Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 3087044 A1 2019/08/01

(21) 3 087 044

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2019/01/28
(87) Date publication PCT/PCT Publication Date: 2019/08/01
(85) Entrée phase nationale/National Entry: 2020/06/24
(86) N° demande PCT/PCT Application No.: US 2019/015434
(87) N° publication PCT/PCT Publication No.: 2019/148109
(30) Priorité/Priority: 2018/01/26 (US62/622,547)

(51) Cl.Int./Int.Cl. *A01N 43/04* (2006.01), *A01N 63/00* (2020.01), *A01N 65/00* (2009.01)

(71) Demandeur/Applicant:
UNIVERSITY OF PITTSBURGH - OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION, US

(72) Inventeurs/Inventors:
DELGOFFE, GREG M., US;
RIVADENEIRA, DAYANA, US;
SAMPATH, PADMAVATHI, US;
THORNE, STEPHEN H., US

(74) Agent: SMART & BIGGAR LLP

(54) Titre : EXPRESSION DE MODULATEURS METABOLIQUES DANS UN MICROENVIRONNEMENT TUMORAL POUR AMELIORER UNE THERAPIE TUMORALE

(54) Title: EXPRESSION OF METABOLIC MODULATORS IN TUMOR MICROENVIRONMENT TO IMPROVE TUMOR THERAPY

(57) Abrégé/Abstract:
Recombinant oncolytic viruses (OVs) that express one or more metabolic modulator proteins, such as an adipokine (e.g., leptin or chemerin), insulin, and/or IGF- 1, and methods of their use to treat cancer, for example in immunotherapy anti-cancer treatments. In some examples, such recombinant OVs and methods increase T cell infiltration into the tumor or tumor microenvironment.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
01 August 2019 (01.08.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/148109 A1

(51) International Patent Classification:
A01N 43/04 (2006.01) *A01N 65/00* (2009.01)
A01N 63/00 (2006.01)

(21) International Application Number:
PCT/US2019/015434

(22) International Filing Date:
28 January 2019 (28.01.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/622,547 26 January 2018 (26.01.2018) US

(71) Applicant: **UNIVERSITY OF PITTSBURGH - OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION** [US/US]; 1st Floor Gardner Steel Conference Center, 130 Thackeray Avenue, Pittsburgh, PA 15260 (US).

(72) Inventors; and
(71) Applicants *(for US only)*: **DELGOFFE, Greg, M.** [US/US]; 5560 Bartlett St. Unit 14, Pittsburgh, PA 15217 (US). **RIVADENEIRA, Dayana** [US/US]; 616 South Dallas Ave., Pittsburgh, PA 15217 (US). **SAMPATH, Padmavathi** [US/US]; 6902 McClure Ave., Pittsburgh, PA 15218 (US). **THORNE, Stephen, H.** [US/US]; 610 Whispering Pines Dr., Pittsburgh, PA 15238 (US).

(74) Agent: **RYBAK, Sheree, Lynn**; Klarquist Sparkman, LLP, One World Trade Center, Suite 1600, 121 SW Salmon Street, Portland, OR 97204 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2019/148109 A1

(54) Title: EXPRESSION OF METABOLIC MODULATORS IN TUMOR MICROENVIRONMENT TO IMPROVE TUMOR THERAPY

(57) Abstract: Recombinant oncolytic viruses (OVs) that express one or more metabolic modulator proteins, such as an adipokine (e.g., leptin or chemerin), insulin, and/or IGF- 1, and methods of their use to treat cancer, for example in immunotherapy anti-cancer treatments. In some examples, such recombinant OVs and methods increase T cell infiltration into the tumor or tumor microenvironment.

EXPRESSION OF METABOLIC MODULATORS IN TUMOR MICROENVIRONMENT TO IMPROVE TUMOR THERAPY

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. provisional application No. 62/622,547 filed January 26, 2018, herein incorporated by reference in its entirety.

FIELD

The preset disclosure provides recombinant oncolytic viruses (OVs) that express one or more metabolic modulator proteins, such as leptin, kits including the same, and methods of their use to treat cancer.

BACKGROUND

The successes associated with immunotherapy as a cancer treatment have resulted in a major shift in both cancer research and clinical practice, with a dominant focus on understanding and modulating immune activity at the tumor site. However, the reality of single agent immunotherapies is that the majority of patients will not experience long-term durable benefits. This resistance likely occurs for multiple reasons, but one is a failure to recruit T cells to the tumor and other, more dominant immunosuppressive mechanisms which limit T cell function in the tumor microenvironment (Sharma *et al.*, Cell 2017; 168(4):707-23). Therefore, there is the need for new therapeutic modalities that could overcome these resistance mechanisms.

Leptin is a canonical adipokine with potent metabolic reprogramming functions such as the promotion of glucose and fatty oxidation as well as mitochondrial biogenesis. However, to date the study of leptin in immunity has not been ascertained therapeutically, much less in the context of cancer therapy.

SUMMARY

The infiltrate of aggressive melanomas induced by oncolytic *Vaccinia* virus is provided herein. It is shown that while oncolytic viruses promote the infiltration of a robust tumor infiltrate, it is ultimately ineffective at promoting complete responses, due in part to metabolic insufficiency. The utility of leptin as a tool to overcome the observed metabolic insufficiency by promoting the metabolic reprogramming of tumor-infiltrating T cells is demonstrated, in some embodiments of this disclosure. Using a novel melanoma model in which leptin is locally elevated in the tumor microenvironment, potent T cell activation and tumor control that was linked to metabolic

reprogramming was observed. Further, the *Vaccinia* virus used was engineered to genetically express and deliver leptin to the tumor microenvironment. This therapy resulted in complete therapeutic responses compared to wild type virus. Leptin expressing vaccinia virus simultaneously lyses tumor cells, leading to stimulation of new T cell infiltration, while also metabolically supporting the activity of that infiltrate through the local secretion of leptin.

Provided herein, in some embodiments, are recombinant oncolytic viruses (OVs) that can include a nucleic acid molecule that can code for one or more proteins capable of inducing metabolic reprogramming of T cells into, *e.g.,* resulting in enhanced infiltration of T cells in tumors, or are capable of modulating an anti-tumor immune response in the tumor microenvironment. For instance, in some cases, the OVs can include one or more nucleic acid molecules that can code for one or more metabolic modulating proteins (*e.g.*, hormones such as an adipokine (*e.g.*, leptin, adiponectin, apelin, chemerin, interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1), plasminogen activator inhibitor-1 (PAI-1), retinol binding protein 4 (RBP4), tumor necrosis factor-alpha (TNFα), visfatin, omentin, vaspin, progranulin or CTRP-4), insulin, and/or insulin-like growth factor 1 (IGF-1)). In some examples, the nucleic acid can be operably linked to a promoter. Examples of OVs include a herpes simplex virus (HSV) (such as T-VEC), vaccinia virus (such as Western Reserve strain), adenovirus, poxvirus, reovirus, poliovirus, coxsackie virus, measles virus, vesicular stomatitis virus (VSV), Seneca valley virus, ECHO virus, Newcastle disease virus, chicken anemia virus, or parovirus. In some examples, the recombinant OV expresses a recombinant protein having at least 80%, at least 90%, or at least 95% sequence identity to SEQ ID NO: 2, 4, 6, or 8. In some examples the recombinant OV includes a nucleic acid molecule encoding a metabolic modulating protein having at least 80%, at least 90%, or at least 95% sequence identity to SEQ ID NO:1, 3, 5 or 7. In some examples the metabolic modulating protein(s) expressed by the recombinant OV is part of a fusion protein, such as a fusion protein that includes two portions, (1) a metabolic modulating protein and (2) second protein (such as a cytokine (*e.g.*, IL-2 or IL-15), chemokine, an interferon, an interleukin, a lymphokine, and/or a tumor necrosis factor). In one example, the fusion protein has the metabolic modulating protein at its N-terminus, and a cytokine at its C-terminus. In some examples, the two portions of the fusion protein are joined by a linker (such as a GSG peptide). In a specific example, the recombinant OV expresses a fusion protein that includes leptin-linker-IL-2, leptin-IL-2, leptin-linker-IL-15, or leptin-IL-15.

Also provided are methods of treating a tumor in a subject, increasing T cell infiltration into a tumor or tumor microenvironment, or both. In some examples, such methods include administering a therapeutically effective amount of one or more recombinant OVs disclosed herein

to the subject, thereby treating the tumor. In some examples, such methods include administering a therapeutically effective amount of one or more metabolic modulating proteins (such as leptin, insulin, chemerin, and/or insulin-like growth factor 1) or nucleic acid molecules encoding the protein(s) to the subject, thereby treating the tumor. In some examples, the tumor is a cancer, such as a cancer of the lung, breast, prostate, liver, pancreas, skin, colon, head and neck, kidney, cervix, or ovary. In a specific example, the cancer is melanoma. In a specific example, the cancer is breast cancer. Such methods can further include administering a therapeutically effect amount of one or more further therapies, such as additional anti-cancer agents, such as chemotherapy, radiotherapy, a biologic, surgery, or combinations thereof. In one example, the anti-cancer agent includes one or more immunomodulatory agents, such as, an antagonist of PD-1, an antagonist of PD-L1, a CTLA4 antagonist, and a T cell agonist (such as an agonist of 4-1BB, an agonist of OX40, an agonist of glucocorticoid-induced tumor necrosis factor (TNF) receptor (GITR)), or combinations thereof. In one example, the anti-cancer agent includes a T cell agonist, such as an agonist of 4-1BB, an agonist of OX40, or an agonist of GITR (such as a monoclonal antibody (mAb) specific for an immune check point protein, such as one of the proteins listed above, a ligand of one of these proteins, or an aptamer of one of these proteins).

Also provided are kits and compositions that can include (1) one or more recombinant OVs provided herein, and (2) one or more of an immunomodulatory agent (*e.g.*, an immune checkpoint inhibitor), such as an antagonist of PD-1, an antagonist of PD-L1, a CTLA4 antagonist, and a T cell agonist (such as an agonist of 4-1BB, an agonist of OX40, or an agonist of GITR). The components of a kit can be in separate containers. In some examples, the kit includes (1) one or more recombinant OVs provided herein, and (2) a T cell agonist (such as an agonist of 4-1BB, an agonist of OX40, an agonist of GITR, or a combination thereof). In some examples, the composition includes (1) one or more recombinant OVs provided herein, and (2) one or more a T cell agonists (such as an agonist of 4-1BB, an agonist of OX40, or an agonist of GITR). Such a composition can further include a pharmaceutically acceptable carrier. In some examples, such as composition is lyophilized. In some examples, such as composition is present in a container, such as a glass or plastic container.

The foregoing and other objects and features of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1C. Generation of Clone 24 from Ptenf/fBrafLSL-V600ETyrCre.ERT2 mice, and depletion of CD8+ T cells from CL24lep or CL24hygro bearing mice. (A) C57BL/6J mice were from mice injected with CL24. Representative flow cytogram and tabulated flow cytometric data for CD8+ T cells from LN and TIL. (B) C57BL/6J mice were from mice injected with CL24. Tumors were then treated with anti-PD1 every other day and tumor growth monitored. Representative flow cytogram of CD8 and CD4 staining in LN and TIL. (C) C57BL/6J mice were treated every other day with anti-CD8 (200ug). At day 6 mice were injected with either CL24hygro or CL24leptin.

FIGS. 2A-2C. Oncolytic Vaccinia virus has potent immunostimulatory activity that can be enhanced through engineering leptin expression. (A) C57BL/6J mice were injected subdermally with CL24 cells. 5-7 days after tumor cell injection tumors were treated intratumorally with PBS, control *Vaccinia* virus ($VV^{control}$) at $2.5x10^6$ PFU and tumor growth monitored. Each line represents an individual mouse. (B) Single-cell RNA-seq data for 4000 cells CD45+ sorted cells treated as in (A). Cells were extracted on day 7. Data was generated by unsupervised clustering through Seurat program. (C) t-SNE analysis of PBS and VV^{ctrl} treated mice. Data represents n=2 per condition.

FIGS. 3A-3C. Single cell RNA sequencing analysis from TIL treated with oncolytic vaccinia virus. (A, B) Feature plots of genes defining different lymphocyte populations. Intensity of purple color indicated the normalized level of gene expression. (C) Transcriptome from CD45+ TIL after PBS, VVcontrol, or VV^{leptin} treatment (n=2 per condition) clustered using Seurat (SLM clustering). Each column represent a cell with the give genes most differentially expressed between each cluster.

FIGS. 4A-4D. The leptin receptor is upregulated in tumor infiltrating T cells and leptin is capable of metabolic reprogramming C57BL/6J mice were injected subdermally with CL24 cells. 5-7 days after tumor cell injection tumors were treated intratumorally with PBS, control *Vaccinia* virus (VV^{ctrll}). (A) CD8 and CD4 expression analysis on LN and TIL from mice treated as in (A). Representative flow cytogram of CD8 and CD4 staining in LN and TIL and tabulated flow cytometric data. (B) Expression of inhibitory molecules PD1 and TIM3 from mice treated as in (A). Representative flow cytogram of PD1 and Tim3 staining in LN and TIL and tabulated flow cytometric data. (C) Representative histogram PD1 expression on CD8+ T cells and tabulated data. (D) Mitochondrial content analyzed by mitotracker staining in CD8+ T cells from mice treated as in (A). Representative flow cytogram of mitotracker against 2NBDG staining in LN and TIL and tabulated flow cytometric data. Data represents at least 3 independent experiments *p <0.05, **p <0.01, ***p <0.001 by paired t-test. Error bars indicate s.e.m.

FIGS. 5A-5E. The leptin receptor is upregulated in tumor infiltrating T cells and leptin is capable of metabolic reprogramming. (A) Representative OCR trace and tabulated OCR and Spare Respiratory Capacity (SRC) of CD8+ T cells activated with 3ug/m: immobilized anti-CD3 in the presence of antiCD28 (2ug/mL) for 24h. Cells were treated with 0.0, 0.1, and 1.0nM of mouse recombinant leptin for 24h. (B) Representative ECAR trace for cells treated as (A). (C) Mitochondrial content analyzed by mitotracker staining and glucose uptake by 2NBDG staining in CD8+ T cells from mice treated as in (A). Representative flow cytogram of mitotracker against 2NBDG staining in LN and TIL and tabulated flow cytometric data. (D) Leptin receptor staining of CD8+ T cells from mouse lymph nodes (LN) and tumor infiltrating CD8+ T cells (TIL) from tumor bearing mice. (E) Leptin receptor expression staining of PD1 and Tim3 in CD8+ T cells in LN and TIL. Data represents at least 3 independent experiments *p <0.05 by unpaired t-test. Error bars indicate s.e.m. Data represents at least 3 independent experiments *p <0.05, **p <0.01, ***p <0.001 by paired t-test. Error bars indicate s.e.m.

FIGS. 6A-6F. Characterization of leptin overexpression in tumor cells and effects in the tumor microenvironment. (A) Immunoblot analysis of mouse leptin protein expression of CL24 cell line stably transduced with control plasmid ($CL24^{hygro}$) and mouse leptin gene plasmid ($CL24^{leptin}$). (B) ELISA analysis of leptin in the media of cells transduced with control plasmid and leptin gene. (C) In vitro growth analysis between $CL24^{hygro}$ and $CL24^{leptin}$ cell lines. (H) NK1.1 and B220 analysis for natural killer cells and B cells respectively on LN and TIL from mice injected with $CL24^{hygro}$ and $CL24^{leptin}$. (D) C57BL/6J mice were treated every other day with anti-CD8 (200ug). At day 6 mice were injected with either $CL24^{hygro}$ or $CL24^{leptin}$ and tumor growth was monitored. CD8 and CD4 expression analysis in lymph node (LN). (E) Representative flow cytogram for NK1.1 and B220 staining in LN and TIL and tabulated flow cytometric data are shown. (F) Representative flow cytogram and tabulated flow cytometric data for CD8+ T cells from LN and TIL from mice injected with $CL24^{hygro}$ and $CL24^{leptin}$ analyzed for pSTAT3, pAKT and pp38MAPK expression. Data represents at least 3 independent experiments *p <0.05, **p <0.01, ***p <0.001 by two-way ANOVA. Error bars indicate s.e.m.

FIGS. 7A-7G. Expression of leptin in cancer cells results in immune-mediated tumor control and metabolically improves the function of tumor infiltrating lymphocytes. (A) CL24hygro and CL24leptin were injected subdermally on C57BL/6J mice and tumor growth monitored. Each line represents an individual mouse. (B) Survival plot of mice treated as in (A). (C) C57BL/6J mice were treated every other day with anti-CD8 (200ug). At day 6 mice were injected with either CL24hygro or CL24leptin and tumor growth was monitored. (D) CD8 and CD4 expression analysis on LN and TIL from mice injected with CL24hygro and CL24leptin. Representative flow cytogram

of CD8 and CD4 staining in LN and TIL and tabulated flow cytometric data. (E) Representative flow cytogram of LN and TIL from mice injected with CL24hygro and CL24leptin cells were stimulated overnight with PMA and ionomycin for cytokine production analysis by staining for IFNγ and TNFα of CD8+ T cells. Tabulated flow cytometric data are shown. (F) Representative flow cytogram and tabulated flow cytometric data for CD8+ T cells from LN and TIL from mice injected with CL24hygro and CL24leptin analyzed for Ki67 expression and metabolic markers Mitotracker FM staining and 2NBDG uptake (G). Data represents at least 3 independent experiments *p <0.05, **p <0.01, ***p <0.001 by paired t-test. Error bars indicate s.e.m.

FIGS. 8A-8F. Leptin-engineered oncolytic *Vaccinia* virus induces leptin secretion *in vitro* and *in vivo* without affecting infectivity. (A) Immunoblot analysis of mouse leptin protein expression of CL24 cell line treated with VVleptin at 2.5x106 PFU in vitro 24h and 48h. (B) ELISA analysis of leptin in the media of CL24 cells treated with VVleptin. (C) ELISA analysis of leptin in interstitial fluid of tumors treated with VVcontrol or VVleptin. Interstitial fluid from white adipose tissue (WA) used as control. Data represents at least 3 independent experiments *p <0.05 by two-way ANOVA. Error bars indicate s.e.m. (D) C57BL/6J mice were injected subdermally with CL24 cells. 5-7 days after tumor cell injection tumors were treated intratumorally with PBS, VVcontrol, or VVleptin 24h later mice were injected with luciferin (30mg/ml) IP for 10min and conducted In Vivo Bioluminescence Imaging. (E) C57BL/6J mice were injected subdermally with CL24 cells. 5-7 days after tumor cell injection tumors were treated intratumorally with PBS, VVcontrol, or VVleptin. On day 10 after treatment lymphocytes were isolated from TIL. Representative flow cytogram and tabulated flow cytometric data for leptin receptor (leptinR) expression. Error bars indicate s.e.m. (F) Representative flow cytogram and tabulated flow cytometric data for CD4+ Foxp3+T cells (T regulatory cells) from LN and TIL from mice injected with PBS, VVcontrol, or VVleptin. Data represents at least 3 independent experiments *p <0.05, **p <0.01, ***p <0.001 by two-way ANOVA. Error bars indicate s.e.m.

FIGS. 9A-9E. Leptin-engineered Vaccinia virus promotes the accumulation of memory-like T cell clones. (A) C57BL/6J mice were injected subdermally with CL24 cells. 5-7 days after tumor cell injection tumors were treated intratumorally with PBS, VVcontrol, or VVleptin at 2.5x106 PFU and tumor growth monitored. Each line represents an individual mouse. On day 10 after treatment lymphocytes were isolated from TIL and LN. (B)) Representative flow cytogram and tabulated flow cytometric data for CD8 and CD4 expression. (C-D) TCR sequencing of genomic DNA extracted from CL24 bearing mice treated intratumorally with PBS, VVcontrol, or VVleptin at 2.5x106 PFU. (n=5 each treatment) (C) Total templates and productive rearrangements. (D) Analysis of sample clonality and mean frequency. (E) Mice were treated as in

(A). Representative hystograms and tabulated flow cytometric data of CD8+ T cells stained for CD127 expression. Data represents at least 3 independent experiments *p <0.05, **p <0.01, ***p <0.001 by two-way ANOVA. Error bars indicate s.e.m.

FIGS. 10A-10C. Leptin qualitatively improves the oncolytic virally induced T cell infiltrate through metabolic reprogramming. C57BL/6J mice were injected subdermally with CL24 cells. 5-7 days after tumor cell injection tumors were treated intratumorally with PBS, $VV^{control}$, or VV^{leptin}. On day 10 after treatment lymphocytes were isolated from TIL and LN. (A) Representative hystograms and tabulated flow cytometric data of CD8+ T cells isolated from LN and TIL were analyzed for mitochondrial protein VDAC. (B) Isolated lymphocytes were stimulated overnight with PMA and ionomycin. Representative flow cytogram and tabulated flow cytometric data for cytokine production analysis by staining for IFNγ and TNFα. (C) Representative hystograms and tabulated flow cytometric data for CD8+ T cells from LN and TIL from mice treated as in (A) analyzed for Ki67 expression. Data represents at least 3 independent experiments *p <0.05, **p <0.01, ***p <0.001 by two-way ANOVA. Error bars indicate s.e.m.

SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. The sequence listing filed here with, generated on January 28, 2018, 32 kb, is herein incorporated by reference.

SEQ ID NOS: 1 and 2: Exemplary human leptin nucleic acid and protein sequence, respectively (see GenBank® Accession Nos. NM_000230.2 and NP_000221.1, respectively). Coding sequence is nt 58-561 of SEQ ID NO: 1. Signal peptide is aa 1-21, and the mature peptide is aa 22-167 of SEQ ID NO: 2.

SEQ ID NOS: 3 and 4: Exemplary human insulin nucleic acid and protein sequences, respectively (see GenBank® Accession Nos. AH002844.2 and AAA59172.1, respectively). Coding sequence is nt 2424..2610,3397..3542 of SEQ ID NO: 3. Signal peptide is aa 1-24, and the mature peptide is aa 25-110 of SEQ ID NO: 4.

SEQ ID NOS: 5 and 6: Exemplary human chemerin (also known as retinoic acid receptor responder protein 2) nucleic acid and protein sequences, respectively (see GenBank® Accession Nos. NM_002889.3 and NP_002880.1, respectively). Coding sequence is nt 118-609 of SEQ ID NO: 5. Signal peptide is aa 1-20, and the mature peptide is aa 21-157 of SEQ ID NO: 6.

SEQ ID NOS: 7 and 8: Exemplary human insulin like growth factor 1 (IGF-1) nucleic acid and protein sequences, respectively (see GenBank® Accession Nos. NM_001111283.2 and NP_001104753.1, respectively). Coding sequence is nt 265-741 of SEQ ID NO: 7. Signal peptide is aa 1-21, and the mature peptide is aa 49-118 of SEQ ID NO: 8.

DETAILED DESCRIPTION

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology can be found in Benjamin Lewin, *Genes VII*, published by Oxford University Press, 1999; Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994; and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995; and other similar references.

As used herein, the singular forms "a," "an," and "the," may refer to both the singular as well as plural, unless the context clearly indicates otherwise. As used herein, the term "comprises" can mean "includes." Thus, "comprising a nucleic acid molecule" may mean "including a nucleic acid molecule" without excluding other elements. It is further to be understood that any and all base sizes given for nucleic acids are approximate, and are provided for descriptive purposes, unless otherwise indicated. Although many methods and materials similar or equivalent to those described herein can be used, particular suitable methods and materials are described below. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. All references, including patent applications and patents, and sequences associated with the GenBank® Accession Numbers listed (as of January 26, 2018) are herein incorporated by reference.

In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided:

Administration: To provide or give a subject an agent, such as a disclosed recombinant OV or other therapeutic agent (such as an anti-cancer agent), by any effective route. Exemplary routes of administration include, but are not limited to, injection (such as subcutaneous, subdermal, intramuscular, intradermal, intraperitoneal, intratumoral, and intravenous), transdermal, intranasal, oral, vaginal, rectal, and inhalation routes.

Cancer: A malignant tumor characterized by abnormal or uncontrolled cell growth. Other features often associated with cancer include metastasis, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels and

suppression or aggravation of inflammatory or immunological response, invasion of surrounding or distant tissues or organs, such as lymph nodes, etc. "Metastatic disease" refers to cancer cells that have left the original tumor site and migrate to other parts of the body for example *via* the bloodstream or lymph system.

Chemerin: (*e.g.*, OMIM 601973): Also known as retinoic acid receptor responder protein 2 (RARRES2). Chemerin is a chemoattractant protein that acts as a ligand for the G protein-coupled receptor CMKLR1. Due to its role in adipocyte differentiation and glucose uptake, chemerin is classified as an adipokine. Human chemerin is encoded by the *RARRES2* gene on chromosome 7, and the native protein is about 14kDa, which is secreted in an inactive form as prochemerin and is activated through cleavage of the C-terminus by inflammatory and coagulation serine proteases. Chemerin sequences are publically available, for example from the GenBank® sequence database (*e.g.*, Accession Nos. NP_002880.1 and NP_001013445.1 provide exemplary chemerin protein sequences, while Accession Nos. NM_002889.3 and NM_001013427.1 provide exemplary chemerin nucleic acid sequences). One of ordinary skill in the art can identify additional chemerin nucleic acid and protein sequences, including chemerin variants, such as those having at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 98%, or at least 99% sequence identity to these GenBank® sequences, such as at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 98%, or at least 99% sequence identity to the peptide aa 21-157 of SEQ ID NO: 6.

Contact: Placement in direct physical association, including a solid or a liquid form. Contacting can occur *in vitro* or *ex vivo*, for example, by adding a reagent to a sample (such as one containing tumor cells), or *in vivo* by administering to a subject.

Effective amount (or therapeutically effective amount): The amount of an agent (such as recombinant OVs disclosed herein, as well as other anti-cancer agents) that is sufficient to effect beneficial or desired results.

An effective amount (also referred to as a therapeutically effective amount) may vary depending upon one or more of: the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The beneficial therapeutic effect can include enablement of diagnostic determinations; amelioration of a disease, symptom, disorder, or pathological condition; reducing or preventing the onset of a disease, symptom, disorder or condition; and generally counteracting a disease, symptom, disorder or pathological condition.

In one embodiment, an "effective amount" (*e.g.*, of a leptin, insulin, chemerin, or IGF-1 protein, recombinant OVs disclosed herein expressing such a protein) may be an amount sufficient to reduce the volume/size of a tumor, the weight of a tumor, the number/extent of metastases, reduce the volume/size of a metastasis, the weight of a metastasis, or combinations thereof, for example by at least about 10%, at least about 20%, at least about 25%, at least about 50%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, or at least about 99% (as compared to no administration of the therapeutic agent). In one embodiment, an "effective amount" (*e.g.*, of leptin, insulin, chemerin, or IGF-1 or a OV expressing such a protein) may be an amount sufficient to increase T cell infiltration, for example into a tumor or tumor microenvironment, by at least about 10%, at least about 20%, at least about 25%, at least about 50%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 99%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500%, or at least about 600% (as compared to no administration of the therapeutic agent).

Fusion (or chimeric) protein: A protein containing amino acid sequence from at least two different (heterologous) proteins or peptides. Fusion proteins can be generated, for example, by expression of a nucleic acid sequence engineered from nucleic acid sequences encoding at least a portion of two different (heterologous) proteins. To create a fusion protein, the nucleic acid sequences are typically in the same reading frame and contain no internal stop codons. In one example, a fusion protein including a metabolic modulating protein (such as an adipokine (*e.g.*, leptin or chemerin), insulin, or IGF-1), and a cytokine (such as IL-2 or IL-15) can be expressed by an OV provided herein.

Fusion proteins include a first portion and a second portion, which can be joined directly or via a linker (such as a peptide linker). In some examples, the first portion is a metabolic modulating protein (such as an adipokine (*e.g.*, leptin or chemerin), insulin, or IGF-1), and the second portion is a cytokine (such as IL-2 or IL-15). In some examples, the first portion is N-terminal and the second portion is C-terminal. In some examples, the first portion is C-terminal and the second portion is N-terminal.

Insulin: (*e.g.*, OMIM 176730): A hormone produced by beta cells of pancreatic islet cells. It regulated the metabolism of carbohydrates, fats and protein, for example by promoting the absorption of carbohydrates, especially glucose from the blood into liver, fat and skeletal muscle cells. Human insulin is encoded by the *Ins* gene on chromosome 11. Within vertebrates, the amino acid sequence of insulin is strongly conserved. Bovine insulin differs from human in only three amino acid residues, and porcine insulin in on. Insulin sequences are publically available, for

example from the GenBank® sequence database (*e.g.*, Accession Nos. AAA59172.1 and AAA41439.1 provide exemplary insulin protein sequences, while Accession Nos. AH002844.2 and V01242.1provide exemplary insulin nucleic acid sequences). One of ordinary skill in the art can identify additional insulin nucleic acid and protein sequences, including insulin variants, such as those having at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 98%, or at least 99% sequence identity to these GenBank® sequences, such as at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 98%, or at least 99% sequence identity to the peptide aa 25-110 of SEQ ID NO: 4.

Insulin-like growth factor 1 (IGF-1): (*e.g.*, OMIM 147440): Also called somatomedin C, is a hormone predominantly made by the liver, which is stimulated by growth hormone. Human IGF-1 is encoded by the *IGF1* gene on chromosome 12, and the native protein is 70 amino acids. IGF-1 sequences are publically available, for example from the GenBank® sequence database (*e.g.*, Accession Nos. NP_001104753.1 (mature peptide aa 49-118), and NP_034642.2 (mature peptide aa 49-116) provide exemplary IGF-1 protein sequences, while Accession Nos. NM_000618.4, NM_001111283.2 and NM_010512.5 provide exemplary IGF-1 nucleic acid sequences). One of ordinary skill in the art can identify additional IGF-1 nucleic acid and protein sequences, including IGF-1 variants, such as those having at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 98%, or at least 99% sequence identity to these GenBank® sequences, such as at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 98%, or at least 99% sequence identity to the peptide aa 49-118 of SEQ ID NO: 8.

Leptin: (*e.g.*, OMIM 164160): A hormone predominantly made by adipose cells, which helps to regulate energy balance by inhibiting hunger. Human leptin is encoded by the Lep gene on chromosome 7, and the native protein is 16kDa and 167 amino acids. Leptin sequences are publically available, for example from the GenBank® sequence database (*e.g.*, Accession Nos. NP_000221.1, NP_001003070.1, NP_999005.1, NP_037208.1, NP_032519.1, and NP_001036220.1 provide exemplary leptin protein sequences, while Accession Nos. NM_000230.2, NM_001003070.1, NM_213840.1 and NM_013076.3 provide exemplary leptin nucleic acid sequences). One of ordinary skill in the art can identify additional leptin nucleic acid and protein sequences, including leptin variants, such as those having at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 98%, or at least 99% sequence identity to these GenBank® sequences such as at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 98%, or at least 99% sequence identity to the peptide aa 22-167 of SEQ ID NO: 2.

Increase or Decrease: A statistically significant positive or negative change, respectively, in quantity from a control value (such as a value representing no therapeutic agent). An increase is

a positive change, such as an increase at least 50%, at least 100%, at least 200%, at least 300%, at least 400% or at least 500% as compared to the control value. A decrease is a negative change, such as a decrease of at least 20%, at least 25%, at least 50%, at least 75%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 100% decrease as compared to a control value. In some examples the decrease is less than 100%, such as a decrease of no more than 90%, no more than 95%, or no more than 99%.

Isolated: An "isolated" biological component (such as an OV, a nucleic acid molecule, or a protein) has been substantially separated, produced apart from, or purified away from other biological components in the cell or tissue of an organism in which the component occurs, such as other cells (*e.g.*, RBCs), chromosomal and extrachromosomal DNA and RNA, and proteins. Nucleic acids and proteins that have been "isolated" include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids and proteins. Isolated recombinant OVs in some examples are at least 50% pure, such as at least 75%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 100% pure.

Linker: A moiety or group of moieties that joins or connects two or more discrete separate peptide or proteins, such as monomer domains, for example to generate a fusion protein. In one example a linker is a substantially linear moiety. Exemplary linkers that can be used to generate the fusion proteins provided herein include but are not limited to: peptides, nucleic acid molecules, peptide nucleic acids, and optionally substituted alkylene moieties that have one or more oxygen atoms incorporated in the carbon backbone. A linker can be a portion of a native sequence, a variant thereof, or a synthetic sequence. Linkers can include naturally occurring amino acids, non-naturally occurring amino acids, or a combination of both. In one example a linker is composed of at least 5, at least 10, at least 15 or at least 20 amino acids, such as 5 to 10, 5 to 20, or 5 to 50 amino acids. In one example the linker is a poly alanine. The linker can be a flexible linker (*e.g.*, (GGGGS)n), rigid linker (*e.g.*, (EAAAK)n), or a cleavable linker (*e.g.*, disulfide, protease sensitive).

Metabolic modulator protein: A protein that can increase or decrease the metabolic activity of a cell, such as a T cell, such as a T cell in a subject with cancer. In one example, a metabolic modulator protein increases the metabolic activity of a T cell, such as a tumor infiltrating T cell. In some examples, the metabolic modulator protein increases the metabolic activity of a cell, such as a T cell, such as a tumor infiltrating T cell, by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 100%, at least about 200%,

at least about 300%, at least about 400%, at least about 500% or at least about 600%, for example relative to the absence of the metabolic modulator protein (such as the absence of administering an OV expressing a metabolic modulator protein). In some examples, the metabolic modulator protein increases the mitochondrial function of a cell, such as a T cell, such as a tumor infiltrating T cell, by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500% or at least about 600%, for example relative to the absence of the metabolic modulator protein (such as the absence of administering an OV expressing a metabolic modulator protein). In some examples, the metabolic modulator protein increases the oxidative phosphorylation by a cell, such as a T cell, such as a tumor infiltrating T cell, by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500% or at least about 600%, for example relative to the absence of the metabolic modulator protein (such as the absence of administering an OV expressing a metabolic modulator protein). In some examples, the metabolic modulator protein increases memory T cells by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500% or at least about 600%, for example relative to the absence of the metabolic modulator protein (such as the absence of administering an OV expressing a metabolic modulator protein). In some examples, the metabolic modulator protein increases T cell clonal expansion in a tumor (*e.g.*, cancer) by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500% or at least about 600%, for example relative to the absence of the metabolic modulator protein (such as the absence of administering an OV expressing a metabolic modulator protein). In some examples, combinations of one or more of these affects may be achieved.

In one example, the metabolic modulator protein is an adipokine. An adipokine can be a cytokine secreted by adipose tissue. Examples of adipokines include, but are not limited to: leptin, adiponectin, apelin, chemerin, interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1), plasminogen activator inhibitor-1 (PAI-1), retinol binding protein 4 (RBP4), tumor necrosis factor-

alpha (TNFα), visfatin, omentin, vaspin, progranulin and CTRP-4. In one example, the metabolic modulator protein is insulin. In one example, the metabolic modulator protein is IGF-1.

Oncolytic virus (OV): A virus that preferentially infects and kills cancer cells. OVs can access cells through binding to receptors on their surface or through fusion with the plasma membrane and establish a lytic cycle in tumors, while leaving normal tissue essentially unharmed. As the infected cancer cells are destroyed by oncolysis, they release new infectious virus particles or virions to help destroy the remaining tumor. Exemplary oncolytic viruses include but are not limited to herpes simplex virus (HSV), vaccinia virus, adenovirus, poxvirus, reovirus, poliovirus, coxsackie virus, measles virus, vesicular stomatitis virus (VSV), Seneca valley virus, ECHO virus, Newcastle disease virus, chicken anemia virus, and parovirus. Specific examples of oncolytic viruses include the ECHO-7 strain enterovirus RIGVIR, a genetically modified adenovirus named H101, and talimogene laherparepvec (T-VEC).

A **recombinant OV** is an OV that includes non-native sequences, such as a nucleic acid molecule encoding a metabolic modulating protein, such as one or more of leptin, insulin, chemerin, and insulin-like growth factor 1, as well as a fusion protein including such and a cytokine.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence (such as a leptin, insulin, chemerin, or insulin-like growth factor 1 coding sequence). Generally, operably linked sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers useful in this invention are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of a therapeutic agent, such as recombinant OV disclosed herein.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Promoter: An array of nucleic acid control sequences which direct transcription of a nucleic acid. A promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription.

Examples of promoters that can used with the disclosed recombinant OVs include, but are not limited to viral promoters, such as 7.5 promoter, SV40 promoter, CMV enhancer-promoter, and the CMV enhancer/β-actin promoter. Both constitutive and inducible promoters can be used (see *e.g.*, Bitter *et al.*, *Methods in Enzymology* 153:516-544, 1987). Also included are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the gene. Promoters produced by recombinant DNA or synthetic techniques can also be used to provide for transcription of the nucleic acid sequences.

Recombinant: A recombinant nucleic acid molecule is one that has a sequence that is not naturally occurring (*e.g.*, an OV with a non-native sequence, such as a mammalian leptin, insulin, chemerin, or insulin-like growth factor 1 coding sequence) or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination can be accomplished by routine methods, such as chemical synthesis or by the artificial manipulation of isolated segments of nucleic acids, such as by genetic engineering techniques. Similarly, a recombinant protein is one encoded for by a recombinant nucleic acid molecule. Similarly, a recombinant or transgenic cell is one that contains a recombinant nucleic acid molecule (such as a recombinant OV) and expresses a recombinant protein.

Sequence identity: The similarity between amino acid (or nucleotide) sequences is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar the two sequences are.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman and Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988; Higgins and Sharp, *Gene* 73:237, 1988; Higgins and Sharp, *CABIOS* 5:151, 1989; Corpet *et al.*, *Nucleic Acids Research* 16:10881, 1988; and Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988. Altschul *et al.*, *Nature Genet.* 6:119, 1994, presents a detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, *J. Mol. Biol.* 215:403, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. A description of how to determine sequence identity using this program is available on the NCBI website on the internet.

Variants of a native leptin, insulin, chemerin, or IGF-1 protein or coding sequences are typically characterized by possession of at least about 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity counted over the full length alignment with the amino acid sequence using the NCBI Blast 2.0, gapped blastp set to default parameters. For comparisons of amino acid sequences of greater than about 30 amino acids, the Blast 2 sequences function is employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 95%, at least 98%, or at least 99% sequence identity. When less than the entire sequence is being compared for sequence identity, homologs and variants will typically possess at least 80% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% or at least 95% depending on their similarity to the reference sequence. Methods for determining sequence identity over such short windows are available at the NCBI website on the internet. One of skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologs could be obtained that fall outside of the ranges provided.

Thus, a variant leptin, insulin, chemerin, or IGF-1 protein or nucleic acid sequence can have at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to any of the sequences shown in the GenBank® Accession Nos. provided herein (such as SEQ ID NO: 1, 2, 3, 4, 5, 6, 7 , or 8).

Subject: A vertebrate, such as a mammal, for example a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. In one embodiment, the subject is a non-human mammalian subject, such as a monkey or other non-human primate, mouse, rat, rabbit, pig, goat, sheep, dog, cat, horse, or cow. In some examples, the subject has a tumor, such as a cancer, that can be treated using the recombinant OVs disclosed

herein. In some examples, the subject is a laboratory animal/organism, such as a mouse, rabbit, or rat.

T cells: White blood cells containing a T cell receptor on their cell surface, which play a role in cell-mediated immunity.

Therapeutic agent: Refers to one or more molecules or compounds that confer some beneficial effect upon administration to a subject. The beneficial therapeutic effect can include enablement of diagnostic determinations; amelioration of a disease, symptom, disorder, or pathological condition; reducing or preventing the onset of a disease, symptom, disorder or condition; and generally counteracting a disease, symptom, disorder or pathological condition.

Transduced and Transformed: A virus or vector "transduces" a cell when it transfers nucleic acid into the cell. A cell is "transformed" or "transfected" by a nucleic acid transduced into the cell when the nucleic acid molecule becomes stably replicated by the cell, either by incorporation of the nucleic acid into the cellular genome, or by episomal replication.

Numerous methods of transfection are known to those skilled in the art, such as: chemical methods (*e.g.*, calcium-phosphate transfection), physical methods (*e.g.*, electroporation, microinjection, particle bombardment), fusion (*e.g.*, liposomes), receptor-mediated endocytosis (*e.g.*, DNA-protein complexes, viral envelope/capsid-DNA complexes) and by biological infection by viruses such as recombinant viruses {Wolff, J. A., ed, Gene Therapeutics, Birkhauser, Boston, USA (1994)}.

Transgene: An exogenous gene supplied by a vector, such as a recombinant OV. In one example, a transgene includes one or more leptin, insulin, chemerin, or IGF-1 coding sequences.

Treating, Treatment, and Therapy: Any success or indicia of success in the attenuation or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement, remission, diminishing of symptoms or making the condition more tolerable to the patient, slowing in the rate of degeneration or decline, making the final point of degeneration less debilitating, improving a subject's physical or mental well-being, or prolonging the length of survival. The treatment may be assessed by objective or subjective parameters; including the results of a physical examination, blood and other clinical tests (such as imaging), and the like. In some examples, treatment with the disclosed methods results in a decrease in the number, volume, and/or weight of a tumor and/or metastases.

Tumor, neoplasia, malignancy or cancer: A neoplasm is an abnormal growth of tissue or cells which results from excessive cell division. Neoplastic growth can produce a tumor. The amount of a tumor in an individual is the "**tumor burden**" which can be measured as the number, volume, or weight of the tumor. A "**non-cancerous tissue**" is a tissue from the same organ

wherein the malignant neoplasm formed, but does not have the characteristic pathology of the neoplasm. Generally, noncancerous tissue appears histologically normal. A "**normal tissue**" is tissue from an organ, wherein the organ is not affected by cancer or another disease or disorder of that organ. A "**cancer-free**" subject has not been diagnosed with a cancer of that organ and does not have detectable cancer.

Exemplary tumors, such as cancers, that can be treated using the disclosed recombinant OVs include solid tumors, such as breast carcinomas (*e.g.* lobular and duct carcinomas, such as a triple negative breast cancer), sarcomas, carcinomas of the lung (*e.g.*, non-small cell carcinoma, large cell carcinoma, squamous carcinoma, and adenocarcinoma), mesothelioma of the lung, colorectal adenocarcinoma, stomach carcinoma, prostatic adenocarcinoma, ovarian carcinoma (such as serous cystadenocarcinoma and mucinous cystadenocarcinoma), ovarian germ cell tumors, testicular carcinomas and germ cell tumors, pancreatic adenocarcinoma, biliary adenocarcinoma, hepatocellular carcinoma, bladder carcinoma (including, for instance, transitional cell carcinoma, adenocarcinoma, and squamous carcinoma), renal cell adenocarcinoma, endometrial carcinomas (including, *e.g.*, adenocarcinomas and mixed Mullerian tumors (carcinosarcomas)), carcinomas of the endocervix, ectocervix, and vagina (such as adenocarcinoma and squamous carcinoma of each of same), tumors of the skin (*e.g.*, squamous cell carcinoma, basal cell carcinoma, malignant melanoma, skin appendage tumors, Kaposi sarcoma, cutaneous lymphoma, skin adnexal tumors and various types of sarcomas and Merkel cell carcinoma), esophageal carcinoma, carcinomas of the nasopharynx and oropharynx (including squamous carcinoma and adenocarcinomas of same), salivary gland carcinomas, brain and central nervous system tumors (including, for example, tumors of glial, neuronal, and meningeal origin), tumors of peripheral nerve, soft tissue sarcomas and sarcomas of bone and cartilage, head and neck squamous cell carcinoma, and lymphatic tumors (including B-cell and T- cell malignant lymphoma).. In one example, the tumor is an adenocarcinoma.

The disclosed recombinant OVs can also be used to treat liquid tumors, such as a lymphatic, white blood cell, or other type of leukemia. In a specific example, the tumor treated is a tumor of the blood, such as a leukemia (for example acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), hairy cell leukemia (HCL), T-cell prolymphocytic leukemia (T-PLL), large granular lymphocytic leukemia, and adult T-cell leukemia), a lymphoma (such as Hodgkin's lymphoma or non-Hodgkin's lymphoma), or a myeloma.

Under conditions sufficient for: A phrase that is used to describe any environment that permits a desired activity. In one example the desired activity is increased expression or activity of

one or more of leptin, insulin, chemerin, or IGF-1, for example in a tumor cell infected with a recombinant OV expressing the protein. In one example the desired activity is treatment of a tumor *in vivo*, for example using the disclosed recombinant oncolytic viruses.

Vector: A nucleic acid molecule as introduced into a host cell (such as a tumor cell), thereby producing a transformed host cell. A vector may include nucleic acid sequences that permit it to replicate in the host cell, such as an origin of replication. A vector may also include one or more leptin, insulin, chemerin, and IGF-1 coding sequences, for example in combination other sequences. A vector can transduce, transform or infect a cell, thereby causing the cell to express nucleic acids and/or proteins other than those native to the cell. A vector optionally includes materials to aid in achieving entry of the nucleic acid into the cell, such as a viral particle, liposome, protein coating or the like.

Overview

Immunotherapy can reinvigorate dormant responses to cancer, but response rates remain low due to several resistance mechanisms, including immunologic ignorance/exclusion and metabolically harsh microenvironments. Oncolytic viruses (OVs), which can replicate in cancer cells, may induce tumor lysis and immune priming. It is demonstrated herein that oncolytic *Vaccinia* virus induces substantial remodeling of the tumor microenvironment, dominated by influx of effector T cells. However, the inventors observed that responses to oncolytic viruses were incomplete, possibly be due to metabolic insufficiencies induced by the tumor microenvironment. The adipokine leptin is identified as a potent metabolic reprogramming agent that supports antitumor responses. Leptin metabolically reprograms T cells *in vitro*, and melanoma cells expressing leptin are immunologically controlled *in vivo*. Engineering oncolytic viruses to express leptin in tumor cells induced complete responses in tumor-bearing mice and promoted a functionally superior tumor infiltrate. Leptin treatment of tumor bearing animals increased T cell infiltration and potent metabolic reprogramming. Tumors engineered to overexpress leptin were controlled more effectively by the immune system and are more metabolically sufficient than wild-type tumors. Thus, leptin leverages an axis that promotes antitumor immunity by increasing the metabolic activity of tumor infiltrating T cells. Thus, leptin and other metabolic reprogramming agents (such as other adipokines such as chemerin, or other proteins such as insulin or IGF-1) can provide metabolic support to tumor immunity and oncolytic viruses represent a platform to deliver metabolic therapy.

Among the many challenges encountered by the immune response in solid tumors is the poor capacity to infiltrate as well as being able to carry out their effector function appropriately in a

hostile microenvironment. The data herein show that both obstacles can be overcome by engineering an OV that can deliver metabolic modulation (*e.g.*, the adipokine leptin) directly to the microenvironment, consequently improving therapeutic efficacy. Recent studies have explored the genetic signature defined by OVs in the tumor and determining targets that can be expressed in OVs (Zamarin *et al., Nat Commun* 2017; 8:14340). The data herein is the first to portray the changes in the immune landscape after oncolytic viral treatments utilizing single cell RNA-seq analysis. These findings reveal striking changes in tumor infiltrate at an early time-point when tumors are not yet regressing. These data indicate that OVs do not simply lyse a portion of tumor cells and promote some immunogenic cell death, but rather have the capacity to completely remodel the tumor immune microenvironment. The data show not only an increased infiltration in the T cell compartment, which is likely central to the observed antitumor immunity, but a wide array of changes in the myeloid population. These results shed new light on the potent immunity induced by OVs and demonstrates that this immune response can be bolstered in specific ways to promote more durable responses.

Improving T cell metabolic function in the tumor microenvironment may allow for a better therapeutic response. This disclosure provides novel methods that employ leptin (or other metabolic modulator of the immune response, such as other adipokines or other hormones such as insulin), especially in cancer. Furthermore, the disclosed methods utilize OVs as an effective delivery system for molecules that can modulate specifically the tumor microenvironment and improve therapeutic response.

Previous studies have shown that immune cells express the leptin receptor and that leptin as a cytokine can have pro-inflammatory functions in innate and adaptive immune responses (Loffreda *et al., FASEB J* 1998;12(1):57-65; La Cava *et al., Nat Rev Immunol* 2004;4(5):371-9; Santos-Alvarez *et al., Cell Immunol 1*999;194(1):6-11). Regarding the adaptive immune response, leptin can activate and enhance proliferation of human T lymphocytes (Martin-Romero *et al., Cell Immunol* 2000; 199(1):15-24). Although there are some observations that leptin might inhibit regulatory T cell proliferation and function in models of inflammation and autoimmunity (Feuerer *et al., Nat Med* 2009; 15(8):930-9), the data herein using oncolytics indicate that Treg cells are not stimulated in a leptin-rich tumor environment.

Little is known about the role of leptin or the leptin receptor in cancer, particularly in the tumor microenvironment. The findings herein demonstrate that there is an increase leptin receptor expression in T cells in the tumor microenvironment compared to those in the secondary lymphoid organs. Leptin can metabolically enhance tumor infiltrating T cell effector function through the persistence of mitochondrial function and an increase in oxidative phosphorylation. Previous

studies indicated that leptin can promote fatty acid oxidation in skeletal muscle. CD4+ T cells from leptin deficient mice showed a reduction in glucose uptake along with decreased proliferation and cytokine production. The data herein show that leptin signals through the activation of STAT3 and PI3K, and can increase mitochondrial content and quality. Leptin can promote PGC1α activation and promote oxidative phosphorylation as well as promote mitochondrial fusion through the expression of mitofusin 1 (Roman *et al., Mol Cell Endocrinol* 2010; 314(1):62-9; Hsu *et al., Int J Obes (Lond)* 2015; 39(12):1750-6.). As tumor infiltrating T cells repress the expression of PGC1α (Scharping *et al., Immunity* 2016; 45(3):701-3), leptin may support TIL function through maintenance of that axis.

The disclosed analysis of the T cell infiltrate of both wild-type and leptin-engineered oncolytic Vaccinia shed light on the immune populations that were more predominant in the tumors treated with leptin-expressing Vaccinia virus. An increase in proportions of memory T cells was observed, which explains the sustained therapeutic response observed. Memory T cells are superior antitumor T cells, have a higher mitochondrial content and oxidative phosphorylation capacity (van der Windt *et al., Immunity* 2012; 36(1):68-78; Sukumar *et al., Cell Metab* 2016; 23(1):63-76), in agreement with the data herein showing an increase in mitochondrial content. TCR sequencing analysis further demonstrated the effects of OVs on tumor infiltrating lymphocytes. While oncolytics induced new T cell clones to infiltrate the tumor, T cell clonal expansion in tumors treated with leptin-expressing *Vaccinia* virus was observed.

There are benefits to metabolically enhancing mitochondrial function in tumor infiltrating lymphocytes. The methods provided herein increases the repertoire of metabolic modulators that can be delivered directly into the tumor. One method of therapeutic delivery of these metabolic modulators is the utilization of OVs, which can deliver genetically encoded payload directly to the tumor microenvironment. Until now, the majority of oncolytic-delivered genes have been immunologic in nature (*e.g.*, cytokines, costimulatory molecules, etc.). However, the disclosed methods provide the first metabolic modulator delivered by OVs. While *Vaccinia* is demonstrated herein to be effective, other oncolytics like HSV, Newcastle Disease Virus, adenovirus, and VSV can also be used. While scRNA-seq revealed that oncolytics have potent immune-stimulatory potential early after infection, to achieve durable, complete responses, metabolic support can provide the strong early effector response into long-lived memory capable of mediating robust antitumor effects.

Based on these observations, provided herein are compositions methods that increase or enhance T cell metabolism, thereby increasing antitumor immunity, increasing a tumor's response to immunotherapy, or both. By modulating tumor microenvironment metabolism, for example by

providing metabolism modulating proteins in the tumor microenvironment, for example by expressing such proteins from a recombinant OV, enhances anti-tumor effects. Such methods can be used in combination with other anti-cancer therapies, such as with a T cell agonist (*e.g.*, with one or more agonists of 4-1BB, OX40, or GITR).

In some examples, leptin (or other metabolic modulating protein such as another adipokine (*e.g.*, chemerin), insulin, or IGF-1) is administered (for example as a protein, or as a nucleic acid encoding the protein, for example via a vector, such as a viral vector) in therapeutic amounts, for example in combination with other anti-cancer therapy, such as immunotherapies like a T cell agonist, such as one or more agonists of 4-1BB, OX40, and GITR. In some examples, recombinant OVs that are express or overexpress leptin (or other metabolic modulating protein such as another adipokine (*e.g.*, chemerin), insulin, or IGF-1) in tumor cells are used.

OVs that express leptin (or other metabolic modulating protein such as another adipokine (*e.g.*, chemerin), insulin, or IGF-1) can kill tumor cells and stimulate the immune system, but the release of leptin (or other metabolic modulating protein such as another adipokine (*e.g.*, chemerin), insulin, or IGF-1) also improve T cell metabolism at the tumor site. Thus, these recombinant OVs become a potent type of self-bolstering immunotherapy. Recombinant leptin (or other metabolic modulating protein such as another adipokine (*e.g.*, chemerin), insulin, or IGF-1) increases T cell infiltration.

Recombinant Oncolytic Viruses (OVs)

Provided herein are recombinant OVs that can be used to improve cellular immunotherapy, such as cancer immunotherapy. For example, the disclosure provides recombinant OVs containing a nucleic acid molecule that encodes one or more metabolic modulatory proteins, such as an adipokine (*e.g.*, leptin or chemerin), chemerin, or IGF-1. In some examples the metabolic modulatory protein (*e.g.*, an adipokine (*e.g.*, leptin or chemerin), chemerin, or IGF-1) is part of a fusion protein expressed by the OV, such as a fusion protein including the metabolic modulatory protein and a gamma chain cytokine (such as IL-2 or IL-15). Expression of the protein in a tumor cell infected with the OVs may result in increased expression of one or more of these proteins, and thus increased activity of these proteins, thereby increasing anti-tumor activity.

The OV can be any OV, such as a naturally occurring OV or a genetically engineered OV. Examples include herpes simplex virus (HSV), vaccinia virus, adenovirus, poxvirus, reovirus, poliovirus, coxsackie virus, measles virus, vesicular stomatitis virus (VSV), Seneca valley virus, ECHO virus, Newcastle disease virus, chicken anemia virus, or parovirus. In a specific example, the OV can be talimogene laherparepvec (T-VEC). In a specific example, the OV can be Western

Reserve strain *Vaccinia* virus. In a specific example, the OV can be vaccinia, and the one or more metabolic modulating proteins can be leptin. In a specific example, the OV can be Western Reserve strain *Vaccinia* virus, and the one or more metabolic modulating proteins is leptin. In a specific example, the OV is can be Reserve strain *Vaccinia* virus, and the one or more metabolic modulating proteins can be a leptin-IL-2 or leptin-IL-15 fusion protein (wherein the leptin and cytokine may be joined by a linker).

The nucleic acid molecule encoding the one or more metabolic modulating proteins in some examples is operably linked to a promoter, such as a constitutive or regulatable promoter. In one example, the promoter may not be native to the protein. For example, the promoter can be one from the virus, such as the 7.5 promoter.

In one example, the one or more metabolic modulating proteins can comprise at least about 80%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2, 4, 6, or 8. In one example the a nucleic acid molecule encoding the one or more metabolic modulating proteins can comprise at least about 80%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:1, 3, 5 or 7. In one example, the one or more metabolic modulating proteins can be a fusion protein including a first protein and a second protein, wherein the first protein comprises at least 80%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO: 2, 4, 6, or 8. In one example the nucleic acid molecule encoding the one or more metabolic modulating proteins can comprise at least about 80%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:1, 3, 5 or 7. In one example the nucleic acid molecule encoding the one or more metabolic modulating proteins can encode a fusion protein that includes a metabolic protein, wherein the fusion protein can include a first protein and a second protein, wherein the first protein may be encoded by a nucleic acid molecule comprising at least about 80%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:1, 3, 5 or 7. In some examples, the recombinant OV may further express one or more immune stimulatory proteins, such as a costimulatory molecule, cytokine, a chemokine, such as one or more of IL-2, IL-12, IL-15, IL-18, IFN-α/β, TNF-α, and GM-CSF, or combinations thereof.

In some examples, upon infection of a tumor cell by the disclosed recombinant OVs, expression and/or activity of an adipokine in the infected tumor cells may increase by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500% or at least

about 600%, for example relative to the absence of administering the OV, or relative to administration of an OV not expressing an adipokine. In some examples, upon infection of a tumor cell by the disclosed recombinant OVs, leptin expression and/or activity in the infected tumor cells may increase by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500% or at least about 600%, for example relative to the absence of administering the OV, or relative to administration of an OV not expressing leptin. In some examples, upon infection of a tumor cell by the disclosed recombinant OVs, insulin expression and/or activity in the infected tumor cells may increase by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500% or at least about 600%, for example relative to the absence of administering the OV, or relative to administration of an OV not expressing insulin. In some examples, upon infection of a tumor cell by the disclosed recombinant OVs, chemerin expression and/or activity in the infected tumor cells can increase by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500% or at least about 600%, for example relative to the absence of administering the OV, or relative to administration of an OV not expressing chemerin. In some examples, upon infection of a tumor cell by the disclosed recombinant OVs, IGF-1 expression and/or activity in the infected tumor cells may be increased by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500% or at least about 600%, for example relative to the absence of administering the OV, or relative to administration of an OV not expressing IGF-1.

In some examples, expressing an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 in the tumor cells from the OV may increase T cell infiltration into the tumor or tumor microenvironment by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500% or at least about 600%, for example relative to the absence of

administering the OV, or relative to administration of an OV not expressing a metabolic modulatory protein. In some examples, expressing an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1, in the tumor cells from the OV may increase mitochondrial activity in T cells at the site of the tumor by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500% or at least about 600%, for example relative to the absence of administering the OV, or relative to administration of an OV not expressing a metabolic modulatory protein. In some examples, expressing an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1, in the tumor cells from the OV may increase T cell oxidative phosphorylation by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500% or at least about 600%, for example relative to the absence of administering the OV, or relative to administration of an OV not expressing a metabolic modulatory protein. In some examples, expressing an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1, in the tumor cells from the OV may increase T cell clonal expansion in a tumor by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500% or at least about 600%, for example relative to the absence of administering the OV, or relative to administration of an OV not expressing a metabolic modulatory protein. In some examples, combinations of these effects may be achieved.

1. Metabolic Modulating Proteins

The metabolic modulating protein, such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1, coding sequence (which can be one part of a fusion protein coding sequence) in the OV can be wild-type (*e.g.*, non-mutated) or variant sequence. In a specific example, the metabolic modulating protein is leptin. In a specific example, the metabolic modulating protein is chemerin. In a specific example, the metabolic modulating protein is insulin. In a specific example, the metabolic modulating protein is IGF-1.

For example, wild-type leptin, insulin, chemerin, and IGF-1 sequences are provided herein via GenBank® Accession Nos. (and sequences are provided in SEQ ID NOS: 1-8). Thus, in some examples, the recombinant OV introduced into a tumor cell can include a native leptin, insulin, chemerin, and/or IGF-1 coding sequence. In some examples, the recombinant OV introduced into the tumor cell includes a non-native adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 coding sequence, but encodes a native adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 protein sequence (*e.g.*, a coding sequence that is degenerate). In some examples, the adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 protein expressed by the recombinant OV includes the signal sequence.

Variant adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 proteins, including variants of the protein sequences provided above via GenBank® Accession Nos., can contain one or more mutations, such as a single insertion, a single deletion, a single substitution. In some examples, the variant adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 protein includes 1-20 insertions, 1-20 deletions, 1-20 substitutions, and/or any combination thereof (*e.g.*, single insertion together with 1-19 substitutions). In some examples, the disclosure provides a variant of any native adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 protein having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 additional amino acid changes, wherein the protein retains native or increased biological activity. In some examples, a variant adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 protein includes 1-8 insertions, 1-15 deletions, 1-10 substitutions, and/or any combination thereof (*e.g.*, 1-15, 1-4, or 1-5 amino acid deletions together with 1-10, 1-5 or 1-7 amino acid substitutions). In some examples, a variant adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 protein has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 amino acid changes. In one example, such variant peptides are produced by manipulating the nucleotide sequence encoding a peptide using standard procedures such as site-directed mutagenesis or PCR.

One type of modification includes the substitution of amino acids for amino acid residues having a similar biochemical property, that is, a conservative substitution (such as 1-4, 1-8, 1-10, or 1-20 conservative substitutions). Typically, conservative substitutions have little to no impact on the activity of a resulting peptide. For example, a conservative substitution is an amino acid substitution in any native adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 protein sequence, which does not substantially affect the native function of the protein. An alanine scan can be used to identify which amino acid residues in a protein can tolerate an amino acid substitution. In one example, the native function of adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 is not altered by more than 25%, for example not more than 20%, for example not more than 10%, when an alanine, or other conservative amino acid, is substituted for 1-4, 1-8, 1-10, or 1-20 native amino acids. Examples of amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative substitutions include: Ser for Ala; Lys, Gln, or Asn for Arg; Gln or His for Asn; Glu for Asp; Ser for Cys; Asn for Gln; Asp for Glu; Pro for Gly; Asn or Gln for His; Leu or Val for Ile; Ile or Val for Leu; Arg or Gln for Lys; Leu or Ile for Met; Met, Leu or Tyr for Phe; Thr for Ser; Ser for Thr; Tyr for Trp; Trp or Phe for Tyr; and Ile or Leu for Val.

More substantial changes can be made by using substitutions that are less conservative, *e.g.*, selecting residues that differ more significantly in their effect on maintaining: (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation; (b) the charge or hydrophobicity of the polypeptide at the target site; or (c) the bulk of the side chain. The substitutions that in general are expected to produce the greatest changes in polypeptide function are those in which: (a) a hydrophilic residue, *e.g.*, serine or threonine, is substituted for (or by) a hydrophobic residue, *e.g.*, leucine, isoleucine, phenylalanine, valine or alanine; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, *e.g.*, lysine, arginine, or histidine, is substituted for (or by) an electronegative residue, *e.g.*, glutamic acid or aspartic acid; or (d) a residue having a bulky side chain, *e.g.*, phenylalanine, is substituted for (or by) one not having a side chain, *e.g.*, glycine. The effects of these amino acid substitutions (or other deletions and/or additions) can be assessed by analyzing the function of the variant leptin, insulin, chemerin, or IGF-1 protein by analyzing the native function of the protein.

The metabolic modulatory protein(s) expressed by the OV can be part of a fusion protein. Thus, an OV expressing a fusion protein that includes an adipokine (*e.g.*, leptin, chemerin,

adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1, is encompassed by this disclosure, and can be used in the disclosed methods for treating a tumor/cancer. In some embodiments, a fusion protein expressed by the OV can include at least two portions, a metabolic modulatory protein and a further protein. In a specific example, the metabolic modulating protein of the fusion protein can be leptin, chemerin, insulin, and/or IGF-1. In some examples, the further protein can be a cytokine protein, such as a chemokine, an interferon, an interleukin, a lymphokine, a tumour necrosis factor, or a fusion protein comprising any combinations thereof. The metabolic modulatory protein portion of the fusion protein can be a native or a mutated metabolic modulatory protein (such a protein having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 2, 4, 6, or 8). In one example, the further protein is a cytokine protein, such as a native or a mutated cytokine, for example a gamma chain cytokine, such as a native or mutated protein belonging to the IL-2 subfamily of cytokine proteins (such as a native IL-2 or a Super IL-2 (*e.*g., see Levin *et al.*, *Nature* 484:529-33, 2012), the IL-1 family of cytokine protein (*e.g.*, IL-18), the IFN subfamily of cytokine proteins, the IL-17 family of cytokine proteins, the TGF superfamily of cytokine proteins (*e.g.*, TGF-β1, TGF-β2, TGF-β3), IL-4, IL-10, IL-13, IL-7, IL-9, IL-15, IL-21, TNFα, IFN-γ, or any combinations thereof. In some cases, the cytokine protein can be a native human protein. In a specific example, the cytokine protein portion of the fusion protein can be IL-2 (*e.g.*, GenBank® Accession No. AAB46883.1), Super IL-2 (*e.*g., see Levin *et al.*, *Nature* 484:529-33, 2012), or IL-15 (*e.g.*, GenBank® Accession No. AAI00963.1 or aa 10-127 of GenBank® Accession No. AAI00963.1). In specific examples, the fusion protein can include leptin and IL-2, or leptin and IL-15. In some examples, the metabolic modulatory protein of the fusion protein is directly attached to a cytokine protein, such as at either the N-terminus or the C-terminus. In some examples (*e.g.*, oncolytic viruses comprising a nucleic acid that can code for a fusion protein comprising a metabolic modulatory protein and a cytokine), the nucleic acid encoding the metabolic modulatory protein portion and the nucleic acid encoding the further portion (*e.g.*, cytokine) can be linked indirectly through the use of a nucleic acid that codes for a linker, such as a peptide linker composed of at least 5, at least 10, at least 15 or at least 20 amino acids, such as 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids. In some examples the linker can be flexible. In one example the linker can be a polyalanine. In one example the linker can be a flexible linker, such as one that includes Gly and Ser residues (*e.g.*, GSG, GSGSGS or GGSGGGGSGG). In specific examples, the fusion protein is leptin-GSG-IL-2, or leptin-GSG-IL-15.

2. Exemplary Oncolytic Viruses

The disclosed recombinant OVs can be generated from any OV. OVs are viral strains that can infect and kill malignant cells (oncolysis) while sparing their normal counterparts. Oncolysis can be either a natural property of the virus (naturally occurring OVs, *e.g.*, reovirus) or a consequence of manipulation of the viral genome (genetically engineered OVs, *e.g.*, adenovirus).

In one example, a recombinant OV is a DNA virus, such as a double stranded DNA (*e.g.*, Herpes simplex virus (HSV) (such as HSV-1), vaccinia virus, or adenovirus) or single strand DNA virus (*e.g.*, parovirus and chicken anemia virus, such as H-1PV). Exemplary HSV OVs include T-VEC (*e.g.*, to treat melanoma), G207 (*e.g.*, to treat glioma), NV1020 (*e.g.*, to treat CRC), HFA10 (*e.g.*, to treat breast, head and neck and pancreatic cancer). Exemplary vaccinia OVs include vvDD (TK mutant strain), JX-594 (TK mutant /GM-CSF expressing strain) (*e.g.*, to treat HCC, CRC), and GL-ONC1 (TK mutant /HA expressing strain) (*e.g.*, to treat solid tumors). In one example, a vaccinia virus that includes a genetic deletion of thymidine kinase (TK) and growth factor genes (VGF) is used, such as the Western Reserve laboratory strain *Vaccinia* virus (see for example Zeh *et al.*, *Mol. Ther* 23:202-14, 2015). Exemplary adenovirus OVs include ONYX (E1B55 mutant), Ad5-D24, CFAd, DNX-2401, Ad5/3 D24-GMCSF and CGTG-102, ColoAd1, and Ad5/d hTERT and CD40 ligand expressing strain (*e.g.*, to treat glioma and solid tumors).

In one example, a recombinant OV is an RNA virus, such as a double stranded RNA (*e.g.*, reovirus) or single strand RNA virus (*e.g.*, coxsackie virus, measles virus, Newcastle disease virus, vesicular stomatitis virus, Seneca valley virus, or ECHO).

Other exemplary OVs that can be used in the compositons and methods provided herein include those provided in Fountzilas *et al.* (*Oncotarget*, 8:102617-39, 2017) Jhawar *et al.* (*Front. Oncol.*, 7:202, 2017) and Guo *et al.* (*Front. Oncol.*, 8:555, 2017) (all herein incorporated by reference in their entireties). In some examples, the recombinant OV can be a lentivirus, a mengovirus, or a myxomavir.

Methods of Using Recombinant Oncolytic Viruses

The recombinant OVs provided herein, for example generated using the disclosed methods, can be used in cancer immunotherapy, for example to treat a tumor *in vivo*. In some examples, the cancer can comprise melanoma, hepatocellular carcinoma, breast cancer, lung cancer, peritoneal cancer, prostate cancer, bladder cancer, ovarian cancer, leukemia, lymphoma, renal carcinoma, pancreatic cancer, epithelial carcinoma, gastric cancer, colon carcinoma, duodenal cancer, pancreatic adenocarcinoma, mesothelioma, glioblastoma multiform, astrocytoma, multiple myeloma, prostate carcinoma, hepatocellular carcinoma, cholangiosarcoma, pancreatic

adenocarcinoma, head and neck squamous cell carcinoma, colorectal cancer, intestinal-type gastric adenocarcinoma, cervical squamous-cell carcinoma, osteosarcoma, epithelial ovarian carcinoma, acute lymphoblastic lymphoma, myeloproliferative neoplasms, or sarcoma. In some examples, the cancer cell can be present in an organ of the subject selected from the group consisting of: the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestine, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In some examples, the cancer can be metastatic.

Solid and liquid tumors can be treated with the disclosed methods. Specific examples of tumors that can be treated include, but are not limited to, a leukemia, lymphoma, pancreatic cancer, colorectal cancer, melanoma, cervical cancer, lung cancer, ovarian cancer, bladder cancer, breast cancer, prostate cancer, HCC, RCC, or head and neck cancer. In one example, the cancer is melanoma. In one example, the cancer is breast cancer. In one example, the cancer is an adenocarcinoma. Other examples are provided herein.

Provided herein are methods of treating a tumor (such as a cancer) in a subject (such as a mammalian subject, such as a human or veterinary subject), increasing T cell infiltration into a tumor (or tumor microenvironment), increasing the metabolic activity of T cells in a tumor or tumor microenvironment, or combinations thereof. Such methods can include administering a therapeutically effective amount of one or more recombinant OVs disclosed herein to the subject (for example systemically or intratumorally), thereby treating the tumor. For example, expression of a metabolic modulating protein (such as one or more of leptin, insulin, chemerin, and IGF-1) by the OVs in tumor cells infected by the recombinant OVs, can kill the tumor cells and increase T cell activity in the vicinity of the tumor. In some examples, instead of using a recombinant OV, the methods can include administering (for example systemically or intratumorally) a therapeutically effective amount of one or more metabolic modulating proteins or nucleic acid molecules encoding the protein(s) to the subject, thereby treating the tumor. For example, expression of the metabolic modulating protein (such as one or more of leptin, insulin, chemerin, and IGF-1) in the vicinity of tumor cells can have anti-tumor effects, for example by increasing T cell activity in the vicinity of the tumor.

In some examples, such methods further include administering a therapeutically effect amount of one or more additional anti-cancer agents, such as chemotherapy (*e.g.*, an alkylating agent, antimetabolite, a hormone, or a hormone antagonist), radiotherapy, a biologic (*e.g.*, monoclonal antibody, such as one that specifically binds and antagonizes PD-1 or PD-L1, or a T cell agonist, such as mAb agonist of 4-1BB, OX40, or GITR), surgery, or combinations thereof. In some examples, such subjects are also administered an effective amount of IL-2 (such as 10,000 to

100,000 units/kg body weight) to the subject before, after, or both before and after, administering the disclosed recombinant OVs (or metabolic modulating protein or nucleic acid molecule encoding the protein).

For example, an effective amount of the disclosed recombinant OVs (such as at least $1x10^6$ pfu recombinant OVs, at least $2x10^6$ pfu recombinant OVs, at least $5x10^6$ pfu recombinant OVs, or at least $1x10^7$ pfu recombinant OVs) are administered to the subject, thereby treating a tumor (such as a primary tumor and/or a metastasis) in the subject. In some embodiments, an effective amount of a recombinant OV of this disclosure, administered to a subject can comprise from about 1 x 10^3 to about 1 x 10^{12} PFU recombinant OVs, or from about 1 x 10^5 and 1 x 10^{10} PFU recombinant OVs, or from about 1 x 10^5 and 1 x 10^8 PFU recombinant OVs, or from about 1 x 10^8 and 1 x 10^{10} PFU recombinant OVs, about 1 x 10^{11} PFU recombinant OVs, 1 x 10^{12} PFU recombinant OVs, 1 x 10^{13} PFU recombinant OVs, 1 x 10^{14} PFU recombinant OVs, or 1 x 10^{15} PFU recombinant OVs.

In some examples, the recombinant OVs are administered intravenously. In some examples, the recombinant OVs are administered intratumorally. In some examples, the recombinant OVs are administered subdermally. In some examples, the recombinant OVs are administered via routes such as rectal, intraurethral, intravaginal, intranasal, intrathecal, or intraperitoneal.

In some examples the subject administered the disclosed recombinant OVs was previously treated unsuccessfully with a chemotherapy, radiation therapy, biologic therapy, or combinations thereof (*e.g.*, the tumor in the subject did not significantly decrease in size or even increased in size, and/or metastasized). In some examples the subject has a tumor that was not responsive to a PD-1 antagonist or a PD-L1 antagonist (*e.g.*, the tumor in the subject did not significantly decrease in size or even increased in size, and/or metastasized), such as an antibody that specifically binds and antagonizes PD-1 or PD-L1, such as Atezolizumab, MPDL3280A, BNS-936558 (Nivolumab), Pembrolizumab, Pidilizumab, CT011, AMP-224, AMP-514, MEDI-0680, BMS-936559, BMS935559, MEDI-4736, MPDL-3280A, MSB-0010718C, MGA-271, Indoximod, Epacadostat, BMS-986016, MEDI-4736, MEDI-4737, MK-4166, BMS-663513, PF-05082566 (PF-2566), Lirilumab, and Durvalumab. In some examples the subject has a tumor that was not responsive to a T cell agonist, such as an agonist of 4-1BB, OX40, or GITR (*e.g.*, the tumor in the subject did not significantly decrease in size or even increased in size, and/or metastasized) (particular examples of such reagents are provided herein).

In some examples, the method includes monitoring T cells in the tumor microenvironment, for example determining the number of cells, determining or measuring the mitochondrial activity (*e.g.*, oxidative metabolism), and determining or measuring the mitochondrial mass of the TILs.

In some examples, the method includes monitoring tumor growth in response to treatment.

Coding Sequences

A vector, including a recombinant OV, can be used to express a metabolic modulating protein in the area of a tumor (or even in a tumor cell), wherein the vector that includes a nucleic acid molecule encoding one or more metabolic modulating proteins (such as one or more of leptin, insulin, chemerin, and IGF-1, which may be part of a fusion protein that includes a cytokine). Examples of vectors that can be used include plasmids, viral vectors, such as an OV.

Nucleic acid molecules include DNA, cDNA and RNA sequences which encode a peptide. Silent mutations in the coding sequence result from the degeneracy (*i.e.*, redundancy) of the genetic code, whereby more than one codon can encode the same amino acid residue. Thus, for example, leucine can be encoded by CTT, CTC, CTA, CTG, TTA, or TTG; serine can be encoded by TCT, TCC, TCA, TCG, AGT, or AGC; asparagine can be encoded by AAT or AAC; aspartic acid can be encoded by GAT or GAC; cysteine can be encoded by TGT or TGC; alanine can be encoded by GCT, GCC, GCA, or GCG; glutamine can be encoded by CAA or CAG; tyrosine can be encoded by TAT or TAC; and isoleucine can be encoded by ATT, ATC, or ATA.

Codon preferences and codon usage tables for a particular species can be used to engineer isolated nucleic acid molecules encoding a metabolic modulating protein (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1; in one example one or more of leptin, insulin, chemerin, and IGF-1) that take advantage of the codon usage preferences of that particular species. For example, the metabolic modulating protein (such as one or more of leptin, insulin, chemerin, and IGF-1) expressed from the vector(s) can be designed to have codons that are preferentially used by a particular organism of interest (*e.g.*, in one whom the therapy is introduced).

A nucleic acid encoding a metabolic modulating protein (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1], which may be part of a fusion protein that includes a cytokine) can be cloned or amplified by *in vitro* methods, such as the polymerase chain reaction (PCR), the ligase chain reaction (LCR), the transcription-based amplification system (TAS), the self-sustained sequence

replication system (3SR) and the Qβ replicase amplification system (QB). A wide variety of cloning and *in vitro* amplification methodologies are known. In addition, nucleic acids encoding sequences encoding a metabolic modulating protein (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1], which may be part of a fusion protein that includes a cytokine) can be prepared by cloning techniques. Examples of appropriate cloning and sequencing techniques, and instructions are found in Sambrook *et al.* (ed.), Molecular Cloning: A Laboratory Manual 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring, Harbor, N.Y., 1989, and Ausubel et al., (1987) in "Current Protocols in Molecular Biology," John Wiley and Sons, New York, N.Y..

Nucleic acid sequences encoding a metabolic modulating protein (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1], which may be part of a fusion protein that includes a cytokine) can be prepared by any suitable method including, for example, cloning of appropriate sequences or by direct chemical synthesis by methods such as the phosphotriester method of Narang *et al., Meth. Enzymol.* 68:90-99, 1979; the phosphodiester method of Brown *et al., Meth. Enzymol.* 68:109-151, 1979; the diethylphosphoramidite method of Beaucage *et al., Tetra. Lett.* 22:1859-1862, 1981; the solid phase phosphoramidite triester method described by Beaucage & Caruthers, *Tetra. Letts.* 22(20):1859-1862, 1981, for example, using an automated synthesizer as described in, for example, Needham-VanDevanter *et al., Nucl. Acids Res.* 12:6159-6168, 1984; and, the solid support method of U.S. Patent No. 4,458,066. Chemical synthesis produces a single stranded oligonucleotide. This can be converted into double stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. While chemical synthesis of DNA is generally limited to sequences of about 100 bases, longer sequences may be obtained by the ligation of shorter sequences.

In one example, a metabolic modulating protein (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1], which may be part of a fusion protein that includes a cytokine) is prepared by inserting the cDNA which encodes the protein into a vector. The insertion can be made so that the protein(s) is read in frame so that the protein(s) is produced. Techniques for preparing recombinant vectors (*e.g.*, plasmid or virus) containing a heterologous nucleic acid sequence encoding the protein are known.

The nucleic acid coding sequence for a metabolic modulating protein (such as one or more of leptin, insulin, chemerin, and IGF-1, which may be part of a fusion protein that includes a further protein, such as a cytokine) can be inserted into an expression vector including, but not limited to a plasmid, virus or other vehicle that can be manipulated to allow insertion or incorporation of sequences and can be expressed (*e.g.*, in a tumor cell). Methods of expressing coding sequences from a vector are known. The expression vector can contain additional elements necessary for the transfer and subsequent replication of the expression vector containing the metabolic modulating protein (such as one or more of leptin, insulin, chemerin, and IGF-1, which may be part of a fusion protein that includes a cytokine) coding sequence in the cell. Examples of such elements include, but are not limited to, origins of replication and selectable markers, such as a thymidine kinase gene or an antibiotic resistance marker.

Nucleic acid sequences encoding metabolic modulating protein (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1], which may be part of a fusion protein that includes a cytokine) can be operatively linked to expression control sequences. An expression control sequence operatively linked to a metabolic modulating protein (such as one or more of leptin, insulin, chemerin, and IGF-1, which may be part of a fusion protein that includes a cytokine) coding sequence is ligated such that expression of the metabolic modulating protein (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1]) protein coding sequence is achieved under conditions compatible with the expression control sequences. Exemplary expression control sequences include, but are not limited to appropriate promoters, enhancers, transcription terminators, a start codon (*i.e.*, ATG) in front of a metabolic modulating protein (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1], which may be part of a fusion protein that includes a cytokine)-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. Examples of expression control elements that can be used include, but are not limited to, lac system, operator and promoter regions of phage lambda, and promoters derived from polyoma, adenovirus, retrovirus or SV40. Additional operational elements include, but are not limited to, leader sequence, termination codons, polyadenylation signals and any other sequences necessary for the appropriate transcription and subsequent translation of the nucleic acid sequence encoding

the metabolic modulating protein (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1], which may be part of a fusion protein that includes a cytokine) protein in the cell. In one example, the promoter is a 7.5 promoter. In one example, an IRES is used to drive expression. In some examples, two promoters are used.

Viral vectors can be prepared that encode a metabolic modulating protein (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1], which may be part of a fusion protein that includes a cytokine) protein. Exemplary viral vectors that can be used include, but are not limited to, polyoma, SV40, adenovirus, vaccinia virus, adeno-associated virus, herpes viruses including HSV and EBV, Sindbis viruses, alphaviruses and retroviruses of avian, murine, and human origin. Baculovirus (Autographa californica multinuclear polyhedrosis virus; AcMNPV) vectors can also be used. Other suitable vectors include orthopox vectors, avipox vectors, fowlpox vectors, capripox vectors, suipox vectors, lentiviral vectors, alpha virus vectors, and poliovirus vectors. Specific exemplary vectors are poxvirus vectors such as vaccinia virus, fowlpox virus and a highly attenuated vaccinia virus (MVA), adenovirus, baculovirus and the like. Pox viruses of use include orthopox, suipox, avipox, and capripox virus. Orthopox include vaccinia, ectromelia, and raccoon pox. One example of an orthopox of use is vaccinia. Avipox includes fowlpox, canary pox and pigeon pox. Capripox include goatpox and sheeppox. In one example, the suipox is swinepox. Other viral vectors that can be used include other DNA viruses such as herpes virus and adenoviruses, and RNA viruses such as retroviruses and polio.

Administration of a Metabolic Modulating Protein

In some examples, instead of using a recombinant OV to express the metabolic modulating protein (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1], which may be part of a fusion protein that includes a cytokine), metabolic modulating protein or nucleic acid molecules encoding the protein is administered to the subject.

In one example, a vector is used to express a metabolic modulating protein in the area of a tumor, wherein the vector that includes a nucleic acid molecule encoding one or more metabolic modulating proteins (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1,

PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1], which may be part of a fusion protein that includes a cytokine). Examples of vectors that can be used include plasmids, viral vectors, such as a lentiviral vector or retrovirus. In another example, a naked nucleic acid molecule encoding for a metabolic modulating protein (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1], which may be part of a fusion protein that includes a cytokine) is administered.

Nucleic acid molecules encoding a native or variant metabolic modulating protein (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1], which may be part of a fusion protein that includes a cytokine) can be incorporated into a vector. Nucleic acid sequences coding for a native or variant a metabolic modulating protein (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1], and IGF-1, which may be part of a fusion protein that includes a cytokine) such as those having at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to those shown in a GenBank® Accession No. provided herein (such as SEQ ID NO: 1, 3, 5, or 7), can be generated. In addition, one of skill can readily construct a variety of clones containing functionally equivalent nucleic acids, such as nucleic acids which differ in sequence but which encode the same protein sequence. In some examples, such a sequence is optimized for expression in a host cell, such as a host tumor used to express the desired protein(s).

Additional Therapies

The subject treated with the disclosed recombinant oncolytic viruses can receive one or more additional therapies, such as one or more of an effective amount of chemotherapy, an effective amount of radiotherapy (for example administration of radioactive material or energy (such as external beam therapy) to the tumor site to help eradicate the tumor or shrink it), an effective amount of a biologic (such as a therapeutic monoclonal antibody, ligand, or aptamer), and surgery (for example surgical resection of the cancer or a portion of it). Thus, in some examples, kits that include one or more of the disclosed recombinant oncolytic viruses and one or more ant-cancer agents (such as a chemotherapeutic or biologic), are provided.

In one example, the subject is further treated with one or more chemotherapeutic agents. Chemotherapeutic agents include any chemical agent with therapeutic usefulness in the treatment of diseases characterized by abnormal cell growth, such as cancer. One of skill in the art can readily identify a chemotherapeutic agent of use (see for example, Slapak and Kufe, *Principles of Cancer Therapy*, Chapter 86 in Harrison's Principles of Internal Medicine, 14th edition; Perry *et al.*, *Chemotherapy*, Ch. 17 in Abeloff, Clinical Oncology 2nd ed., © 2000 Churchill Livingstone, Inc; Baltzer, L., Berkery, R. (eds): *Oncology Pocket Guide to Chemotherapy*, 2nd ed. St. Louis, Mosby-Year Book, 1995; Fischer, D.S., Knobf, M.F., Durivage, H.J. (eds): *The Cancer Chemotherapy Handbook*, 4th ed. St. Louis, Mosby-Year Book, 1993; Chabner and Longo, *Cancer Chemotherapy and Biotherapy: Principles and Practice* (4th ed.). Philadelphia: Lippincott Willians & Wilkins, 2005; Skeel, *Handbook of Cancer Chemotherapy* (6th ed.). Lippincott Williams & Wilkins, 2003). Combination chemotherapy is the administration of more than one agent to treat cancer.

Examples of chemotherapeutic agents that can be used include alkylating agents, antimetabolites, natural products, or hormones and their antagonists. Examples of alkylating agents include nitrogen mustards (such as mechlorethamine, cyclophosphamide, melphalan, uracil mustard or chlorambucil), alkyl sulfonates (such as busulfan), nitrosoureas (such as carmustine, lomustine, semustine, streptozocin, or dacarbazine). Specific non-limiting examples of alkylating agents are temozolomide and dacarbazine. Examples of antimetabolites include folic acid analogs (such as methotrexate), pyrimidine analogs (such as 5-FU or cytarabine), and purine analogs, such as mercaptopurine or thioguanine. Examples of natural products include vinca alkaloids (such as vinblastine, vincristine, or vindesine), epipodophyllotoxins (such as etoposide or teniposide), antibiotics (such as dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin, or mitocycin C), and enzymes (such as L-asparaginase). Examples of miscellaneous agents include platinum coordination complexes (such as cis-diamine-dichloroplatinum II also known as cisplatin), substituted ureas (such as hydroxyurea), methyl hydrazine derivatives (such as procarbazine), and adrenocrotical suppressants (such as mitotane and aminoglutethimide). Examples of hormones and antagonists include adrenocorticosteroids (such as prednisone), progestins (such as hydroxyprogesterone caproate, medroxyprogesterone acetate, and magestrol acetate), estrogens (such as diethylstilbestrol and ethinyl estradiol), antiestrogens (such as tamoxifen), and androgens (such as testerone proprionate and fluoxymesterone).

Examples of commonly used chemotherapy drugs that can be used in combination with the disclosed OVs that express one or more metabolic modulatory proteins include Adriamycin, Alkeran, Ara-C, BiCNU, Busulfan, CCNU, Carboplatinum, Cisplatinum, Cytoxan, Daunorubicin, DTIC, 5-fluoruracil (5-FU), Fludarabine, Hydrea, Idarubicin, Ifosfamide, Methotrexate,

Mithramycin, Mitomycin, Mitoxantrone, Nitrogen Mustard, Taxol (or other taxanes, such as docetaxel), Velban, Vincristine, VP-16, while some more newer drugs include Gemcitabine (Gemzar), Herceptin, Irinotecan (Camptosar, CPT-11), Leustatin, Navelbine, Rituxan STI-571, Taxotere, Topotecan (Hycamtin), Xeloda (Capecitabine), Zevelin and calcitriol. Non-limiting examples of immunomodulators that can be used include AS-101 (Wyeth-Ayerst Labs.), bropirimine (Upjohn), gamma interferon (Genentech), GM-CSF (granulocyte macrophage colony stimulating factor; Genetics Institute), IL-2 (Cetus or Hoffman-LaRoche), human immune globulin (Cutter Biological), IMREG (from Imreg of New Orleans, La.), SK&F 106528, and TNF (tumor necrosis factor; Genentech).

Additional therapeutic agents that can be used in combination with the disclosed OVs that express one or more metabolic modulatory proteins include microtubule binding agents, DNA intercalators or cross-linkers, DNA synthesis inhibitors, DNA and/or RNA transcription inhibitors, antibodies, enzymes, enzyme inhibitors, gene regulators, angiogenesis inhibitors. These agents (which are administered at a therapeutically effective amount) and treatments can be used alone or in combination. Methods and therapeutic dosages of such agents are known, and can be determined by a skilled clinician.

Microtubule binding agents refers to agents that interact with tubulin to stabilize or destabilize microtubule formation thereby inhibiting cell division. Examples of microtubule binding agents that can be used in conjunction with the disclosed therapies include, without limitation, paclitaxel, docetaxel, vinblastine, vindesine, vinorelbine (navelbine), the epothilones, colchicine, dolastatin 15, nocodazole, podophyllotoxin and rhizoxin. Analogs and derivatives of such compounds also can be used. For example, suitable epothilones and epothilone analogs are described in International Publication No. WO 2004/018478. Taxoids, such as paclitaxel and docetaxel, as well as the analogs of paclitaxel taught by U.S. Pat. Nos. 6,610,860; 5,530,020; and 5,912,264 can be used.

Suitable DNA and/or RNA transcription regulators, including, without limitation, actinomycin D, daunorubicin, doxorubicin and derivatives and analogs thereof also are suitable for use in combination with the disclosed therapies. DNA intercalators and cross-linking agents that can be administered to a subject include, without limitation, cisplatin, carboplatin, oxaliplatin, mitomycins, such as mitomycin C, bleomycin, chlorambucil, cyclophosphamide and derivatives and analogs thereof. DNA synthesis inhibitors suitable for use as therapeutic agents include, without limitation, methotrexate, 5-fluoro-5'-deoxyuridine, 5-fluorouracil (5-FU) and analogs thereof. Examples of suitable enzyme inhibitors include, without limitation, camptothecin, etoposide, formestane, trichostatin and derivatives and analogs thereof. Suitable compounds that

affect gene regulation include agents that result in increased or decreased expression of one or more genes, such as raloxifene, 5-azacytidine, 5-aza-2'-deoxycytidine, tamoxifen, 4-hydroxytamoxifen, mifepristone and derivatives and analogs thereof.

The disclosed methods can further include administering to the subject a therapeutically effective amount of an immunotherapy. Non-limiting examples of immunomodulators that can be used include AS-101 (Wyeth-Ayerst Labs.), bropirimine (Upjohn), gamma interferon (Genentech), GM-CSF (granulocyte macrophage colony stimulating factor; Genetics Institute), IL-2 (Cetus or Hoffman-LaRoche), human immune globulin (Cutter Biological), IMREG (from Imreg of New Orleans, La.), SK&F 106528, and TNF (tumor necrosis factor; Genentech). The immunotherpautic agent can be a PD-1 antagonist or a PD-L1 antagonist, such as an antibody (such as a monoclonal antibody) that specifically binds PD-1 or PD-L1, such as Atezolizumab, MPDL3280A, BNS-936558 (Nivolumab), Pembrolizumab, Pidilizumab, Durvalumab, CT011, AMP-224, AMP-514, MEDI-0680, BMS-936559, BMS935559, MEDI-4736, MPDL-3280A, MSB-0010718C. The immunotherpautic agent can also be a CTLA-4, LAG-3, or B7-H3 antagonist, such as Tremelimumab, BMS-986016, and MGA271.

In some examples, the additional therapeutic agent administered is a T cell agonist, such as an agonist of 4-1BB (CD137), OX40, and/or GITR. OX40 is a type 1 transmembrane glycoprotein. The human OX40 sequence includes an extracellular N-terminal portion of 191 aa, and an intracellular region of 36 aa. OX40L is a type II transmembrane glycoprotein. In one example, an OX40 agonist is an anti-OX40 antibody, such as a monoclonal antibody (mAb) (*e.g.*, PF-04518600, MEDI-6469, MEDI-0562, MEDI-6383, MOXR-0916, BMS 986178, or GSK3174998). Mimicking the natural OX40 ligand (OX40L), anti-OX40 monoclonal antibody selectively binds to and activates the OX40 receptor. In one example, an OX40 agonist is an OX40 ligand, OX40L, such as a natural ligand (such as a human OX40L). In one example, an OX40 agonist is a OX40 aptamer. 4-1BB (CD137/TNFSF9) belongs to the TNF receptor family, which includes multiple T cell co-stimulatory receptors. It is found on T cells, including CD8 and CD4 T cells. 4-1BB's expression on both T cells and antigen presenting cells, coupled with its capacity to promote survival, expansion, and enhanced effector function of activated T cells, makes it target for tumor immunotherapy. In one example, a 4-1BB agonist is a 4-1BB agonist antibody, such as a mAb. Specific agonist mAbs that can be used with the disclosed methods include PF-05082566 (utomilumab), and BMS-663513 (Urelumab). In one example, a 4-1BB agonist is a 4-1BB ligand (4-1BBL), such as a natural 4-1BBL (such as the human 4-1IBBL) or a streptavidinated 4-1BBL (SA-4-1BBL) complex. In one example, a 4-1BB agonist is a 4-1BB aptamer. GITR (glucocorticoid-induced tumor necrosis factor (TNF) receptor, or TNFRSF18) is a type I

transmembrane protein with homology to other TNF receptor family members such as OX40, CD27, and 4-1BB. GITR is normally expressed at low levels on resting CD4+foxp3- and CD8+ T cells, but is constitutively expressed at high levels on CD4+CD25+foxp3+ regulatory T cells (Tregs). Its ligand, GITRL (TNFSF18) is also a member of the TNF superfamily and is predominantly expressed by activated antigen presenting cells (APCs), including DCs, macrophage and activated B cells. In one example, a GITR agonist is a GITR agonist antibody, such as a mAb. Specific GITR agonist mAbs that can be used with the disclosed methods include DTA-1, TRX518, MK-4166, MK-1248, AMG 228, INCAGN01876, GWN323 (from Novartis), CK-302 (from Checkpoint Therapeutics) and BMS-986156. In one example, a GITR agonist is a GITR ligand (GITRL), such as a natural GITRL or a multivalent GITR ligand fusion protein. In one example, the GITR agonist is MEDI1873, a hexameric GITRL molecule with a human IgG1 Fc domain. In one example, GITR agonist is a GITR aptamer.

Non-limiting examples of anti-angiogenic agents include molecules, such as proteins, enzymes, polysaccharides, oligonucleotides, DNA, RNA, and recombinant vectors, and small molecules that function to reduce or even inhibit blood vessel growth. Examples of suitable angiogenesis inhibitors that can be used with the disclosed methods include, without limitation, angiostatin K1-3, staurosporine, genistein, fumagillin, medroxyprogesterone, suramin, interferon-alpha, metalloproteinase inhibitors, platelet factor 4, somatostatin, thromobospondin, endostatin, thalidomide, and derivatives and analogs thereof. For example, in some embodiments the anti-angiogenesis agent is an antibody that specifically binds to VEGF (*e.g.*, Avastin, Roche) or a VEGF receptor (*e.g.*, a VEGFR2 antibody). In one example the anti-angiogenic agent includes a VEGFR2 antibody, or DMXAA (also known as Vadimezan or ASA404; available commercially, *e.g.*, from Sigma Corp., St. Louis, MO) or both. The anti-angiogenic agent can be bevacizumab, sunitinib, an anti-angiogenic tyrosine kinase inhibitors (TKI), such as sunitinib, xitinib and dasatinib. These can be used individually or in any combination.

Exemplary kinase inhibitors that can be used with the disclosed methods include Gleevac, Iressa, and Tarceva, sunitinib, sorafenib, anitinib, and dasatinib that prevent phosphorylation and activation of growth factors. Antibodies that can be used include Herceptin and Avastin that block growth factors and the angiogenic pathway. These can be used individually or in combination.

In some examples, the additional therapeutic agent administered is a biologic, such as a monoclonal antibody, for example, 3F8, Abagovomab, Adecatumumab, Afutuzumab, Alacizumab , Alemtuzumab, Altumomab pentetate, Anatumomab mafenatox, Apolizumab, Arcitumomab, Bavituximab, Bectumomab, Belimumab, Besilesomab, Bevacizumab, Bivatuzumab mertansine, Blinatumomab, Brentuximab vedotin, Cantuzumab mertansine, Capromab pendetide,

Catumaxomab, CC49, Cetuximab, Citatuzumab bogatox, Cixutumumab, Clivatuzumab tetraxetan, Conatumumab, Dacetuzumab, Detumomab, Ecromeximab, Eculizumab, Edrecolomab, Epratuzumab, Ertumaxomab, Etaracizumab, Farletuzumab, Figitumumab, Galiximab, Gemtuzumab ozogamicin, Girentuximab, Glembatumumab vedotin, Ibritumomab tiuxetan, Igovomab, Imciromab, Intetumumab, Inotuzumab ozogamicin, Ipilimumab, Iratumumab, Labetuzumab, Lexatumumab, Lintuzumab, Lorvotuzumab mertansine, Lucatumumab, Lumiliximab, Mapatumumab, Matuzumab, Mepolizumab, Metelimumab, Milatuzumab, Mitumomab, Morolimumab, Nacolomab tafenatox, Naptumomab estafenatox, Necitumumab, Nimotuzumab, Nofetumomab merpentan, Ofatumumab, Olaratumab, Oportuzumab monatox, Oregovomab, Panitumumab, Pemtumomab, Pertuzumab, Pintumomab, Pritumumab, Ramucirumab, Rilotumumab, Rituximab, Robatumumab, Satumomab pendetide, Sibrotuzumab, Sonepcizumab, Tacatuzumab tetraxetan, Taplitumomab paptox, Tenatumomab, TGN1412, Ticilimumab (tremelimumab), Tigatuzumab, TNX-650, Trastuzumab, Tremelimumab, Tucotuzumab celmoleukin, Veltuzumab, Volociximab, Votumumab, Zalutumumab, or combinations thereof. In some examples, the therapeutic antibody is specific for PD-1 or PDL-1.

In some examples, the subject is also administered an effective amount of nonmyeloablative chemotherapy or radiotherapy. For example, the subject may receive an effective amount of nonmyeloablative chemotherapy, such as administration of one or more of cisplatin, fludarabine, idarubicin, melphalan, ara-C, 2-chlorodeoxyadenosine, antithymocyte globulin, and cyclophosphamide (such as 10 to 50 mg/kg body weight). In some examples, the subject receives an effective amount of solid tumor irradiation, thymic irradiation, or total body irradiation (*e.g.*, 2 Gy), or combinations thereof.

In some examples, following administration of the recombinant oncolytic virus, the subject is administered one or more of an effective amount of tacrolimus, cyclosporine, and/or methotrexate.

In some examples, the recombinant OVs and the one or more additional therapies can be, for example, and not by way of limitation, can be administered concurrently to the subject being treated, or can be administered at the same time or sequentially in any order or at different points in time.

The additional therapy can be administered, in various examples, in a liquid dosage form, a solid dosage form, a suppository, an inhalable dosage form, an intranasal dosage form, in a liposomal formulation, a dosage form comprising nanoparticles, a dosage form comprising microparticles, a polymeric dosage form, or any combinations thereof. In certain cases, the additional therapy can be administered over a period of about 1 week to about 2 weeks, about 2

weeks to about 3 weeks, about 3 weeks to about 4 weeks, about 4 weeks to about 5 weeks, about 6 weeks to about 7 weeks, about 7 weeks to about 8 weeks, about 8 weeks to about 9 weeks, about 9 weeks to about 10 weeks, about 10 weeks to about 11 weeks, about 11 weeks to about 12 weeks, about 12 weeks to about 24 weeks, about 24 weeks to about 48 weeks, about 48 weeks or about 52 weeks, or longer. The frequency of administration of the additional therapy can be, in certain instances, once daily, twice daily, once every week, once every three weeks, once every four weeks (or once a month), once every 8 weeks (or once every 2 months), once every 12 weeks (or once every 3 months), or once every 24 weeks (once every 6 months). In certain cases, a method of treating a subject having a cancer can include administering, to the subject, an effective amount of a recombinant OV, *e.g.,* a recombinant vaccinia virus, of this disclosure, comprising one or more nucleic acids that can code for one or more metabolic modulating proteins.

Clinical Response

Such methods can treat the tumor in the subject by reducing the volume or weight of the tumor, reducing the number of metastases, reducing the size or weight of a metastasis, or combinations thereof. In some examples a metastasis is cutaneous or subcutaneous. Thus, in some examples, administration of a disclosed recombinant oncolytic virus (alone or in combination with another anti-cancer therapy) treats a tumor in a subject by reducing the size or volume of the tumor by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 95%, at least 98% or at least 99%, for example as compared to no administration of a disclosed recombinant oncolytic virus or administration of a recombinant oncolytic virus not containing a metabolic modulating protein coding sequence (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1]). In some examples, administration of a disclosed recombinant oncolytic virus treats a tumor in a subject by reducing the weight of the tumor by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 95%, at least 98% or at least 99%, for example as compared to no administration of a disclosed recombinant oncolytic virus or administration of a recombinant oncolytic virus not containing a metabolic modulating protein coding sequence (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1]). In some examples, administration of a disclosed

recombinant oncolytic virus treats a tumor in a subject by reducing the size or volume of a metastasis by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 95%, at least 98% or at least 99%, for example as compared to no administration of a disclosed recombinant oncolytic virus or administration of a recombinant oncolytic virus not containing a metabolic modulating protein coding sequence (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1]). In some examples, administration of a disclosed recombinant oncolytic virus treats a tumor in a subject by reducing the number of metastases by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 95%, at least 98% or at least 99% for example as compared to no administration of a disclosed recombinant oncolytic virus or administration of a recombinant oncolytic virus not containing a metabolic modulating protein coding sequence (such as an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4), insulin, and/or IGF-1 [such as one or more of leptin, insulin, chemerin, and IGF-1]). In some examples, combinations of these effects are achieved.

Compositions and Kits

Also provided are compositions and kits that can be used with the disclosed methods. In some examples, the composition or kit includes one or more disclosed recombinant oncolytic viruses that expresses a metabolic modulating protein, for example with a pharmaceutically acceptable carrier. In one example, the OV expresses an adipokine (*e.g.*, leptin, chemerin, adiponectin, apelin, IL-6, MCP-1, PAI-1, RBP4, TNFα, visfatin, omentin, vaspin, progranulin and/or CTRP-4). In one example, the OV expresses insulin and/or IGF-1. In one example, the OV expresses leptin. The adipokine, insulin and/or IGF-1 can be part of a fusion protein that further includes a second protein, such as a gamma chain cytokine (*e.g.*, L-2 or IL-15). In some examples the second protein can be a cytokine protein, such as a chemokine, an interferon, an interleukin, a lymphokine, a tumour necrosis factor, or a fusion protein comprising any combinations thereof. Other examples are provided above.

In one example, the OV expresses leptin, insulin, chemerin, or IGF-1 (which can be part of a fusion protein that further includes a gamma chain cytokine, such as IL-2 or IL-15). In a specific example, the OV is talimogene laherparepvec (T-VEC). In a specific example, the OV is Western Reserve strain *Vaccinia* virus. In a specific example, the OV is vaccinia, and the one or more

metabolic modulating proteins is leptin. In a specific example, the OV is Western Reserve strain *Vaccinia* virus, and the one or more metabolic modulating proteins is leptin. In a specific example, the OV is Western Reserve strain *Vaccinia* virus, and the one or more metabolic modulating proteins is a leptin-IL-2 or leptin-IL-15 fusion protein (wherein the leptin and cytokine may be joined by a linker).

The kits can include additional reagents, such as one or more anti-cancer reagents, such as a chemotherapeutic, biologic, or combination thereof. In some examples, in a kit, the OV and anti-cancer reagents are present in separate containers.

In one example, the kit further includes a biologic, such as a PD-1 antagonist; a PD-L1 antagonist; a CTLA4 antagonist; a T cell agonist; or combinations thereof. In one example, the PD-1 antagonist, PD-L1 antagonist, CTLA4 antagonist, and T cell agonist, are mAbs. Exemplary T cell agonists include agonists of 4-1BB, agonists of OX40, and agonists GITR, such as a mAb, aptamer, or ligand for these receptors. Exemplary agonists of 4-1BB that can be included in the kit include mAbs, such as PF-05082566 (utomilumab) or BMS-663513 (Urelumab), or a ligand (*e.g.*, 4-1BBL or SA-4-1BBL). Exemplary agonists of OX40 that can be included in the kit include a mAb (*e.g.*, PF-04518600, MEDI6469, MEDI0562, MEDI6383, MOXR0916, BMS 986178, or GSK3174998), or a ligand (e.g., OX40L). Exemplary agonists of agonists GITR that can be included in the kit include a GITR agonist mAb, such as DTA-1, TRX518, MK-4166, MK-1248, AMG 228, INCAGN01876, GWN323 (from Novartis), CK-302 (from Checkpoint Therapeutics) or BMS-986156. Exemplary agonists of GITR that can be included in the kit include a GITR ligand (GITRL), such as a natural GITRL or a multivalent GITR ligand fusion protein, such as MEDI1873.

Also provided are containers that include a composition disclosed herein, such as an OV provided herein. In some embodiments, the container is a syringe. In some examples, the syringe includes a needle. The plunger in a syringe can have a stopper to prevent the plunger from being accidentally removed during aspiration. Disposable syringes generally contain a single dose of vaccine. The syringe can have a tip cap to seal the tip prior to attachment of a needle. In non-limiting examples, the tip cap is made of rubber, such as a butyl rubber.

In other embodiments, the container is a vial. In some examples, the vial is made of glass, such as a colorless glass, for example borosilicate. In other examples, the vial is made of plastic. The vial can include a stopper, such as a rubber stopper, or a cap, such as cap adapted to enable insertion of a syringe. In some examples, the vial includes a single dose of the composition. In other examples, the vial includes multiples doses of the composition, such as 2, 3, 4, 5, 6, 7, 8, 9 or

10 or more doses of the composition. Generally, the vial is sterilized prior to adding the composition.

Also provided are kits that include a container disclosed herein. In some embodiments, the kits includes a vial (such as a vial containing the composition), a syringe (for example, an empty syringe or a syringe containing the composition), a needle, or any combination thereof. The compositions can be in a suspension, such as in PBS or other pharmaceutically acceptable carrier. Alternatively, the compositions can be in a dried or powered form, such as lyophilized or freeze dried, which can then be reconstituted by an end user (for example with PBS or other pharmaceutically acceptable carrier). In some examples the containers can include a pharmaceutically acceptable carrier, such as PBS, or the pharmaceutically acceptable carrier, such as PBS, can be in a separate container (for example if the compositions are freeze-dried or lyophilized). In some examples, the containers in the kit further include one or more stabilizers. In some examples, the kits also include a device that permits administration of the composition to a subject. Examples of such devices include a syringe. A kit can be packaged (for example, in the same box) with a leaflet including details of the composition, such as instructions for administration and/or details of the OVs within the composition.

Example 1

Materials and Methods

This example describe materials and methods used to generate the results described in the Examples below.

Mice

C57/BL6 mice and *Pten$^{f/f}$Braf^{V600E}Tyr$^{Cre.ER}$* mice were obtained from Jackson Laboratories and bred in house.

Tumor lines

Tumor experiments were conducted using a single-cell clone derived from a melanoma tumor formed from a *Pten$^{f/f}$Braf^{V600E}Tyr$^{Cre.ER}$* painted with tamoxifen (clone 24). The cDNA for leptin was obtained from OriGene and transfected into clone 24 followed by hygromycin selection (an empty vector plasmid was used as a control). Single cell clones were selected and grown as cell line CL24^{hygro} for control plasmid and CL24^{leptin} for leptin expressing cell line.

Tumor models

C57BL/6J mice were injected with $CL24^{hygro}$ or $CL24^{leptin}$ melanoma cell line (250,000 cells intradermally) on day 0 and followed until tumors reach 15 mm in any direction. Tumors were measured every other day with digital calipers and tumor size was calculated by LxW. Tumors were treated with PBS, VV^{ctrl} or VV^{leptin} (2.5x106 PFU) intratumorally when tumors reached approximately a $20mm^2$ and tumor growth was monitored until tumors treated with PBS reached 15mm in any direction. For CD8 depletion experiments mice were injected every other day starting at day 0 with anti-CD8 (YTS) at 200ug per mouse. On day 7 mice were injected with $CL24^{hygro}$ or $CL24^{leptin}$ melanoma cell line (250,000 cells intradermally) and followed until tumor reach 15mm in any direction.

Oncolytic virus production

The wild-type *Vaccinia* virus Western Reserve (WR) strain was obtained from the American Type Culture Collection (BEI Resources). WR.TK–.Luc+ were described previously (Kirn *et al., PLoS Med* 2007; 4(12):e353) and were constructed for this work, with the pSC65 plasmid (from Prof. Bernie Moss, NIH) cloned to express firefly luciferase from the viral pSE/L promoter and mouse leptin (*Lep*) from the p7.5 promoter. This was recombined into the viral TK gene. *Vaccinia* virus expressing leptin was generated by cloning in the leptin gene using Gibson Cloning (New England BioLabs) into the *Vaccinia* plasmid. Leptin gene was cloned from a mouse leptin ORF mammalian expression plasmid (Sino Biological Inc.).

T cell isolations from lymph node and tumor

Spleen and lymph node $CD8^+$ T cells were isolated from wild-type mice. Tissue was harvested, mechanically disrupted, and incubated with a biotinylated antibody cocktail consisting of antibodies (BioLegend) to B220, CD11b, CD11c, CD16/32, CD19, CD25, CD105, NK1.1, TCRγδ, and CD4. After a wash step, cells were incubated with streptavidin-coated magnetic nanoparticles (BioLegend). After washing, $CD8^+$ cells were isolated by applying a magnetic field and removing untouched cells.

To obtain single-cell suspensions of tumor infiltrating lymphocytes, tumor bearing mice were sacrificed and tumors were harvested. Excised, whole tumors were injected repeatedly using 20G needles with 2mg/mL collagenase type VI, 2U/mL hyluronidase (Dispase), and 10U/mL DNAse I (Sigma) in buffered RPMI with 10% FBS and incubated for 30 min at 37°C. Tumors were then mechanically disrupted between frosted glass slides and filtered to remove particulates, then vortexed for 2 minutes. In many experiments (especially prior to sorting), tumor homogenates were debulked of tumor cells using CD105-biotin mediated magnetic depletion.

Metabolic Assays

T cell metabolic output was measured by Seahorse technology as previously described (Scharping *et al., Cancer Immunol. Res.* 2017; 5:9-16). Briefly, 100,000 T cells were seeded into Cell-Tak-coated XFe96 plates in minimal unbuffered assay media containing 25 mM glucose, 2 mM glutamine, and 1 mM sodium pyruvate. Cells received sequential injections of 2 μM oligomycin, 2 μM FCCP, 10 mM 2-deoxyglucose, and 0.5 μM rotenone/antimycin A..

Single-cell metabolic capacity was assayed by flow cytometry. Specifically, 2-NBD-glucose (Cayman Chemical) and MitoTracker FM dyes (ThermoFisher) were used to assay the propensity of cells to take up glucose or generate intermediates via their mitochondria. Nondraining and draining lymph node or tumor preparations were pulsed with 20 μM 2-NBDG in 5% FBS-containing media for 30 min at 37°C. Cells were surface stained and loaded with MitoTracker FM dyes to measure mitochondrial mass and function.

Immunoblotting

Immunoblotting was performed as previously described (Delgoffe *et al., Mol. Immunol.* 2009; 46(13):2694-8). Leptin antibody was obtained from Mouse Leptin/OB antibody (R&D system BAF498).

ELISA

ELISA plate was coated with 50uL capture antibody (1:1000 in PBS) and put at 4°C overnight. Next day plate was washed 3 times with Wash Buffer (1L PBS + 0.05% Tween 20). Plate was Blocked with 200uL blocking buffer (200mL PBS + 1% BSA) for 1 hour at room temperature. Samples were added (50 μl) in blocking buffer to the wells together with Standard Curve samples. Plate was incubated at room temperature for 2 hours. Secondary antibody was added (1:2000 in blocking buffer) and incubated at room temperature for 1 hour. After one hour HRP streptavidin (1:2000 in blocking buffer) was added and incubated at room temperature for 30min. 40 μL TMB substrate A and 40 μL TMB substrate B were added to develop samples. Plate was read at 450nm in a plate reader. Antibodies used for leptin Elisa experiment: Capture Mouse Leptin/OB antibody (R&D systems AF498) and detection antibody Mouse Leptin/OB antibody (R&D system BAF498).

TCR Sequencing

CL24 tumors with different treatments were excised and processed for genomic DNA extraction (DNeasy QIAGEN kit). TCR sequencing was then performed following the immunoSEQ assay (Adaptive Biotechnologies) (Robins *et al., Blood* 2009; 114(19):4099-107).

Single cell RNA sequencing analysis

CL24 tumors were treated with PBS, VV^{ctrl} or VV^{leptin} ($2.5x10^6$ PFU) intratumorally for 7 days. Tumor infiltrating lymphocytes were isolated and sorted for $CD45^+$ lymphocytes. $CD45^+$ cell were loaded into the Chromium instrument (10X Genomics, Pleasanton, CA), and the resulting barcoded cDNAs were used to construct libraries. The libraries from each sample were then RNA-sequenced. Cell-gene unique molecular identifier counting matrices were generated and analyzed using Seurat (Satija *et al.,* Nat Biotechnol 2015;33(5):495-502) and were hierarchically clustered using Cluster 3.0 (de Hoon *et al.,* Bioinformatics 2004; 20(9):1453-4).

Example 2

Oncolytic Vaccinia *virus treatment of tumors remodels tumor immune microenvironment*

While oncolytic viruses like T-vec are FDA approved immunotherapy for cancer treatment (*e.g.*, see Andtbacka *et al., J Clin Oncol* 2015; 33(25):2780-8), the immune consequences of these agents are unclear. The immune infiltrate induced by oncolytic virus infection was systematically profiled. One major limitation of oncolytic virus therapy is that many viruses, including T-VEC, do not replicate efficiently in hypoxia (Friedman *et al., Transl Oncol* 2012; 5(3):200-7; Pipiya *et al., Gene Ther* 2005; 12(11):911-7). Thus, oncolytic *Vaccinia* virus, which is easily engineered, encodes its own polymerase, and, maintains replicative function in hypoxic tumor cells, was used. The Western Reserve laboratory strain *Vaccinia* virus was used. This virus harbors a genetic deletion of thymidine kinase and *Vaccinia* growth factor genes generating a potent oncolytic viral agent (Buller *et al., Nature* 1985; 317(6040):813-5; Whitman *et al., Surgery* 1994; 116(2):183-8; Puhlmann *et al., Cancer Gene Ther* 2000; 7(1):66-73). A melanoma cell line termed clone 24 (CL24), generated from a single-cell of a $Pten^{f/f}Braf^{LSL.V600E}Tyr2^{Cre.ER}$ mouse that developed melanoma after tamoxifen administration (Dankort *et al., Nat Genet* 2009; 41(5):544-52) was used (**FIG. 1A**). This cell line is syngeneic to C57/BL6 mice, carries driver mutations common in human melanoma (as opposed to the often used B16). Additionally, this CL24 cell line is poorly infiltrated (**FIG. 1B**), and is completely insensitive to anti-PD1 monotherapy (**FIG. 1C**).

Vaccinia virus was injected intratumorally when tumors reached 4 mm in size, which resulted in substantial tumor regression, but no complete responses (**FIG. 2A**). To determine the

character of the tumor infiltrate induced by oncolytic viruses, single cell RNA-sequencing of the CD45$^+$ tumor infiltrating leukocytes of PBS or *Vaccinia* infected clone 24 tumors was determined by 10X Genomics profiling. Unsupervised clustering data analysis was used to separate the CD45+ cells into distinct groups of immune populations (**FIG. 2B**). These immune populations were then classified based on the expression of known markers for each population (**FIGS. 3A-3C**). Importantly, these analyses were conducted when tumors had not yet regressed. This data identified known immune cell populations when analyzed in aggregate, however subsetting based on treatment group revealed that *Vaccinia* virus oncolytic immunotherapy induced striking changes in the tumor immune microenvironment (**FIG. 2C**). *Vaccinia* infected tumors showed a massive influx of new, effector and effector like CD8$^+$ T cells, M1 macrophages, and a loss of dysfunctional or suppressive cells like MDSC, M2 macrophages, exhausted T cells, and regulatory T cells (**FIG. 2C**). Thus, oncolytic *Vaccinia* virus induces a dramatic remodeling of the tumor immune microenvironment, but one that ultimately succumbs to tumor-induced immune suppression and eventual outgrowth (**FIG. 2A**).

Example 3

Oncolytic Vaccinia *virus promotes non-exhausted T cell infiltration with severe metabolic deficiencies*

Flow cytometric analysis of the TIL from oncolytic virus treated mice confirmed that the influx of new immune cells appeared to be dominated by CD8$^+$ T cells while we observe a decrease of T regulatory T cells (**FIG. 4A**). Analysis of the co-inhibitory marker expression of these cells show an influx of CD8$^+$ T cells that have expression of Tim3 alone (**FIG. 4B**) as well as a low to mid expression of PD1 (**FIG. C**) indicating these cells are not reinvigorated tumor residents but rather new immigrants and are not yet fully exhausted T cells. While co-inhibitory molecule expression is associated with T cell dysfunction, metabolic insufficiency, too, can predict T cell function. Mitochondrial content was analyzed as a marker for metabolic sufficiency, revealing that despite the 'non-exhausted' co-inhibitory molecule pattern of expression, TIL from oncolytic virus-treated tumors still succumbed to metabolic exhaustion (**FIG. 4D**).

Example 4

Leptin metabolically reprograms activated T cells

Given that oncolytic viruses stimulated new immune infiltrate that still succumbed to metabolic insufficiency, ways to bolster those new T cells such that they would be more competitive in the tumor microenvironment were identified. The goal was to utilize a genetically

encoded payload, so that such an agent could be encoded in the viral vector. Leptin is a cytokine that modulates energy homeostasis as well as promotes an inflammatory response. To determine the metabolic reprogramming functions of leptin on T cells, activated $CD8^+$ T cells isolated from peripheral lymph nodes (LN) were cultured in increasing concentrations of leptin.

Leptin induced increases in both basal oxygen consumption rate as well as spare respiratory capacity (a measure of mitochondrial reserve that defines long-lived memory T cells (20)) of $CD8^+$ T cells (**FIG. 5A**), but had little effect on activated T cells' ability to perform glycolysis as measured by extracellular acidification (**FIG. 5B**). Flow cytometry analysis reinforces this data showing an increase in mitochondrial mass under leptin exposure indicative of higher oxidative phosphorylation, while observing no changes in glucose uptake (**FIG. 5C**). Thus, leptin can stimulate T cells to increase their oxidative activity and capacity, a metabolic reprogramming event highly desirable in the tumor microenvironment.

The expression of the leptin receptor in was confirmed in murine T cells, as previously observed (18) (**FIG. 5D**). Furthermore, melanoma tumor infiltrating lymphocytes (TIL) express a higher level of the leptin receptor compared to T cells in the lymph nodes (**FIG. 5D**). Categorizing the TIL according to their expression levels of co-inhibitory molecules, higher expression of the leptin receptor was observed in activated or exhausted T cells with high expression of PD1 and Tim3 (**FIG. 5E**). Thus, leptin can promote metabolic reprogramming in T cells, and tumor-infiltrating T cells bear its receptor.

Example 5

Elevating local leptin levels in the tumor microenvironment enables antitumor immunity

The therapeutic effects of leptin in the context of tumor infiltrating lymphocytes have not been previously investigated. It was hypothesized that leptin can enhance the metabolic capacity of tumor infiltrating lymphocytes, consequently enhancing their function in the tumor.

Initial studies treating tumor-bearing mice with recombinant leptin showed that systemic delivery, even at relatively high doses, cannot substantially improve leptin levels in the tumor interstitial fluid. So to first test the metabolic reprogramming functions of leptin in isolation (outside of oncolytic virus infection), CL24 were engineered cells to express an empty vector ($CL24^{hygro}$), or leptin ($CL24^{leptin}$). $CL24^{leptin}$ cells expressed leptin intracellularly (**FIG. 6A**) and released it into culture supernatant (**FIG. 6B**). *In vitro*, $CL24^{leptin}$ showed a comparable growth kinetics with CL24-expressing a control plasmid ($CL24^{hygro}$) (**FIG. 6C**). However, when $CL24^{leptin}$ cells were injected subdermally into C57BL/6J mice, they grew at a substantially slower rate

compared to CL24^{hygro} controls (**FIG. 7A**) and have significantly prolonged survival (**FIG. 7B**) indicating leptin may stimulate host immunity. Indeed, depletion of CD8$^+$ T cells (**FIG. 6D**) revealed that the controlled tumor growth observed in CL24^{leptin} tumors required functional immunity (**FIG. 7C**). Thus, locally elevating leptin in the tumor microenvironment induced immune-mediated tumor growth control. Analysis of the tumor infiltrating lymphocytes at day ten (when tumors were of comparable size between groups) showed an increased infiltration of CD8$^+$ T cells in the tumors expressing leptin compared to control tumors (**FIG. 7D**). Increased infiltration of natural killer cells, but not other immune populations such as B cells, was observed (**FIG. 6E**).

Example 6

Leptin metabolically improves the function of tumor infiltrating lymphocytes

It was observed that leptin functionally improved T cells. CD8$^+$ T cells infiltrating leptin-overexpressing tumors synthesized elevated levels of IFNγ and TNFα upon restimulation with PMA and ionomycin (**FIG. 7E**). Additionally, CD8$^+$ T cells that infiltrate leptin expressing tumors are more proliferative *in situ* as measured by Ki67 staining (**FIG. 7F**). Leptin can activate downstream signals via the leptin receptors through the JAK-Stat3 and MAPK pathway, and indeed, T cells infiltrating leptin-overexpressing tumors had higher steady-state phosphorylation of AKT, STAT3 and p38-MAPK (**FIG. 6F**). Thus, while these cells may appear more phenotypically 'exhausted', leptin-induced metabolic support allowed cells to be polyfunctional, proliferative, and mediate tumor control.

Example 7

Leptin expressing oncolytic Vaccinia virus induces superior antitumor responses

To generate a leptin expressing *Vaccinia* virus, the leptin gene (*Lep)* was cloned in the luciferase expressing pSC65 vector under the control of the *Vaccinia* p7.5 promoter. Leptin containing recombinant vaccinia virus (VV^{leptin}) and control luciferase expressing virus (VV^{ctrl}) were generated and used to infect CL24 cells. Expression of leptin in CL24 cells was analyzed 24 and 48 hour post infection (**FIG. 8A**) as well as the release of leptin in the media (**FIG. 8B**). Mice harboring CL24 tumors were treated with VV^{ctrl} or VV^{leptin} with a dose of 2.5x10^6 PFU intratumorally, which was sufficient to induce luciferase expression specifically in the tumor (**FIG. 8D**) and detect free leptin in the tumor interstitial fluid; white adipose (WA) tissue interstitial fluid acted as a positive control (**FIG. 8C**). Furthermore, analysis of leptin receptor (LeptinR) expression in TIL show no changes between the three treatment conditions (**FIG. 8E**).

Consistent with previous results, all mice injected with control virus experienced partial responses, leading to eventual tumor outgrowth. The partial response to control virus was especially exciting as this aggressive melanoma line is completely resistant to anti-PD1 immunotherapy (**FIG. 1C**). In contrast to mice treated with VV^{ctrl}, those injected with the same dose of VV^{leptin} had larger regressions, including a substantial proportion of complete responses (**FIG. 9A**).

The transcriptomic profiles were confirmed with analysis of the tumor infiltrate. Consistent with the scRNAseq data, analysis of immune infiltrate in tumors treated with VV^{ctrl} and VV^{leptin} showed that both oncolytic viruses induced an increase in T cell infiltration at the tumor site (**FIG. 9B).** As oncolytic viruses have been purported to induce new T cell priming to viral and tumor antigens (Brown *et al., Sci Transl Me*d 2017; 9(408); Russell *et al., Cancer Cell* 2018;33(4):599-605), the effects of the treatments on the T cell repertoire at the tumor site was examined. TCR sequencing revealed that while PBS treated tumors had few infiltrating T cells dominated by an oligoclonal population, treatment with *Vaccinia* resulted in a substantial influx of new T cells with a polyclonal repertoire (**FIG. 9C**). Leptin-engineered *Vaccinia* had a slightly less clonal population, suggesting at this time point (7 days after viral treatment) some clones were preferentially expanding (**FIG. 9D**). The clonal expansion could be indicative of expansion of some memory precursors, and indeed leptin-engineered VV induced a greater percentage of $CD127^{hi}$ memory precursors (**FIG. 9E**).

Leptin inhibits regulatory T cells (T_{reg}) and modulates the inflammatory response in autoimmune diseases. Furthermore, oncolytic virus therapy can reduce the infiltration of T_{reg} cells (Barve *et al., J Clin Oncol* 2008; 26(27):4418-25; Ricca J *et al., Mol Ther* 2018; 26(4):1008-19). Consistently, after oncolytic virus treatment of VV^{ctrl} and VV^{leptin} a decrease in percentage of the T_{reg} population compared to PBS treatment was observed with comparable levels between VV^{ctrl} and VV^{leptin} (**FIG. 8F**) indicating that leptin was not necessarily acting at the level of T_{reg} cell modulation.

Memory T cells have increased mitochondrial reserve and depend on that reserve for their memory function (van der Windt *et al., PNAS* 2013; 110(35):14336-41). As such, leptin may metabolically support $CD8^+$ T cells. $CD8^+$ T cells in tumors treated with VV^{leptin} exhibited an increase in mitochondrial mass as measured by VDAC staining (**FIG. 10A**). Furthermore, analysis of $CD8^+$ T cells infiltrating treated tumors revealed VV^{leptin} induced a qualitatively superior tumor infiltrate: increased T cell activity at the tumor site shown by an increase in cytokine competency (**FIG. 10B**), as well as increased proliferative capacity (**FIG. 10C**).

In summary, these data demonstrate that by providing metabolic support to newly infiltrated T cells induced by oncolytic virus treatment, memory precursor populations with superior anti-tumor capabilities can preferentially expand and mediate complete responses.

In view of the many possible embodiments to which the principles of the disclosed invention may be applied, it should be recognized that the illustrated embodiments are only examples of the invention and should not be taken as limiting the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. A recombinant oncolytic virus, comprising:
a nucleic acid molecule encoding one or more metabolic modulating proteins.

2. The recombinant oncolytic virus of claim 1, wherein the nucleic acid is operably linked to a promoter.

3. The recombinant oncolytic virus of claim 1 or 2, wherein the oncolytic virus is a herpes simplex virus (HSV), vaccinia virus, adenovirus, poxvirus, reovirus, poliovirus, coxsackie virus, measles virus, vesicular stomatitis virus (VSV), Seneca valley virus, ECHO virus, Newcastle disease virus, chicken anemia virus, or parovirus.

4. The recombinant oncolytic virus of any one of claims 1 to 3, wherein the oncolytic virus is a herpes simplex virus (HSV), poxvirus, or a vaccinia virus.

5. The recombinant oncolytic virus of claim 4, wherein the oncolytic virus is HSV.

6. The recombinant oncolytic virus of claim 5, wherein the HSV is talimogene laherparepvec (T-VEC).

7. The recombinant oncolytic virus of claim 4, wherein the oncolytic virus is a poxvirus.

8. The recombinant oncolytic virus of claim 7, wherein the poxvirus is vaccinia virus.

9. The recombinant oncolytic virus of claim 8, wherein the vaccinia virus is Western Reserve strain *Vaccinia* virus.

10. The recombinant oncolytic virus of any of claims 1 to 9, wherein the one or more metabolic modulating proteins comprise an adipokine, insulin, insulin-like growth factor 1, or any combinations thereof.

11. The recombinant oncolytic virus of claim 10, wherein the adipokine is leptin, adiponectin, apelin, chemerin, interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1), plasminogen

activator inhibitor-1 (PAI-1), retinol binding protein 4 (RBP4), tumor necrosis factor-alpha (TNFα), visfatin, omentin, vaspin, progranulin or CTRP-4.

12. The recombinant oncolytic virus of claim 11, wherein the adipokine is leptin.

13. The recombinant oncolytic virus any of claims 8 to 12, wherein the oncolytic virus is the vaccinia virus, and the one or more metabolic modulating proteins comprise leptin.

14. The recombinant oncolytic virus of any of claims 1 to 13, wherein the one or more metabolic modulating proteins comprises a fusion protein, wherein the fusion protein comprises a first protein and a second protein, wherein the first protein comprises the one of the one or more metabolic modulating proteins.

15. The recombinant oncolytic virus of claim 14, wherein the second protein is a cytokine, chemokine, an interferon, an interleukin, a lymphokine, and/or a tumour necrosis factor.

16. The recombinant oncolytic virus of claim 14 or 15, wherein the second protein is a cytokine.

17. The recombinant oncolytic virus of claim 15 or 16, wherein the cytokine is IL-1, IL-2, IL-17, IL-18, TGF-β1, TGF-β2, TGF-β3, IL-4, IL-10, IL-13, IL-7, IL-9, IL-15, IL-21, TNFα, IFN-γ, or any combinations thereof.

18. The recombinant oncolytic virus of any one of claims 14 to 17, wherein the first protein is leptin, insulin, chemerin, or insulin-like growth factor 1 and the second protein is interleukin (IL)-2 or IL-15.

19. The recombinant oncolytic virus of any one of claims 14 to 18, wherein the fusion protein comprises a linker between the first protein and the second protein.

20. The recombinant oncolytic virus of claim 19, wherein the oncolytic virus is vaccinia, the first protein is an N-terminal leptin, the second protein is a C-terminal IL-2 or IL-15, and wherein the first protein and second protein are joined by the linker.

21. The recombinant oncolytic virus of any of claims 1 to 20, wherein the one or more metabolic modulating proteins comprises at least about 80%, at least about 90%, or at least about 95% sequence identity to SEQ ID NO: 2, 4, 6, or 8.

22. The recombinant oncolytic virus of any of claims 1 to 21, wherein the nucleic acid molecule encoding the one or more metabolic modulating proteins comprises at least about 80%, at least about 90%, or at least about 95% sequence identity to SEQ ID NO:1, 3, 5 or 7.

23. The recombinant oncolytic virus any of claims 2 to 22, wherein the promoter is not native to the one or more metabolic modulating proteins.

24. The recombinant oncolytic virus any of claims 2 to 23, wherein the promoter is a constitutive promoter.

25. A method of treating a tumor in a subject, comprising

administering a therapeutically effective amount of the recombinant oncolytic virus any of claims 1 to 24 to the subject, thereby treating the tumor; or

administering a therapeutically effective amount of one or more metabolic modulating proteins or nucleic acid molecules encoding the protein(s) to the subject, thereby treating the tumor.

26. A method of increasing T cell infiltration into a tumor, comprising

administering a therapeutically effective amount of the recombinant oncolytic virus any of claims 1 to 24 to a subject with the tumor, thereby increasing T cell infiltration into the tumor; or

administering a therapeutically effective amount of one or more metabolic modulating proteins or nucleic acid molecules encoding the protein(s) to the subject with the tumor, thereby increasing T cell infiltration into the tumor.

27. The method of claim 25 or 26, wherein the tumor is a cancer.

28. The method of claim 27, wherein the cancer is a cancer of the lung, breast, prostate, liver, pancreas, skin, colon, head and neck, kidney, cervix, or ovary.

29. The method of claim 28, wherein the cancer is melanoma.

30. The method of any of claims 25 to 29, wherein the administering comprises a systemic administration.

31. The method of any of claims 25 to 29, wherein the administering comprises an intratumoral administration.

32. The method of any of claims 22 to 31, wherein the subject is a mammalian subject.

33. The method of claim 32, wherein the mammalian subject is a human.

34. The method of any of claims 25 to 33, further comprising administering a therapeutically effect amount of one or more additional therapies.

35. The method of claim 34, wherein the one or more additional therapies comprises administering one or more additional anti-cancer agents.

36. The method of claim 35, wherein the one or more additional anti-cancer agents comprise chemotherapy, radiotherapy, a biologic, surgery, or combinations thereof.

37. The method of claim 36, wherein the chemotherapy comprises one or more of an alkylating agent, antimetabolite, a hormone, or a hormone antagonist.

38. The method of claim 36 or 37, wherein the biologic comprises:

a PD-1 antagonist;

a PD-L1 antagonist;

a CTLA4 antagonist;

a T cell agonist; or

combinations thereof.

39. A composition or kit, comprising:

(a) the recombinant oncolytic virus of any of claims 1 to 24; and

(b) a PD-1 antagonist, a PD-L1 antagonist, a CTLA4 antagonist, a T cell agonist; or combinations thereof.

40. The method of claim 38 or the composition or kit of claim 39, wherein the T cell agonist is an agonist of 4-1BB, an agonist of OX40, an agonist of glucocorticoid-induced tumor necrosis factor (TNF) receptor (GITR), or combinations thereof.

41. The method or composition or kit of claim 40, wherein the T cell agonist is a monoclonal antibody, an aptamer, or a ligand.

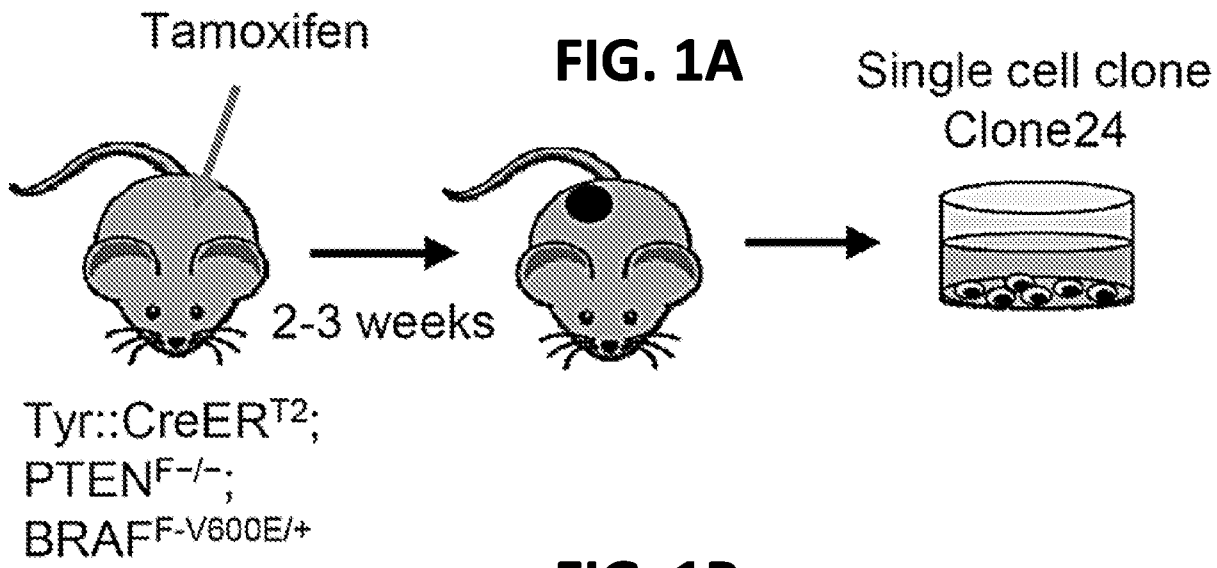

Tamoxifen
2-3 weeks
Single cell clone
Clone24
Tyr::CreER^{T2};
PTEN$^{F-/-}$;
BRAF$^{F-V600E/+}$

FIG. 1A

FIG. 1B

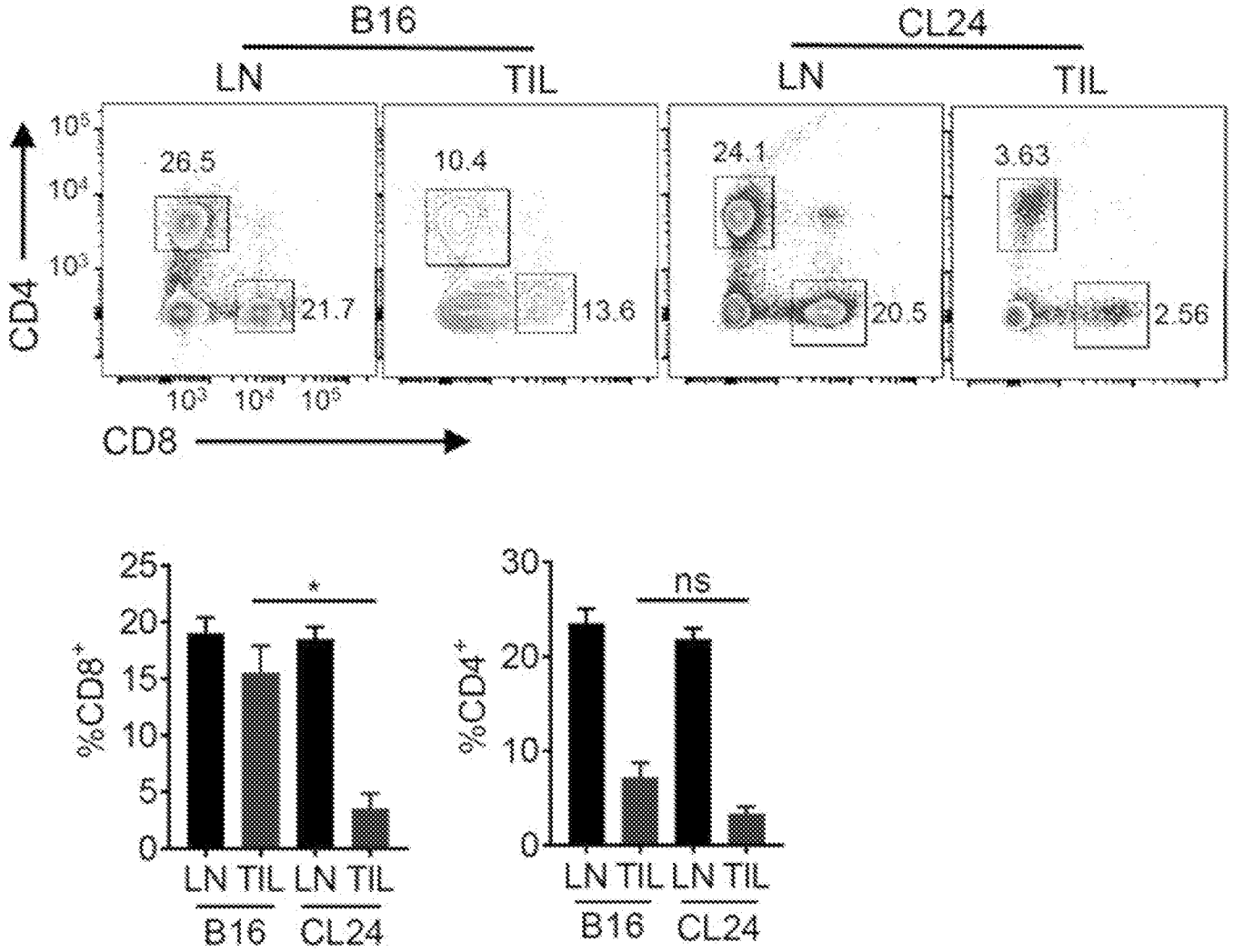

B16
CL24
LN
TIL
LN
TIL
CD4
CD8
26.5
21.7
10.4
13.6
24.1
20.5
3.63
2.56
%CD8+
%CD4+
*
ns
LN TIL LN TIL
B16 CL24

FIG. 1C

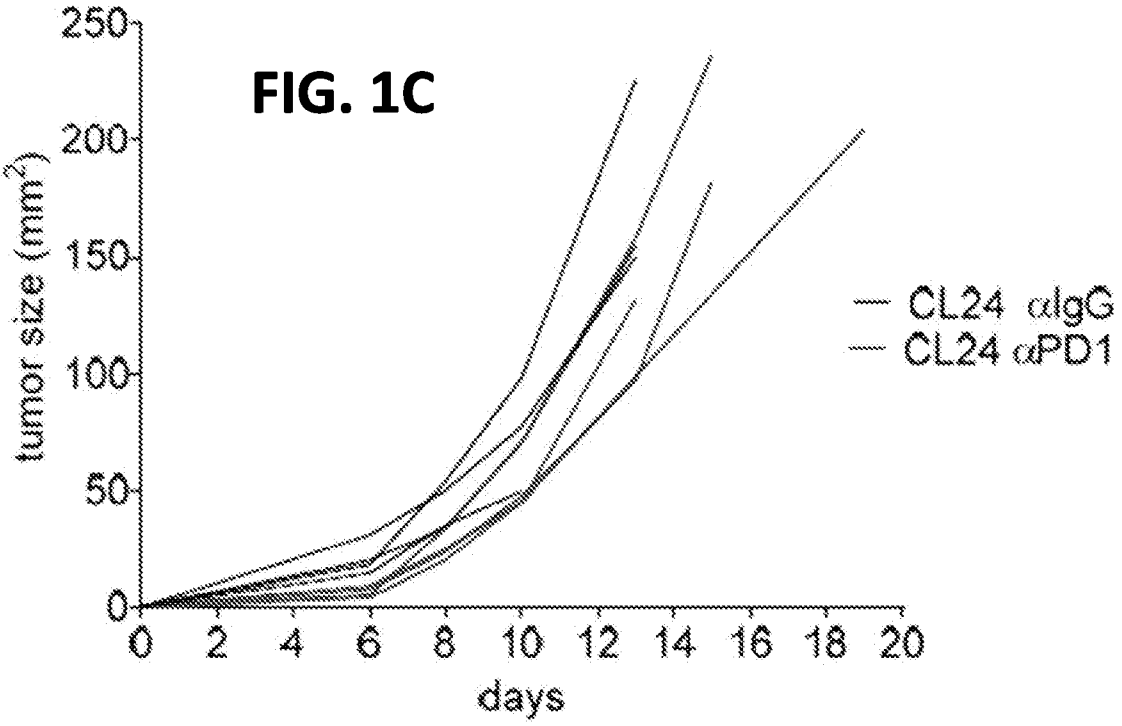
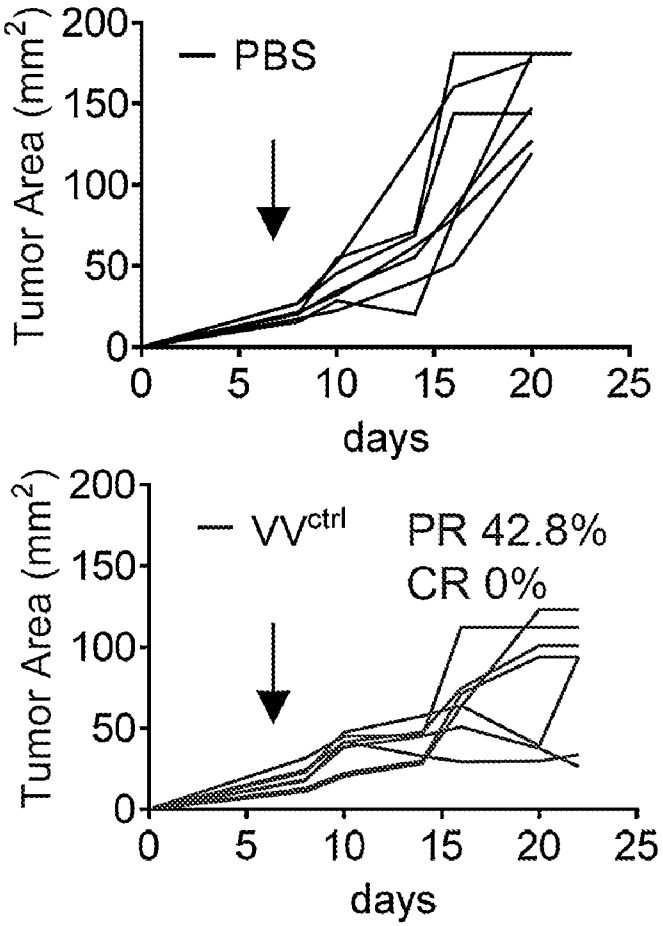

250
200
150
100
50
0
tumor size (mm2)
0 2 4 6 8 10 12 14 16 18 20
days
CL24 αIgG
CL24 αPD1

FIG. 2A


200
150
100
50
0
Tumor Area (mm2)
PBS
0 5 10 15 20 25
days
200
150
100
50
0
Tumor Area (mm2)
VVctrl
PR 42.8%
CR 0%
0 5 10 15 20 25
days

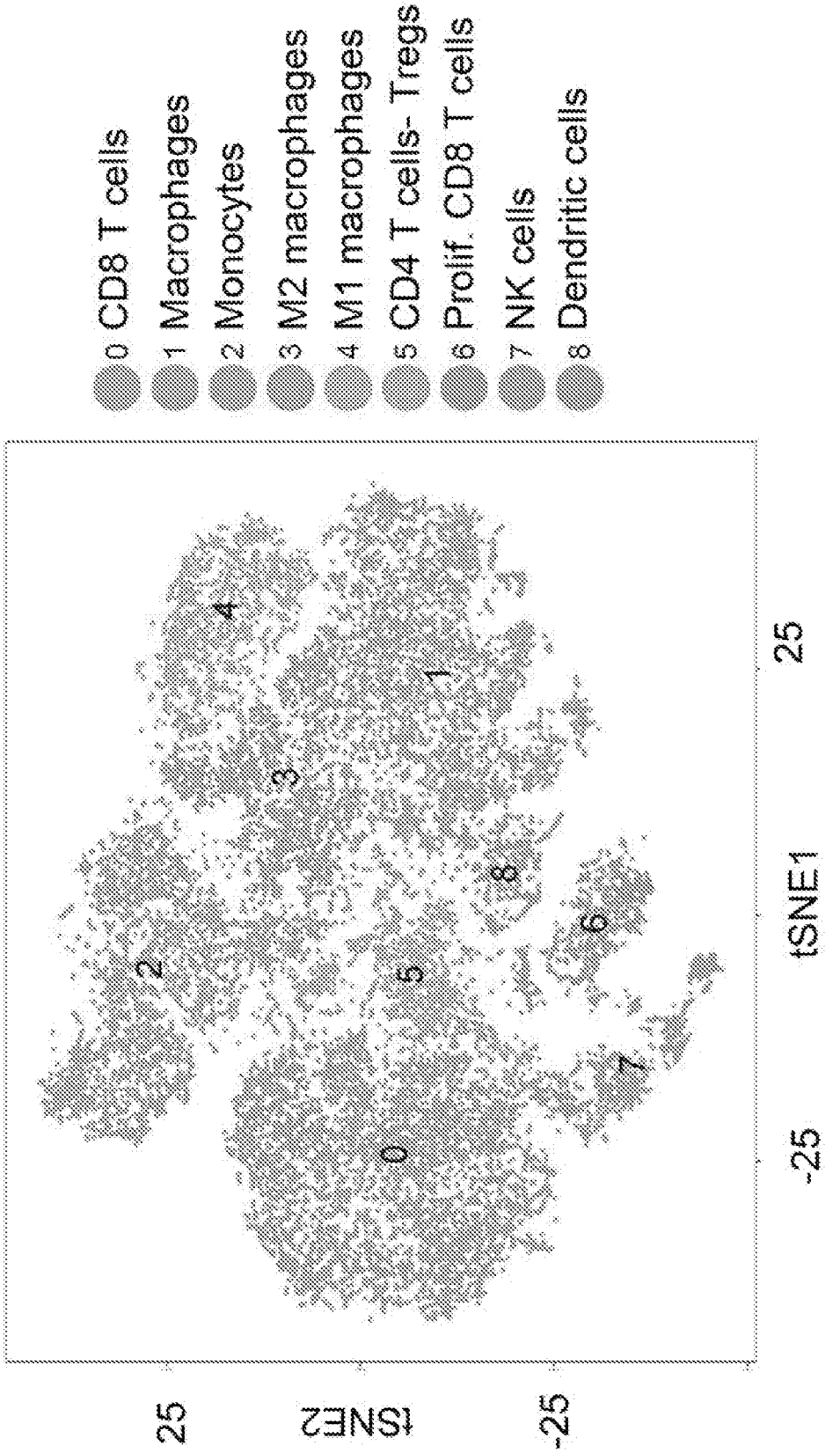

0 CD8 T cells
1 Macrophages
2 Monocytes
3 M2 macrophages
4 M1 macrophages
5 CD4 T cells- Tregs
6 Prolif. CD8 T cells
7 NK cells
8 Dendritic cells
25
tSNE2
-25
-25
tSNE1
25

FIG. 2B

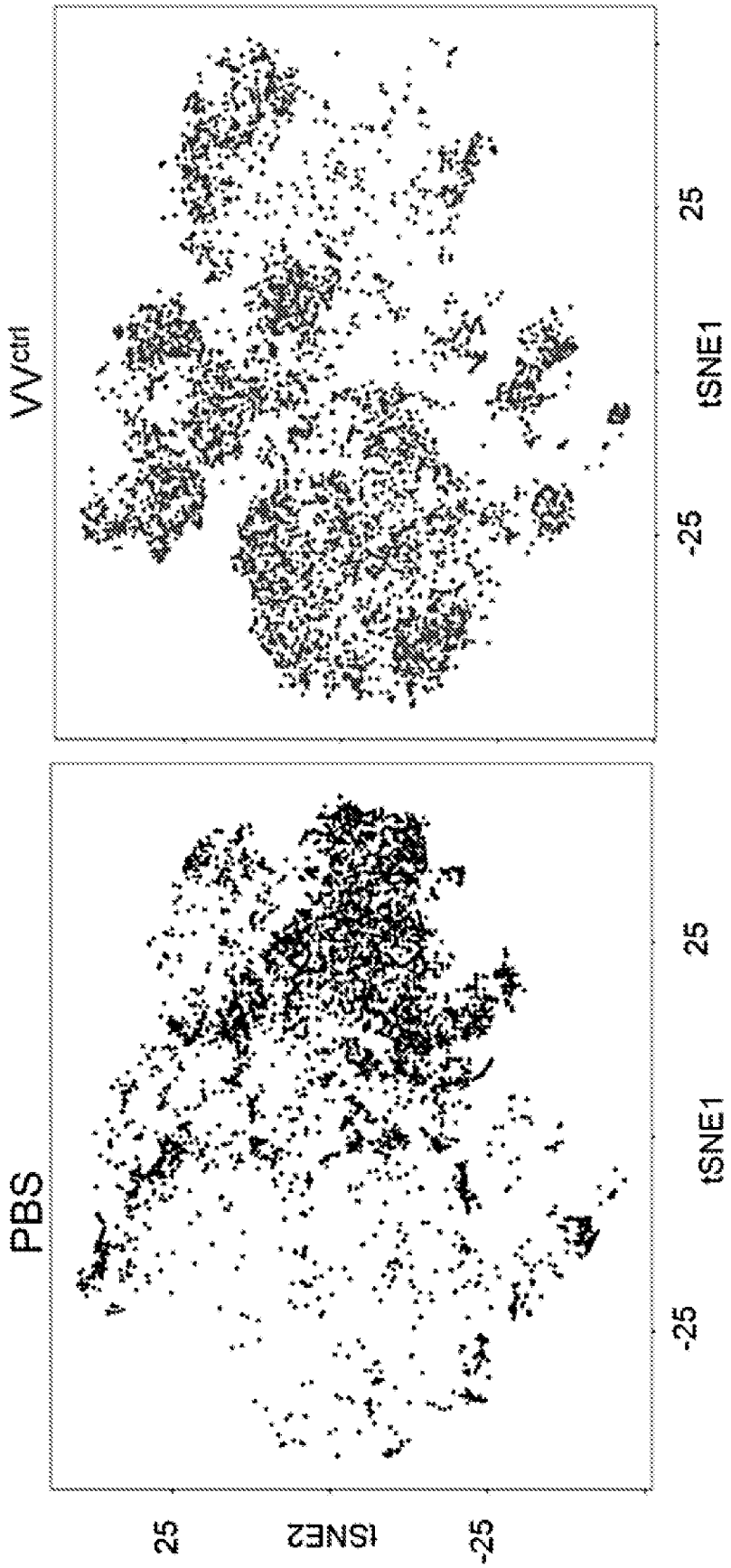


FIG. 2C

FIG. 3A

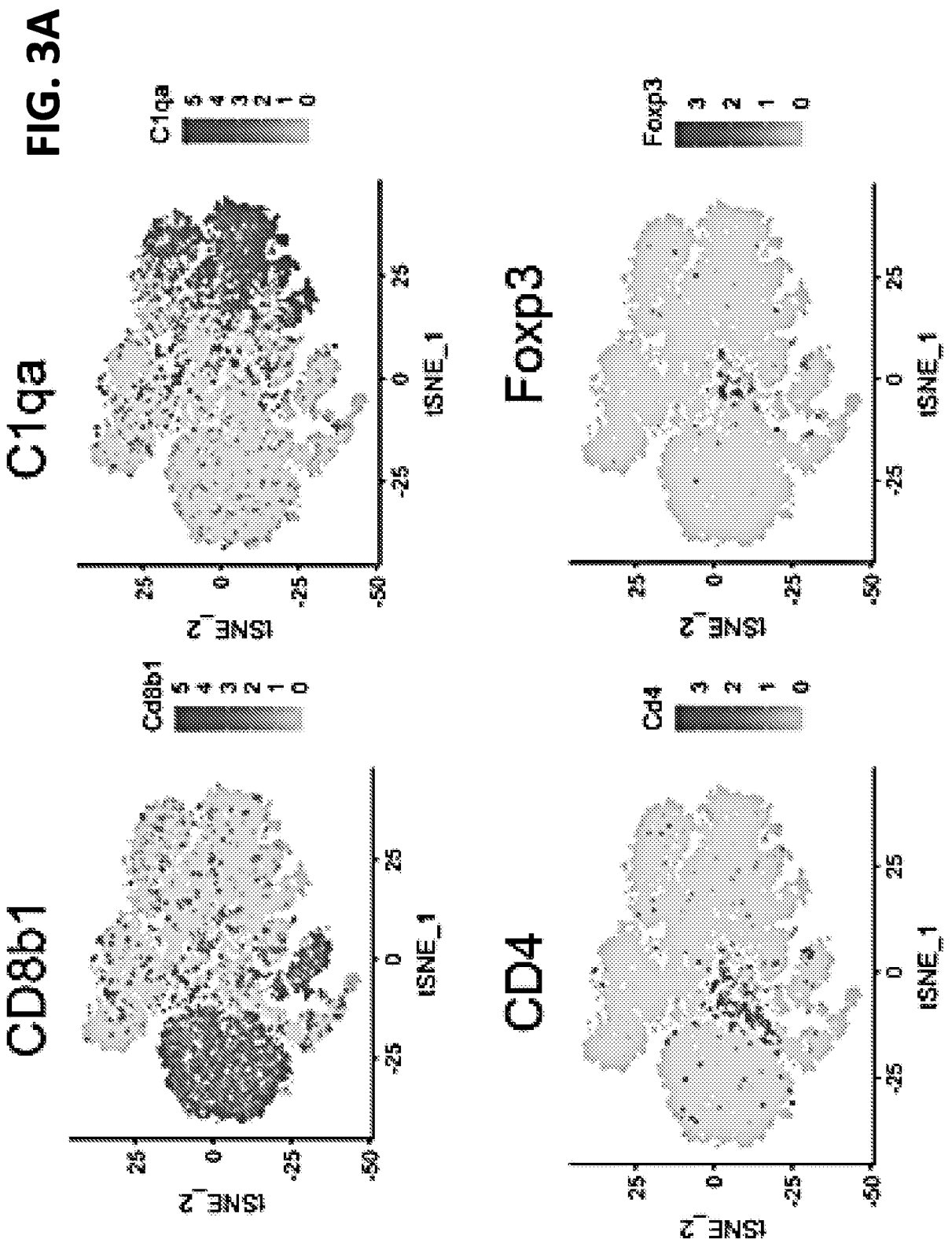

C1qa
C1qa
5 4 3 2 1 0
tSNE_2
tSNE_1
25 0 -25 -50
25 0 -25
Foxp3
Foxp3
3 2 1 0
CD8b1
Cd8b1
5 4 3 2 1 0
CD4
Cd4
3 2 1 0

FIG. 3B

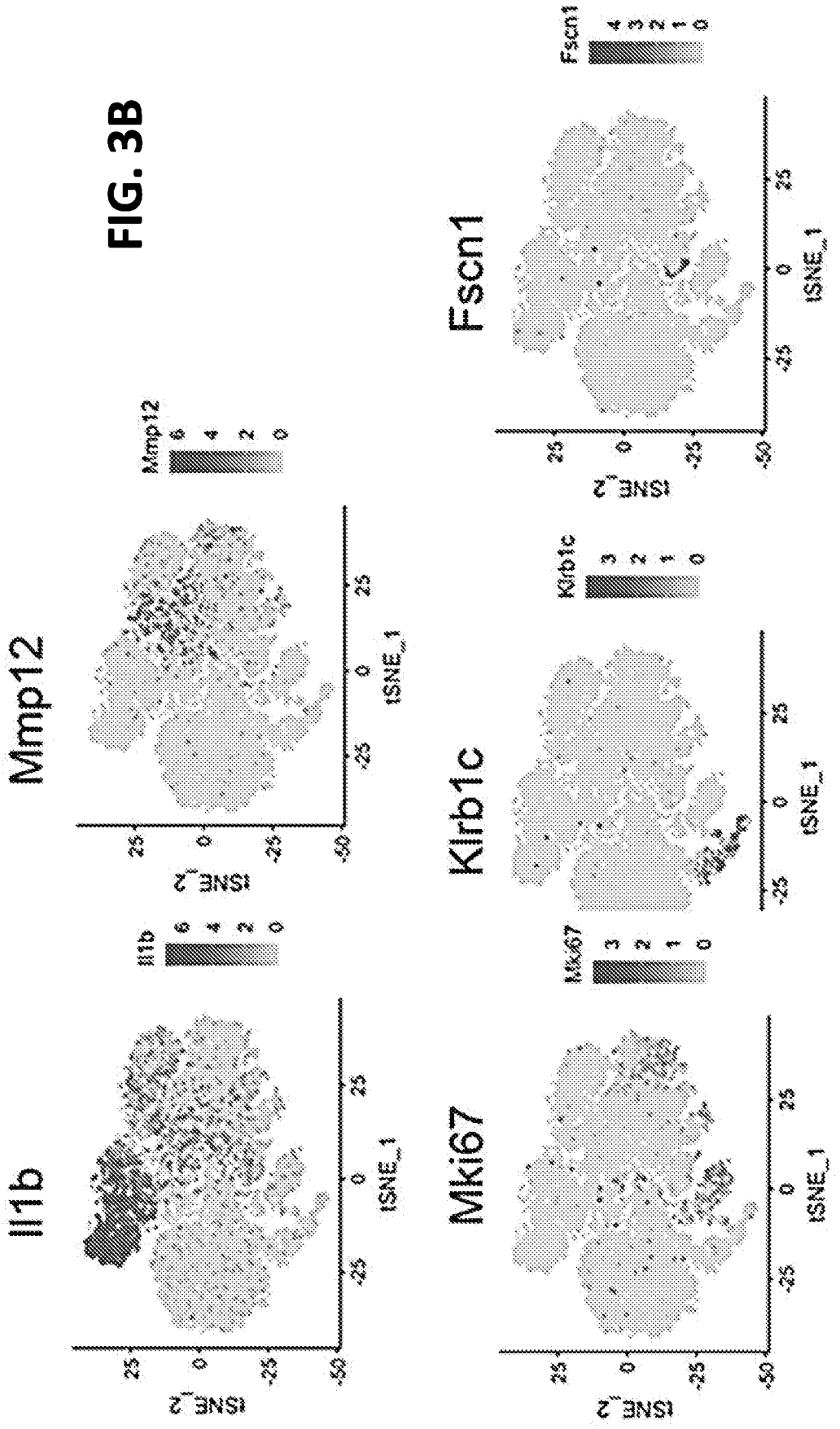


FIG. 3C

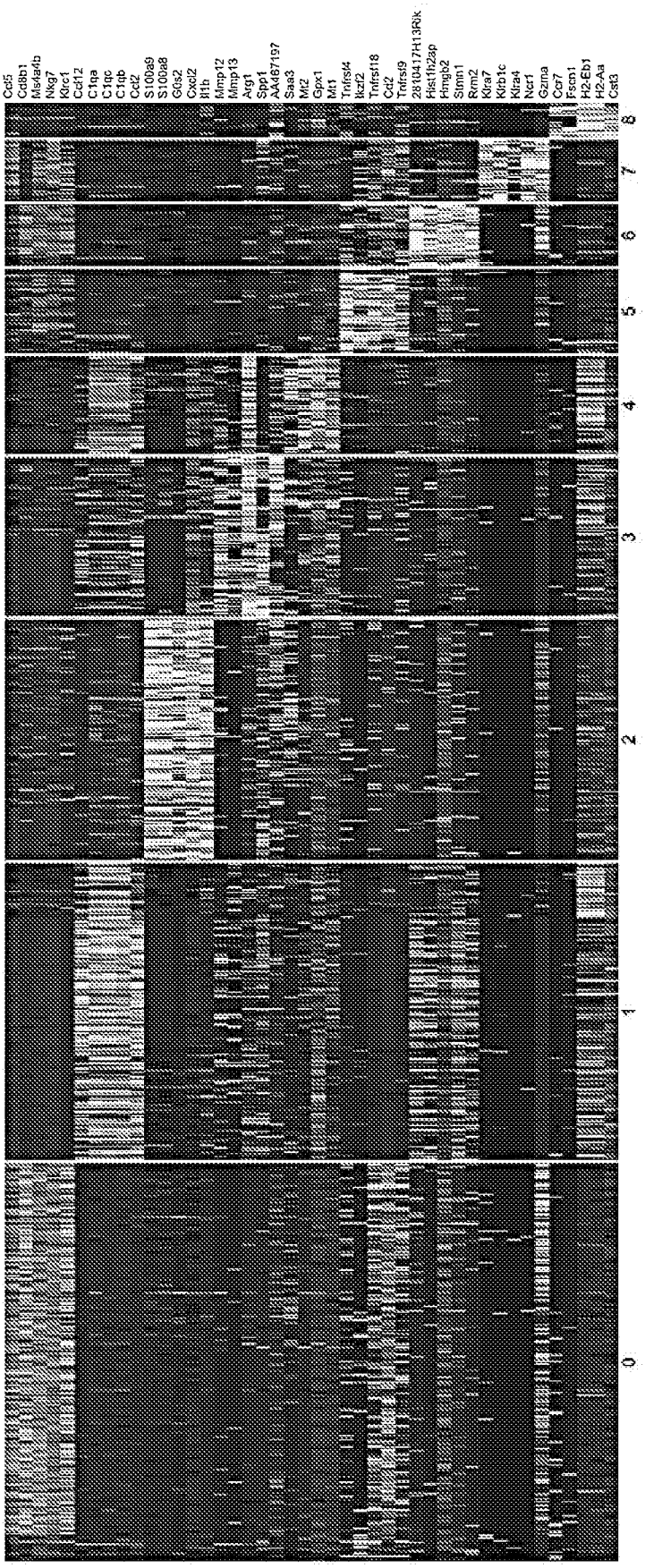
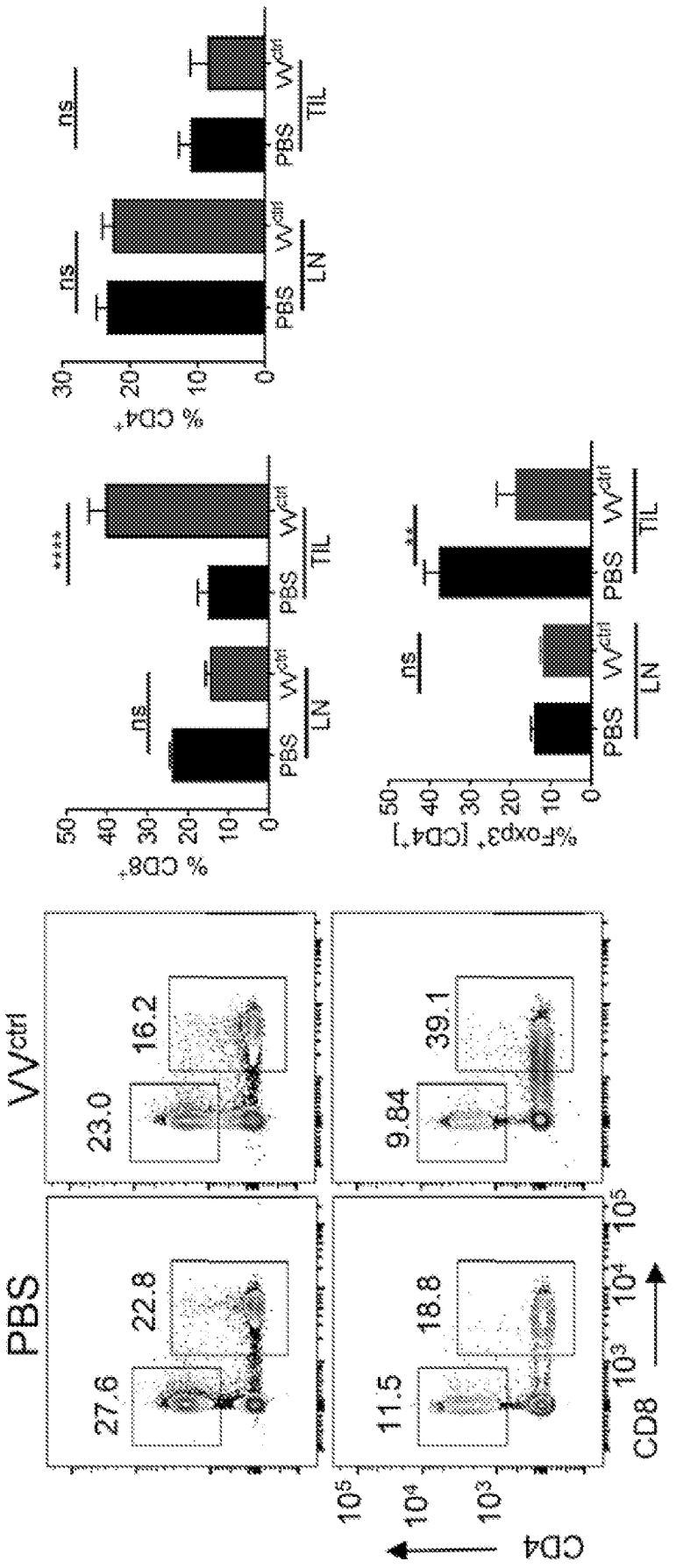

Ccl5
Cd8b1
Ms4a4b
Nkg7
Klrc1
Ccl12
C1qa
C1qc
C1qb
Ccl2
S100a9
S100a8
Gbp2
Cxcl2
Il1b
Mmp12
Mmp13
Arg1
Spp1
AA467197
Saa3
Mt2
Gpx1
Mt1
Tnfrsf4
Icam2
Tnfrsf18
Cd2
Tnfrsf9
2810417H13Rik
Hist1h2ap
Hmgb2
Stmn1
Rrm2
Klra7
Klrb1c
Klra4
Ncr1
Gzma
Cst7
Fscn1
H2-Eb1
H2-Aa
Cst3
0
1
2
3
4
5
6
7
8

FIG. 4A


PBS
VVctrl
27.6
22.8
23.0
16.2
11.5
18.8
9.84
39.1
CD4
CD8
% CD8+
% CD4+
%Foxp3+ [CD4+]
LN
TIL
PBS
ns
****
**

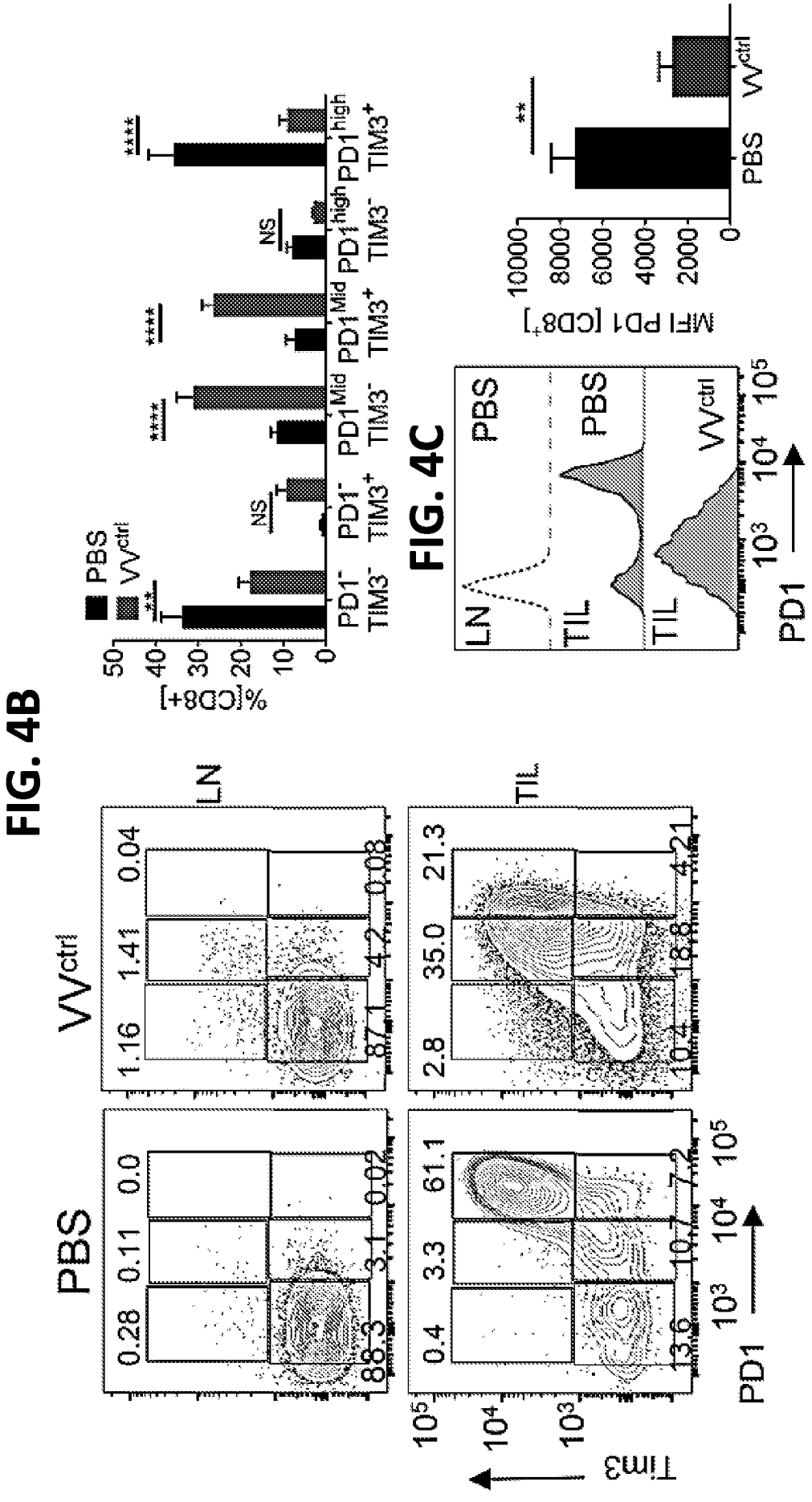


FIG. 4B
PBS
VVctrl
LN
TIL
Tim3
PD1
0.28
0.11
0.0
88.3
3.1
0.02
0.4
3.3
61.1
13.6
10.7
7.2
1.16
1.41
0.04
87.1
4.2
0.08
2.8
35.0
21.3
10.4
18.8
4.21
%[CD8+]
PD1- TIM3-
PD1- TIM3+
PD1Mid TIM3-
PD1Mid TIM3+
PD1high TIM3-
PD1high TIM3+
NS
****
**
FIG. 4C
LN
TIL
PBS
VVctrl
PD1
MFI PD1 [CD8+]
PBS
VVctrl
**

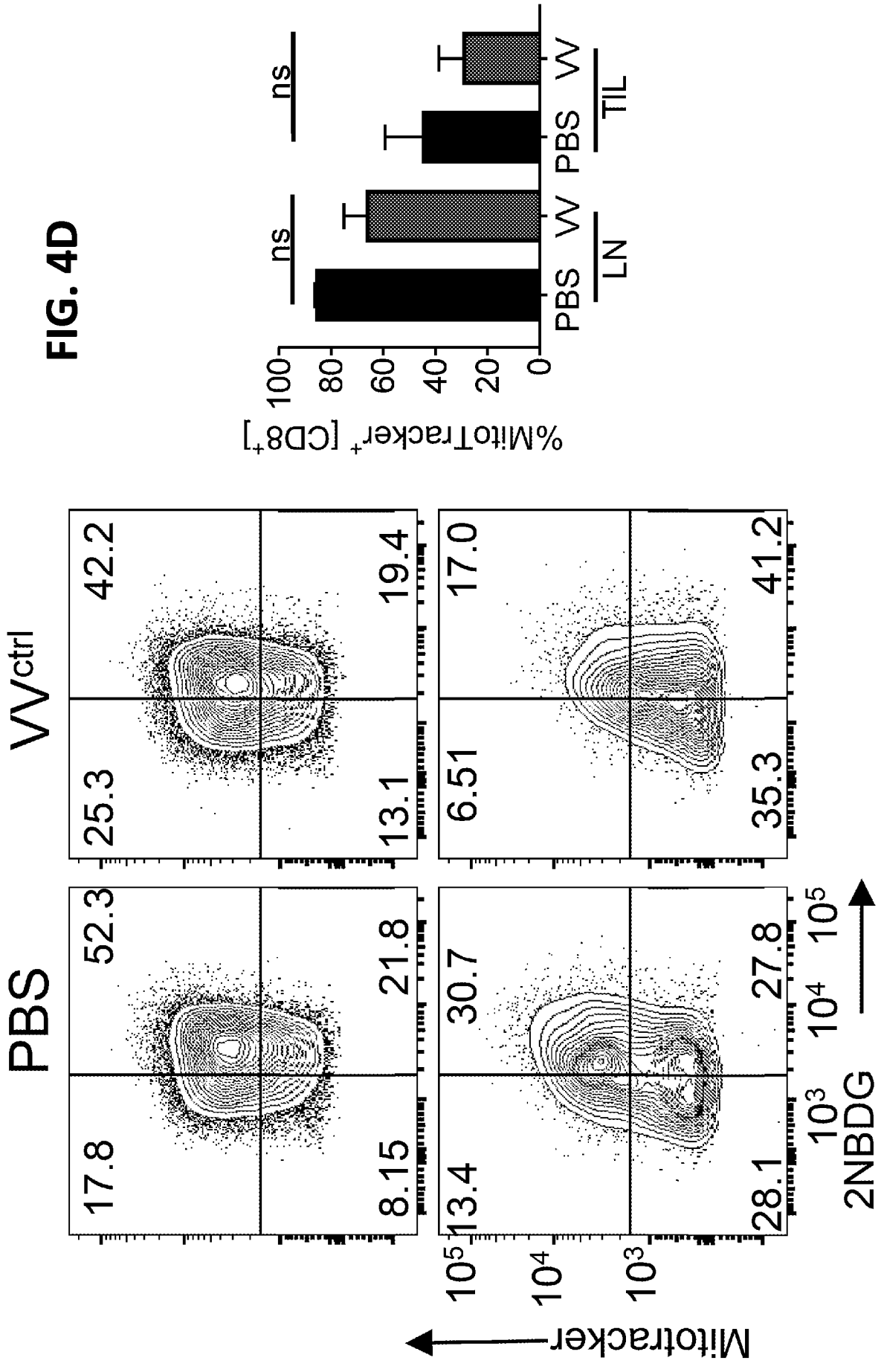
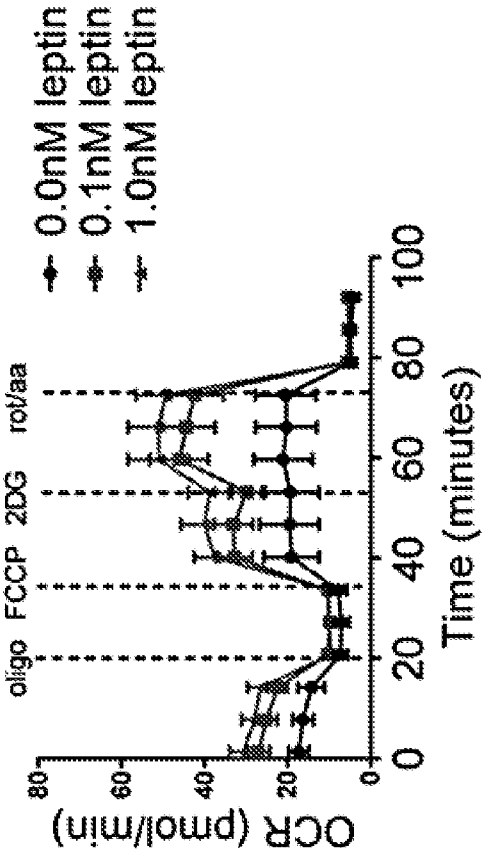


FIG. 4D
%MitoTracker+ [CD8+]
100
80
60
40
20
0
ns
ns
PBS
VV
PBS
VV
LN
TIL
PBS
VVctrl
52.3
21.8
17.8
8.15
42.2
19.4
25.3
13.1
30.7
27.8
13.4
28.1
17.0
41.2
6.51
35.3
Mitotracker
2NBDG
10^5
10^4
10^3
10^3
10^4
10^5

FIG. 5A


0.0nM leptin
0.1nM leptin
1.0nM leptin
oligo
FCCP
2DG
rot/aa
OCR (pmol/min)
Time (minutes)

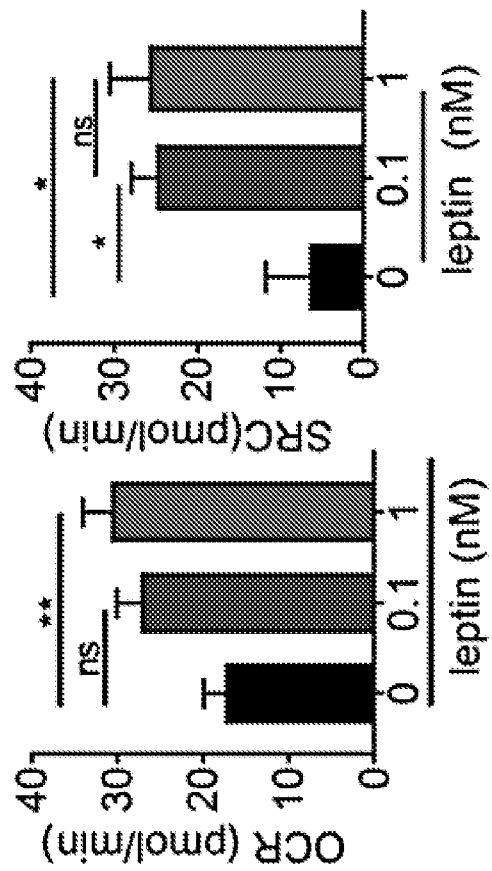

OCR (pmol/min)
SRC(pmol/min)
basal ECAR (mpH/min)
leptin (nM)
ns
*
**

FIG. 5B

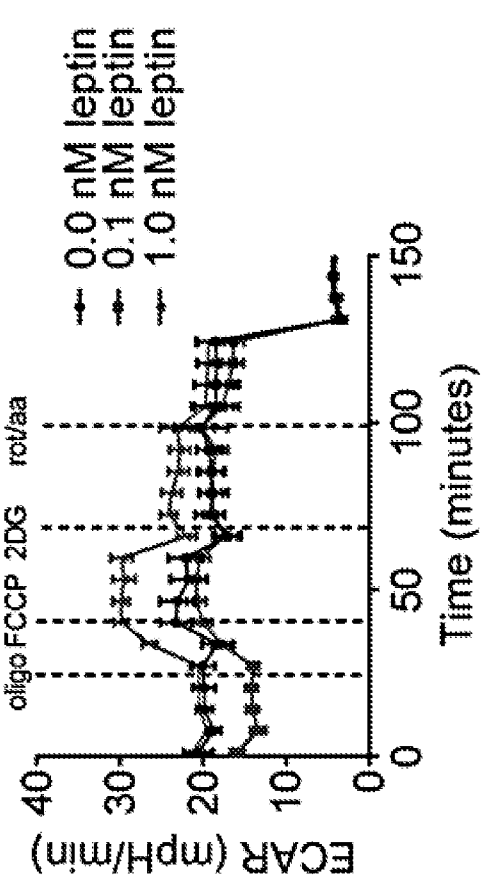

0.0 nM leptin
0.1 nM leptin
1.0 nM leptin
oligo
FCCP
2DG
rot/aa
ECAR (mpH/min)
Time (minutes)

FIG. 5C

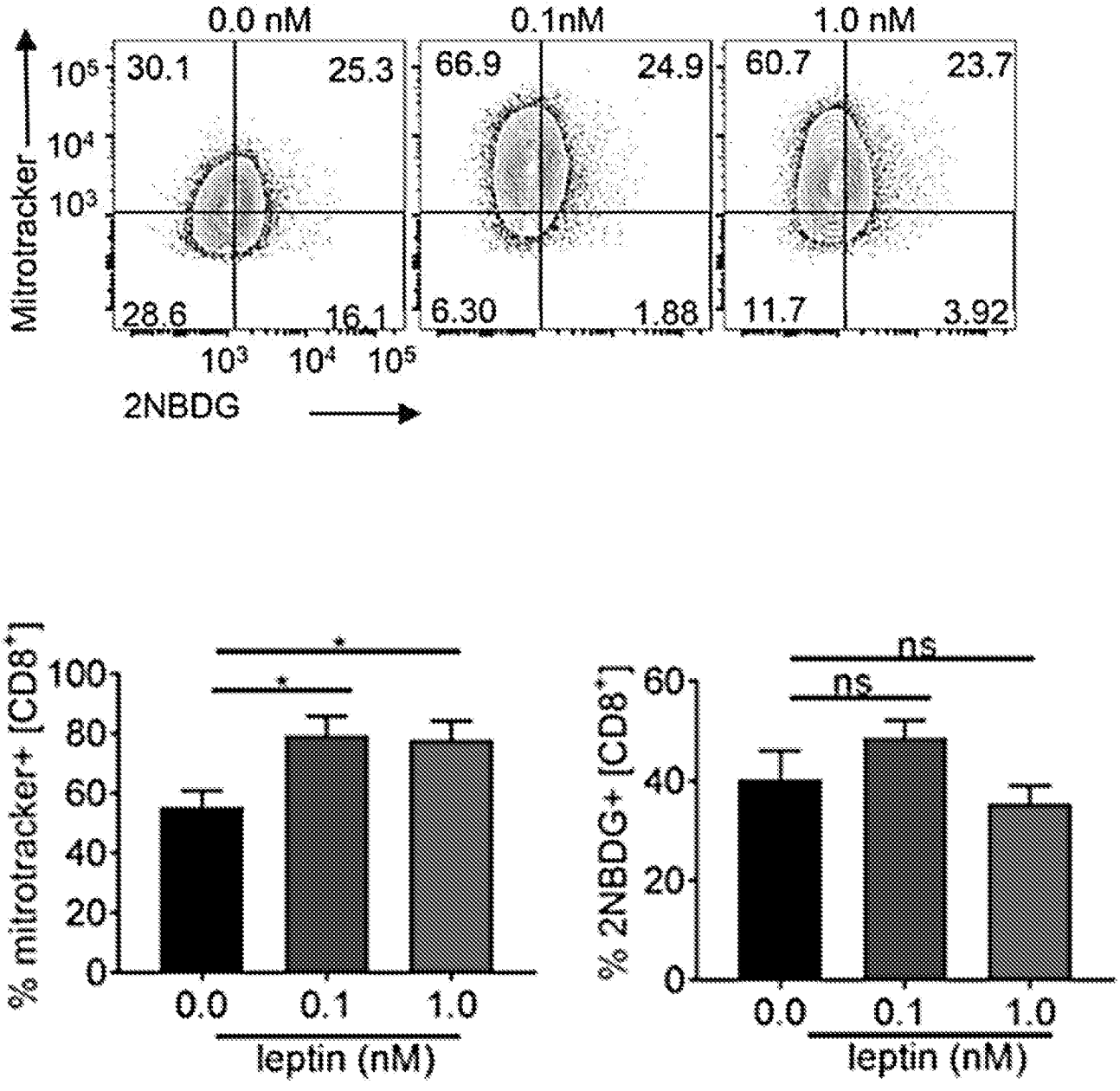

0.0 nM
0.1nM
1.0 nM
Miltrotracker
2NBDG
30.1
25.3
28.6
16.1
66.9
24.9
6.30
1.88
60.7
23.7
11.7
3.92
% miltrotracker+ [CD8+]
% 2NBDG+ [CD8+]
leptin (nM)
ns

FIG. 5D

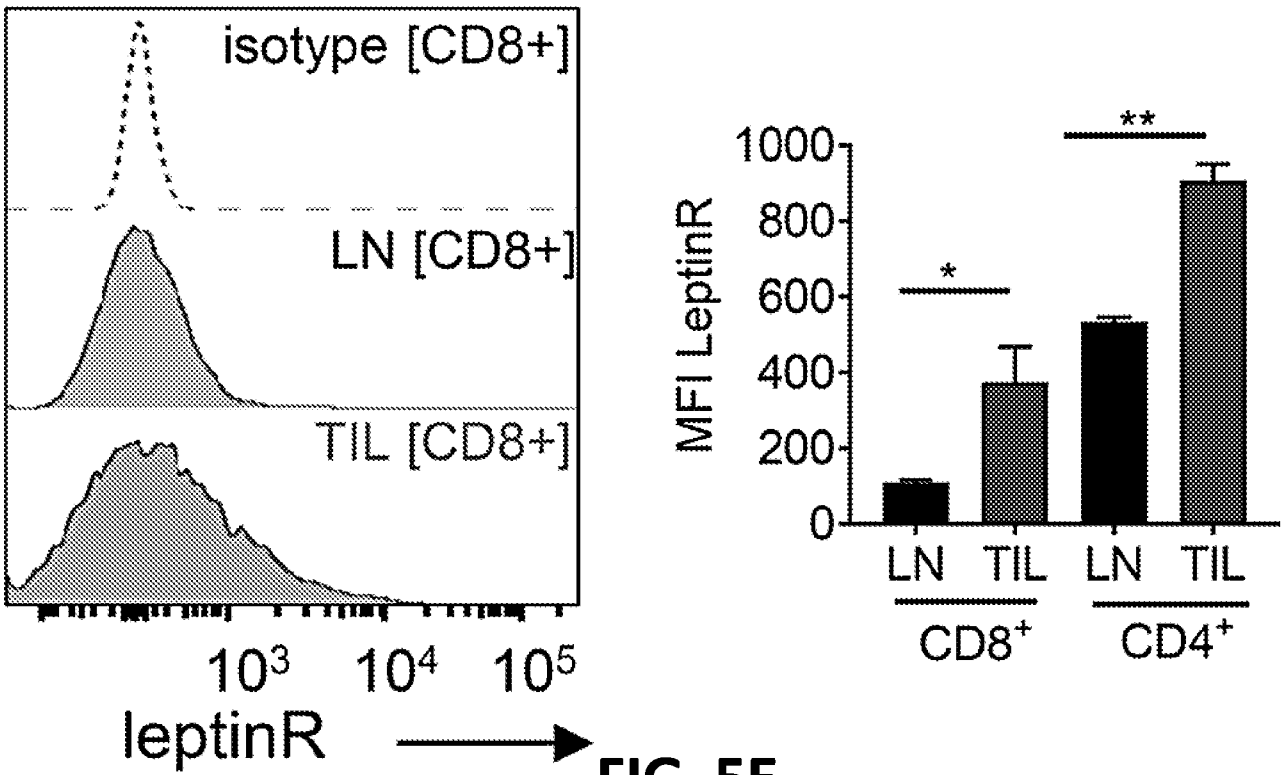

isotype [CD8+]
LN [CD8+]
TIL [CD8+]
10^3 10^4 10^5
leptinR
MFI LeptinR
1000
800
600
400
200
0
*
**
LN TIL LN TIL
CD8+ CD4+

FIG. 5E

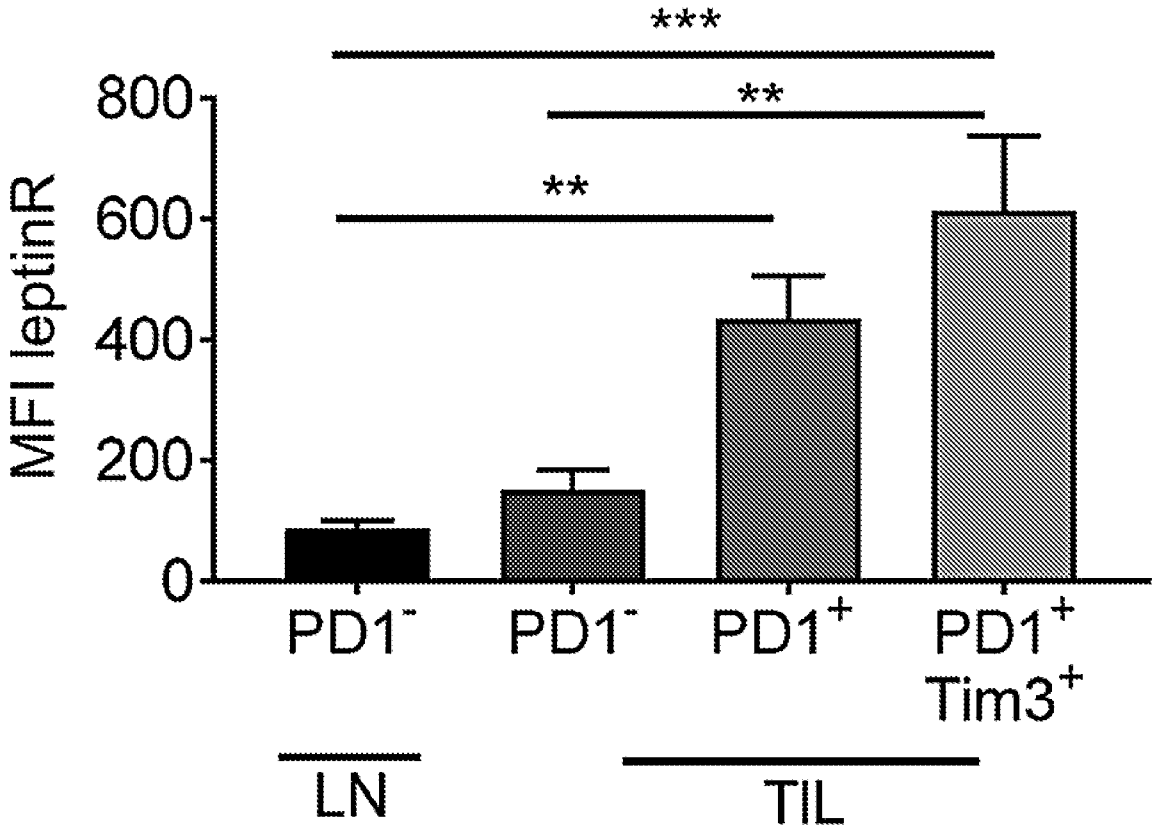

***
**
**
MFI leptinR
800
600
400
200
0
PD1- PD1- PD1+ PD1+ Tim3+
LN
TIL

FIG. 6A

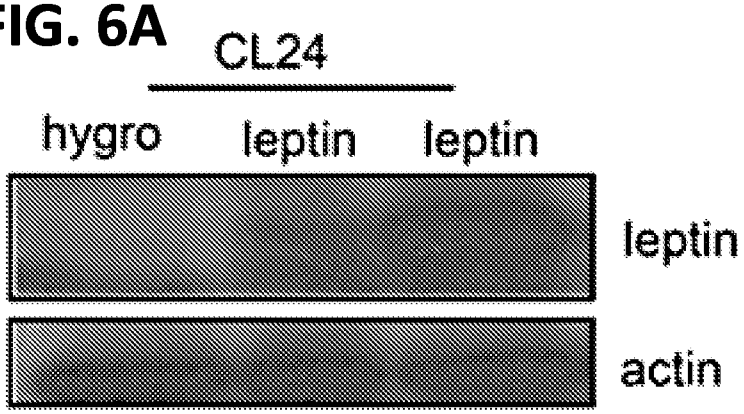


FIG. 6B

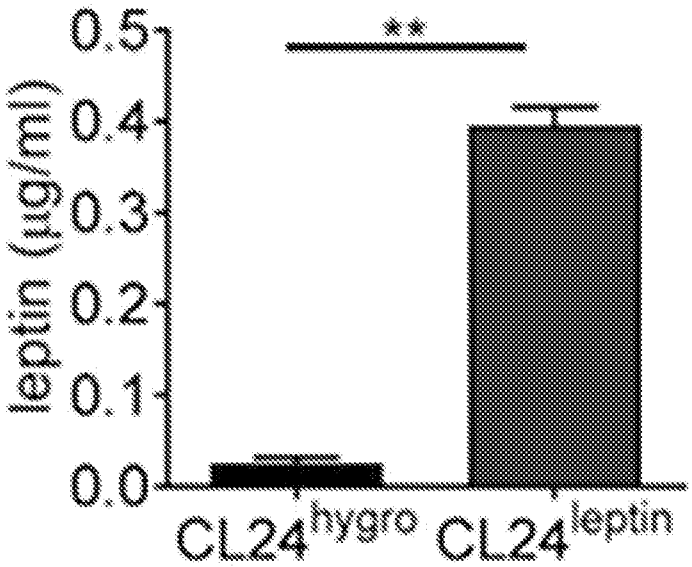


FIG. 6C

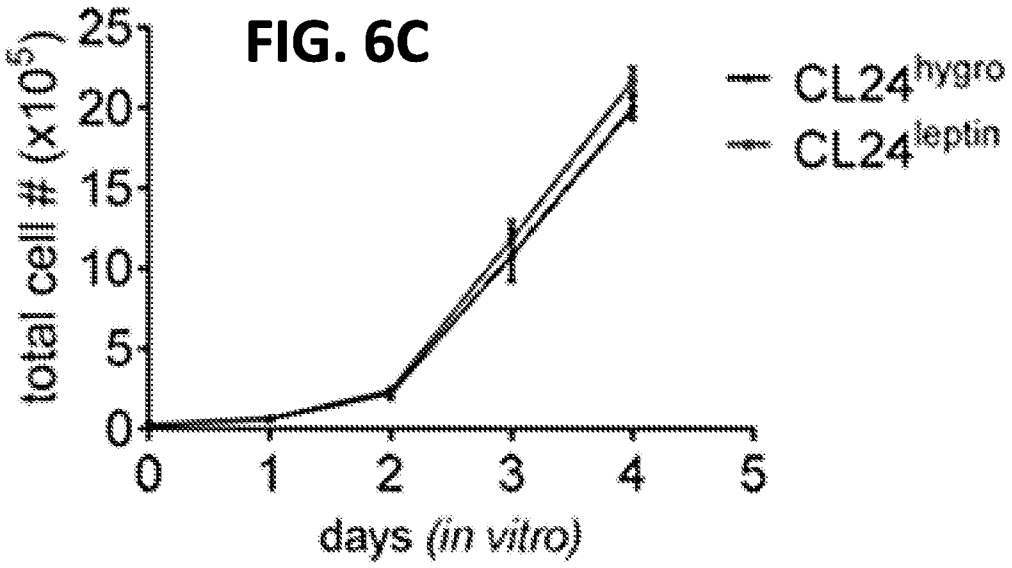


FIG. 6D

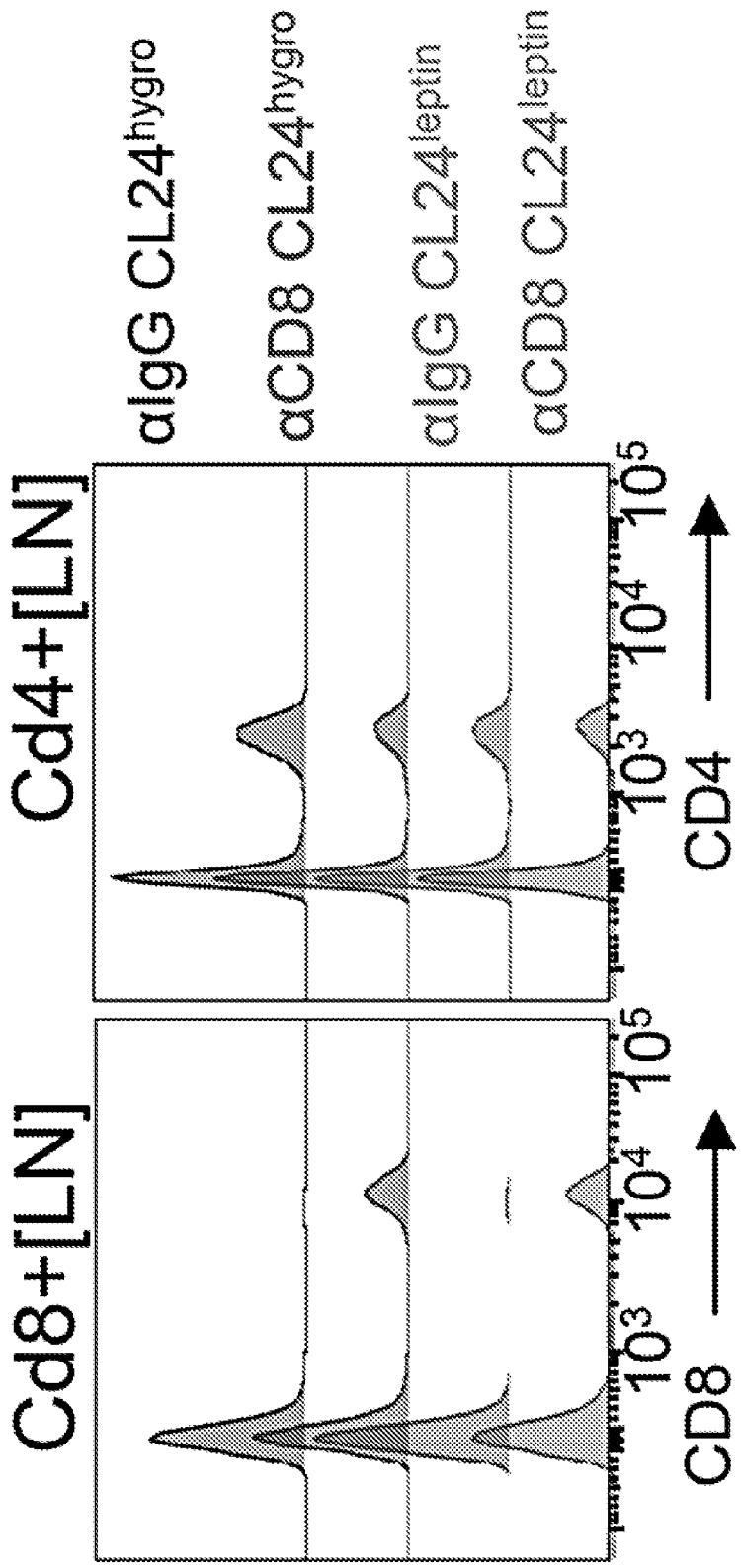

αIgG CL24hygro
αCD8 CL24hygro
αIgG CL24leptin
αCD8 CL24leptin
Cd4+[LN]
Cd8+[LN]
CD4
CD8
10^3
10^4
10^5

FIG. 6E

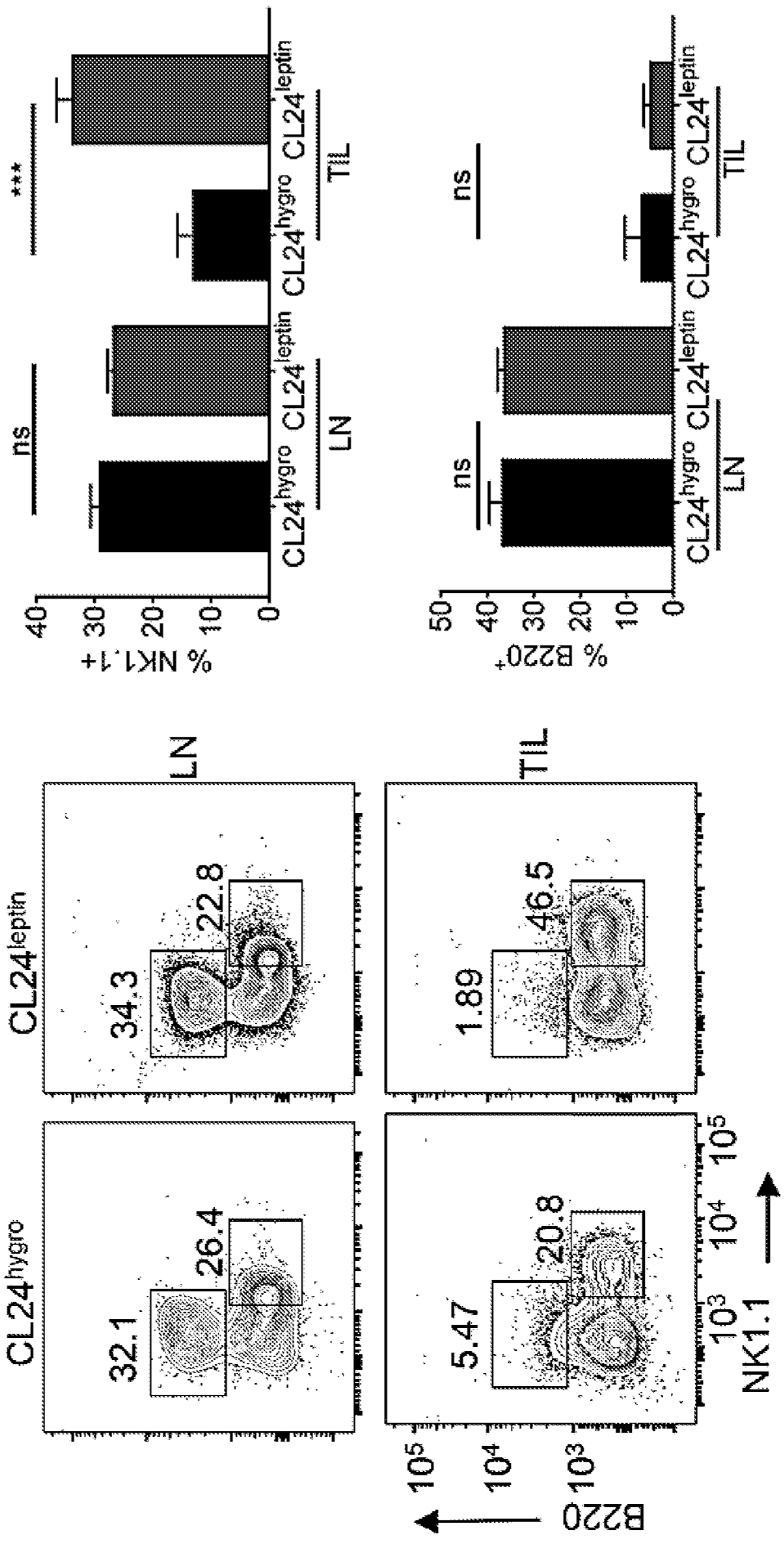

% NK1.1+
ns
***
CL24hygro
CL24leptin
CL24hygro
CL24leptin
LN
TIL
% B220+
ns
ns
CL24hygro
CL24leptin
CL24hygro
CL24leptin
LN
TIL
LN
TIL
CL24leptin
CL24hygro
34.3
22.8
1.89
46.5
32.1
26.4
5.47
20.8
B220
NK1.1

FIG. 6F

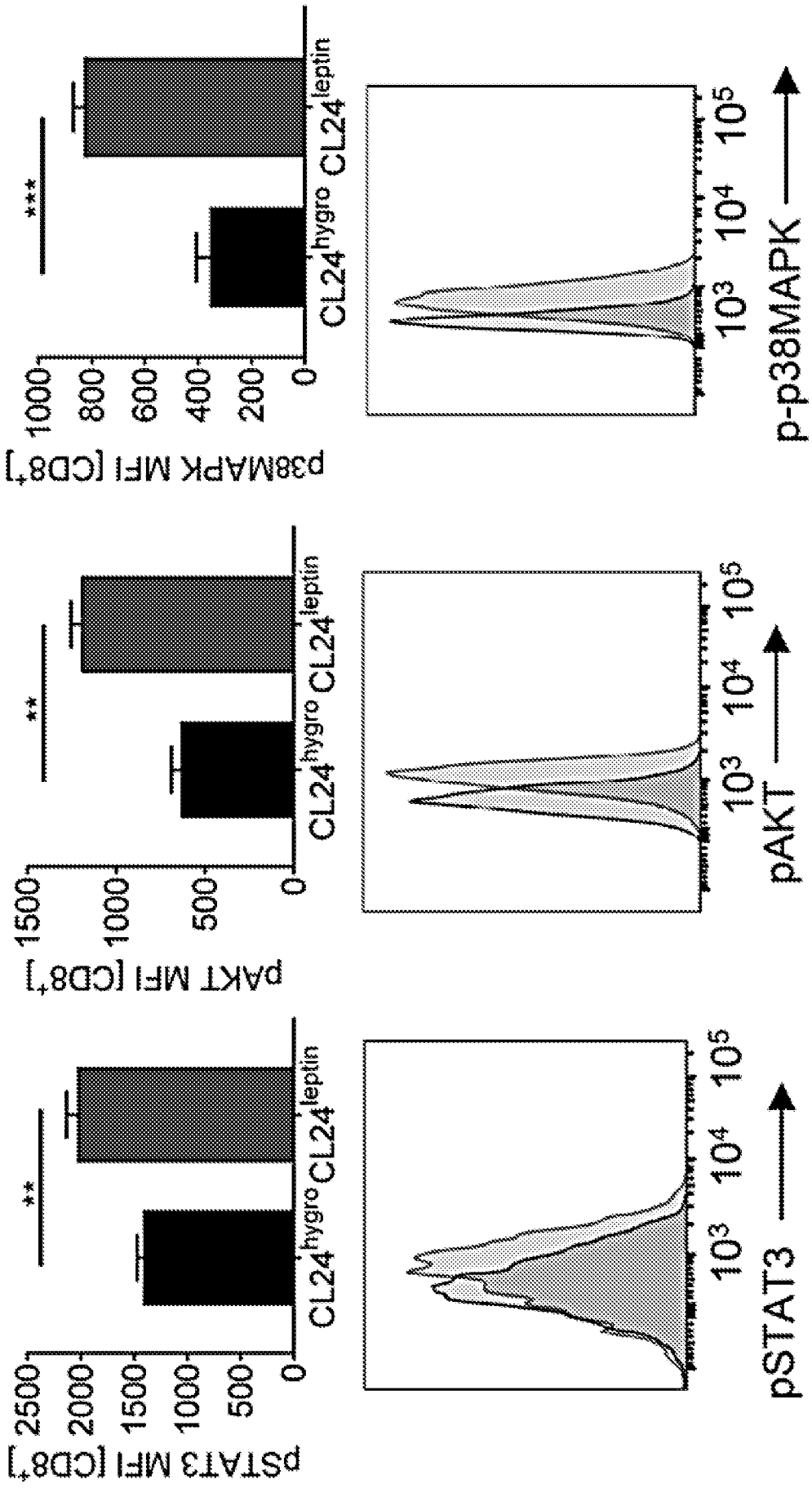

p38MAPK MFI [CD8+]
1000
800
600
400
200
0
***
CL24hygro
CL24leptin
p-p38MAPK
10^3
10^4
10^5
pAKT MFI [CD8+]
1500
1000
500
0
**
CL24hygro
CL24leptin
pAKT
10^3
10^4
10^5
pSTAT3 MFI [CD8+]
2500
2000
1500
1000
500
0
**
CL24hygro
CL24leptin
pSTAT3
10^3
10^4
10^5

FIG. 7A

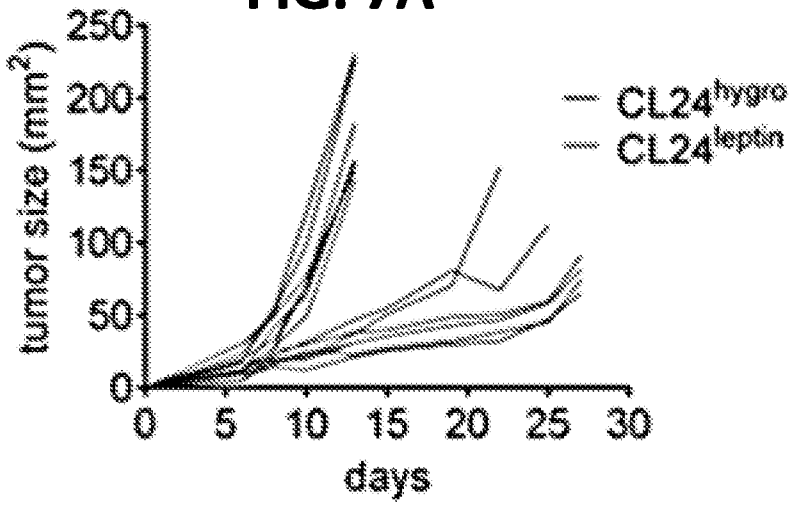


FIG. 7B

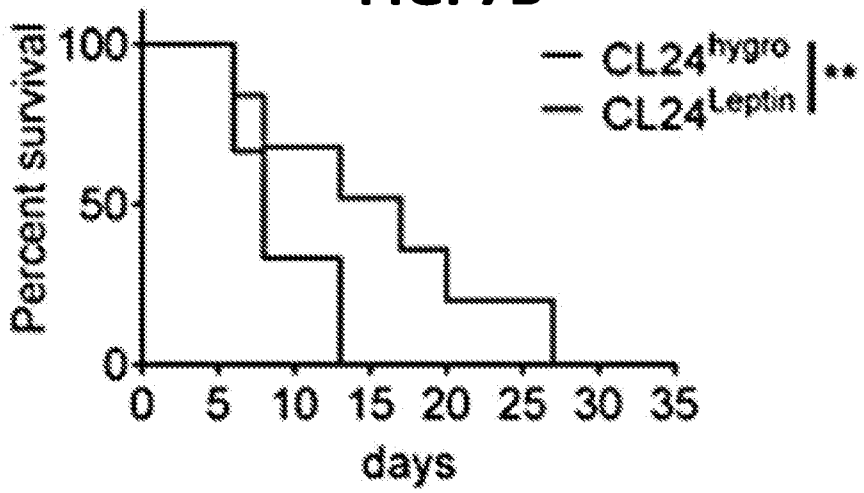


FIG. 7C

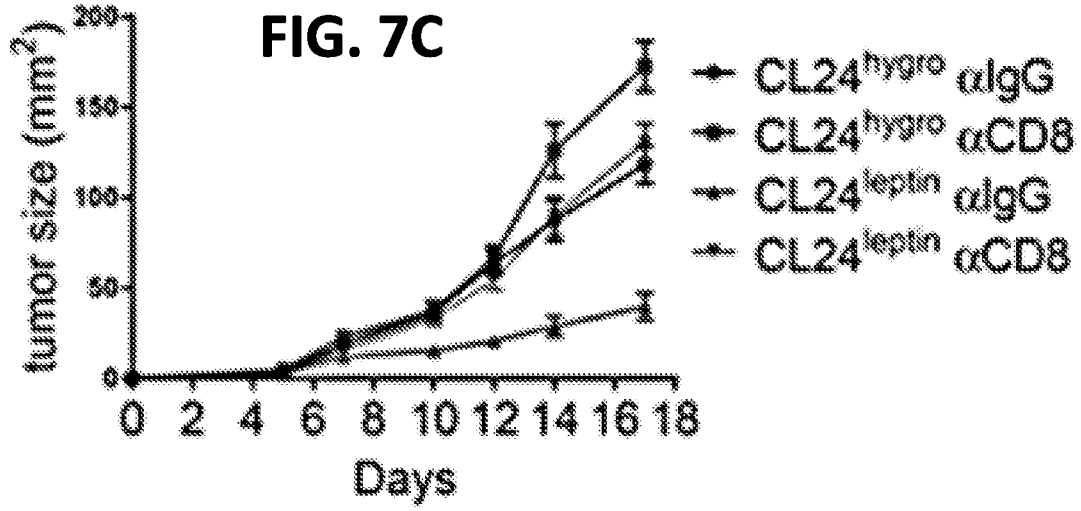


FIG. 7D

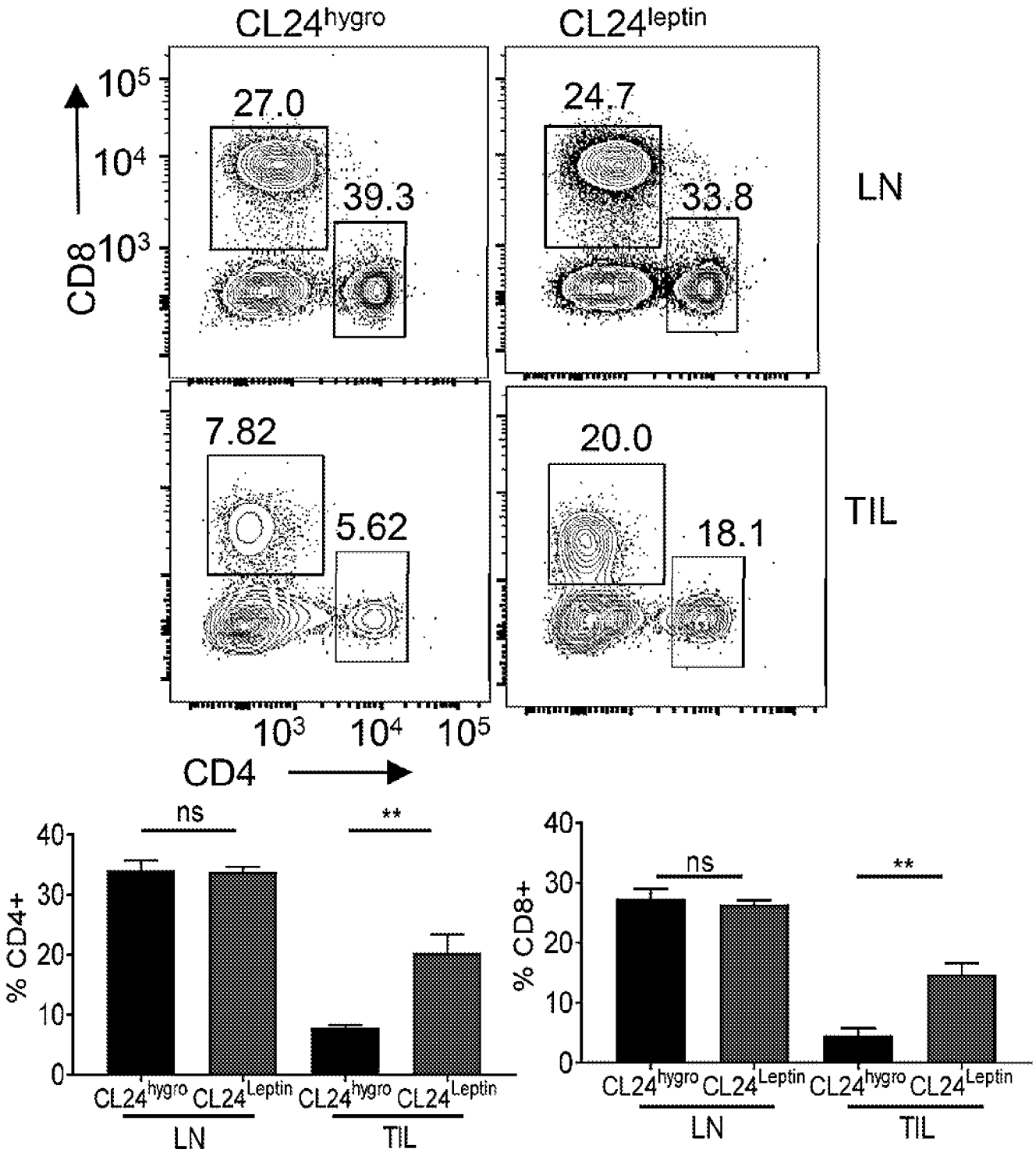

CL24hygro
CL24leptin
CD8
CD4
27.0
39.3
24.7
33.8
LN
7.82
5.62
20.0
18.1
TIL
10^3
10^4
10^5
ns
**
% CD4+
% CD8+
CL24hygro
CL24Leptin
LN
TIL

FIG. 7E

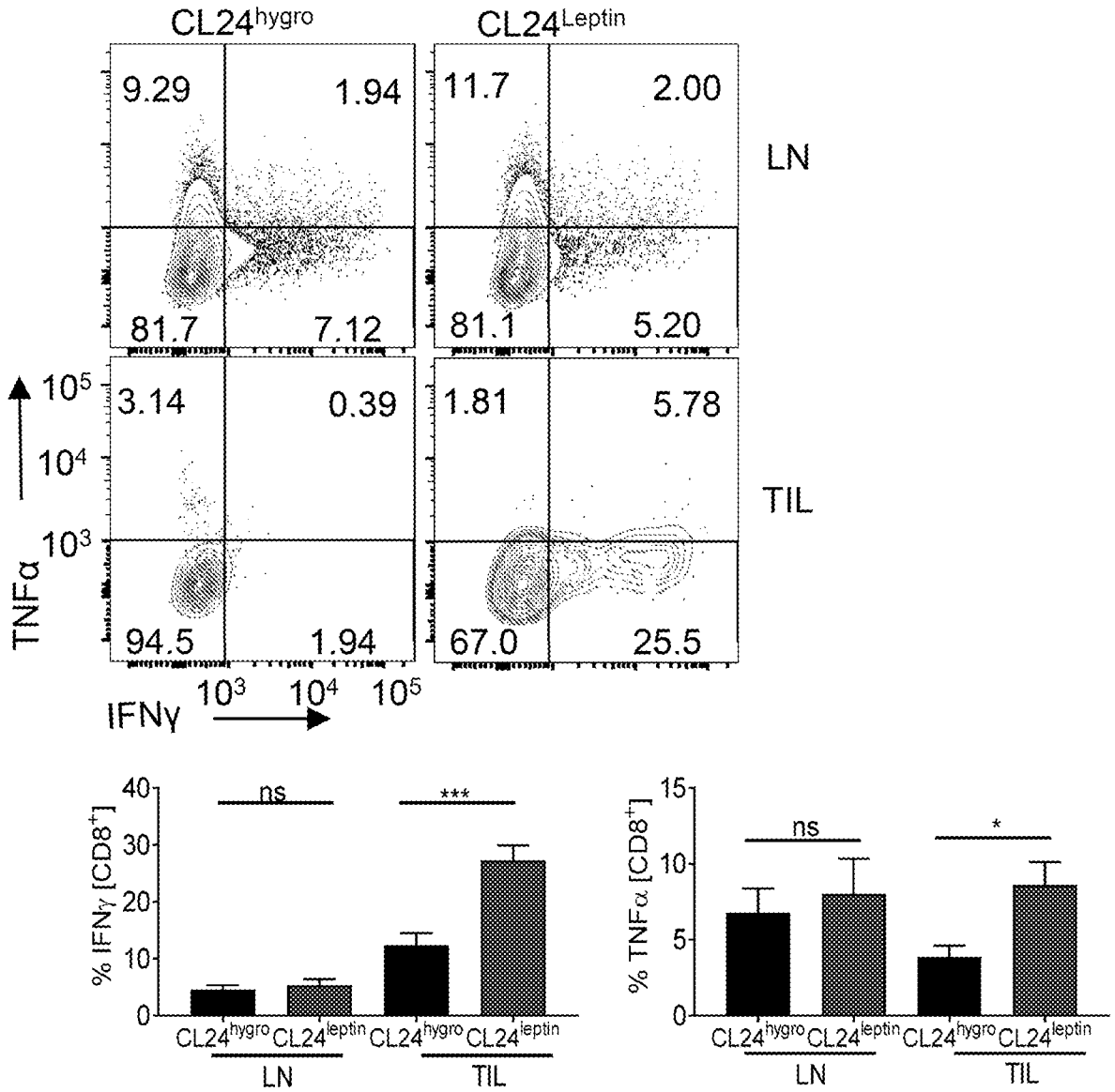
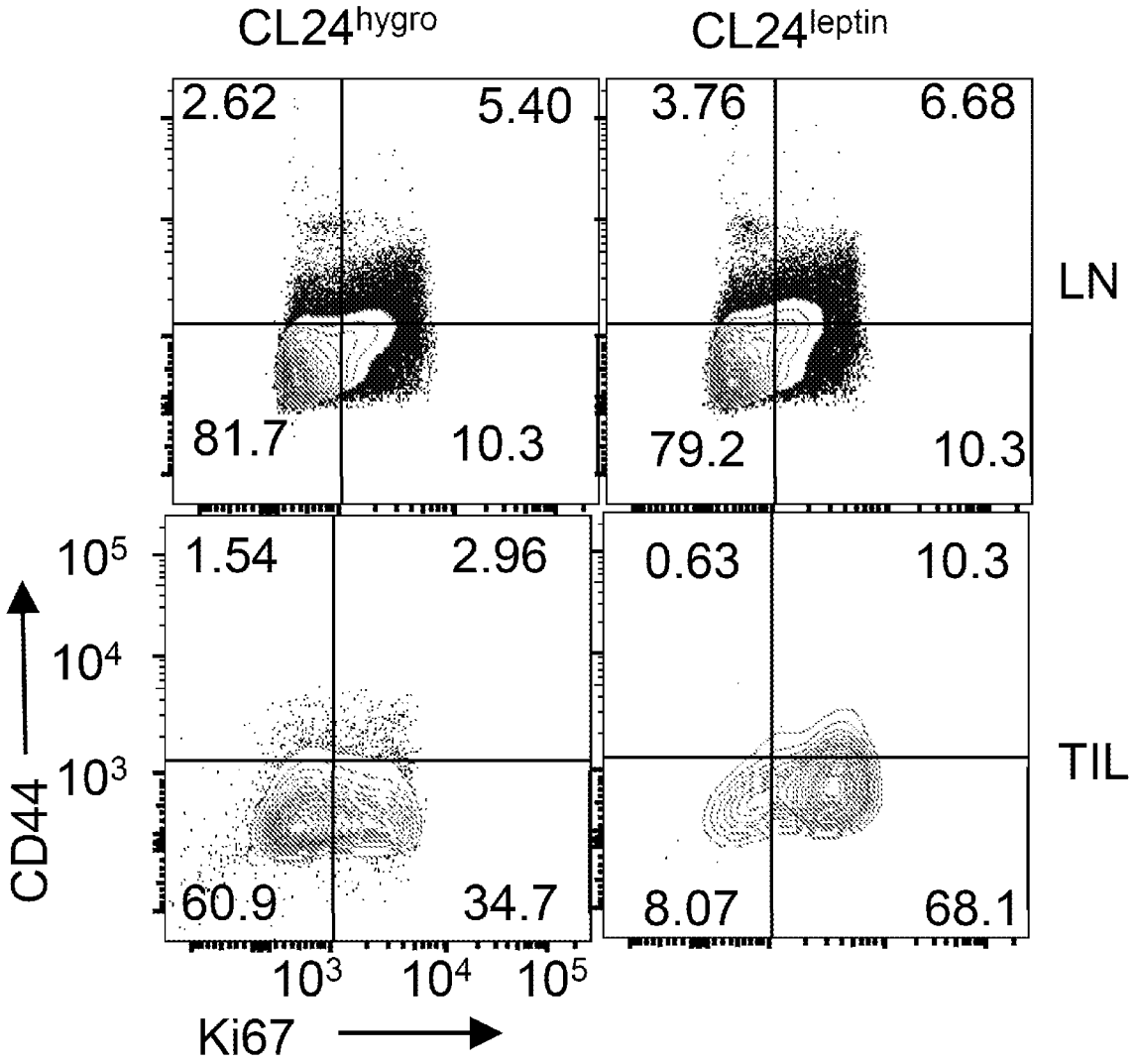

CL24hygro
CL24Leptin
9.29
1.94
81.7
7.12
11.7
2.00
81.1
5.20
LN
3.14
0.39
94.5
1.94
1.81
5.78
67.0
25.5
TIL
TNFα
IFNγ
10^5
10^4
10^3
ns
***
% IFNγ [CD8+]
CL24hygro
CL24leptin
LN
TIL
*
% TNFα [CD8+]

FIG. 7F


CL24hygro
CL24leptin
2.62
5.40
3.76
6.68
LN
81.7
10.3
79.2
10.3
1.54
2.96
0.63
10.3
TIL
60.9
34.7
8.07
68.1
CD44
Ki67
10^3
10^4
10^5

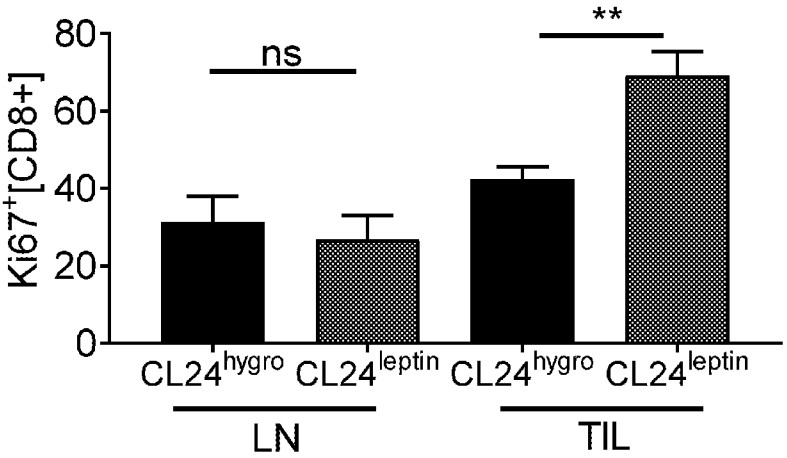

Ki67+[CD8+]
80
60
40
20
0
ns
**
CL24hygro
CL24leptin
CL24hygro
CL24leptin
LN
TIL

FIG. 7G

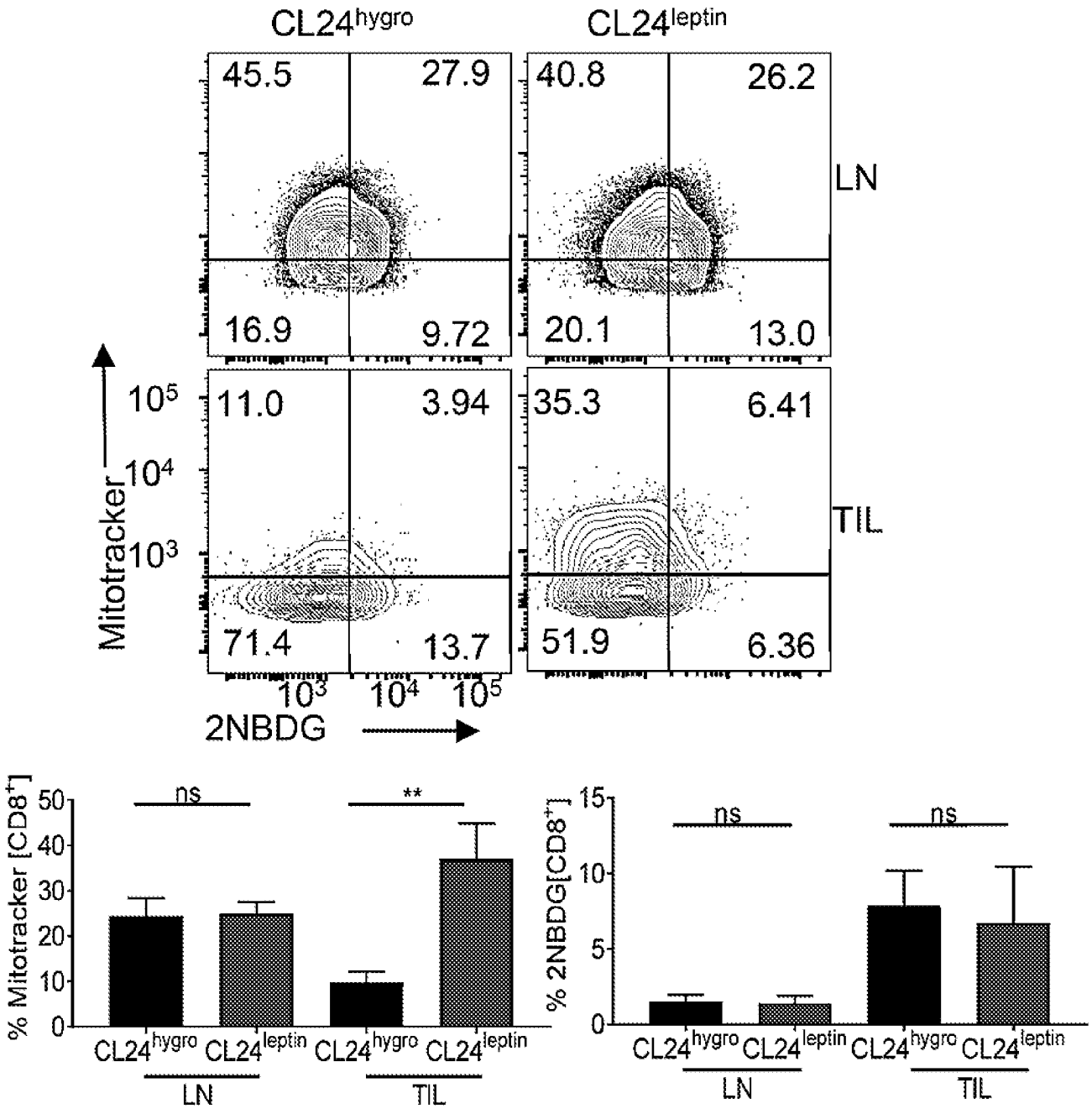

CL24hygro
CL24leptin
45.5
27.9
40.8
26.2
LN
16.9
9.72
20.1
13.0
Mitotracker
10^5
10^4
10^3
11.0
3.94
35.3
6.41
TIL
71.4
13.7
51.9
6.36
10^3
10^4
10^5
2NBDG
% Mitotracker [CD8+]
ns
**
CL24hygro
CL24leptin
LN
TIL
% 2NBDG[CD8+]
ns
ns

FIG. 8A

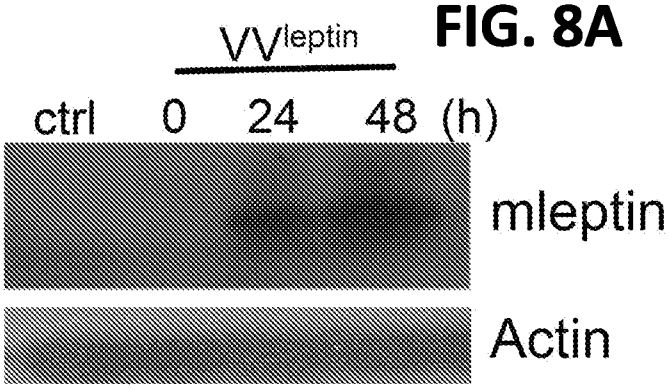


FIG. 8B

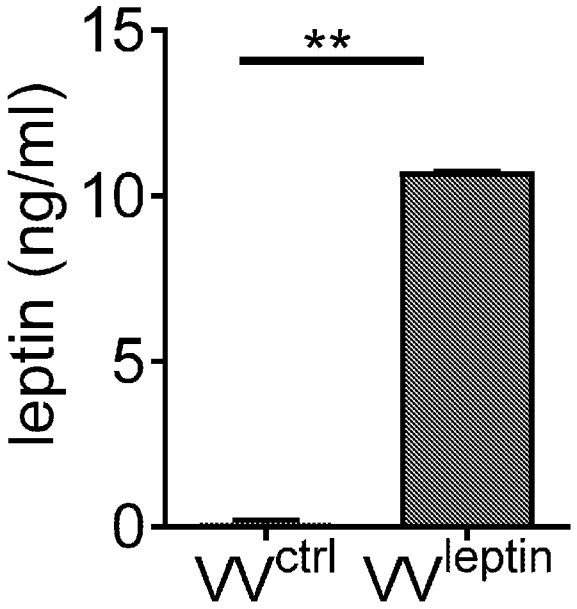


FIG. 8C

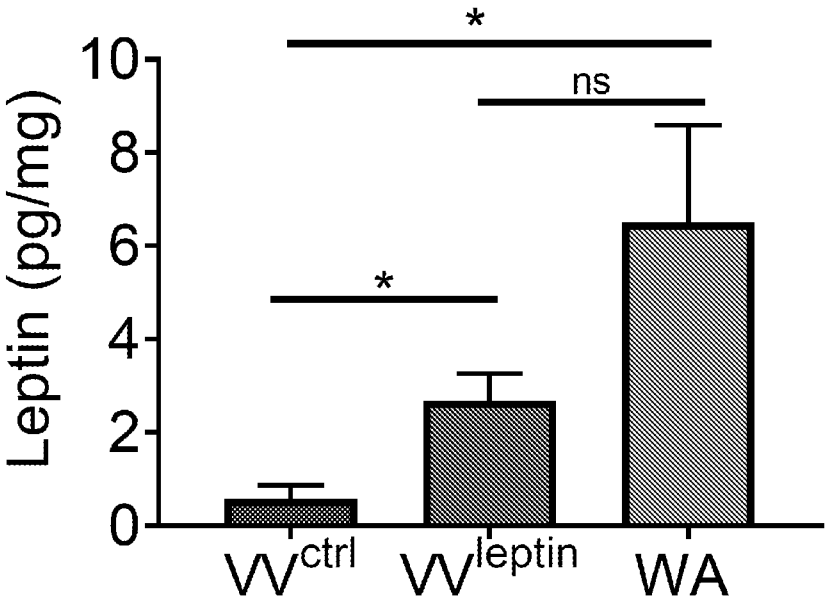
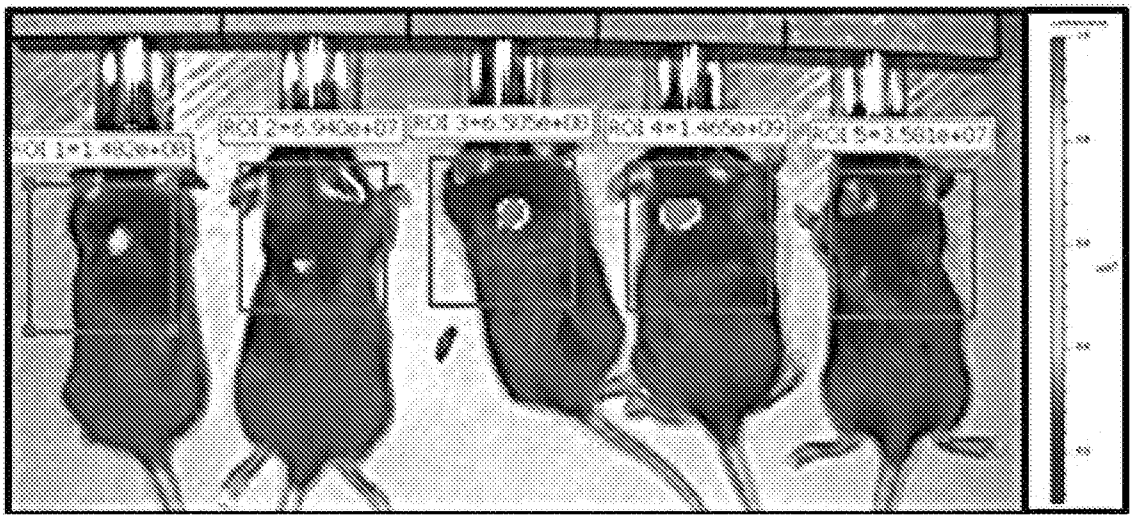


FIG. 8D

VVctrl



VVleptin

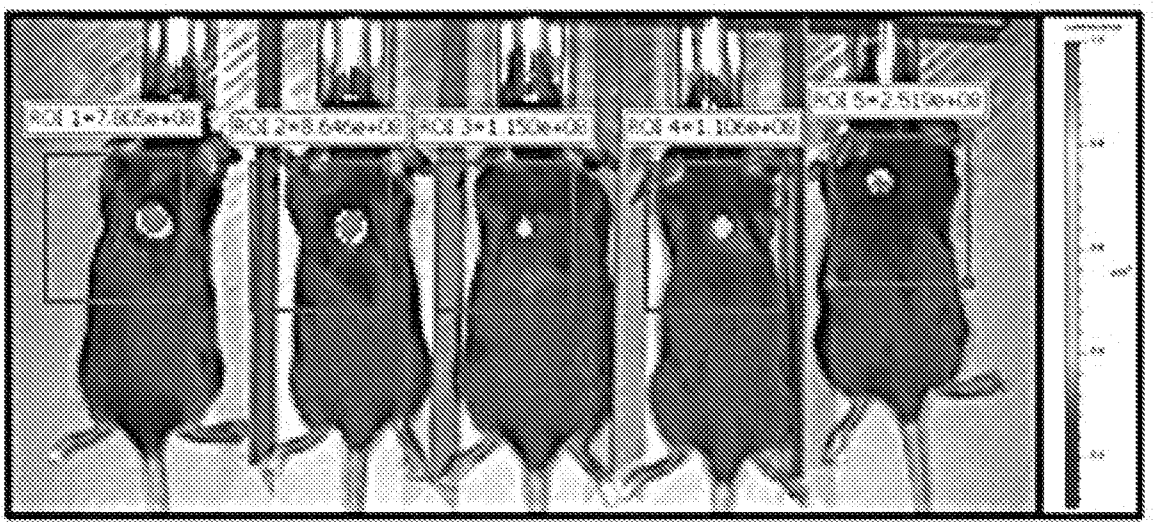


FIG. 8E

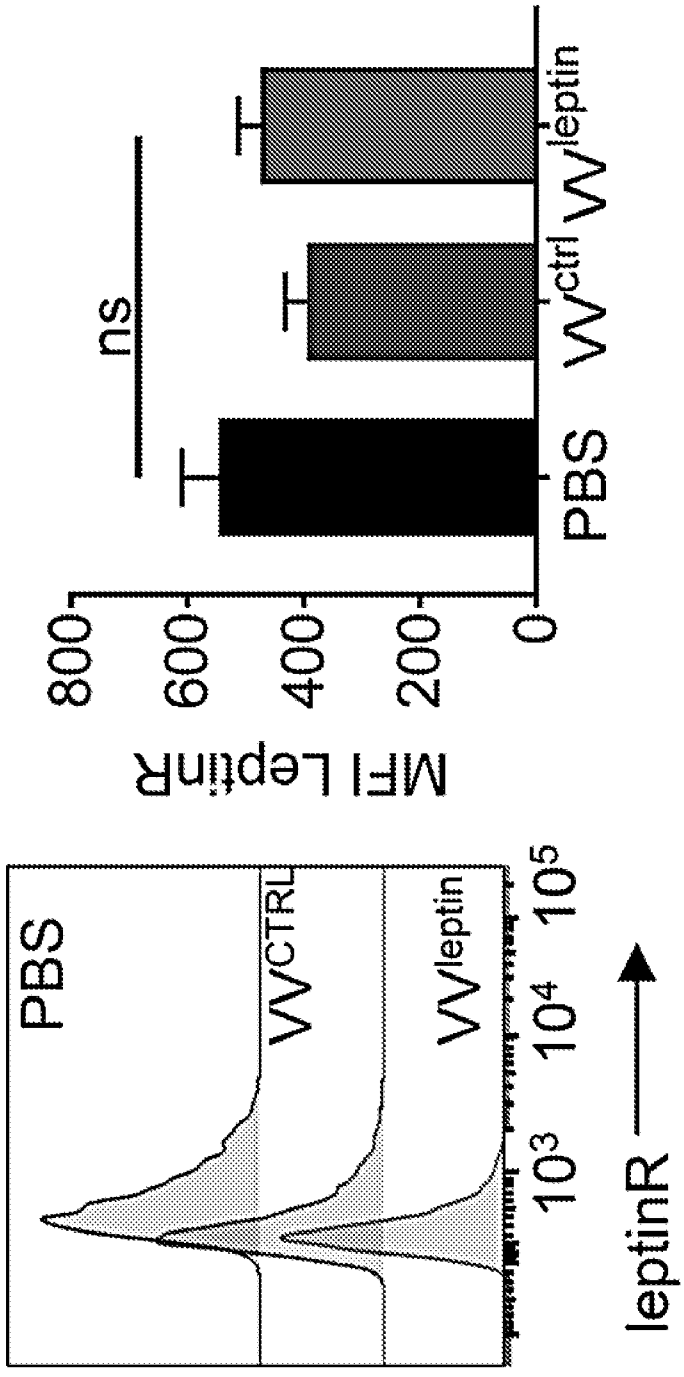
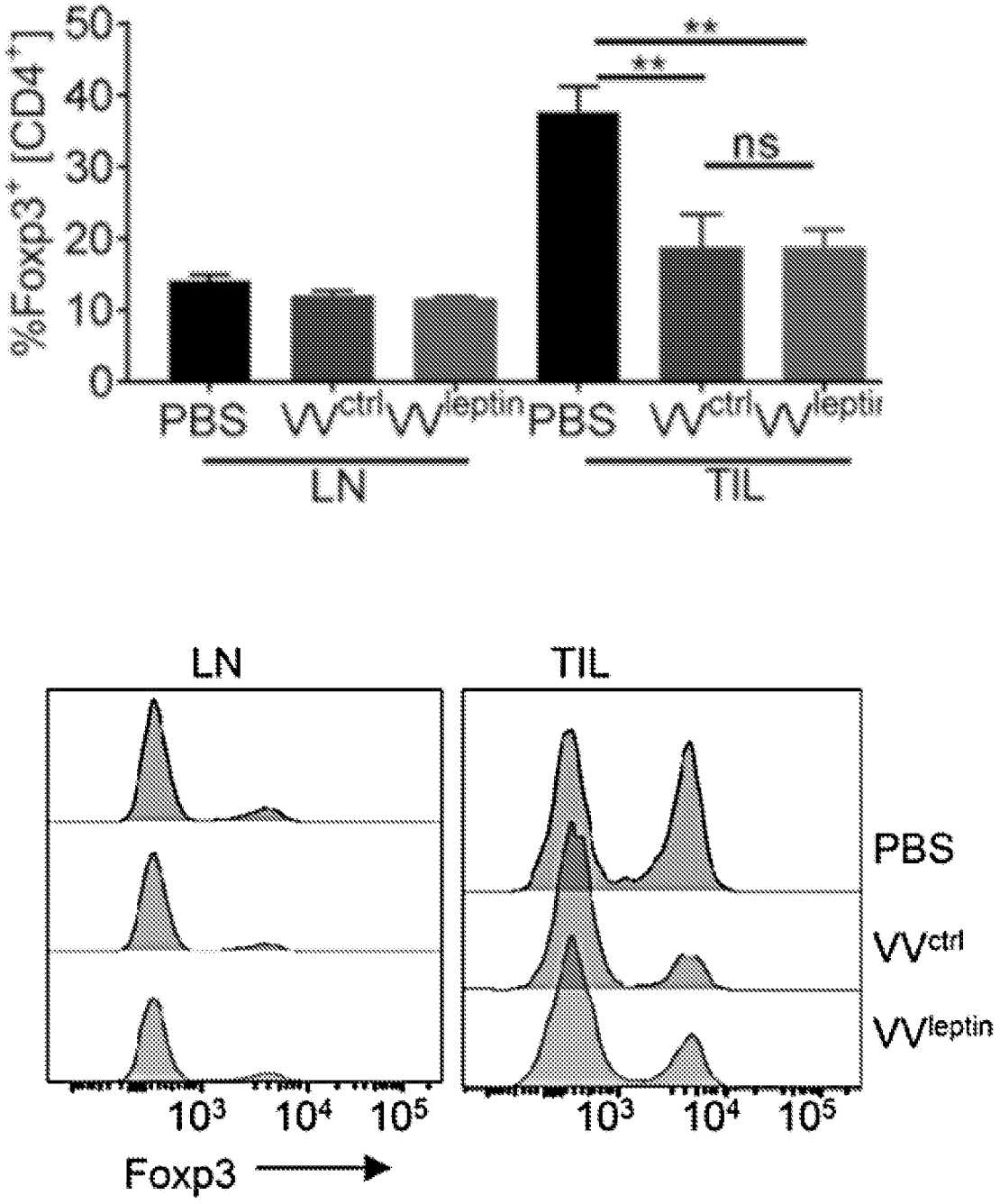

MFI LeptinR
800
600
400
200
0
ns
PBS
VV^ctrl
VV^leptin
PBS
VV^CTRL
VV^leptin
10^3
10^4
10^5
leptinR

FIG. 8F


%Foxp3+ [CD4+]
50
40
30
20
10
0
**
**
ns
PBS
VVctrl
VVleptin
PBS
VVctrl
VVleptin
LN
TIL
LN
TIL
PBS
VVctrl
VVleptin
10^3
10^4
10^5
10^3
10^4
10^5
Foxp3

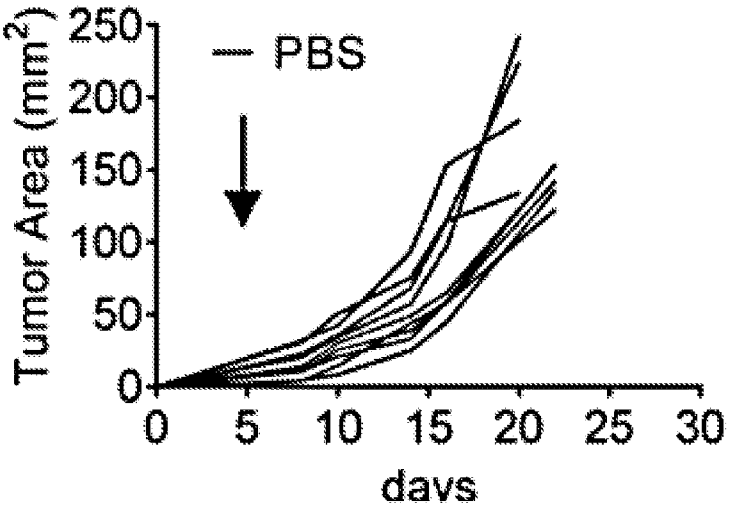
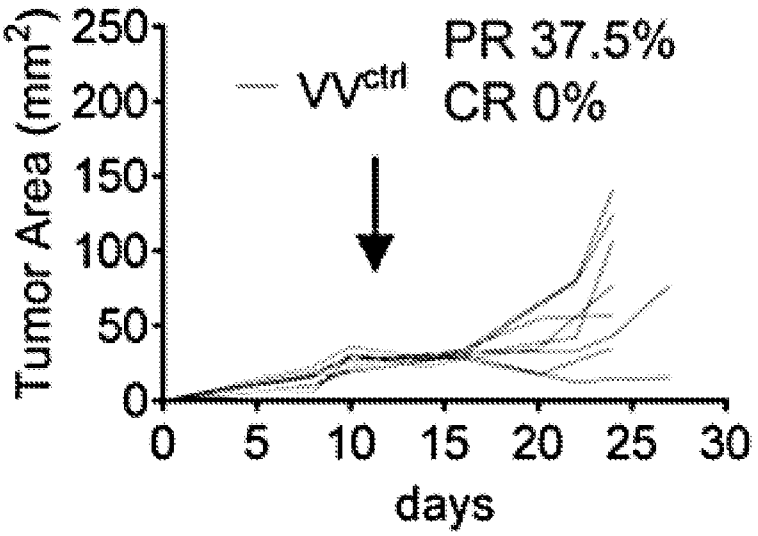
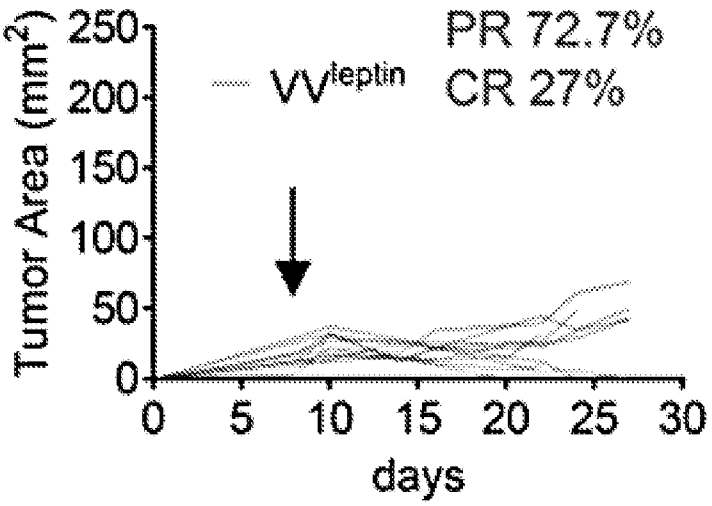

PBS
Tumor Area (mm2)
250
200
150
100
50
0
0 5 10 15 20 25 30
days

FIG. 9A


VVctrl
PR 37.5%
CR 0%
Tumor Area (mm2)
250
200
150
100
50
0
0 5 10 15 20 25 30
days


VVleptin
PR 72.7%
CR 27%
Tumor Area (mm2)
250
200
150
100
50
0
0 5 10 15 20 25 30
days

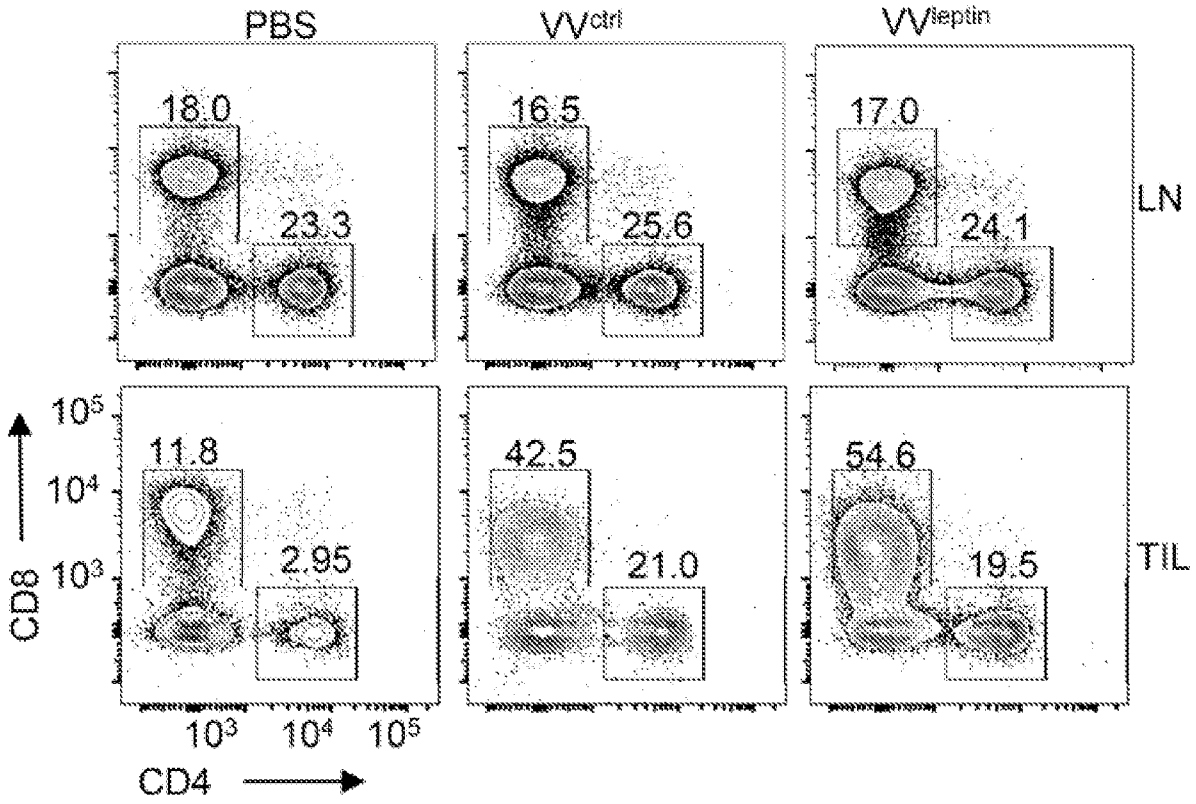
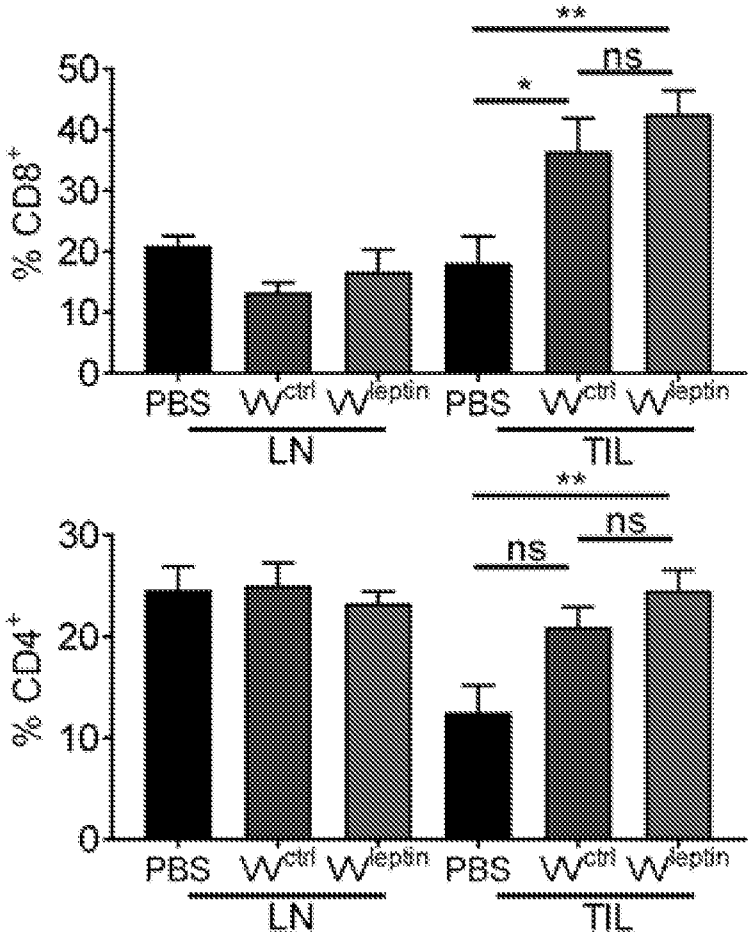
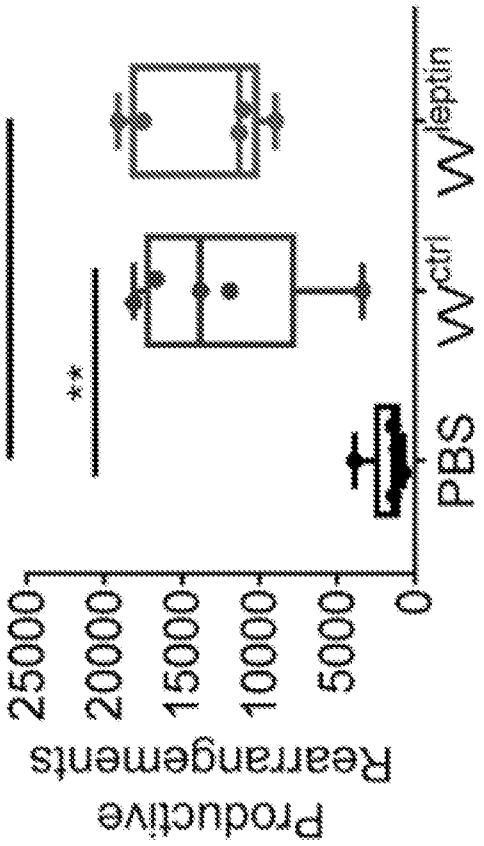

PBS
VVctrl
VVleptin
18.0
23.3
16.5
25.6
17.0
24.1
LN
11.8
2.95
42.5
21.0
54.6
19.5
TIL
CD8
CD4
10^5
10^4
10^3
10^3 10^4 10^5

FIG. 9B


% CD8+
% CD4+
PBS
Wctrl
Wleptin
LN
TIL
**
*
ns


Productive Rearrangements
25000
20000
15000
10000
5000
0
PBS
W^ctrl
W^leptin
**
**
**

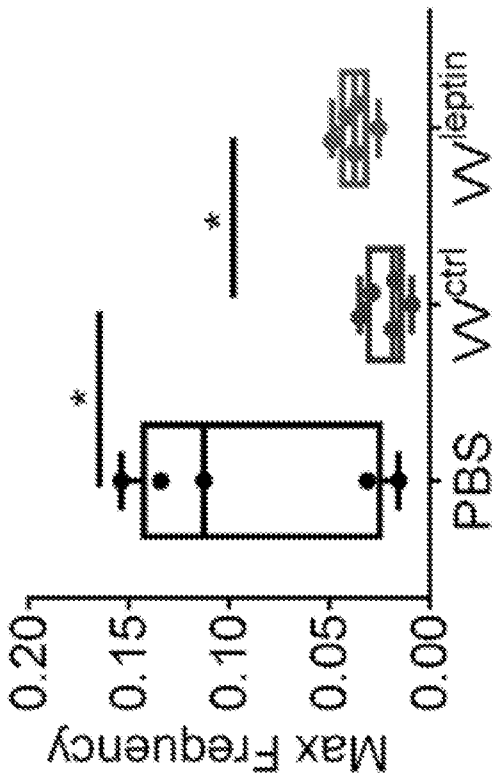

Max Frequency
0.20
0.15
0.10
0.05
0.00
PBS
W^ctrl
W^leptin
*
*

FIG. 9C

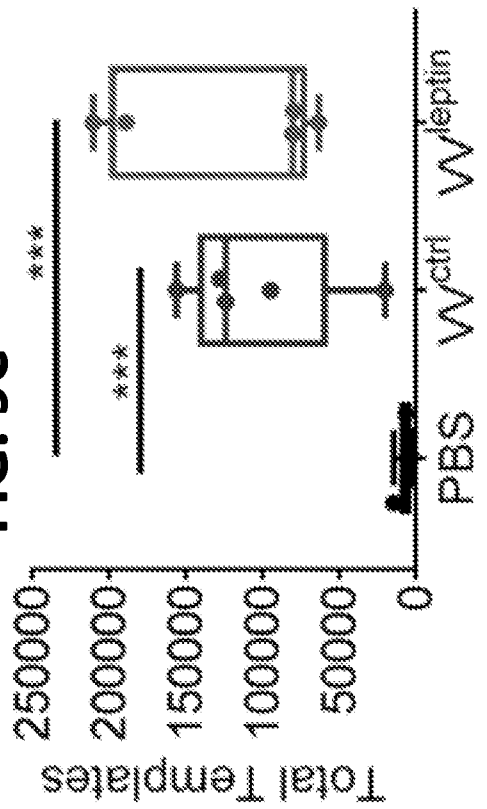

Total Templates
250000
200000
150000
100000
50000
0
PBS
W^ctrl
W^leptin
***
***

FIG. 9D

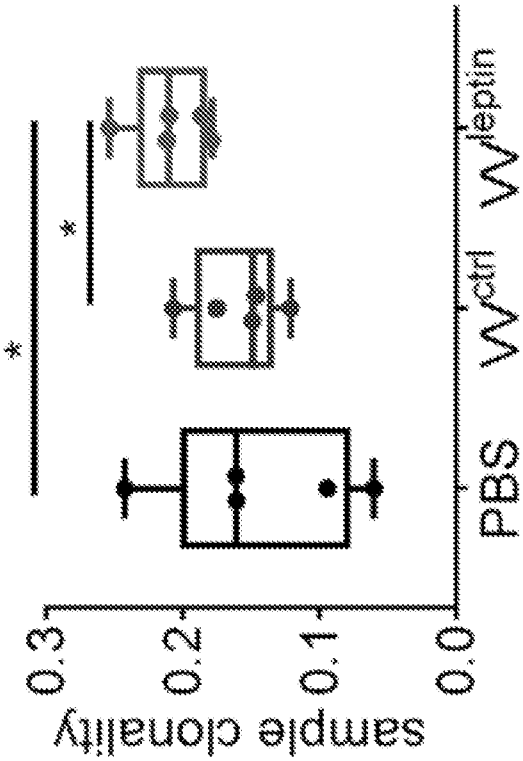
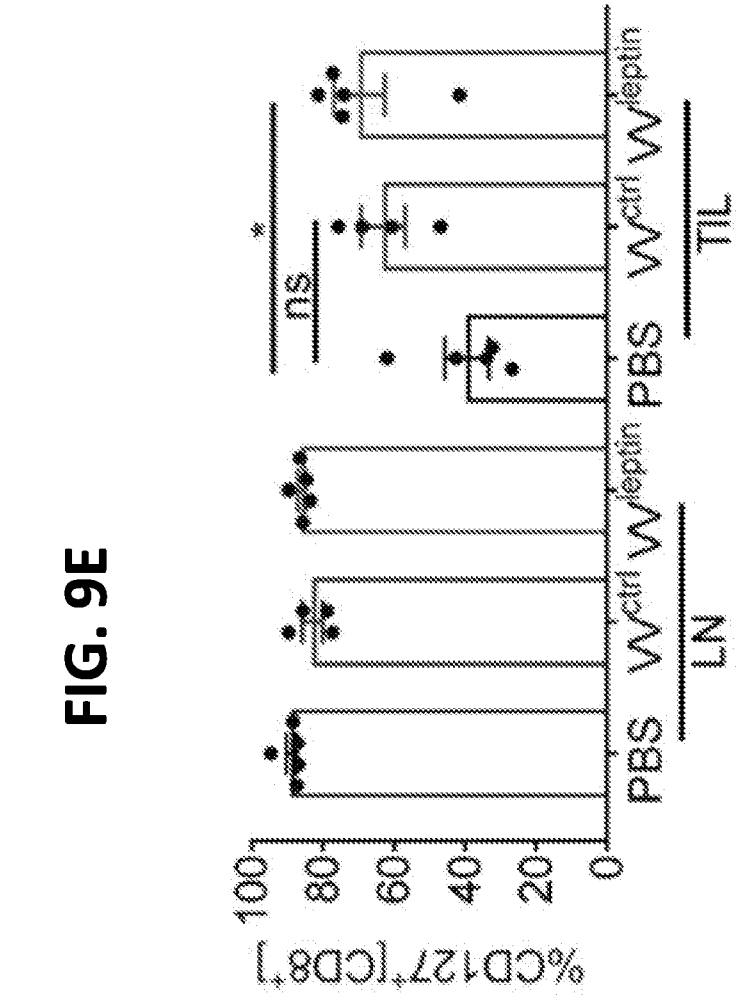

sample clonality
0.3
0.2
0.1
0.0
PBS
W^ctrl
W^leptin
*
*

FIG. 9E


%CD127+(CD8+)
100
80
60
40
20
0
PBS
VVctrl
VVleptin
PBS
VVctrl
VVleptin
LN
TIL
ns
*

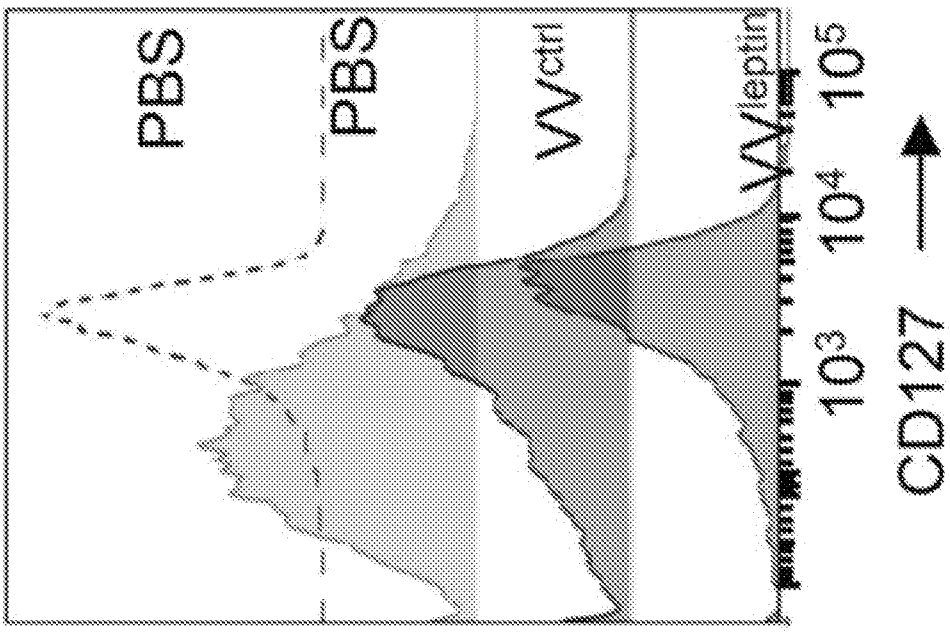
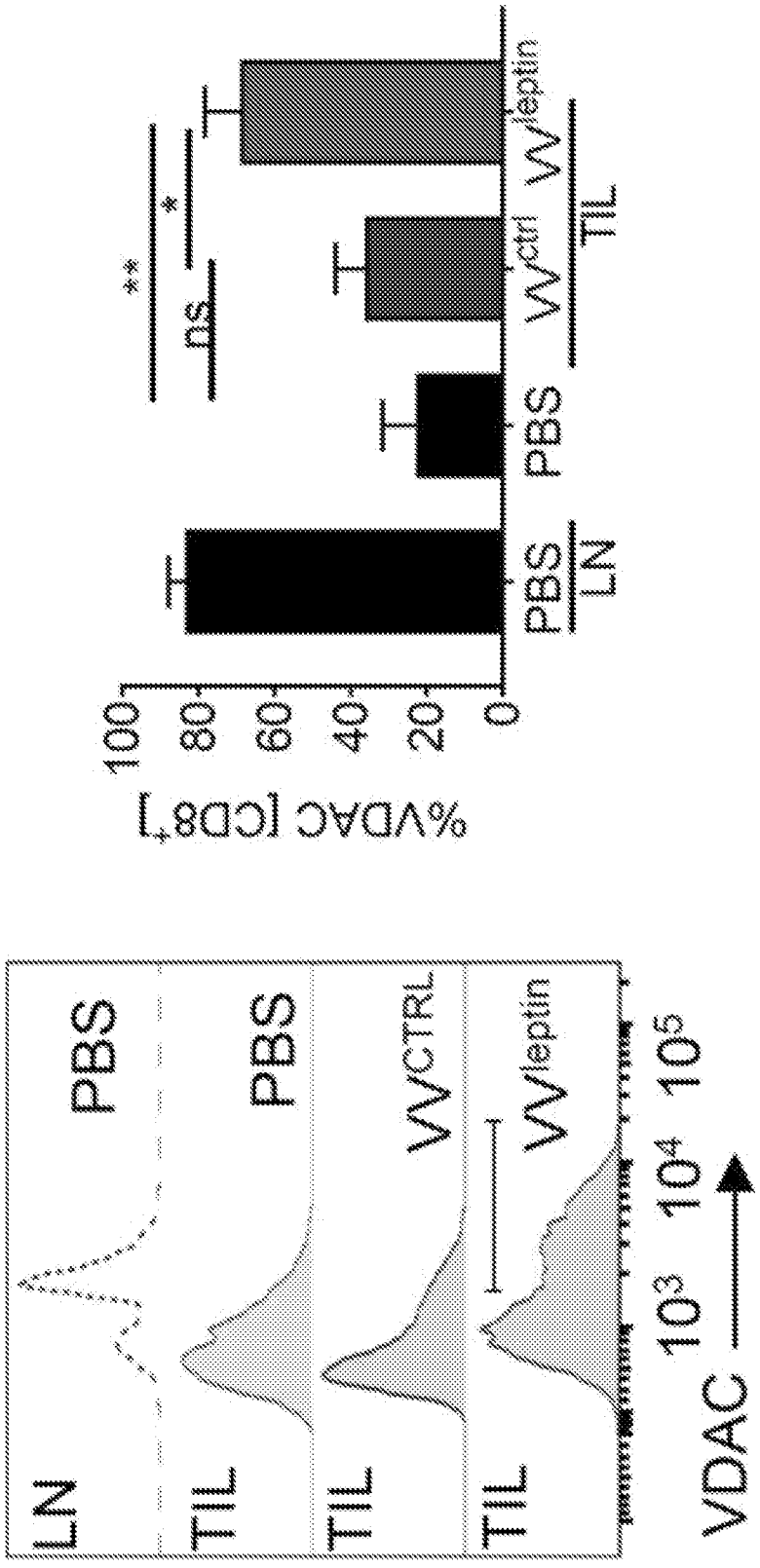

PBS
PBS
VVctrl
VVleptin
10³
10⁴
10⁵
CD127

FIG. 10A


%VDAC [CD8+]
100
80
60
40
20
0
PBS
LN
PBS
TIL
VVctrl
TIL
VVleptin
TIL
ns
*
**
LN
PBS
TIL
PBS
TIL
VVCTRL
TIL
VVleptin
VDAC
10^3
10^4
10^5

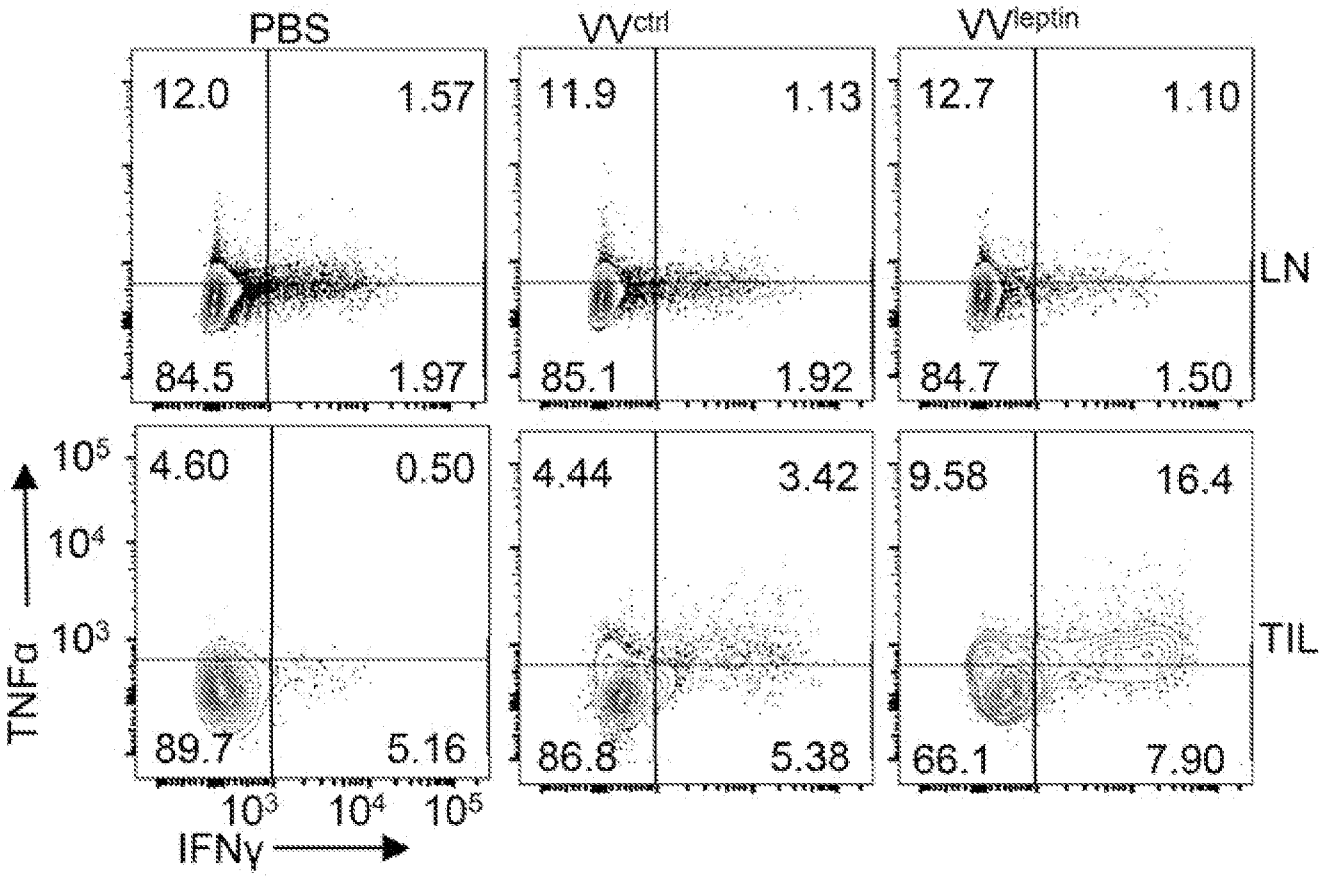
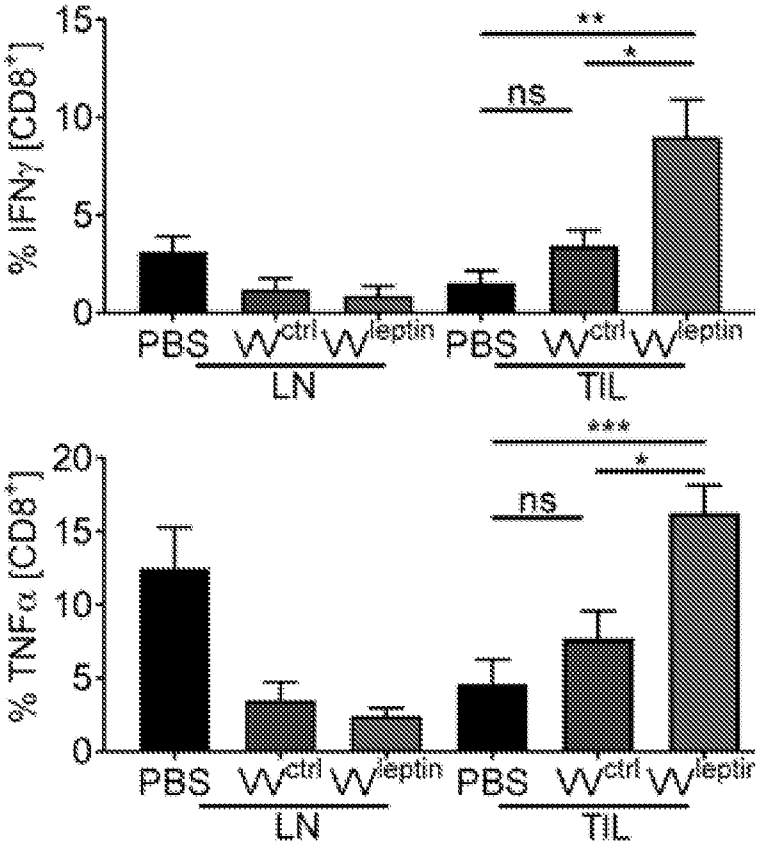

PBS
VVctrl
VVleptin
12.0
1.57
84.5
1.97
11.9
1.13
85.1
1.92
12.7
1.10
84.7
1.50
LN
4.60
0.50
89.7
5.16
4.44
3.42
86.8
5.38
9.58
16.4
66.1
7.90
TIL
TNFα
IFNγ
10^5
10^4
10^3

FIG. 10B


% IFNγ [CD8+]
% TNFα [CD8+]
PBS
VVctrl
VVleptin
LN
TIL
ns
*
**
***

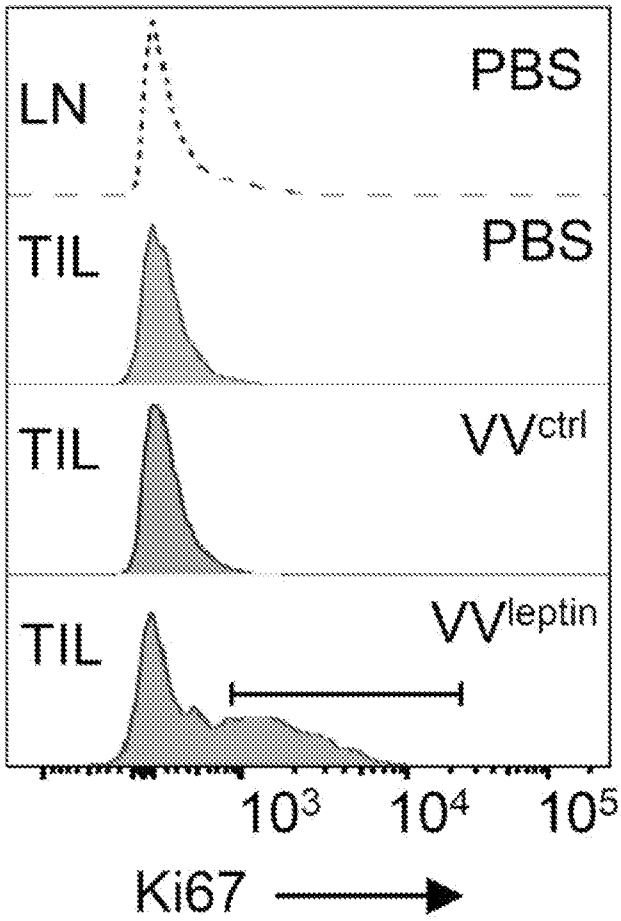
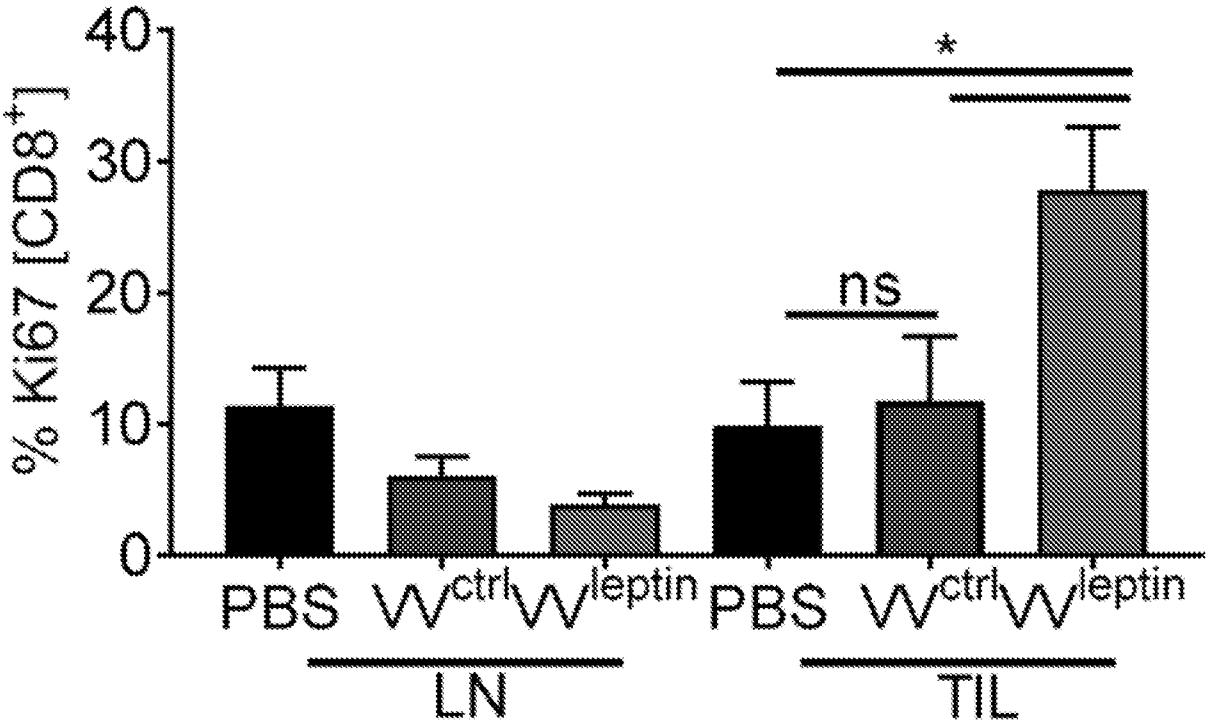

LN
PBS
TIL
PBS
TIL
VVctrl
TIL
VVleptin
10^3
10^4
10^5
Ki67

FIG. 10C


% Ki67 [CD8+]
40
30
20
10
0
*
ns
PBS
Wctrl
Wleptin
PBS
Wctrl
Wleptin
LN
TIL