D1) of SIRPγ.

17. The method of any one of the preceding claims, wherein the SIRPγ binder binds to D1 and Ig Domain 2 (D2) of SIRPγ.

18. The method of any one of the preceding claims , wherein the SIRPγ binder binds to both D1 and D2, optionally at the interface between D1 and D2.

19. The method of any one of the preceding claims, wherein the SIRPγ binder binds to the epitope to which SIRPγ monoclonal antibody OX117 binds.

20. The method of any one of the preceding claims, wherein the SIRPγ binder competes with OX117 for binding to SIRPγ.

21. The method of claim 20 wherein the SIRPγ binder binds to SIRPγ with the same or higher affinity as OX117.

22. The method of any one of the preceding claims, wherein the SIRPγ binder is OX117 or an antigen binding fragment thereof.

23. The method of any one of the preceding claims, wherein the SIRPγ binder forms hydrogen bonds with one or more of amino acid residues Q8, E10, G109, K11, L12, and D149 of SIRPγ.

24. The method of any one of the preceding claims, wherein the SIRPγ binder causes a conformational change of SIRPγ, upon binding to SIRPγ.

25. The method of any one of the preceding claims, wherein the SIRPγ binder simultaneously binds to two SIRPγ molecules or promotes SIRPγ dimerization.

26. The method of any one of the preceding claims, wherein the SIRPγ binder binds to an epitope which does not overlap with the CD47 binding site.

27. The method of any one of the preceding claims, wherein the SIRPγ binder is an antigen-binding protein.

28. The method of claim 27, wherein the antigen-binding protein is an antibody, an antigen-binding antibody fragment, or an antibody protein product.

29. The method of claim 27 or 28, wherein the antigen-binding protein binds at an epitope within the CD47 binding site of SIRPγ.

30. The method of any one of the preceding claims, wherein the subject has hepatocellular carcinoma (HCC), colorectal cancer (CRC), lung cancer, optionally, non-small-cell lung cancer (NSCLC).

31. A method of treating a subject with a tumor or cancer, comprising increasing an immune response against the tumor or cancer in the subject in accordance with any one of the preceding claims.

32. A method of treating a subject with a tumor or cancer, comprising increasing effector activity or reducing suppressive activity of T-cells in the subject in accordance with any one of the preceding claims.

FIGURE 1A

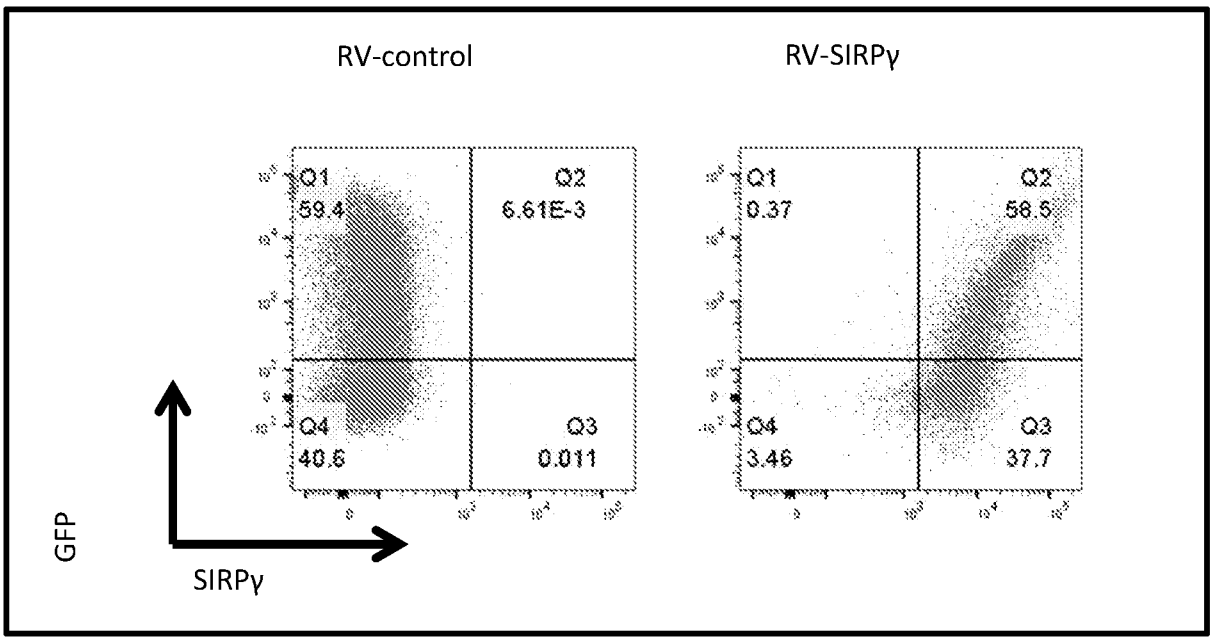


FIGURE 1B

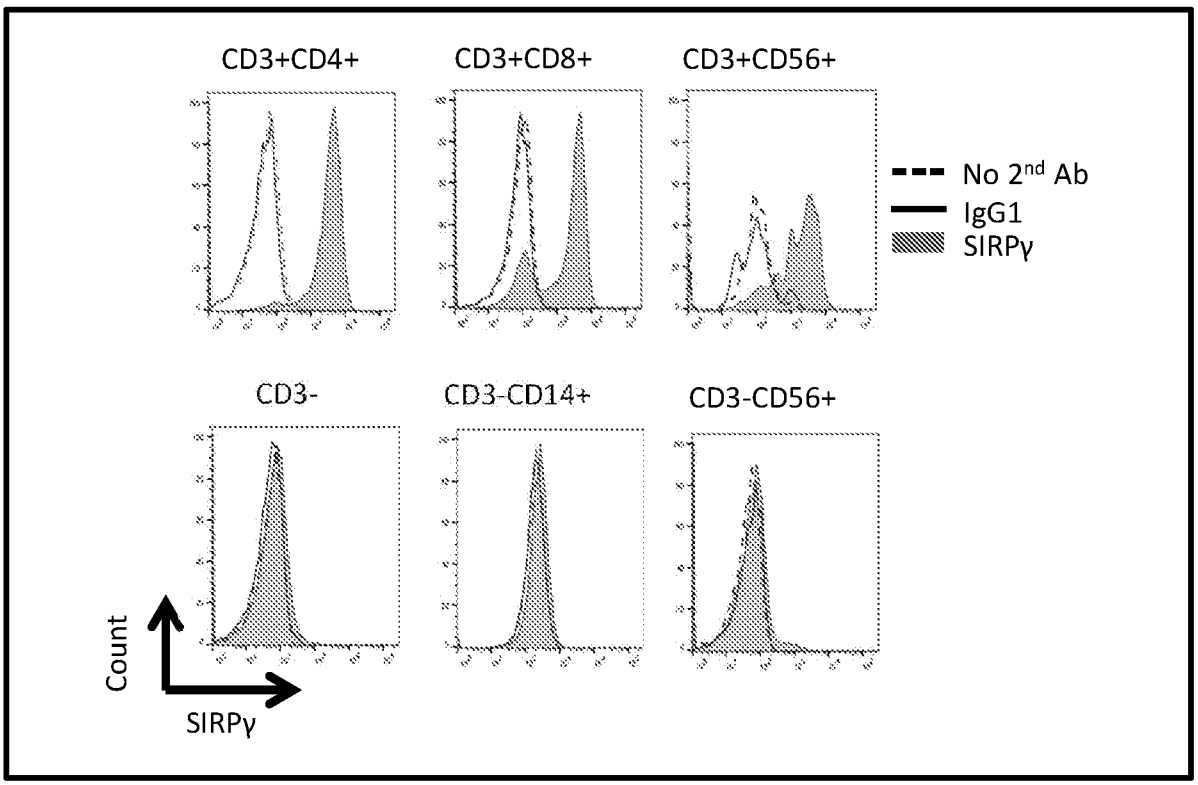


FIGURE 1C

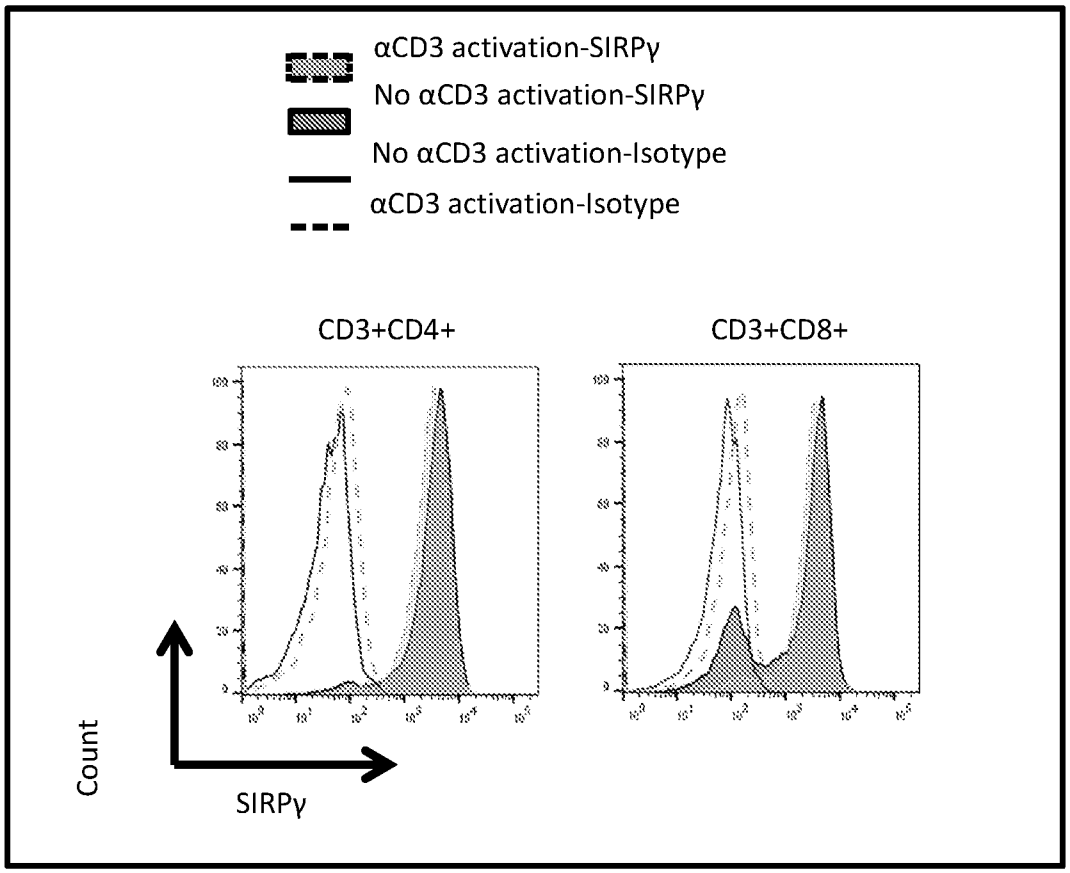

αCD3 activation-SIRPγ
No αCD3 activation-SIRPγ
No αCD3 activation-Isotype
αCD3 activation-Isotype
CD3+CD4+
CD3+CD8+
Count
SIRPγ

FIGURE 2A

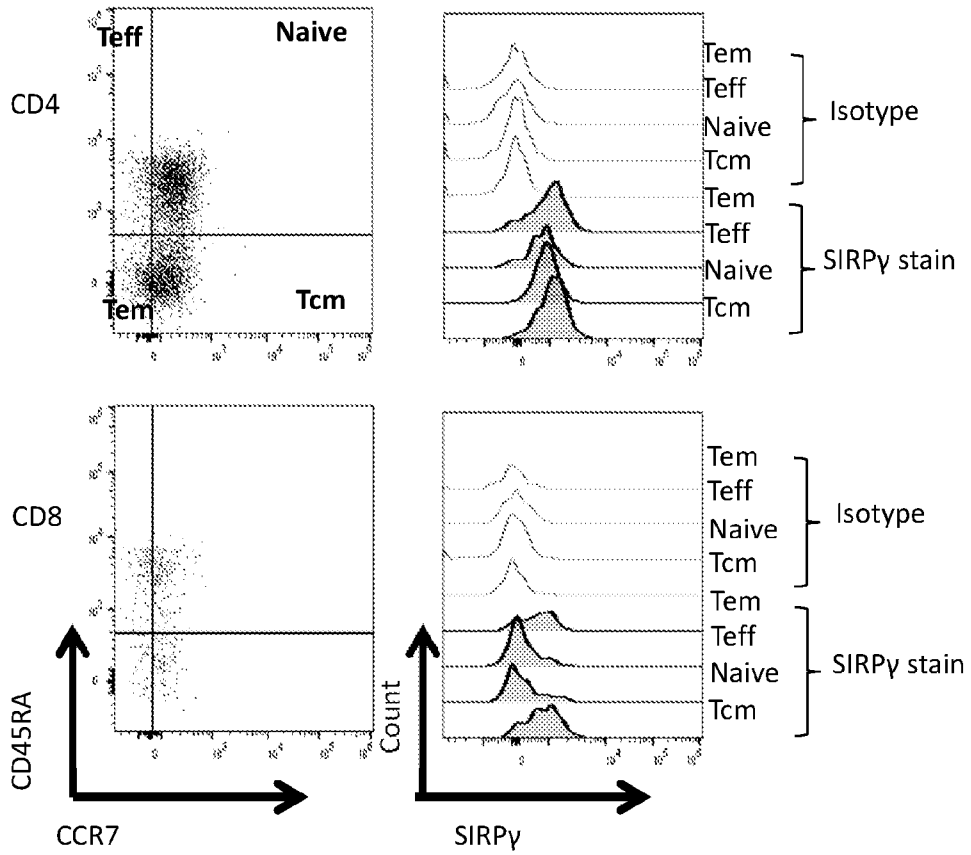


FIGURE 2B

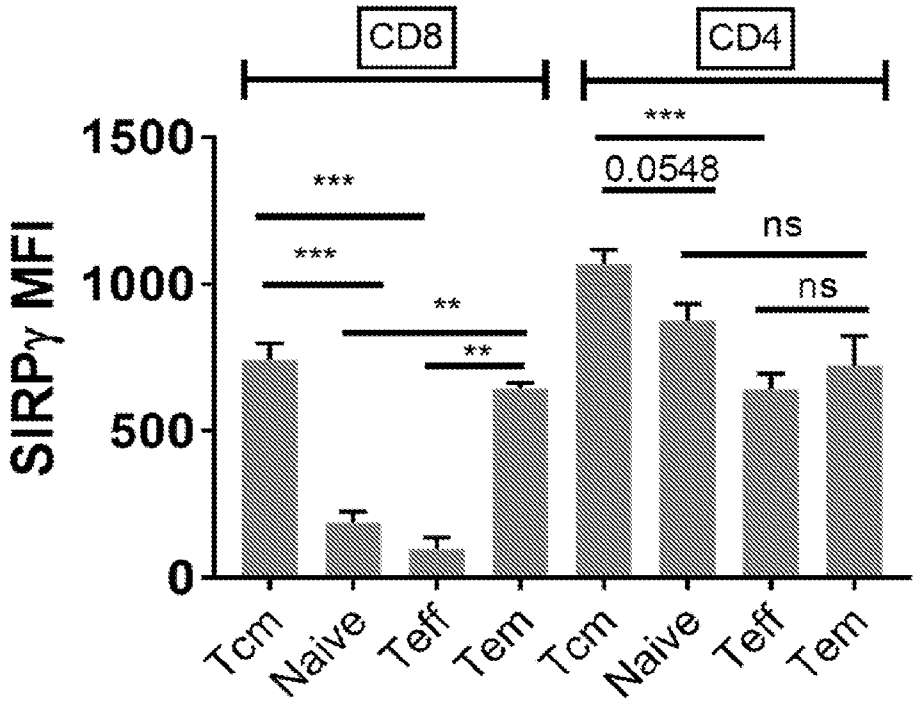


FIGURE 3A
HCC
SIRPγ Expression

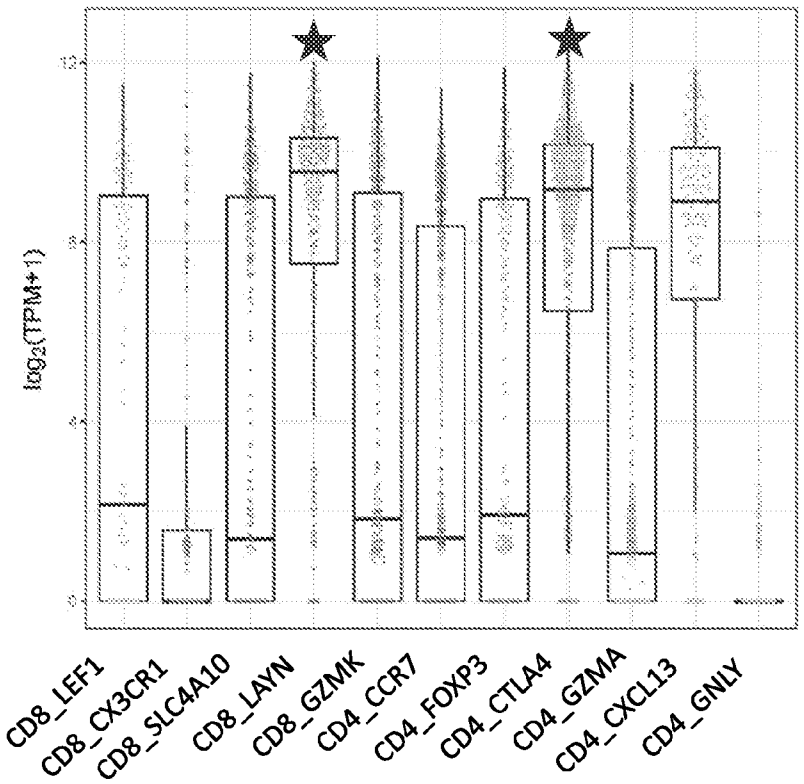


FIGURE 3B
CRC
SIRPγ Expression

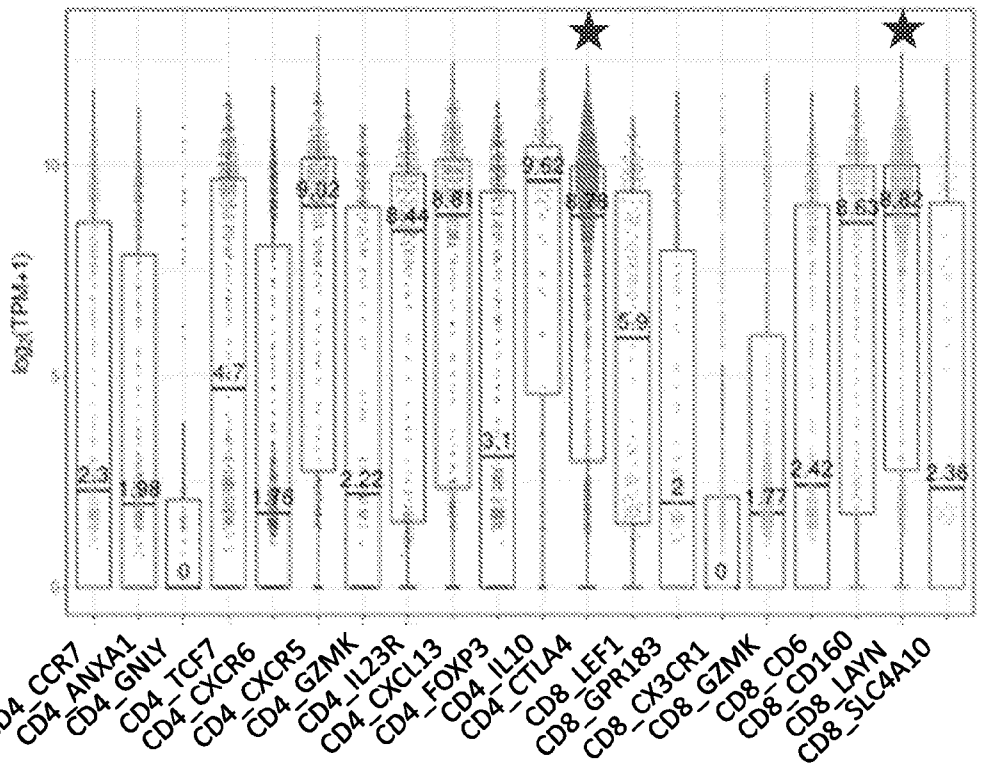


FIGURE 3C
Lung Cancer
SIRPγ Expression

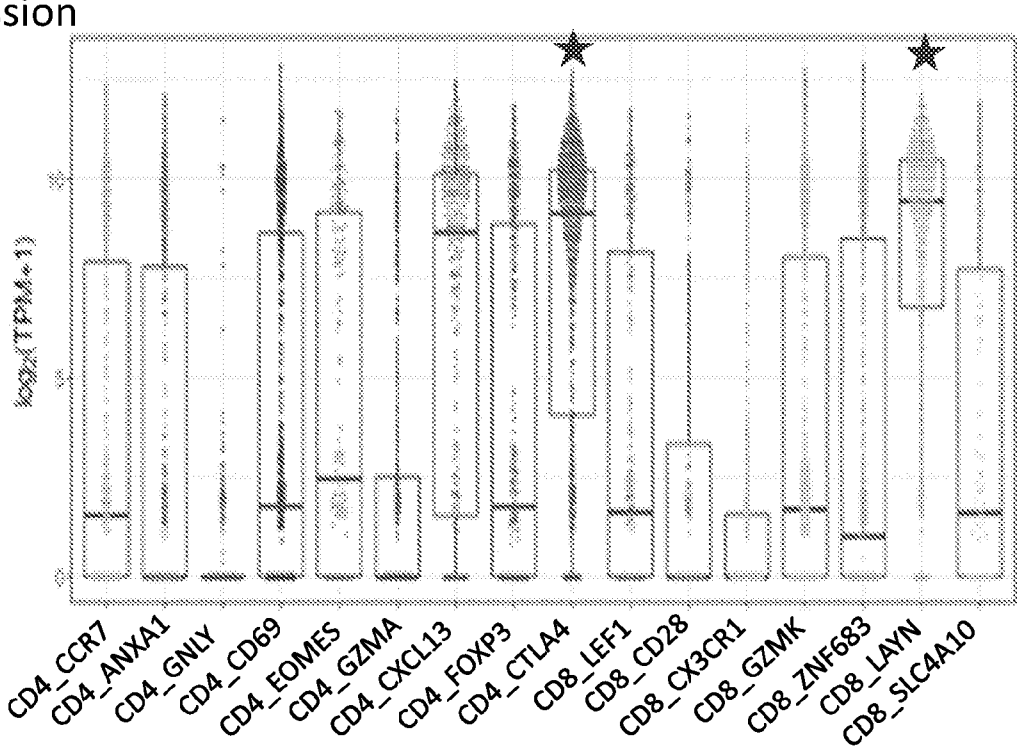


FIGURE 4

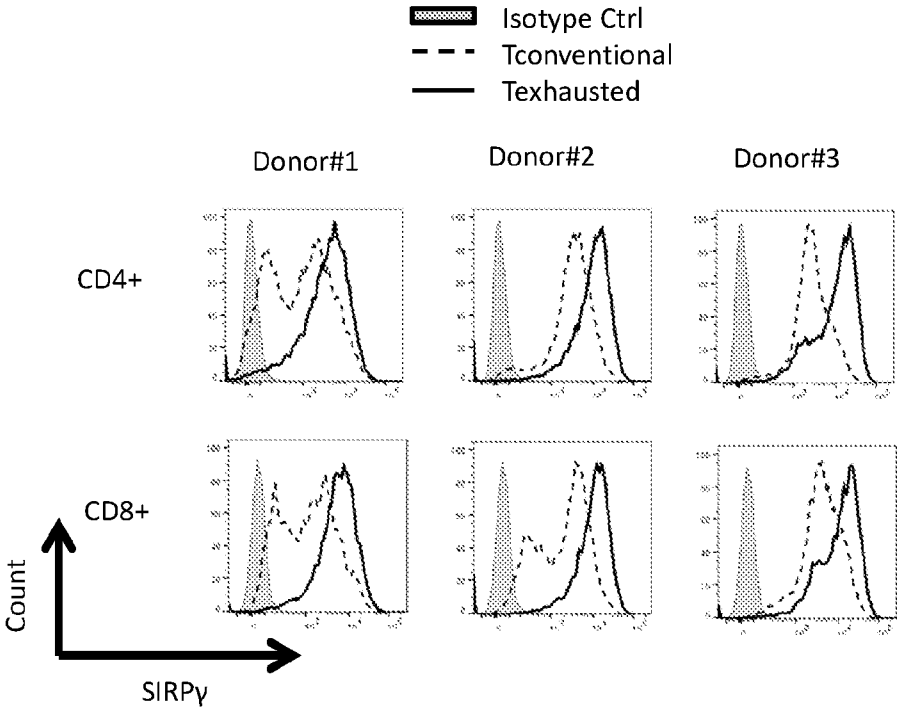


FIGURE 5A

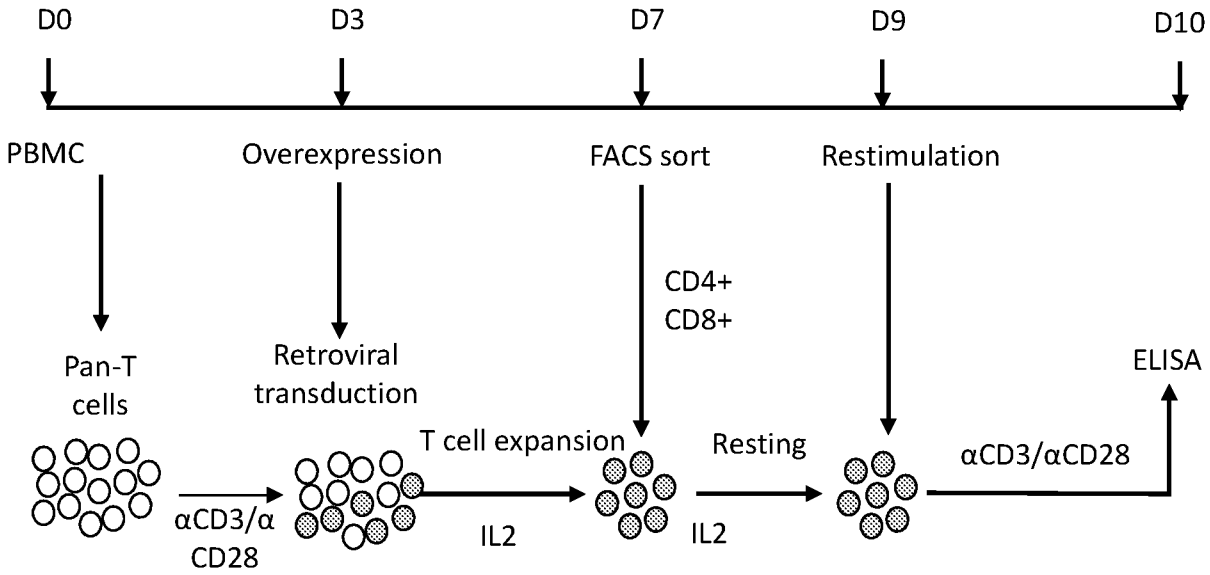


FIGURE 5B

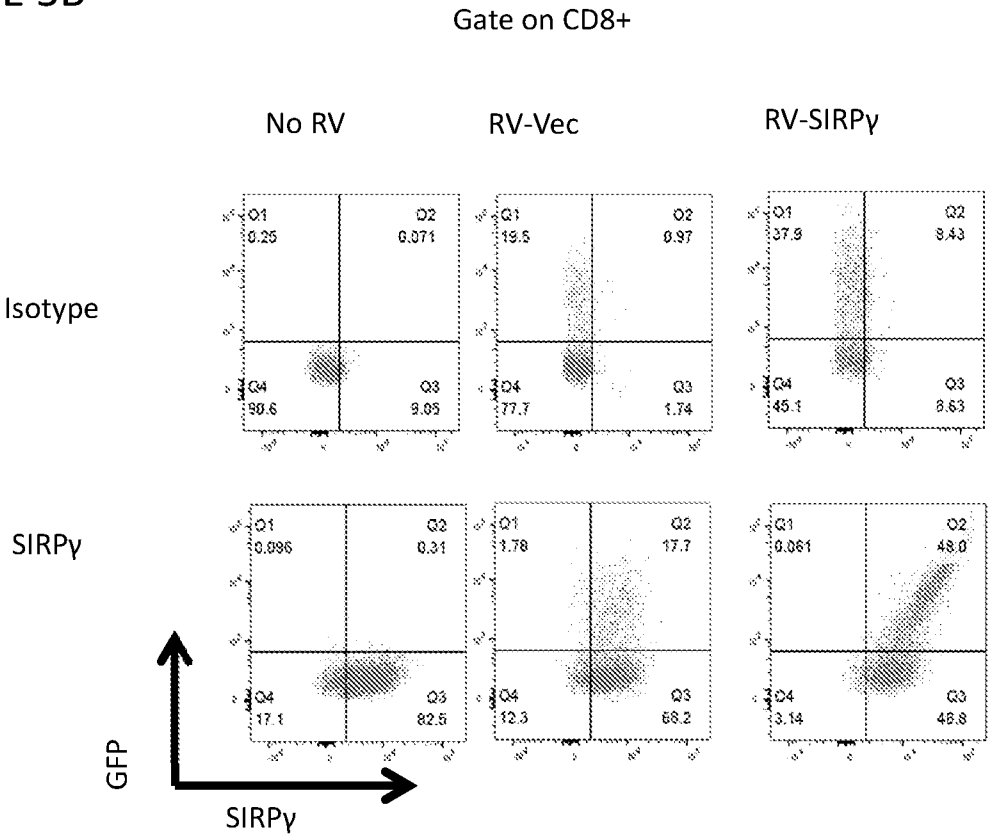


FIGURE 5C

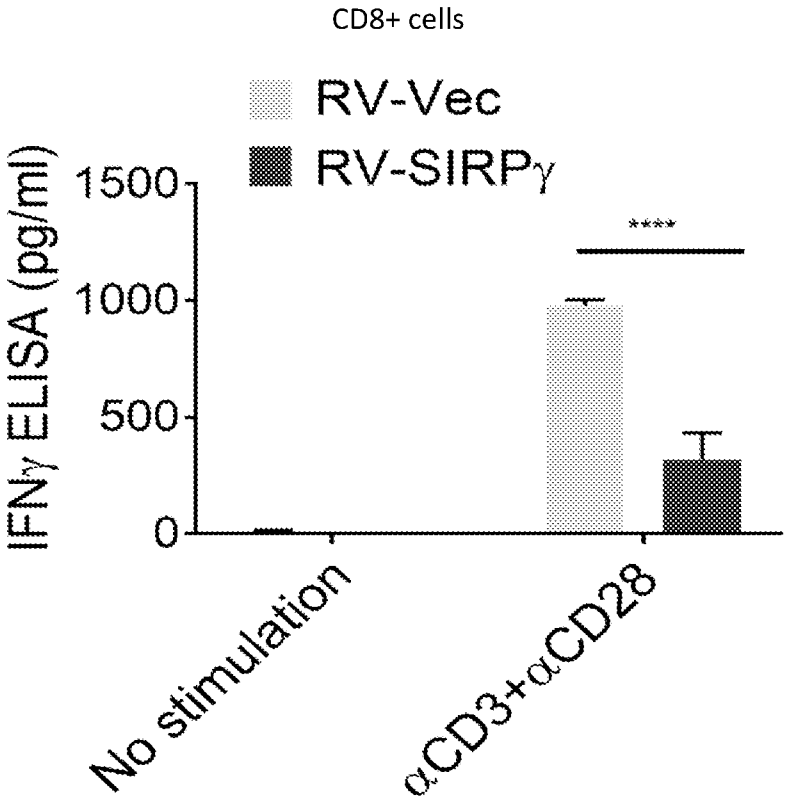


FIGURE 5D

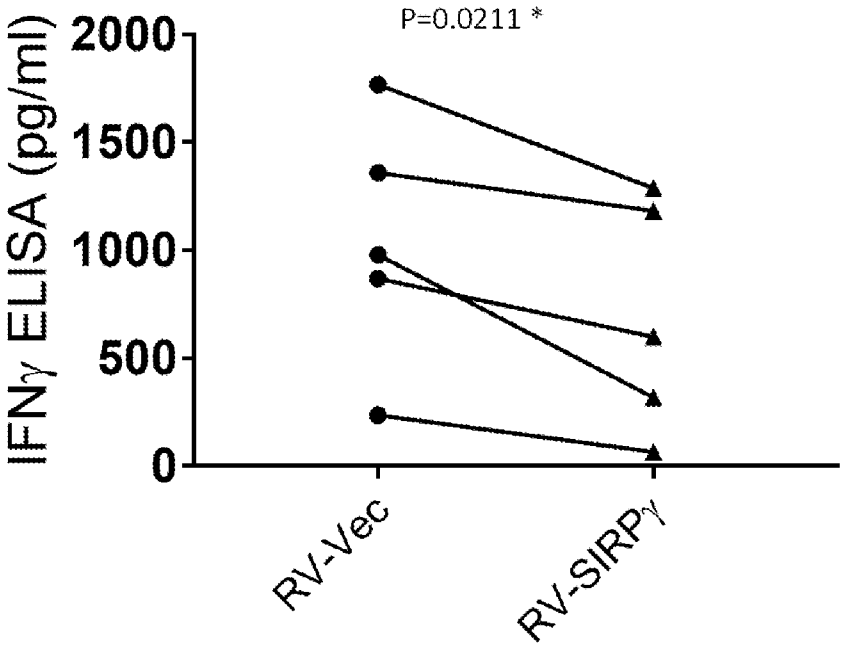


FIGURE 6A

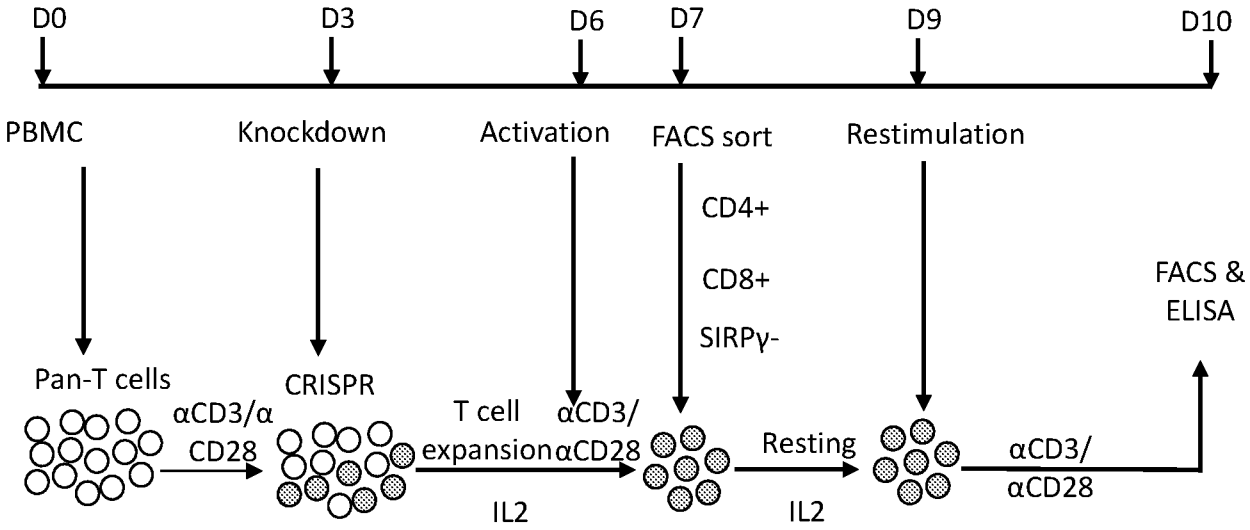


FIGURE 6B

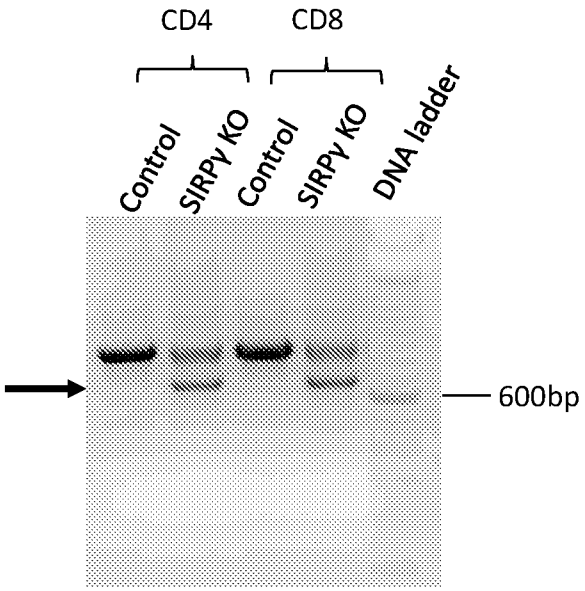
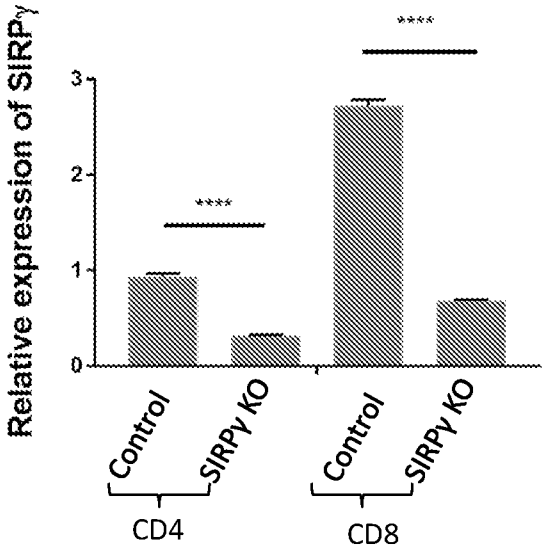


FIGURE 6C



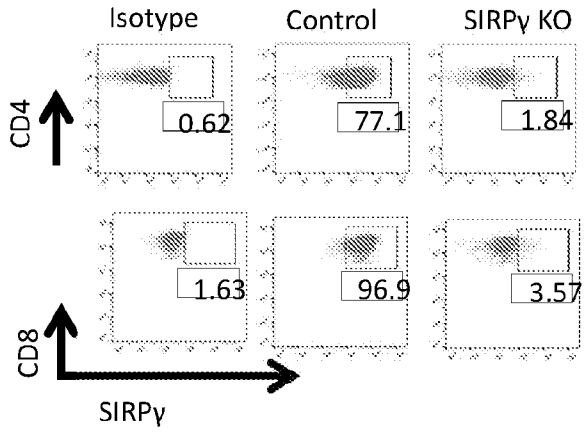


FIGURE 6D

FIGURE 6E

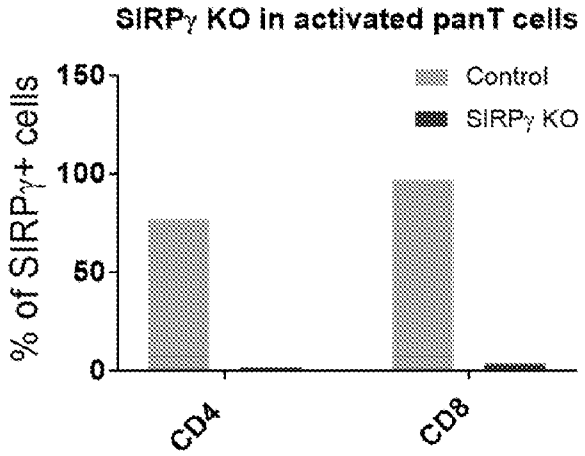


FIGURE 6F

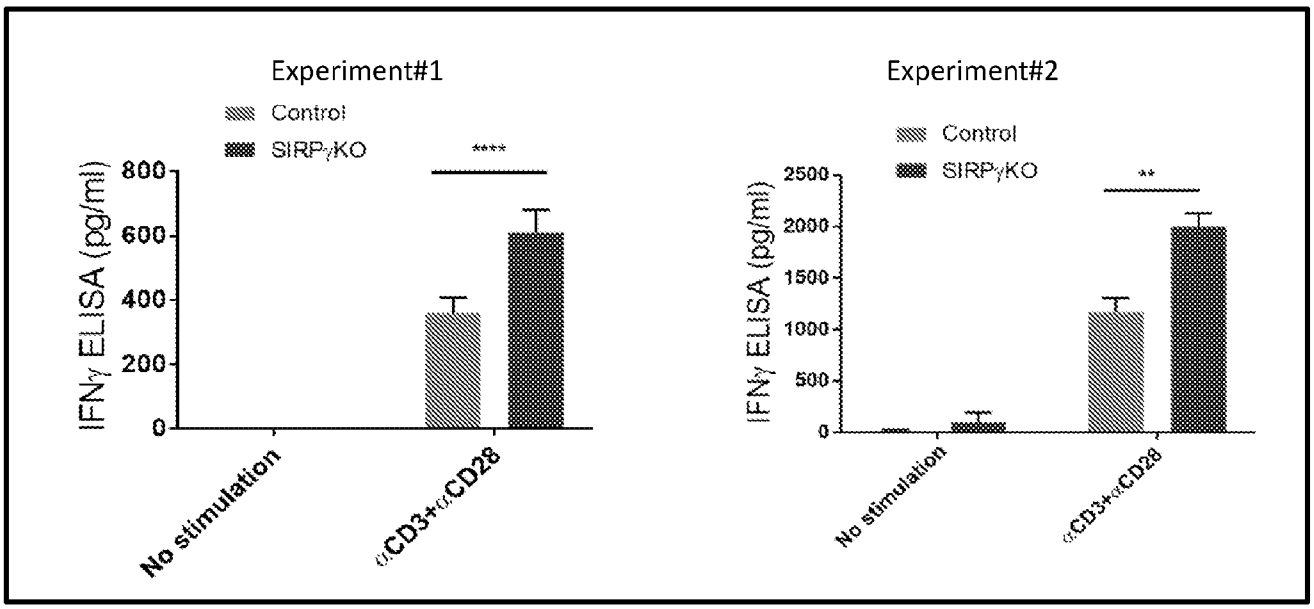


FIGURE 7A

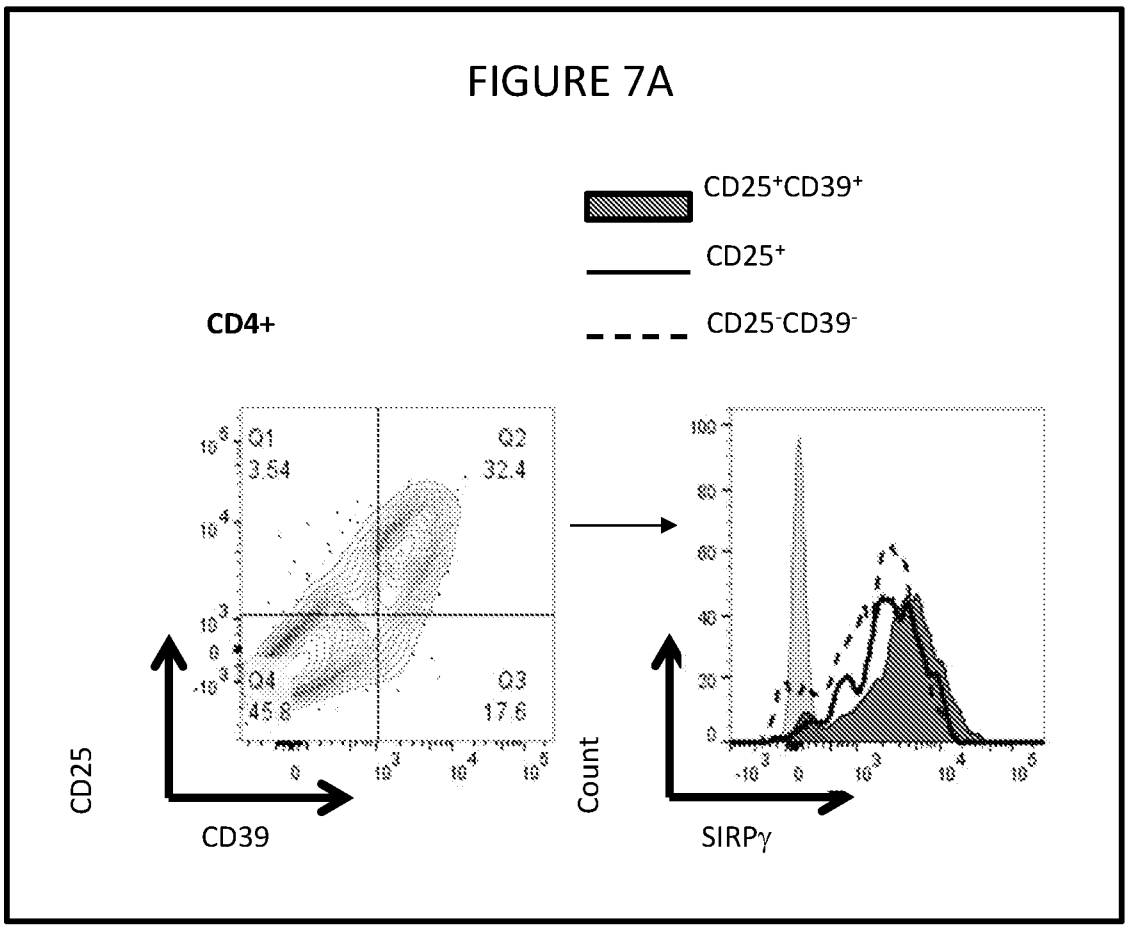


FIGURE 7B

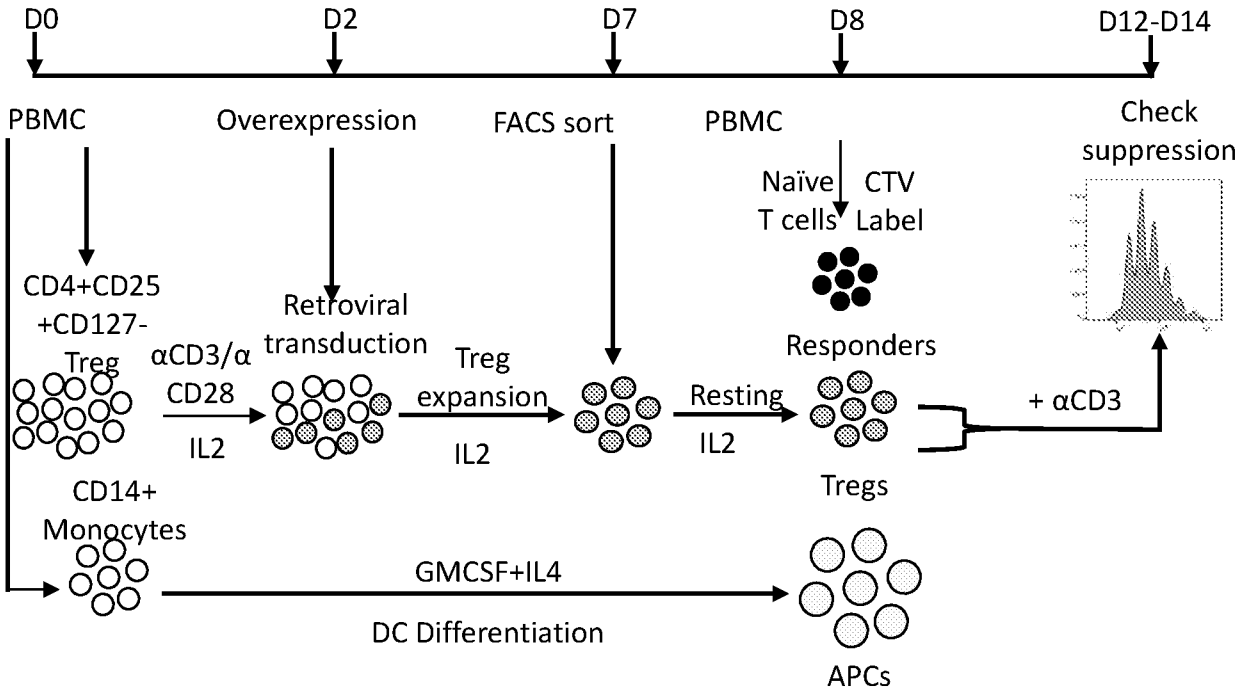


FIGURE 7C

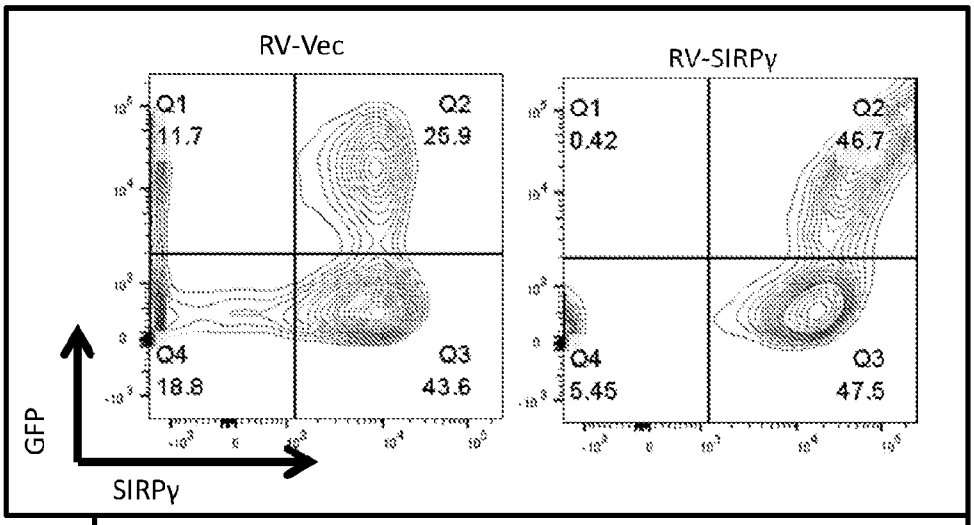


FIGURE 7D

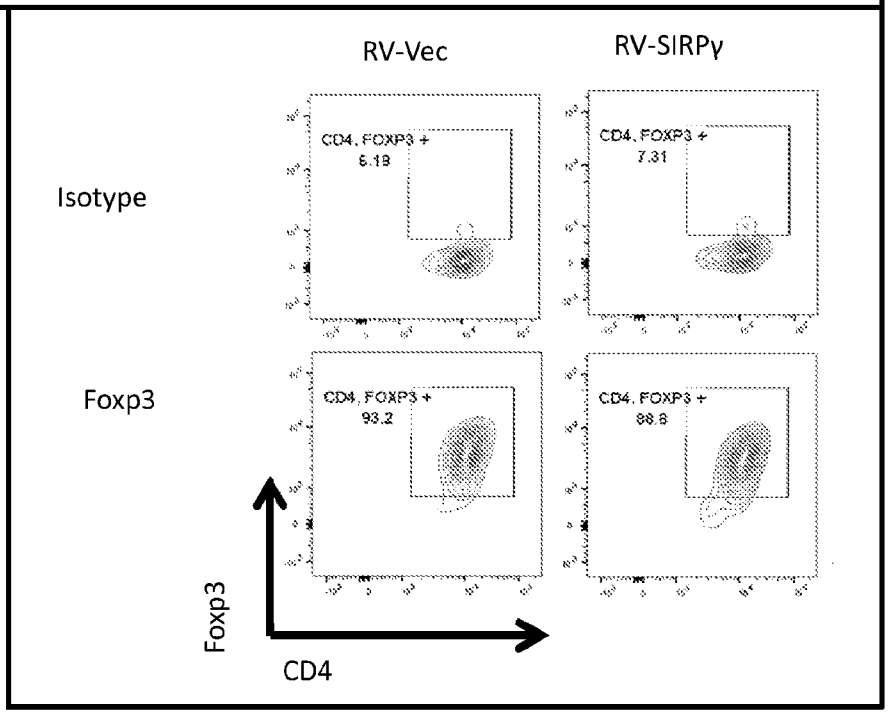


FIGURE 7E

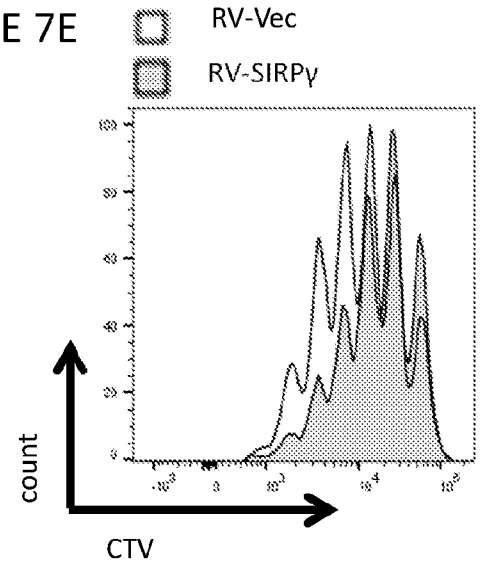


FIGURE 7F

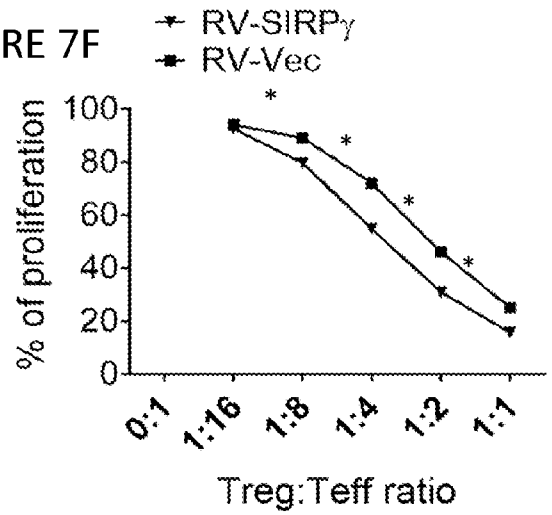


FIGURE 8A

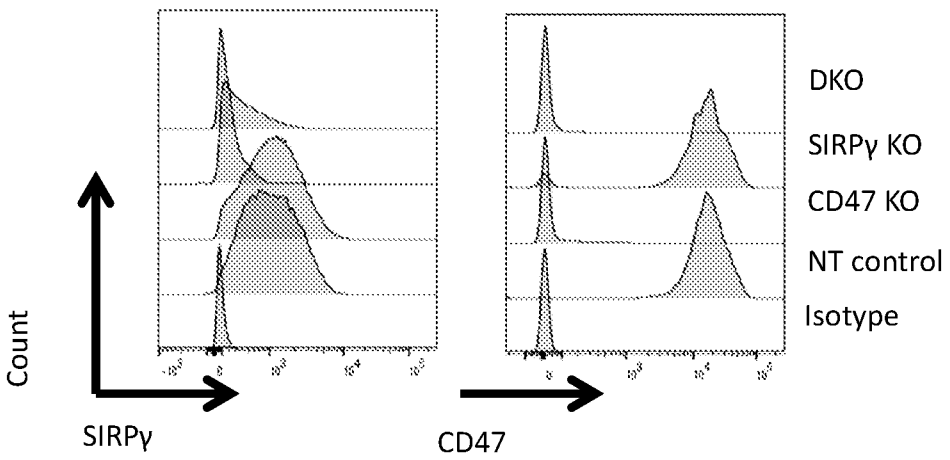


FIGURE 8B

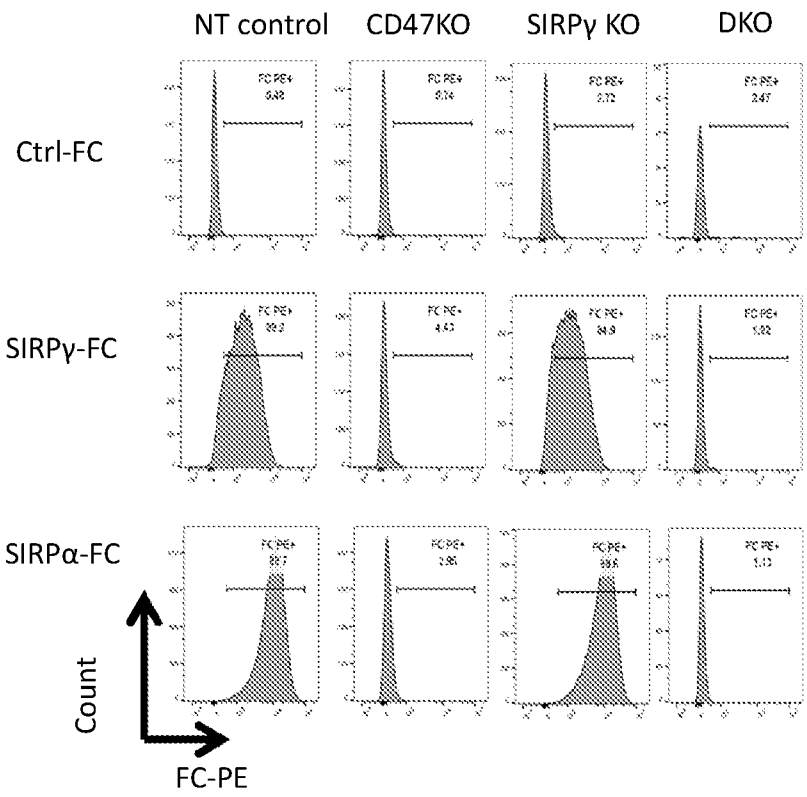


FIGURE 8C

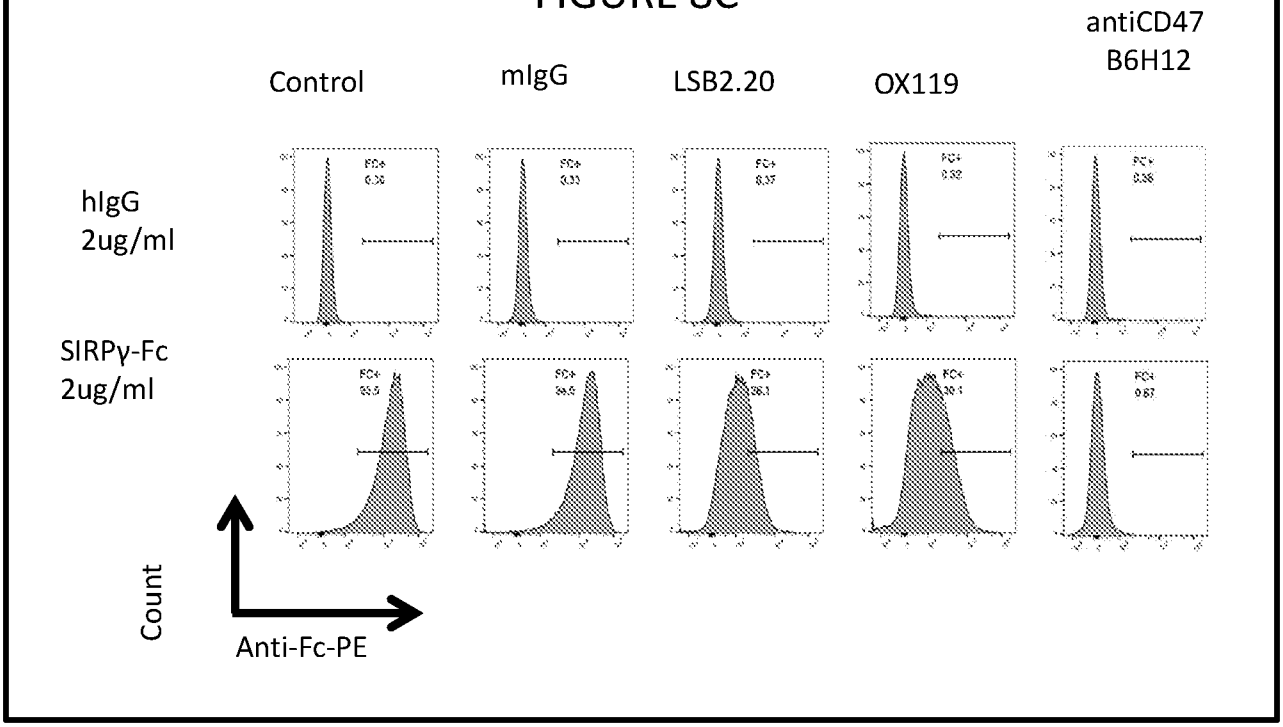


FIGURE 8D

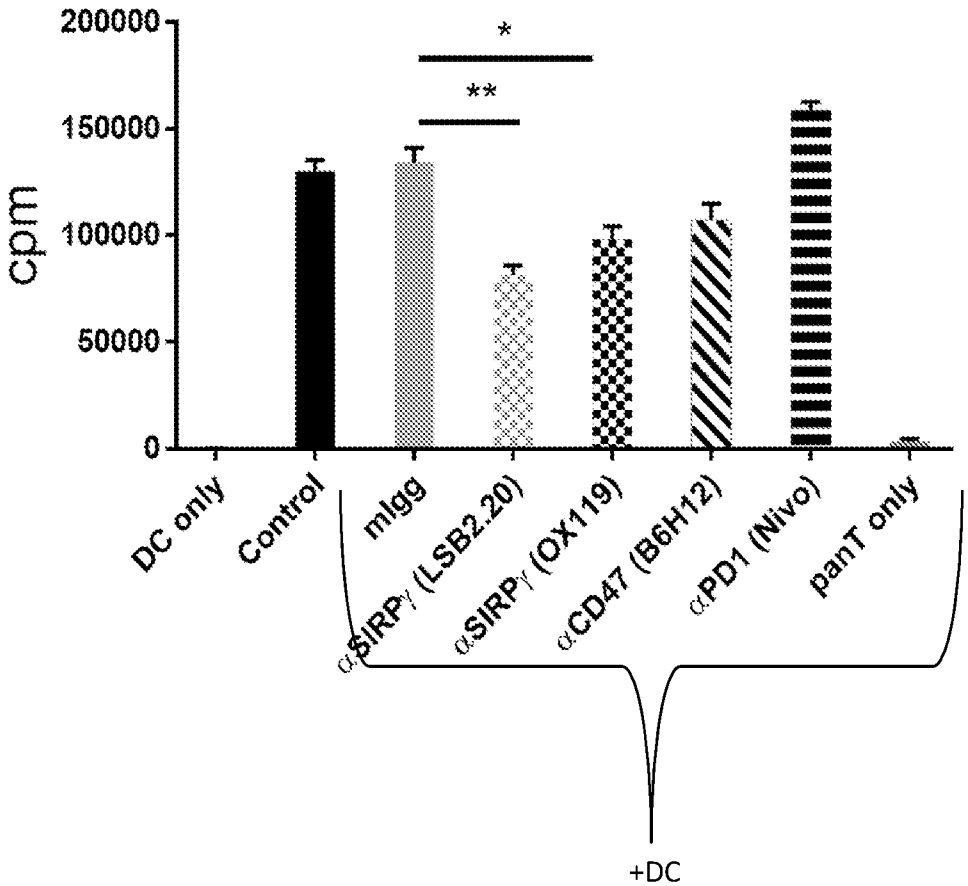


FIGURE 8E

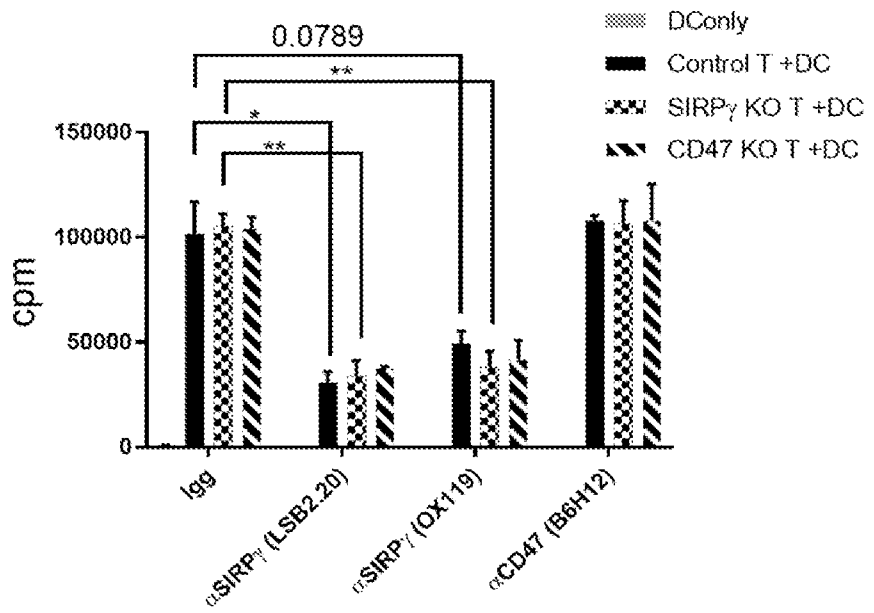


FIGURE 8F

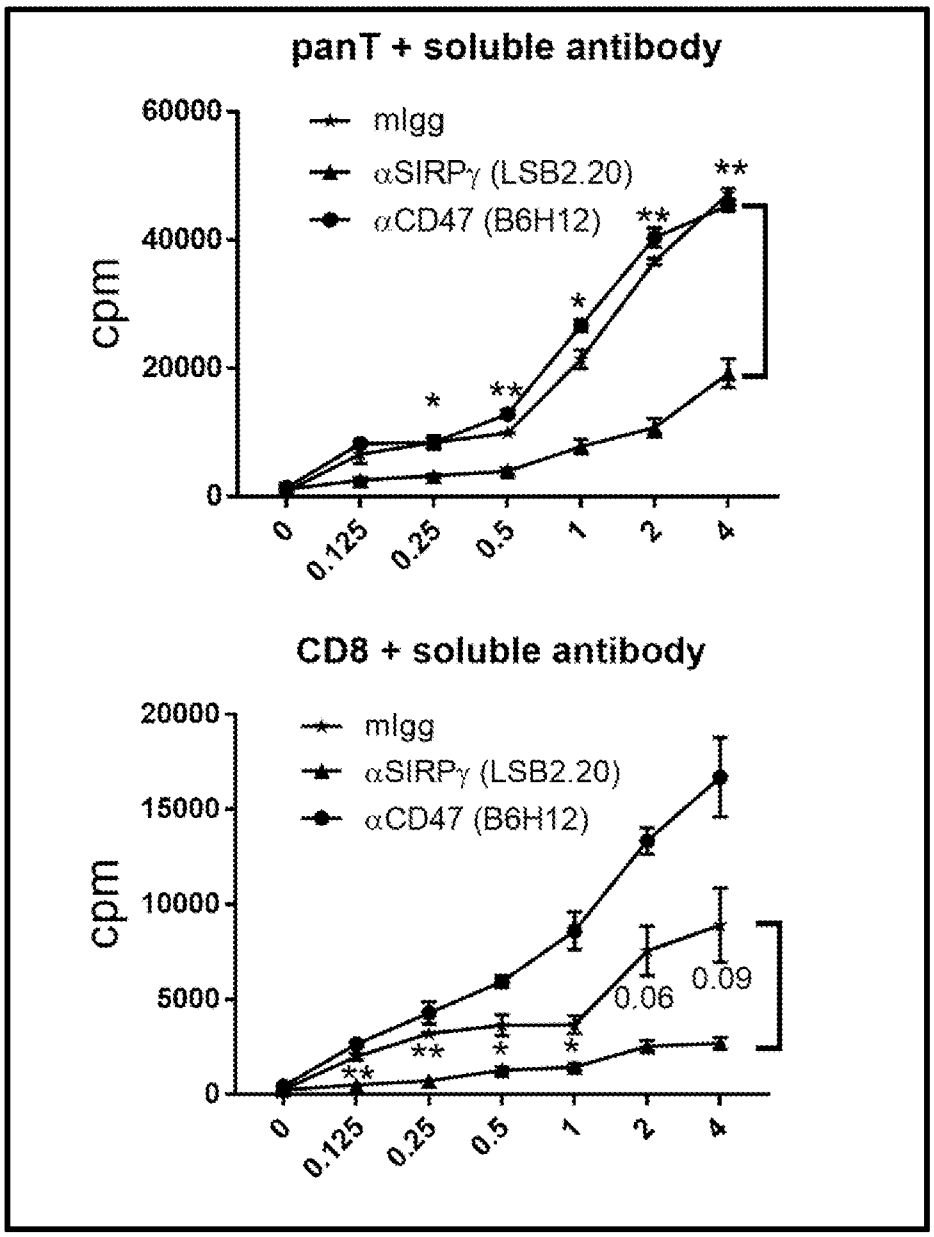


FIGURE 9A

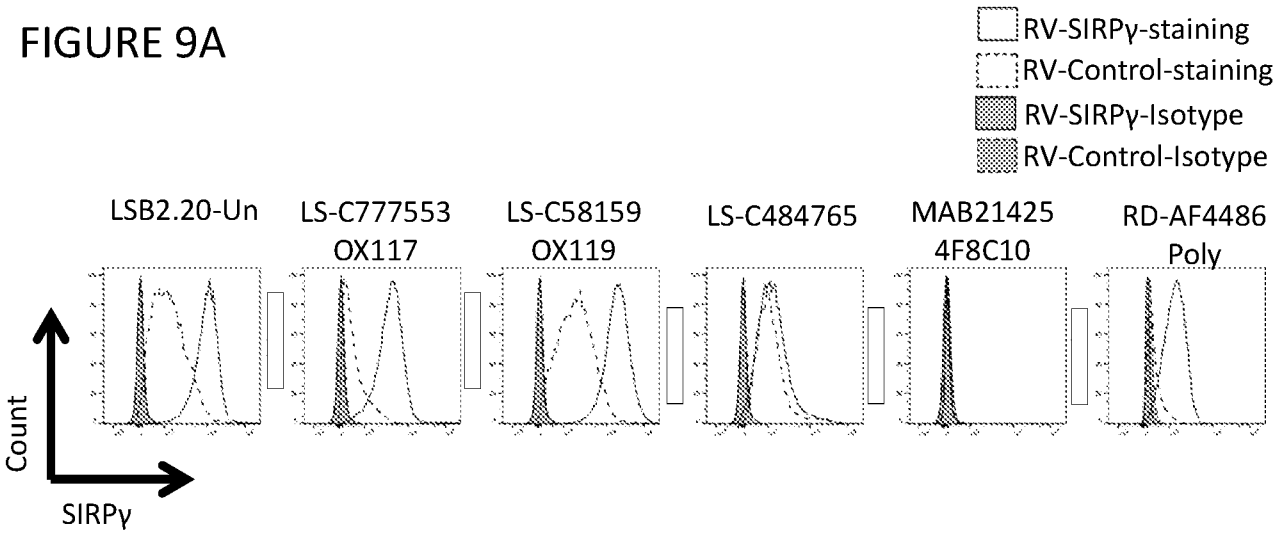


FIGURE 9B

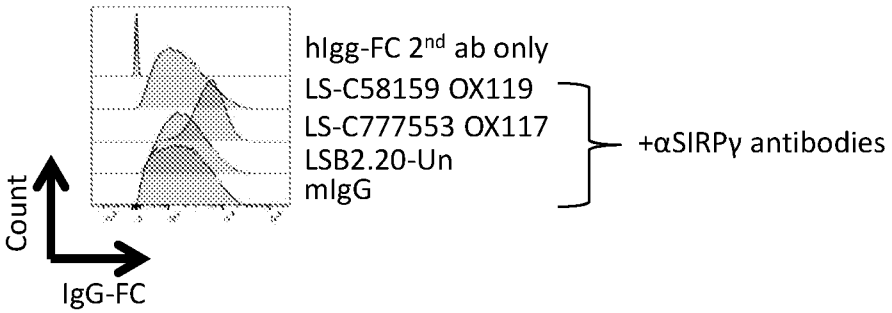


FIGURE 9C

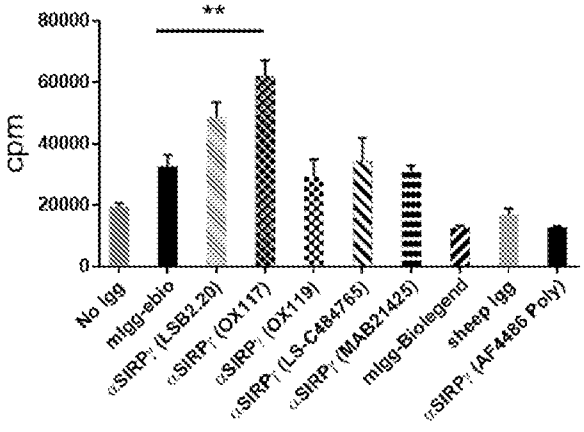


FIGURE 9D

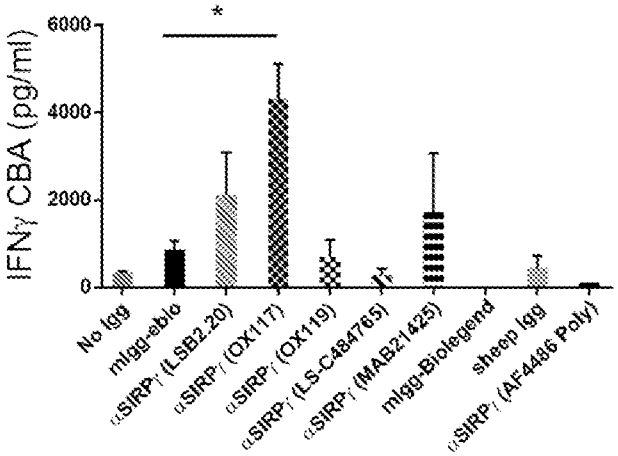
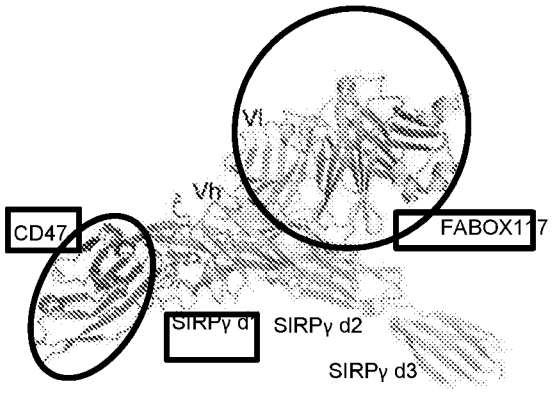


FIGURE 9E



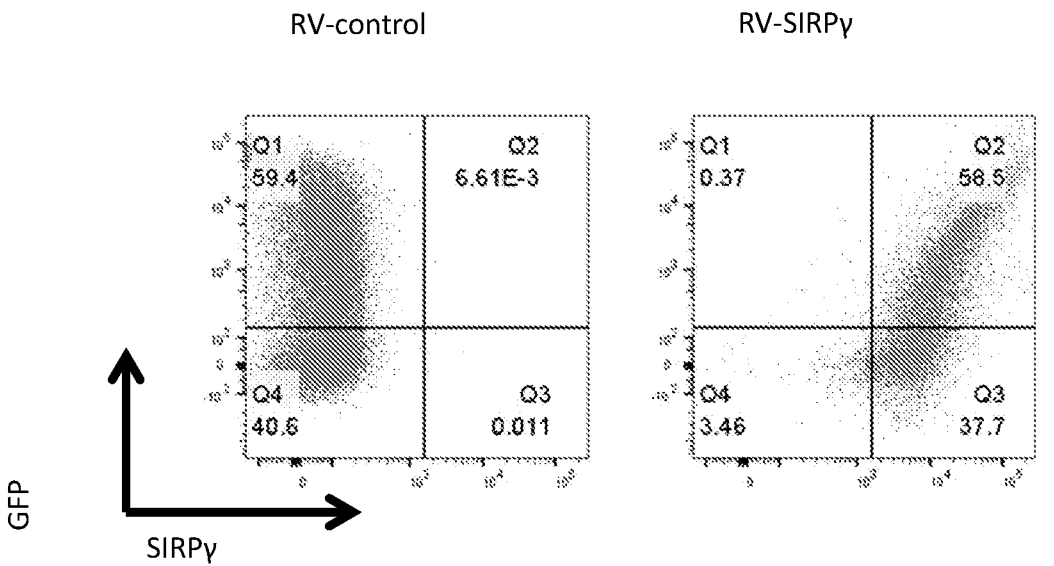
Taken from Nettleship et al., BMC Struct. Biol. 13: 13 (2013)

FIGURE 10

Cat#	Clone#	Species	Vendor	Binding to SIRPγ	Binding domain on SIRPγ	Blocking interaction between SIRPγ and CD47	Enhance T cell proliferation and cytokine secretion
LS-C484765	NA	Mouse	LS bio	Yes	ND	No	No
LS-C777553	OX117	Mouse	LS bio	Yes	D1/D2 of SIRPG	ND	Yes
LS-C58159	OX119	Mouse	LS bio	Yes	D1 of SIRPG	Yes	No
MAB21425	4F8C10	mouse IGG1	R&D aa29-360	No	ND	No	No
336602	LSB2.20	Mouse	Biolegend	Yes	D1 of SIRPG	Yes	No
AF4486	Polyclonal	SHEEP POLY	R&D 64-364	Yes	ND	No	No

ND = not determined

FIGURE 1A


RV-control
RV-SIRPγ
Q1
59.4
Q2
6.61E-3
Q4
40.6
Q3
0.011
Q1
0.37
Q2
56.5
Q4
3.46
Q3
37.7
GFP
SIRPγ