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(54) Titre : IMMUNOTHERAPIE ANTITUMORALE UTILISANT DES VECTEURS VIRAUX SINDBIS ET DES ANTICORPS MONOCLONAUX AGONISTES

(54) Title: TUMOR IMMUNOTHERAPY USING SINDBIS VIRAL VECTORS AND AGONIST MONOCLONAL ANTIBODIES

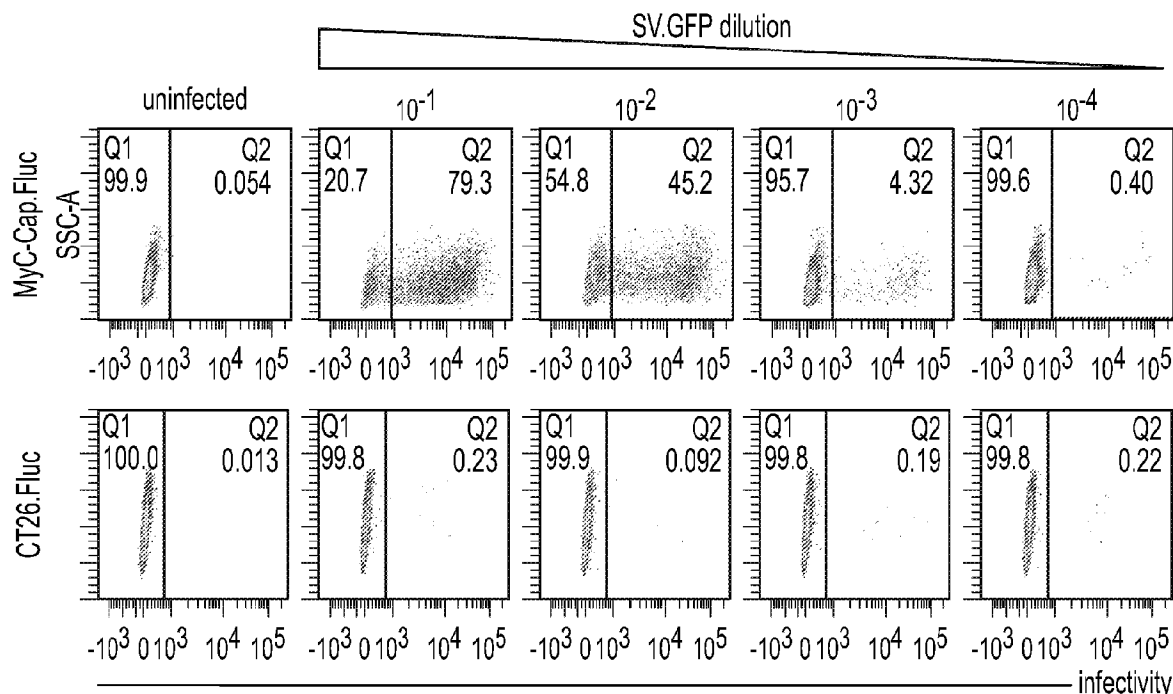


FIG. 4A

(57) **Abrégé/Abstract:**

The present disclosure provides compositions and methods for the treatment of cancer. More specifically, the present disclosure provides compositions and methods utilizing a combination of an oncolytic viruses, such as Sindbis virus, and antibodies directed against a co-stimulatory molecule or to an immune system agonist molecule, such as anti-OX40 antibodies and anti-4-1BB antibodies.

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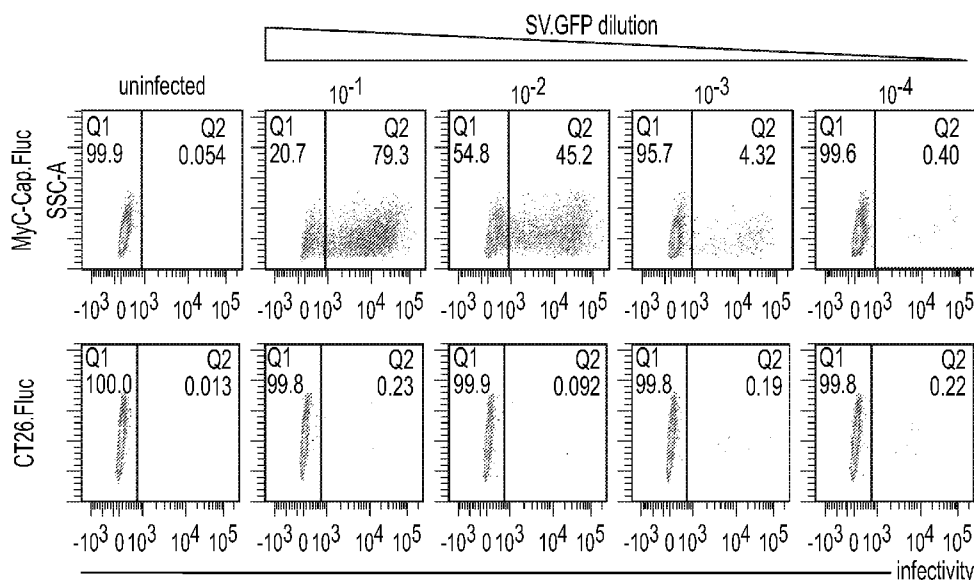


FIG. 4A

(57) Abstract: The present disclosure provides compositions and methods for the treatment of cancer. More specifically, the present disclosure provides compositions and methods utilizing a combination of an oncolytic viruses, such as Sindbis virus, and antibodies directed against a co-stimulatory molecule or to an immune system agonist molecule, such as anti-OX40 antibodies and anti-4-1BB antibodies.

[Continued on next page]

WO 2021/007276 A1

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TUMOR IMMUNOTHERAPY USING SINDBIS VIRAL VECTORS AND AGONIST MONOCLONAL ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/871,675, filed on July 8, 2019, the contents of which is hereby incorporated by reference in their entirety.

INCORPORATION-BY-REFERENCE OF SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 03, 2020, is named "27522-0225PCT Sequence Listing_ST25.txt" and is 34 kilobytes in size.

FIELD OF THE INVENTION

[0003] The present disclosure describes compositions and methods directed to treating cancer where the compositions include utilizing oncolytic viruses, such as Sindbis virus, and antibodies directed against a co-stimulatory molecule or to an immune system agonist molecule, such as OX-40 and 4-1BB (CD137).

BACKGROUND OF THE INVENTION

[0004] Immune checkpoint modulation has shown remarkable promise in treating cancer. Although, high response rates with immune checkpoint blockade have been documented in patients with highly immunogenic tumors, often the proportion of patients that respond to treatment is still low. Major challenges to overcome are the lack of T cell infiltration into the tumor microenvironment as well as the immunosuppressive nature of the tumor, which inhibits the intratumoral immune response. Further, tumors tend to quickly escape the immune response by mutating or losing the expression of drug targets or tumor antigens targeted by the immune response. Thus there is a need in the art for

compositions and methods that overcome these limitations. The present disclosure addresses these needs.

SUMMARY OF THE INVENTION

[0005] The present disclosure provides methods for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of (a) a oncolytic viral vector and (b) an antibody directed against a co-stimulatory molecule or a nucleic acid encoding same; or an antibody to an immunesystem agonist molecule or a nucleic acid encoding same.

[0006] The oncolytic viral vector can be a Sindbis viral vector. The Sindbis viral vector can be replication defective. The Sindbis viral vector can comprise at least one nucleic acid encoding a therapeutic protein. The Sindbis viral vector can comprise at least one nucleic acid encoding an immunostimulatory or an immunomodulatory protein. The immunostimulatory or immunomodulatory protein can be IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-34, IL-35, IL-36, or any combination thereof. In a preferred aspect, the immunostimulatory or immunomodulatory protein is IL-12. The Sindbis viral vector can comprise at least one nucleic acid encoding LacZ, Flue or GFP.

[0007] The antibody can be an anti-OX40 antibody, an anti-4-1BB antibody, an anti-CD28 antibody, an anti-GITR antibody, an anti-CD137 antibody, an anti-CD37 antibody, an anti-HVEM antibody, or a combination thereof.

[0008] The Sindbis viral vector and the antibody can induce an immune response in a tumor associated antigen (TAA) nonspecific manner. The induced and nonspecific immune response can be a first immune response. The first immune response can be followed by a secondary immuneresponse. The secondary immune response can be the result of one or more TAAs released from the dead tumor cells. The secondary immune response can comprise memory T cells directed against one or more TAAs released from the dead tumor cells.

[0009] The present disclosure also provides methods for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of

(a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and (b) an anti-OX40 monoclonal antibody or a nucleic acid encoding same, thereby treating cancer in the subject.

[00010] The Sindbis viral vector can be replication defective. The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 and can further comprise the nucleic acid encoding the anti-OX40 monoclonal antibody. The method can comprise administering a Sindbis viral vector comprising the nucleic acid encoding interleukin-12 and administering a Sindbis viral vector comprising the nucleic acid encoding the anti-OX40 monoclonal antibody. The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit (IL-12 α , IL-12, p35 subunit) and interleukin-12 beta subunit (IL-12 β , IL-12, p40 subunit). The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit of GenBank accession no. M86672 and interleukin-12 beta subunit (IL-12 β , IL-12, p40 subunit) GenBank accession no. M86671. The nucleic acid encoding interleukin-12 alpha subunit comprises the nucleic acid sequence of SEQ ID NO: 1. The nucleic acid encoding interleukin-12 beta subunit comprises the nucleic acid sequence of SEQ ID NO: 2.

[00011] The Sindbis viral vector can comprise a nucleic acid encoding interleukin-12 alpha subunit of amino acid sequence of GenBank accession no. AAA39292.1 and comprise a nucleic acid encoding interleukin-12 beta subunit of amino acid sequence of GenBank accession no. AAA39296.1. The amino acid sequence of the interleukin-12 alpha subunit is of amino acid sequence of SEQ ID NO: 3. The amino acid sequence of the interleukin-12 beta subunit is of amino acid sequence of SEQ ID NO: 4.

[00012] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 5. The nucleic acid sequence encoding the anti-OX40 variable heavy chain is SEQ ID NO: 6. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 7. The nucleic acid sequence encoding the anti-OX40 variable light chain is SEQ ID NO: 8.

[00013] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain of amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain of amino acid sequence of SEQ ID NO: 7. The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain that is at least 70%, 75%,

80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 7.

[00014] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain comprising the amino acid sequence of SEQ ID NO: 9. The nucleic acid sequence encoding the an anti-OX40 antibody heavy chain is SEQ ID NO: 10.

[00015] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 antibody light chain comprising the amino acid sequence of SEQ ID NO: 11. The nucleic acid sequence encoding the anti-OX40 antibody light chain is SEQ ID NO: 12.

[00016] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence of SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain of SEQ ID NO: 10 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 12.

[00017] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 10 and an anti-OX40 antibody light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 12.

[00018] The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[00019] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target

antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[00020] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain amino acid sequence, and an anti-OX40 antibody variable light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain amino acid sequence, and an anti-OX40 antibody light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[00021] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 31. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 32. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 33. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 34.

[00022] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 31. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 32. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 33. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 34.

[00023] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 35. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 36. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light

chain comprising the amino acid sequence of SEQ ID NO: 37. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 38.

[00024] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 35. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 36. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 37. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 38.

[00025] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 39. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 40. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 41. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 42.

[00026] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 39. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 40. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 41. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 42.

[00027] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 43. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 44. The

Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 45. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 46.

[00028] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 43. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 44. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 45. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 46.

[00029] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 47. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 48. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 49. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 50.

[00030] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 47. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 48. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 49. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 50.

[00031] The Sindbis viral vector and the anti-OX40 monoclonal antibody can be administered sequentially or concurrently. The Sindbis viral vector can be administered

systemically. The anti-OX40 monoclonal antibody can be administered systemically. Both the Sindbis viral vector and the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be administered systemically. The Sindbis viral vector can be administered parenterally. The anti-OX40 monoclonal antibody can be administered parenterally. Both the Sindbis viral vector and the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be administered parenterally. The Sindbis viral vector can be administered intraperitoneally. The anti-OX40 monoclonal antibody can be administered intraperitoneally. Both the Sindbis viral vector and the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be administered intraperitoneally.

[00032] An antibody of the present disclosure, or a fragment thereof, can be derived from any species, including, but not limited to, a human, a mouse, a rat, a hamster, a dog, a rabbit, a frog, a sheep, a goat, a cow, a horse, a pig, a bird, a donkey, a chicken, a camel, a llama, a dromedary, an alpaca, a shark, a bovine and a turtle. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a human. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a mouse. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a camel, a llama or an alpaca. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a shark. In some aspects, an antibody of the present disclosure, or a fragment thereof, of the present disclosure is a chimeric antibody that is derived from two or more of the aforementioned species. In a non-limiting example, an antibody of the present disclosure, or fragment thereof, can be a chimeric antibody that is derived from a human and a mouse. In some aspects, an antibody of the present disclosure, or a fragment thereof, can be derived from any species other than human and can be further humanized using standard methods known in the art as to reduce the immunogenicity of the antibody.

[00033] A “monoclonal antibody” as disclosed herein, can be a full-length antibody or an antigen binding fragment thereof, wherein the “antigen binding fragment” is a fragment of the full length antibody that retains binding to the target antigen of the said monoclonal antibody. For example, a monoclonal antibody against OX-40 or an anti-OX40 monoclonal antibody, as described herein, can be a full length antibody against OX40 antibody or an antigen binding fragment thereof, wherein the fragment retains binding to the OX40 receptor on a cell surface. An “antigen-binding fragment” of an anti-OX-40 antibody, as described herein can include any

fragment selected from the group consisting of Fv, Fav, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, scFv-CH₃, scFv-Fc, and diabody fragments.

[00034] The cancer can be a solid cancer or a liquid/hematologic cancer. The cancer can comprise metastatic cancer. The cancer can comprise a solid tumor. The cancer can be a carcinoma, a lymphoma, a blastoma, a sarcoma, a leukemia, a brain cancer, a breast cancer, a blood cancer, a bone cancer, a lung cancer, a skin cancer, a liver cancer, an ovarian cancer, a bladder cancer, a renal cancer, a gastric cancer, a thyroid cancer, a pancreatic cancer, an esophageal cancer, a prostate cancer, a cervical cancer or a colorectal cancer. In one preferred aspect, the cancer is colon cancer. In one preferred aspect, the cancer is prostate cancer. In one preferred aspect, the cancer is ovarian cancer.

[00035] The present disclosure further provides a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a nucleic acid encoding an anti-OX40 monoclonal antibody. The present disclosure also provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and (b) an anti-OX40 monoclonal antibody or a nucleic acid encoding same. The present disclosure also provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and (b) a Sindbis viral vector comprising a nucleic acid encoding an anti-OX40 monoclonal antibody.

[00036] The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit (IL-12 α , IL-12, p35 subunit) and interleukin-12 beta subunit (IL-12 β , IL-12, p40 subunit). The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit of GenBank accession no. M86672 and interleukin-12 beta subunit (IL-12 β , IL-12, p40 subunit) GenBank accession no. M86671. The nucleic acid encoding interleukin-12 alpha subunit comprises the nucleic acid sequence of SEQ ID NO: 1. The nucleic acid encoding interleukin-12 beta subunit comprises the nucleic acid sequence of SEQ ID NO: 2.

[00037] The Sindbis viral vector can comprise a nucleic acid encoding interleukin-12 alpha subunit of amino acid sequence of GenBank accession no. AAA39292.1 and comprise a nucleic acid encoding interleukin-12 beta subunit of amino acid sequence of GenBank accession no. AAA39296.1. The amino acid sequence of the interleukin-12 alpha subunit is of amino acid

sequence of SEQ ID NO: 3. The amino acid sequence of the interleukin-12 beta subunit is of amino acid sequence of SEQ ID NO: 4.

[00038] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 5. The nucleic acid sequence encoding the anti-OX40 variable heavy chain is SEQ ID NO: 6. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 7. The nucleic acid sequence encoding the anti-OX40 variable light chain is SEQ ID NO: 8.

[00039] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain of amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain of amino acid sequence of SEQ ID NO: 7. The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 7.

[00040] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain comprising the amino acid sequence of SEQ ID NO: 9. The nucleic acid sequence encoding the an anti-OX40 antibody heavy chain is SEQ ID NO: 10.

[00041] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 antibody light chain comprising the amino acid sequence of SEQ ID NO: 11. The nucleic acid sequence encoding the anti-OX40 antibody light chain is SEQ ID NO: 12.

[00042] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence of SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain of SEQ ID NO: 10 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 12.

[00043] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%,

97%, 98%, 99% or 100% identical to SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 10 and an anti-OX40 antibody light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 12.

[00044] The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[00045] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[00046] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain amino acid sequence, and an anti-OX40 antibody variable light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain amino acid sequence, and an anti-OX40 antibody light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[00047] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 31. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 32. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 33. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 34.

[00048] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%,

85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 31. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 32. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 33. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 34.

[00049] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 35. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 36. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 37. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 38.

[00050] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 35. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 36. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 37. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 38.

[00051] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 39. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 40. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 41. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 42.

[00052] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 39. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 40. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 41. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 42.

[00053] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 43. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 44. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 45. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 46.

[00054] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 43. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 44. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 45. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 46.

[00055] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 47. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 48. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light

chain comprising the amino acid sequence of SEQ ID NO: 49. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 50.\

[00056] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 47. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 48. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 49. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 50.

[00057] An antibody of the present disclosure, or a fragment thereof, can be derived from any species, including, but not limited to, a human, a mouse, a rat, a hamster, a dog, a rabbit, a frog, a sheep, a goat, a cow, a horse, a pig, a bird, a donkey, a chicken, a camel, a llama, a dromedary, an alpaca, a shark, a bovine and a turtle. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a human. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a mouse. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a camel, a llama or an alpaca. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a shark. In some aspects, an antibody of the present disclosure, or a fragment thereof, of the present disclosure is a chimeric antibody that is derived from two or more of the aforementioned species. In a non-limiting example, an antibody of the present disclosure, or fragment thereof, can be a chimeric antibody that is derived from a human and a mouse. In some aspects, an antibody of the present disclosure, or a fragment thereof, can be derived from any species other than human and can be further humanized using standard methods known in the art as to reduce the immunogenicity of the antibody.

[00058] A “monoclonal antibody” as disclosed herein, can be a full-length antibody or an antigen binding fragment thereof, wherein the “antigen binding fragment” is a fragment of the full length antibody that retains binding to the target antigen of the said monoclonal antibody. For example, a monoclonal antibody against OX-40 or an anti-OX40 monoclonal antibody, as

described herein, can be a full length antibody against OX40 antibody or an antigen binding fragment thereof, wherein the fragment retains binding to the OX40 receptor on a cell surface. An “antigen-binding fragment” of an anti-OX-40 antibody, as described herein can include any fragment selected from the group consisting of Fv, Fav, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, scFv-CH₃, scFv-Fc, and diabody fragments.

[00059] The present disclosure also provides methods for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1, thereby treating cancer in the subject. The present disclosure also provides methods for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a nucleic acid encoding NY-ESO-1, thereby treating cancer in the subject.

[00060] The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit (IL-12 α , IL-12, p35 subunit) and interleukin-12 beta subunit (IL-12 β , IL-12, p40 subunit). The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit of GenBank accession no. M86672 and interleukin-12 beta subunit (IL-12 β , IL-12, p40 subunit) GenBank accession no. M86671. The nucleic acid encoding interleukin-12 alpha subunit comprises the nucleic acid sequence of SEQ ID NO: 1. The nucleic acid encoding interleukin-12 beta subunit comprises the nucleic acid sequence of SEQ ID NO: 2.

[00061] The Sindbis viral vector can comprise a nucleic acid encoding interleukin-12 alpha subunit of amino acid sequence of GenBank accession no. AAA39292.1 and comprise a nucleic acid encoding interleukin-12 beta subunit of amino acid sequence of GenBank accession no. AAA39296.1. The amino acid sequence of the interleukin-12 alpha subunit is of amino acid sequence of SEQ ID NO: 3. The amino acid sequence of the interleukin-12 beta subunit is of amino acid sequence of SEQ ID NO: 4.

[00062] The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of NCBI Reference accession no. NM_001327.1. The nucleic acid encoding NY-ESO-1 comprises the nucleic acid sequence of SEQ ID NO: 14. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of amino acid sequence of NCBI Reference accession no.

NP_001318.1. The amino acid sequence of the NY-ESO-1 comprises the amino acid sequence of SEQ ID NO: 15.

[00063] The Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and the Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 can be administered sequentially or concurrently. The Sindbis viral vector comprising a nucleic acid encoding interleukin-12 can be administered systemically. The Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 can be administered systemically. Both the Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and the Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 can be administered systemically. The Sindbis viral vector comprising a nucleic acid encoding interleukin-12 can be administered parenterally. The Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 can be administered parenterally. Both the Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and the Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 can be administered parenterally. The Sindbis viral vector comprising a nucleic acid encoding interleukin-12 can be administered intraperitoneally. The Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 can be administered intraperitoneally. Both the Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and the Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 can be administered intraperitoneally.

[00064] The cancer can be a solid cancer or a liquid/hematologic cancer. The cancer can comprise metastatic cancer. The cancer can comprise a solid tumor. The cancer can be a carcinoma, a lymphoma, a blastoma, a sarcoma, a leukemia, a brain cancer, a breast cancer, a blood cancer, a bone cancer, a lung cancer, a skin cancer, a liver cancer, an ovarian cancer, a bladder cancer, a renal cancer, a gastric cancer, a thyroid cancer, a pancreatic cancer, an esophageal cancer, a prostate cancer, a cervical cancer or a colorectal cancer. In one preferred aspect, the cancer is colon cancer. In one preferred aspect, the cancer is prostate cancer. In one preferred aspect, the cancer is ovarian cancer.

[00065] The present disclosure further provides a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a nucleic acid encoding NY-ESO-1. The present disclosure also provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and (b) a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1.

[00066] The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit (IL-12 α , IL-12, p35 subunit) and interleukin-12 beta subunit (IL-12 β , IL-12, p40 subunit). The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit of GenBank accession no. M86672 and interleukin-12 beta subunit (IL-12 β , IL-12, p40 subunit) GenBank accession no. M86671. The nucleic acid encoding interleukin-12 alpha subunit comprises the nucleic acid sequence of SEQ ID NO: 1. The nucleic acid encoding interleukin-12 beta subunit comprises the nucleic acid sequence of SEQ ID NO: 2.

[00067] The Sindbis viral vector can comprise a nucleic acid encoding interleukin-12 alpha subunit of amino acid sequence of GenBank accession no. AAA39292.1 and comprise a nucleic acid encoding interleukin-12 beta subunit of amino acid sequence of GenBank accession no. AAA39296.1. The amino acid sequence of the interleukin-12 alpha subunit is of amino acid sequence of SEQ ID NO: 3. The amino acid sequence of the interleukin-12 beta subunit is of amino acid sequence of SEQ ID NO: 4.

[00068] The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of NCBI Reference accession no. NM_001327.1. The nucleic acid encoding NY-ESO-1 comprises the nucleic acid sequence of SEQ ID NO: 14. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of amino acid sequence of NCBI Reference accession no. NP_001318.1. The amino acid sequence of the NY-ESO-1 comprises the amino acid sequence of SEQ ID NO: 15.

[00069] The present disclosure provides methods for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of (a) a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 and (b) an anti-OX40 monoclonal antibody or a nucleic acid encoding same, thereby treating cancer in the subject.

[00070] The Sindbis viral vector can be replication defective. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 and can further comprise the nucleic acid encoding the anti-OX40 monoclonal antibody. The method can comprise administering a Sindbis viral vector comprising the nucleic acid encoding NY-ESO-1 and administering a Sindbis viral vector comprising the nucleic acid encoding the anti-OX40 monoclonal antibody. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of NCBI Reference accession no. NM_001327.1. The nucleic acid encoding NY-ESO-1 comprises the nucleic acid

sequence of SEQ ID NO: 14. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of amino acid sequence of NCBI Reference accession no. NP_001318.1. The amino acid sequence of the NY-ESO-1 comprises the amino acid sequence of SEQ ID NO: 15.

[00071] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 5. The nucleic acid sequence encoding the anti-OX40 variable heavy chain is SEQ ID NO: 6. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 7. The nucleic acid sequence encoding the anti-OX40 variable light chain is SEQ ID NO: 8.

[00072] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain of amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain of amino acid sequence of SEQ ID NO: 7. The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 7.

[00073] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain comprising the amino acid sequence of SEQ ID NO: 9. The nucleic acid sequence encoding the an anti-OX40 antibody heavy chain is SEQ ID NO: 10.

[00074] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 antibody light chain comprising the amino acid sequence of SEQ ID NO: 11. The nucleic acid sequence encoding the anti-OX40 antibody light chain is SEQ ID NO: 12.

[00075] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence of SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain of SEQ ID NO: 10 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 12.

[00076] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 9 and an anti-OX40 antibody

light chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 10 and an anti-OX40 antibody light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 12.

[00077] The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[00078] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[00079] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain amino acid sequence, and an anti-OX40 antibody variable light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain amino acid sequence, and an anti-OX40 antibody light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[00080] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 31. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 32. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 33. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 34.

[00081] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 31. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 32. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 33. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 34.

[00082] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 35. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 36. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 37. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 38.

[00083] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 35. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 36. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 37. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 38.

[00084] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 39. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 40. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light

chain comprising the amino acid sequence of SEQ ID NO: 41. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 42.

[00085] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 39. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 40. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 41. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 42.

[00086] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 43. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 44. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 45. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 46.

[00087] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 43. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 44. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 45. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 46.

[00088] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 47. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 48. The

Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 49. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 50.

[00089] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 47. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 48. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 49. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 50.

[00090] The Sindbis viral vector and the anti-OX40 monoclonal antibody can be administered sequentially or concurrently. The Sindbis viral vector can be administered systemically. The anti-OX40 monoclonal antibody can be administered systemically. Both the Sindbis viral vector and the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be administered systemically. The Sindbis viral vector can be administered parenterally. The anti-OX40 monoclonal antibody can be administered parenterally. Both the Sindbis viral vector and the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be administered parenterally. The Sindbis viral vector can be administered intraperitoneally. The anti-OX40 monoclonal antibody can be administered intraperitoneally. Both the Sindbis viral vector and the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be administered intraperitoneally.

[00091] An antibody of the present disclosure, or a fragment thereof, can be derived from any species, including, but not limited to, a human, a mouse, a rat, a hamster, a dog, a rabbit, a frog, a sheep, a goat, a cow, a horse, a pig, a bird, a donkey, a chicken, a camel, a llama, a dromedary, an alpaca, a shark, a bovine and a turtle. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a human. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a mouse. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a camel, a llama or an alpaca. In

some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a shark. In some aspects, an antibody of the present disclosure, or a fragment thereof, of the present disclosure is a chimeric antibody that is derived from two or more of the aforementioned species. In a non-limiting example, an antibody of the present disclosure, or fragment thereof, can be a chimeric antibody that is derived from a human and a mouse. In some aspects, an antibody of the present disclosure, or a fragment thereof, can be derived from any species other than human and can be further humanized using standard methods known in the art as to reduce the immunogenicity of the antibody.

[00092] A “monoclonal antibody” as disclosed herein, can be a full-length antibody or an antigen binding fragment thereof, wherein the “antigen binding fragment” is a fragment of the full length antibody that retains binding to the target antigen of the said monoclonal antibody. For example, a monoclonal antibody against OX-40 or an anti-OX40 monoclonal antibody, as described herein, can be a full length antibody against OX40 antibody or an antigen binding fragment thereof, wherein the fragment retains binding to the OX40 receptor on a cell surface. An “antigen-binding fragment” of an anti-OX-40 antibody, as described herein can include any fragment selected from the group consisting of Fv, Fav, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, scFv-CH₃, scFv-Fc, and diabody fragments.

[00093] The cancer can be a solid cancer or a liquid/hematologic cancer. The cancer can comprise metastatic cancer. The cancer can comprise a solid tumor. The cancer can be a carcinoma, a lymphoma, a blastoma, a sarcoma, a leukemia, a brain cancer, a breast cancer, a blood cancer, a bone cancer, a lung cancer, a skin cancer, a liver cancer, an ovarian cancer, a bladder cancer, a renal cancer, a gastric cancer, a thyroid cancer, a pancreatic cancer, an esophageal cancer, a prostate cancer, a cervical cancer or a colorectal cancer. In one preferred aspect, the cancer is colon cancer. In one preferred aspect, the cancer is prostate cancer. In one preferred aspect, the cancer is ovarian cancer.

[00094] The present disclosure further provides a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 and a nucleic acid encoding an anti-OX40 monoclonal antibody. The present disclosure also provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 and (b) an anti-OX40 monoclonal antibody or a nucleic acid encoding same. The present disclosure also provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding NY-

ESO-1 and (b) a Sindbis viral vector comprising a nucleic acid encoding an anti-OX40 monoclonal antibody. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of NCBI Reference accession no. NM_001327.1. The nucleic acid encoding NY-ESO-1 comprises the nucleic acid sequence of SEQ ID NO: 14. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of amino acid sequence of NCBI Reference accession no. NP_001318.1. The amino acid sequence of the NY-ESO-1 comprises the amino acid sequence of SEQ ID NO: 15.

[00095] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 5. The nucleic acid sequence encoding the anti-OX40 variable heavy chain is SEQ ID NO: 6. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 7. The nucleic acid sequence encoding the anti-OX40 variable light chain is SEQ ID NO: 8.

[00096] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain of amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain of amino acid sequence of SEQ ID NO: 7. The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 7.

[00097] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain comprising the amino acid sequence of SEQ ID NO: 9. The nucleic acid sequence encoding the an anti-OX40 antibody heavy chain is SEQ ID NO: 10.

[00098] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 antibody light chain comprising the amino acid sequence of SEQ ID NO: 11. The nucleic acid sequence encoding the anti-OX40 antibody light chain is SEQ ID NO: 12.

[00099] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence of SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 11. The Sindbis viral vector

can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain of SEQ ID NO: 10 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 12.

[000100] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 10 and an anti-OX40 antibody light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 12.

[000101] The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000102] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000103] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain amino acid sequence, and an anti-OX40 antibody variable light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain amino acid sequence, and an anti-OX40 antibody light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000104] An antibody of the present disclosure, or a fragment thereof, can be derived from any species, including, but not limited to, a human, a mouse, a rat, a hamster, a dog, a rabbit, a

frog, a sheep, a goat, a cow, a horse, a pig, a bird, a donkey, a chicken, a camel, a llama, a dromedary, an alpaca, a shark, a bovine and a turtle. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a human. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a mouse. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a camel, a llama or an alpaca. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a shark. In some aspects, an antibody of the present disclosure, or a fragment thereof, of the present disclosure is a chimeric antibody that is derived from two or more of the aforementioned species. In a non-limiting example, an antibody of the present disclosure, or fragment thereof, can be a chimeric antibody that is derived from a human and a mouse. In some aspects, an antibody of the present disclosure, or a fragment thereof, can be derived from any species other than human and can be further humanized using standard methods known in the art as to reduce the immunogenicity of the antibody.

[000105] A “monoclonal antibody” as disclosed herein, can be a full-length antibody or an antigen binding fragment thereof, wherein the “antigen binding fragment” is a fragment of the full length antibody that retains binding to the target antigen of the said monoclonal antibody. For example, a monoclonal antibody against OX-40 or an anti-OX40 monoclonal antibody, as described herein, can be a full length antibody against OX40 antibody or an antigen binding fragment thereof, wherein the fragment retains binding to the OX40 receptor on a cell surface. An “antigen-binding fragment” of an anti-OX40 antibody, as described herein can include any fragment selected from the group consisting of Fv, Fav, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, scFv-CH₃, scFv-Fc, and diabody fragments.

[000106] The present disclosure also provides methods for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount (a) a Sindbis viral vector and (b) an anti-4-1BB (CD137) monoclonal antibody or a nucleic acid encoding same, thereby treating cancer in the subject. The present disclosure further provides *in vitro* or *ex vivo* methods for treating cancer or assessing the treatment of cancer in a subject comprising contacting a biological sample from the subject with (a) a Sindbis viral vector and (b) an anti-4-1BB monoclonal antibody or a nucleic acid encoding same. The Sindbis viral vector does not comprise an endogenous nucleic acid encoding any protein.

[000107] The Sindbis viral vector is replication defective. The Sindbis viral vector can comprise a nucleic acid sequence encoding a therapeutic protein, an immunostimulatory protein or an immunomodulatory protein. The Sindbis viral vector can comprise the nucleic acid encoding the therapeutic protein, an immunostimulatory protein or an immunomodulatory protein and further comprise the nucleic acid encoding the anti-4-1BB monoclonal antibody. The Sindbis viral vector can comprise a nucleic acid sequence encoding LacZ (lac operon structural gene lacZ encoding β -galactosidase), Flue (firefly luciferase) or GFP (green fluorescent protein). The Sindbis viral vector can comprise the nucleic acid encoding LacZ, Flue or GFP and further comprise the nucleic acid encoding the anti-4-1BB monoclonal antibody.

[000108] The Sindbis viral vector can comprise a nucleic acid encoding an anti-4-1BB antibody heavy chain comprising the heavy chain complementarity determining region 1 (HCDR1), HCDR2 and HCDR3 amino acid sequences of SEQ ID NOs: 16, 17 and 18, respectively. The Sindbis viral vector can comprise the nucleic acid encoding an anti-4-1BB antibody heavy chain comprising the amino acid sequence of SEQ ID NO: 19. The Sindbis viral vector can comprise a nucleic acid encoding an anti-4-1BB antibody heavy chain comprising the light chain complementarity determining region 1 (LCDR1), LCDR2 and LCDR3 amino acid sequences of SEQ ID NOs: 20, 21 and 22, respectively. The Sindbis viral vector can comprise the nucleic acid encoding an anti-4-1BB antibody light chain comprising the amino acid sequence of SEQ ID NO: 23.

[000109] The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody heavy chain CDR1, CDR2 and CDR3 amino acid sequences that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24. The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody light chain CDR1, CDR2 and CDR3 amino acid sequences that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24. The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody heavy chain amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24. The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody light chain amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24.

[000110] The immunostimulatory or immunomodulatory protein can be IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-34, IL-35, IL-36, or any combination thereof. In a preferred aspect, the immunostimulatory or immunomodulatory protein is IL-12. The anti-4-1BB antibody can be urelumab, utomilumab or a combination thereof. The anti-4-1BB antibody can be InVivoMAb anti-mouse 4-1BB (BioXCell, Clone: LOB12.3, Cat.No. BE0169).

[000111] The Sindbis viral vector and the anti-4-1BB monoclonal antibody can be administered sequentially or concurrently. The Sindbis viral vector can be administered systemically. The anti-4-1BB monoclonal antibody can be administered systemically. Both the Sindbis viral vector and the anti-4-1BB monoclonal antibody, or a nucleic acid encoding same, can be administered systemically. The Sindbis viral vector can be administered parenterally. The anti-4-1BB monoclonal antibody can be administered parenterally. Both the Sindbis viral vector and the anti-4-1BB monoclonal antibody, or a nucleic acid encoding same, can be administered parenterally. The Sindbis viral vector can be administered intraperitoneally. The anti-4-1BB monoclonal antibody can be administered intraperitoneally. Both the Sindbis viral vector and the anti-4-1BB monoclonal antibody, or a nucleic acid encoding same, can be administered intraperitoneally.

[000112] An antibody of the present disclosure, or a fragment thereof, can be derived from any species, including, but not limited to, a human, a mouse, a rat, a hamster, a dog, a rabbit, a frog, a sheep, a goat, a cow, a horse, a pig, a bird, a donkey, a chicken, a camel, a llama, a dromedary, an alpaca, a shark, a bovine and a turtle. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a human. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a mouse. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a camel, a llama or an alpaca. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a shark. In some aspects, an antibody of the present disclosure, or a fragment thereof, of the present disclosure is a chimeric antibody that is derived from two or more of the aforementioned species. In a non-limiting example, an antibody of the present disclosure, or fragment thereof, can be a chimeric antibody that is derived from a human and a mouse. In some aspects, an antibody of the present disclosure, or a fragment thereof, can be derived from any species other

than human and can be further humanized using standard methods known in the art as to reduce the immunogenicity of the antibody.

[000113] A “monoclonal antibody” as disclosed herein, can be a full-length antibody or an antigen binding fragment thereof, wherein the “antigen binding fragment” is a fragment of the full length antibody that retains binding to the target antigen of the said monoclonal antibody. For example, a monoclonal antibody against 4-1BB or an anti-4-1BB monoclonal antibody, as described herein, can be a full length antibody against 4-1BB antibody or an antigen binding fragment thereof, wherein the fragment retains binding to the 4-1BB receptor on a cell surface. An “antigen-binding fragment” of an anti-4-1BB antibody, as described herein can include any fragment selected from the group consisting of Fv, Fav, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, scFv-CH₃, scFv-Fc, and diabody fragments.

[000114] The cancer can be a solid cancer or a liquid/hematologic cancer. The cancer can comprise metastatic cancer. The cancer can comprise a solid tumor. The cancer can be a carcinoma, a lymphoma, a blastoma, a sarcoma, a leukemia, a brain cancer, a breast cancer, a blood cancer, a bone cancer, a lung cancer, a skin cancer, a liver cancer, an ovarian cancer, a bladder cancer, a renal cancer, a gastric cancer, a thyroid cancer, a pancreatic cancer, an esophageal cancer, a prostate cancer, a cervical cancer or a colorectal cancer. In one preferred aspect, the cancer is a lymphoma. In one preferred aspect, the cancer is a B cell lymphoma.

[000115] The present disclosure provides a Sindbis viral vector comprising a nucleic acid encoding encoding a therapeutic protein, an immunostimulatory protein or an immunomodulatory protein and a nucleic acid encoding an anti-4-1BB monoclonal antibody. The present disclosure provides a Sindbis viral vector comprising a nucleic acid encoding encoding LacZ, Flue or GFP and a nucleic acid encoding an anti-4-1BB monoclonal antibody. The present disclosure also provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding a therapeutic protein, an immunostimulatory protein or an immunomodulatory protein and (b) a Sindbis viral vector comprising a nucleic acid encoding an anti-4-1BB monoclonal antibody. The present disclosure also provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding LacZ, Flue or GFP and (b) a Sindbis viral vector comprising a nucleic acid encoding an anti-4-1BB monoclonal antibody. The present disclosure further provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding a therapeutic

protein, an immunostimulatory protein or an immunomodulatory protein and (b) an anti-4-1BB monoclonal antibody or a nucleic acid encoding same. The present disclosure provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding encoding LacZ, Flue or GFP and (b) an anti-4-1BB monoclonal antibody or a nucleic acid encoding same.

[000116] The Sindbis viral vector can comprise a nucleic acid encoding an anti-4-1BB antibody heavy chain comprising the heavy chain complementarity determining region 1 (HCDR1), HCDR2 and HCDR3 amino acid sequences of SEQ ID NOs: 16, 17 and 18, respectively. The Sindbis viral vector can comprise the nucleic acid encoding an anti-4-1BB antibody heavy chain comprising the amino acid sequence of SEQ ID NO: 19. The Sindbis viral vector can comprise a nucleic acid encoding an anti-4-1BB antibody heavy chain comprising the light chain complementarity determining region 1 (LCDR1), LCDR2 and LCDR3 amino acid sequences of SEQ ID NOs: 20, 21 and 22, respectively. The Sindbis viral vector can comprise the nucleic acid encoding an anti-4-1BB antibody light chain comprising the amino acid sequence of SEQ ID NO: 23.

[000117] The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody heavy chain CDR1, CDR2 and CDR3 amino acid sequences that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24. The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody light chain CDR1, CDR2 and CDR3 amino acid sequences that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24. The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody heavy chain amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24. The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody light chain amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24.

[000118] The immunostimulatory or immunomodulatory protein can be IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-34, IL-35, IL-36, or any combination thereof. In a preferred aspect, the immunostimulatory or immunomodulatory protein is IL-12.

[000119] An antibody of the present disclosure, or a fragment thereof, can be derived from any species, including, but not limited to, a human, a mouse, a rat, a hamster, a dog, a rabbit, a frog, a sheep, a goat, a cow, a horse, a pig, a bird, a donkey, a chicken, a camel, a llama, a dromedary, an alpaca, a shark, a bovine and a turtle. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a human. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a mouse. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a camel, a llama or an alpaca. In some aspects, an antibody of the present disclosure, or a fragment thereof, is derived from a shark. In some aspects, an antibody of the present disclosure, or a fragment thereof, of the present disclosure is a chimeric antibody that is derived from two or more of the aforementioned species. In a non-limiting example, an antibody of the present disclosure, or fragment thereof, can be a chimeric antibody that is derived from a human and a mouse. In some aspects, an antibody of the present disclosure, or a fragment thereof, can be derived from any species other than human and can be further humanized using standard methods known in the art as to reduce the immunogenicity of the antibody.

[000120] A “monoclonal antibody” as disclosed herein, can be a full-length antibody or an antigen binding fragment thereof, wherein the “antigen binding fragment” is a fragment of the full length antibody that retains binding to the target antigen of the said monoclonal antibody. For example, a monoclonal antibody against 4-1BB or an anti-4-1BB monoclonal antibody, as described herein, can be a full length antibody against 4-1BB antibody or an antigen binding fragment thereof, wherein the fragment retains binding to the 4-1BB receptor on a cell surface. An “antigen-binding fragment” of an anti-4-1BB antibody, as described herein can include any fragment selected from the group consisting of Fv, Fav, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, scFv-CH₃, scFv-Fc, and diabody fragments.

[000121] Any of the above aspects can be combined with any other aspect.

[000122] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In the Specification, the singular forms also include the plural unless the context clearly dictates otherwise; as examples, the terms “a,” “an,” and “the” are understood to be singular or plural and the term “or” is understood to be inclusive. By way of example, “an element” means one or more element. Throughout the specification the word “comprising,” or

variations such as “comprises” or “comprising,” will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from the context, all numerical values provided herein are modified by the term “about.”

[000123] Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. The references cited herein are not admitted to be prior art to the claimed invention. In the case of conflict, the present Specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting. Other features and advantages of the disclosure will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF DRAWINGS

[000124] **FIG. 1A-1D. SV.IL12 induces a modest therapeutic efficacy and increases OX40 expression on CD4 T cells.** FIG. 1A depicts treatment schema. BALB/c mice were given intraperitoneal (i.p.) injections of SV, IL-12 (50 ng), or SV.IL12 at various times after injection of 7×10^4 CT.26.Fluc on day 0. FIG. 1B depicts survival plots of control and treated mice bearing CT26.Fluc tumors. The x-axis shows days of treatment and y-axis shows percentage survival. Statistical significance between SV.IL12 and all other groups was determined with the Mantel-Cox test. Results are representatives of at least two independent experiments. FIG. 1C-D depict effect on OX40 expression of treatment of CT26 tumor-bearing mice with SV, IL-12 (50 ng), or SV.IL12 on 4 consecutive days (days 1, 2, 3, and 4). On day 7, spleens were excised and a single-cell suspension was stained and analyzed by flow cytometry. As controls, naive and untreated (control) tumor-bearing mice were used. FIG. 1C depicts percentage of OX40 expression by CD4 T cells (left), regulatory T cells (TREG; middle), and CD8 T cells (right). The x-axis shows the various treatment groups and the y-axis shows percentage of OX40+ cells. FIG. 1D depicts representative flow cytometry plots indicating OX40 staining in different T cell subsets. Bars represent means and each symbol represents an individual mouse. Statistical

significance was determined with the Kruskal-Wallis test followed by the Dunns' test or the Mann-Whitney test. Results are representatives of at least two independent experiments.

[000125] FIG. 2A-2C. SV infects monocytes/macrophages in mediastinal lymph nodes and quickly activates T cells. FIG. 2A depicts that tumor free mice were treated i.p. with SV expressing the firefly luciferase (Fluc) protein. 4 hours later, bioluminescent images were taken to monitor Fluc expression from SV. To determine the source of the signal, the mediastinal lymph nodes (LN) and adipose tissue were extracted and imaged separately. FIG. 2B depicts percentage of GFP expression by Ly6G-CD11b+F4/80+ cells. FIG. 2C depicts percentage of CD69 expression by CD4 (left graph) and CD8 (right graph) T cells. Tumor free mice were treated i.p. with SV expressing GFP for 4 consecutive days. On day 5, mediastinal and inguinal LN were extracted and a single cell suspension was stained and analyzed by flow cytometry. As control, naïve mice were used. Statistical significance was determined with the Mann-Whitney test. Results are representatives of at least two independent experiments.

[000126] FIG. 3A-3D. IL-12 and IFN- γ production derived from SV.IL12 infection. FIG. 3A depicts IL-12 levels in supernatant of infected cells measured by ELISA. 5×10^5 MyC-CaP cells were infected with SV.IL12 at various MOI (10; 1; 0.1) for 2 hours. As control, MyC-CaP cells were infected with SV or left uninfected (mock). SV was washed away and replaced with fresh media. After 24 hours incubation, supernatant was collected and IL-12 was measured by ELISA. FIG. 3B depicts IL-12 levels in plasma was measured by ELISA. FIG. 3C depicts percentage of Tbet expression by CD4 T cells in cell suspensions from mediastinal LN stained and analyzed by flow cytometry. FIG. 3D depicts IFN γ enzyme-linked immunospot analysis of splenocytes from control and treated mice as indicated (n=3-10 mice per group). Tumor bearing mice were treated with SV.IL12 on 4 consecutive days (days 1; 2; 3; 4). As control, naïve, untreated (control), IL-12 (50 ng) and SV treated mice were used. On day 7, plasma, spleen and mediastinal LN were collected from each mice. Statistical significance was determined with the Kruskal-Wallis test followed by the he Dunns' test or Mann-Whitney test. Results are representatives of at least two independent experiments.

[000127] FIG. 4A-4B. SV infectivity of MyC-CaP.Fluc tumors. MyC-CaP.Fluc and CT.26.Fluc cells were challenged with serially diluted single round replication SV.GFP (10^{-1} – 10^{-4}) and incubated overnight. 16 hours post infection the percentage of GFP-positive cells was analyzed by flow cytometer for each dilution. FIG. 4A depicts representative flow cytometry

plots of GFP positive MyC-CaP.Fluc and CT.26.Fluc cells per dilution and uninfected controls are shown. FIG. 4B depicts plotted infectivity curve of GFP-positive cells.

[000128] FIG. 5A-5E. SV.IL12 in combination with anti-OX40 antibody cures established tumors in vivo. FIG. 5A depicts the experimental protocol for the prostate and colon cancer model. FVB/NJ or BALB/c mice were given an i.p. injection of SV.IL12 and/or anti-OX40 at various times after injection of 10^5 MyC-CaP.Fluc or 7×10^4 CT26.Fluc cells on day 0, respectively. FIG. 5B depicts CT26.Fluc tumor growth curves shown as fold changes relative to the luminescence on day 0 of the same mouse. Each line represents an individual mouse. Left graphs: control (n = 14) (top) and SV.IL12 (n = 20) (bottom). Right graphs: anti-OX40 (n = 10) (top) and SV.IL12+anti-OX40 (n = 11) (bottom). FIG. 5C depicts the representative bioluminescence images of control and treated CT26.Fluc-bearing mice. FIG. 5D depicts survival plots of control and treated mice bearing peritoneally disseminated CT26.Fluc tumors. FIG. 5E depicts survival plots of control and treated mice bearing peritoneally disseminated MyC-CaP.Fluc tumors. Statistical significances between SV.IL12+anti-OX40 and anti-OX40 or SV.IL12 were determined with the Mantel-Cox test. Results are representatives of at least two independent experiments.

[000129] FIG. 6A-6B. Tumor growth of MyC-CaP.Fluc tumor bearing mice during treatment. FVB/NJ mice were given injection of SV.IL12 and/or anti-OX40 intraperitoneally (i.p.) at various times after injection of 10^5 MyC-CaP.Fluc cells on day 0. FIG. 6A depicts tumor growth curves are shown as fold changes relative to the luminescence on day 0 of the same mouse. Each line represents an individual mouse. Left graphs: Control (n=10) (top) and SV.IL12 (n=10) (bottom). Right graphs: aOX40 (n=10) (top) and SV.IL12+aOX40 (n=10) (bottom). FIG. 6B depicts representative bioluminescence images of control and treated CT.26.Fluc bearing mice. Results are representatives of at least two independent experiments.

[000130] FIG. 7A-7D. The therapeutic efficacy of SV.IL12 in combination with anti-OX40 is maintained at reduced treatment regimen in CT26.Fluc bearing mice. FIG. 7A depicts the treatment schema. BALB/c mice were given i.p. injection of SV.IL12 (day 1 and 8) and/or anti-OX40 (day 2 and 9) of 7×10^4 CT26.Fluc on day 0. FIG. 7B depicts representative bioluminescence images of control and treated CT26.Fluc bearing mice. FIG. 7C depicts tumor growth curves are shown as fold changes relative to the luminescence on day 0 of the same mouse. Each line represents an individual mouse. Left graphs: Control (n=14) (top) and SV.IL12

(n=15) (bottom). Right graphs: aOX40 (n=17) (top) and SV.IL12+aOX40 (n=15) (bottom). FIG. 7D depicts survival plots of control and treated mice bearing CT26.Fluc tumors. Statistical significance between SV.IL12+ α OX40 and SV.IL12 was determined with the Mantel-Cox test. Results are representatives of at least two independent experiments.

[000131] FIG. 8A-8B. Therapeutic efficacy of SV.IL12 in combination with anti-OX40 is dependent on CD4 and CD8 T cells. FIG. 8A depicts that mice injected with anti-CD4 (0.4 mg) or anti-CD8 (0.1 mg) depleting antibody. As a control, rat IgG2b (0.4 mg) isotype control was used. The frequency of CD4 and CD8 T cells were assessed by flow cytometry in splenocytes after 24, 48, 72 and 96 hours. FIG. 8B depicts that BALB/c mice were inoculated with 7×10^4 CT.26.Fluc on day -4. Depletion antibody anti-CD4 or anti-CD8 were injected i.p. on day -3, 1, 5, 9, 13 and 17. Mice were left untreated (control) or were treated with SV.IL12 and anti-OX40 on day 4 and 11. Tumor growth curves are shown as fold changes relative to the luminescence on day 0 of the same mouse. Each line represents an individual mouse.

[000132] FIG. 9A-9I. Combination therapy markedly changes the transcriptome signature of T cells favoring effector T cells with a Th1 type phenotype. FIGs. 9A-9I depict RNA sequencing of T cells isolated from spleens derived from untreated tumor bearing mice (control) compared with mice treated with SV.IL12 and/or anti-OX40 on day 7. FIG. 9A depicts principal component analysis (PCA) of normalized read counts from the CT26.Fluc tumor model. FIG. 9B depicts PCA of normalized read counts from the MyC-CaP.Fluc tumor model. FIG. 9C depicts MA plots of differentially expressed genes (DEG; >2-fold) in T cells of control versus anti-OX40 treated mice (top graph), SV.IL12 treated mice (middle graph) or SV.IL12+anti-OX40 treated mice (bottom graph) in the CT.26 model. Significantly ($p < 0.05$) upregulated and downregulated DEG are depicted in red or blue, respectively. FIG. 9D depicts Pathway and network analysis based on DEG in T cells isolated from CT26.Fluc-bearing mice treated with combination therapy. Downregulated (blue) and upregulated (red) pathways are shown, respectively. FIG. 9E depicts Heatmap analysis of selected genes based on normalized read counts linked to T cell differentiation and activation as well as T cell lineage transcription factors. FIGs. 9F-I depict data from tumor bearing mice that were treated with SV.IL12 and/or anti-OX40. As control, naïve and untreated (control) tumor bearing mice were used. On day 7, spleens were excised and a single cell suspension was stained and analyzed by flow cytometry. FIG. 9F depicts percentage of CD44 or Ki-67 expression by T cells from the CT26.Fluc tumor

model. FIG. 9G depicts percentage of CD44 or Ki-67 expression by T cells from the MyC-CaP.Fluc tumor model. FIG. 9H depicts the percentage of ICOS and T-bet expression by CD4 T cells. FIG. 9I depicts representative flow cytometry plots ICOS and T-bet expression by CD4 T cells. Bars represent means and each symbol represent an individual mouse. Statistical significance was determined with the Kruskal-Wallis test followed by the Dunns' test. Results are representatives of at least two independent experiments.

[000133] FIG. 10A-10B. Combination therapy induces systemic CD4 and CD8 T cell activation. Tumor bearing mice were left untreated or treated with SV.IL12 and/or anti-OX40. On day 7, spleens were excised and a single cell suspension was stained and analyzed by flow cytometry. As control, naïve and untreated (control) tumor bearing mice were used. FIGs. 10A and 10B depict representative flow cytometry plots of CD44 and Ki-67 expression on CD4 and CD8 T cells in the CT.26.Fluc and MyC-CaP.Fluc tumor model, respectively.

[000134] FIG. 11A-11G. SV.IL12 in combination with anti-OX40 promotes metabolic reprogramming of T cells. Tumor bearing mice were left untreated or treated with SV.IL12 and/or anti-OX40. T cells were isolated from spleens on day 7 or otherwise indicated. FIG. 11A depicts selected gene set enrichment analysis (GSEA) of oxidative phosphorylation and glycolysis pathways based on DEG in control versus SV+anti-OX40. FIG. 11B depicts mitochondrial respiration assessed by measuring the median values of oxygen consumption rates (OCR) in T cells of indicated groups using an extracellular flux analyzer. Oligomycin, FCCP, Antimycin A and Rotenone were injected as indicated to identify energetic mitochondrial phenotypes. FIG. 11C depicts Mitotracker Green FM staining of T cells from indicated groups using flow cytometry. FIG. 11D depicts Mitotracker Deep Red FM staining of T cells from indicated groups using flow cytometry. FIG. 11E depicts western blot of c-Myc protein expression in T cells of control or mice treated with anti-OX40, SV.IL12 or SV.IL12+anti-OX40. GAPDH (bottom) is loading control. FIG. 11F depicts baseline extracellular acidification rates (ECAR) in T cells of indicated groups derived from the CT26.Fluc and MyC-CaP.Fluc tumor models. FIG. 11G depicts energy profile (OCR versus ECAR) of T cells from naïve or CT26.Fluc bearing mice treated with SV.IL12+anti-OX40 on day 7, 14 and 40. Error bars indicate SEM. Results are representatives of at least two independent experiments in FIGs. 11B-G.

[000135] FIG. 12A-12D. Combination therapy rewires T cells metabolically. FIGs.

12A and 12B depict the metabolic activity of T cells isolated from spleens of mice bearing CT.26.Fluc or MyC-CaP.Fluc tumor respectively, on day 7. Baseline OCR (left) and respiratory capacity (right) was measured in T cells of indicated groups using an extracellular flux analyzer. FIG. 12C depicts representative flow cytometry plots of Mitotracker Green FM and Mitotracker Deep Red staining in CD4 and CD8 T cells isolated from spleens of CT26.Fluc tumor bearing mice. FIG. 12D depicts energy profile (OCR versus ECAR) of T cells from naïve (bottom graph) or CT26.Fluc bearing mice treated with SV.IL12+anti-OX40 (top graph) on day 7, 14 and 30. Data represent the mean of three different experiments. Bars represent means $\pm$ SEM (A, B, D). Results are representatives of at least two independent experiments.

[000136] FIG. 13A-13G. Reprogrammed T cells in SV+anti-OX40 treated mice display enhanced CD4 mediated cytokine production and anti-tumor activity.

Tumor bearing mice were left untreated or treated with SV.IL12 and/or anti-OX40. Spleens were excised on day 7 for further analysis. FIG. 13A depicts Heatmap analysis of selected genes based on normalized read counts linked to cytokine expression and enhanced anti-tumor activity. FIG. 13B depicts RNA sequencing performed on isolated T cells from CT26.Fluc tumor-bearing mice. FIG. 13C depicts Interferon-gamma (IFN- γ) enzyme-linked immunospot analysis of splenocytes from control and treated mice as indicated (n=5-10 mice per group). Additionally, IFN- γ enzyme-linked immunospot analysis was measured in splenocytes depleted of CD4 or CD8 T cells in the CT26.Fluc (Top) and MyC-CaP.Fluc (Bottom) tumor models. FIG. 13D depicts representative flow cytometry plots of T-bet and granzyme B (GrB) expression by CD4 T cells from indicated groups using flow cytometry. FIG. 13E depicts Percentage of T-bet and granzyme B (GrB) expression by CD4 T cells from indicated groups using flow cytometry of FIG. 13D. FIG. 13F depict cytotoxic activity of T cells from control and treated mice (n=5-10 mice per group) co-cultured at an effector-to-target cell ratio of 10:1 with either CT26.FLUC. FIG. 13G depicts MyC-CaP.Fluc tumor cell lines for 2 days. Additionally, T cells were depleted of CD4 or CD8 T cells and co-cultured as previously described. Cytotoxic activity was assessed based on viability of tumor cells, which was determined by measuring the luciferase activity and is shown relative to naïve T cells. Bars represent means $\pm$ SEM in FIGs. 13B, 13F and 13G, and each symbol represent an individual mouse in FIG. 13E. Statistical significance was determined with the

Kruskal-Wallis test followed by the he Dunns' test. Results are representatives of at least two independent experiments.

[000137] FIG. 14A-14F. CD8 T cells show enhanced cytotoxic potential in mice treated with SV.IL12 and anti-OX40. Tumor bearing mice were left untreated or were treated with SV.IL12 with or without anti-OX40. Mice were sacrificed on day 7 to analyze the T cell immune response in spleen. FIGs. 14A-14B depict percentage of granzyme B and T-bet expression, by CD8 T cells from CT26.Fluc tumor bearing mice. FIGs. 14C-14D depict percentage of granzyme B and T-bet expression, respectively by CD8 T cells from MyC-CaP.Fluc tumor bearing mice. FIG. 14B and 14D depict representative flow cytometry plots. FIGs. 14E and 14F depict percentage of NKG2D (left graph) or T-bet (right graph) expression by CD8 T cells in the mice bearing CT26.Fluc and MyC-CaP.Fluc tumor, respectively. Bars represent means and each symbol represent an individual mouse. Statistical significance was determined with the Kruskal-Wallis test followed by the he Dunns' test. Results are representatives of at least two independent experiments.

[000138] FIG. 15A-15F. Mice treated with SV.IL12 in combination with anti-OX40 display enhanced T cell migration and intratumoral T cell activity. CT26.Fluc bearing mice were left untreated or were treated with SV.IL12 and/or anti-OX40. On day 7, spleens were excised and a single cell suspension was stained and analyzed by flow cytometry. FIG. 15A depicts percentage of CXCR3 expression by CD4 (left graph) and CD8 (right graph) T cells. FIG. 15 B depicts representative flow cytometry plots. Tumors were harvested after 2 weeks of treatment from control and treated mice. FIG. 15C depicts intratumoral gene expression of CXCL9 (Top) and CXCL10 (bottom) analyzed by real time PCR. Data are normalized to GAPDH. FIG. 15D depicts intratumoral T cell immune responses from indicated groups that were assessed by flow cytometry. Percentage of CD4 expression by T cells (left graph), Ki-67 expression (middle graph) and granzyme B expression (right graph) by CD4 T cells. FIG. 15E depicts multiplex immunofluorescence staining of tumors isolated from CT26.Fluc tumor bearing mice. FIG. 15F depicts multiplex immunofluorescence staining of tumors isolated from MyC-CaP.Fluc tumor bearing mice. Representative images of T cell infiltration are shown for control as well as anti-OX40, SV.IL12 and SV.IL12+ anti-OX40. Proteins of interest were stained and are indicated by color in each image: K-i67 (red), CD3 (green), CD8 (magenta) and DAPI nuclear staining appears in blue. Bars represent means $\pm$ SEM in FIG. 15C, and each

symbol represent an individual mouse in FIG. 15A and 15D). Statistical significance was determined with the Kruskal-Wallis test followed by the Dunns' test. Results are representatives of at least two independent experiments

[000139] FIG. 16A-16D. T cells show enhanced migration into tumors and exert anti-tumor activity in mice treated with SV.IL12+anti-OX40. Tumor bearing mice were left untreated or were treated with SV.IL12 with or without anti-OX40. Mice were sacrificed on day 7 and 14 to analyze the T cell immune response in spleen. FIG. 16A-16C depict percentage of CXCR3 expression by CD4 (left graph) and CD8 (right graph) T cells measured by flow cytometry in the CT26.Fluc tumor model on day 14, in the MyC-CaP.Fluc tumor model on day 7 (B) and in the MyC-CaP.Fluc tumor model on day 14, respectively. FIG. 16D depicts percentage of CD8 expression by T cells (left graph), Ki-67 expression (middle graph) and granzyme B expression (right graph) by CD8 T cells. Tumors were harvested after 2 weeks of treatment from control and treated mice. T cell immune responses from indicated groups were assessed by flow cytometry. Bars represent means and each symbol represent an individual mouse. Statistical significance was determined with the Kruskal-Wallis test followed by the he Dunns' test. Results are representatives of at least two independent experiments.

[000140] FIG. 17. Combination therapy stimulates granzyme B expression in MyC-CaP.Fluc tumors. FIG. 17 depicts tumors stained by multiplex immunofluorescence. MyC-CaP.Fluc tumors were harvested after 2 weeks of treatment from control and treated mice. Representative images are shown for untreated (control), anti-OX40, SV.IL12 and SV.IL12+anti-OX40 treated mice. Proteins of interest were stained and are indicated by color in each image: F4/80 (red) and granzyme B (green). DAPI nuclear staining appears in blue.

[000141] FIG. 18A-18B. SV.IL12 triggers innate immune response and induces iNOS expression in MyC-CaP.Fluc tumors. MyC-CaP.Fluc tumors in FIG. 18A and CT26.Fluc tumors in FIG. 18B, were harvested after 2 weeks of treatment from control and treated mice. Tumors were stained by multiplex immunofluorescence. Representative images are shown for untreated (control), anti-OX40, SV.IL12 and SV.IL12+anti-OX40 treated mice. Proteins of interest were stained and are indicated by color in each image: iNOS (Cyan), Arginase 1 (green), and CD11b (magenta). DAPI nuclear staining appears in blue.

[000142] FIG. 19. Treatment schema of C57/Bl6 (female) mice re-injected with Alm5-2Fluc-17 tumor. Alm5-2Fluc-17 tumor reinjection was done from 9 C57/Bl6 mice in 80 mice

(16 cages) on day 0. Treatment started on day 9 after cells implantation. Antibodies (250ug/dose) treatment (blue dots) was done 3 times/week for 3 weeks starting at day 10 after cells re-injected. Sindbis Vector was administered 4 days/week for 4 weeks (red dots) starting day 9 after cells (mornings). IVIS imaging was done on indicated days after tumor implantation.

[000143] FIG. 20. Combination of IL-12 and anti-OX40 expressed by Sindbis viral vectors synergistically enhances survival of subjects with established tumors. FIG. 20. depicts Percentage survival rate of C57/Bl6 (female) mice re-injected with Alm5-2Fluc-17 tumor and treated with SV.IL12 vector; SV.IL-12 vector and anti-OX40 IgG; RepOX40IgG_Rep-IL12 (fragmented SV expressing OX-40 IgG and fragmented SV expressing IL-12, 50% mix of both vectors) or RepOX40IgG_SV-IL12 (fragmented SV expressing OX-40 IgG and full length SV expressing IL-12, 50% mix of both vectors), with n=5 mice in each treatment group. Untreated mice were used as a control. The mice were re-injected with tumor cells and treated according to the scheme in FIG. 19.

[000144] FIG. 21A-21C. A20 lymphoma cells were SV infection resistant. FIG. 21A depicts A20 cells and BHK cells were infected with SV carrying GFP overnight. GFP expression was observed under fluorescent microscope. FIG. 21B depicts SV-GFP infectivity to BHK cells was verified by flow cytometry. FIG. 21C depicts SV-GFP infectivity to A20 cells in vivo were measured by flow cytometry. 10^7 A20 cells (express CD45.2) were inoculated to CByJ.SJL(B6)-Ptpcrca/J (CD45.1 BALB/C) mice. Recipient mice were treated with SV-GFP 4 days later. GFP expression was measured the next day.

[000145] FIG. 22A-22C. Sindbis virus (SV) and α 4-1BB combination completely cured BALB/C mice A20 lymphoma. FIG. 21A depicts representative bioluminescence images of groups as indicated. Intensity scale, day 0, 7, 21, min: 400, max: 7000; day 14, min: 100, max: 1000; day 28, min: 3000, max: 50000. FIG. 2B depicts tumor growth measured by relative firefly luciferase (fLuc) activity (normalized to day 0 fLuc activity). Untreated, n = 16; SV, n = 18; α 4-1BB Ab, n = 13; SV plus α 4-1BB Ab, n = 13. FIG. 2C depicts survival curve of all groups (the ratio is shown as survived number/total number).

[000146] FIG. 23A-23E. SV alone and SV plus α 4-1BB mAb stimulated cell cycle progression, cytokine production, and activation. FIG. 23A depicts the numbers of significant differential (SD) expressed genes (upregulated and downregulated) of SV vs. untreated are as indicated. SD expressed genes were selected based on Deseq2 analysis ($q < 0.05$), $|\text{Log2FC}| \geq 1$.

FIG. 23B depicts the enrichment scores for gene cluster of cell cycle for SV vs. untreated, SV+ α 4-1BB vs. untreated and SV+ α 4-1BB vs. SV respectively (“cell cycle” is the gene cluster with the highest enrichment score for these 3 comparisons). FIG. 23C depicts the heat map representing SD expressed cytokine and chemokine genes (left, SV vs. untreated; right, SV+ α 4-1BB vs. α 4-1BB, Log2FC ≥ 1). Expression values are shown by Z-score. Genes are hierarchically clustered by one minus Pearson correlation. Red arrow, Ccl8, IL4, IL13 and IL21 expression. FIG. 23D depicts the percentage of CD69+ T cells from all groups on day 2 after starting treatment was measured by flow cytometry. FIG. 23E depicts GSEA enrichment plot of KEGG (SV + α 4-1BB vs. untreated) TCR receptor signaling pathway. *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$.

[000147] FIG. 24A-24C. SV infection enhanced cell cycle progression and migration.

FIG. 24A depicts DAVID KEGG analysis. FIG. 24B depicts GSEA enrichment plot of KEGG (SV vs. Untreated) cell cycle pathway (SV vs. Untreated). FIG. 24C depicts cell movement pathway was significantly enhanced by IPA (SV vs. Untreated).

[000148] FIG. 25A-25B. Significant differential (SD) upregulated genes are clustered by DAVID analysis. FIG. 25A depicts enrichment score of gene clusters for SV+ α 4-1BB vs Untreated. FIG. 25B depicts enrichment score of gene clusters for SV+ α 4-1BB vs SV.

[000149] FIG. 26A-26D. Untreated group had low ratio of T cells and high ratio of regulatory T cells on day 28. FIGs. 26A-26C depict the frequency of CD4, CD8, and Treg respectively measured by flow cytometry. FIG. 26D depicts the Treg/CD8 ratio as indicated.

[000150] FIG. 27A-27D. Sindbis virus plus α 4-1BB combination induced higher cytotoxicity. FIG. 27A depicts splenocytes mixed with fLuc-A20 lymphoma cells according to the ratio as indicated (splenocytes: lymphoma cells). Cytotoxicity corresponds to the reduction of normalized Luc activity (fLuc activity of A20 lymphoma cells only is normalized to 1). SV + tumor, α 4-1BB + tumor, SV+ α 4-1BB + tumor: tumor inoculated mice. SV, α 4-1BB, SV+ α 4-1BB: mice without tumor inoculation. FIG. 27B depicts splenocytes harvested from all groups after 7 days treatment. The percentage of NKG2D+ cells was measured by flow cytometry (CD8 T cell gated). FIG. 27C depicts the percentage of granzyme B+ and perforin+ cells was measured by flow cytometry (CD8 T cell gated). FIG. 27D depicts cytotoxicity associated genes upregulated in SV + α 4-1BB treated group. The heat map depicts the relative expression level of cytotoxicity associated genes. Expression values are shown by Z-score. Genes are hierarchically

clustered by one minus Pearson correlation (day 7). Red square, granzyme b and perforin expression. Red arrow, Ifng and Stat4 expression. **, $p < 0.01$; ****, $p < 0.0001$.

[000151] FIG. 28A-28F. Sindbis virus plus $\alpha 4$ -1BB combination induced Th1 differentiation and IFN γ production. FIG. 28A depicts IFN γ Elispot analysis of splenocytes harvested at day 2, 7, 14 and 28 from all groups as indicated. Upper panel, IFN γ Elispot image on day 7 after treatment. 1, 2, 3: three individual mice. Lower panel, IFN γ spots number from indicated groups over the course of treatment (2×10^5 splenocytes per well). No stimulator was added. FIG. 28B depicts IFN γ production from CD4/CD8 T cell population in splenocytes and purified CD4/CD8 T cells. All groups were cultured in media for 5 h in the presence of brefeldin A. FIG. 28C depicts IFN γ production from purified CD4 T cells at different stimulation conditions. FIG. 28D depicts upregulated Th1 pathway gene set under SV, $\alpha 4$ -1BB and SV + $\alpha 4$ -1BB stimulation. Expression values are shown by Z-score. Genes are hierarchically clustered by one minus Pearson correlation (day 7). FIG. 28E depicts T-bet expression for all groups as indicated. FIG. 28F depicts EOMES expression for all groups as indicated. CD8 T cell gated. FIGs. 28E and 28F, day 7 after treatment. *, $p < 0.05$; **, $p < 0.01$, ****, $p < 0.0001$.

[000152] FIG. 29 depicts IFN γ production from splenocytes of all groups with or without tumor inoculation on day 7 after treatment was measured by Elispot. With tumor: tumor was inoculated on day 0. Without tumor: tumor was not inoculated. No stimulator was added in Elispot assay.

[000153] FIG. 30A-30B. IFN γ production measurement. FIG. 30A, IFN γ production (at day 7) by all groups, as indicated, was measured by Elispot. FIG. 30B, IFN γ production of purified T cells (CD8 T cell portion) on day 7 after treatment was measured by flow cytometry.

[000154] FIG. 31A-31I. SV and $\alpha 4$ -1BB mAb stimulated chemotaxis, adhesion and enhanced T cell infiltration and activation in tumor. FIG. 31A depicts heat map of the expression pattern of SV + $\alpha 4$ -1BB upregulated chemokine and chemokine receptor genes (Expression values are shown by Z-score.) Genes are hierarchically clustered by one minus Pearson correlation (day 7). FIG. 31B depicts the percentage of CCR5 $^{+}$ cells was measured by flow cytometry (day 7). FIGs. 31C and 31D depict the percentage of CD11a $^{+}$ and ICAM-1 $^{+}$ cells, respectively measured by flow cytometry. FIG. 31E depicts the relative expression of CD11a (Itgal) and ICAM-1 was shown by heat map measured by RNA-Seq. Expression values are shown by Z score. FIG. 31F depicts the percentage of OX40 $^{+}$ and ICOS $^{+}$ T cells were

measured by flow cytometry. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. FIG. 31G depicts the frequency of CD3 and CD8 T cells to total harvested cells from tumor measured by flow cytometry. FIG. 31H depicts the CD8/Treg ratio of tumor infiltrated T cells. FIG. 31I depicts the percentage of granzyme B+ CD8 T cells as indicated. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

[000155] FIG. 32A-32E. The phenotype of tumor infiltrated T cells. FIGs. 32A-32E depict the percentage of Ki67+, Foxp3+, T-bet+, EOMES+, NKG2D+ T cells respectively, measured by flow cytometry.

[000156] FIG. 33A-33C. Sindbis virus plus $\alpha 4$ -1BB synergistically enhanced T cell glycolysis and oxidative phosphorylation. FIG. 33A depicts GSEA enrichment plot of KEGG (SV + $\alpha 4$ -1BB vs. untreated) glycolysis pathway. FIG. 33B depicts the canonical pathways of SV plus $\alpha 4$ -1BB Ab stimulation clustered by IPA. Red square, oxidative phosphorylation. FIG. 33C depicts both oxygen consumption rate (oxidative phosphorylation) and Extracellular Acidification Rate (glycolysis) measured by seahorse XFe24. All groups are as indicated ($n = 4$).

[000157] FIG. 34A-34B. SV plus low dose $\alpha 4$ -1BB mAb cured A20 tumor bearing mice. FIG. 34A depicts Bioluminescence images of mice showing tumor load in A20 tumor bearing mice treated with SV plus low dose $\alpha 4$ -1BB mAb, as compared to control (untreated) and SV alone. FIG. 34B depicts tumor growth (Relative Luciferase activity) in each treatment group as indicated. Each line is a single mice.

[000158] FIG. 35A-35D. Cured mice are completely protected from A20 lymphoma re-challenge. FIG. 35A depicts Bioluminescence images of groups, previously treated as indicated, were re-challenged with A20 lymphoma cells. FIG. 35B depicts IFN γ production from purified T cells of all groups (To SV + $\alpha 4$ -1BB, 4 months after treatment finished), in the absence or presence of A20 tumor cells (5×10^4 per well), was measured by Elispot assay. FIG. 35C depicts cytotoxicity assay was performed the same as FIG. 27A. Left 2 panels, total splenocytes were used. Right, purified T cells were used. Left upper, A20 Fluc cells and left lower, CT26 Fluc cells were used for co-culture. FIG. 35D depicts significant differential (SD) upregulated gene sets are clustered by DAVID KEGG analysis. *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$

[000159] FIG. 36. Combination of NY-ESO-1 and IL-12 expressed by separate Sindbis viral vectors synergistically enhances survival of subjects with established tumors. FIG. 36 depicts the percentage survival rate of C57/Bl6 (albino-female) mice re-injected with Alm5-

2Fluc-17 tumor cells and treated with SV-IL-12, SV-NY-ESO-1 or a 50% mixture of both the vectors in one injection, SV-IL-12 and SV-NY-ESO-1, as indicated. Untreated mice were used as control. A total of n=5 mice in each group were tested for percentage survival days after tumor transplantation

[000160] FIG. 37. Combination of NY-ESO-1 and IL-12 expressed by the same Sindbis viral vectors synergistically enhances survival of subjects with established tumors. FIG. 37 depicts the percentage survival rate of C57/Bl6 (albino-female) mice re-injected with Alm5-2Fluc-17 tumor cells and treated with SV-IL-12, SV-NY-ESO-1 or a Sindbis viral vector expressing both IL-12 and NY-ESO-1 (SV-NYESO-SGP2-IL12), as indicated. Untreated mice were used as control. A total of n=5 mice in each group were tested for percentage survival days after tumor transplantation.

[000161] FIG. 38. pSP6-R_IL12 Sindbis replicon vector expressing IL12 a and b subunits. FIG. 38 depicts plasmid map with SP6, promoter for *in vitro* transcription; Replicase, SV RNA polymerase; Psg, subgenomic promoter for IL12 expression; linker, joins IL12 a and b subunits; AmpR, ampicillin resistance gene. Numbers show nucleotide positions of genes in the replicon plasmid.

[000162] FIG. 39. Sindbis Replicon vector expressing full length antibody to OX40 IgG2a. FIG. 39 depicts plasmid map with T7 promoter for *in vitro* transcription; Replicase, SV RNA polymerase; Psg subgenomic promoter for expression of anti-OX40 heavy chain IgG2a; 2Psg, second subgenomic promoter for expression of light chain anti-OX40. AmpR, ampicillin resistance gene; ColE1, plasmid origin of replication. Numbers show nucleotide positions of genes in the replicon plasmid.

[000163] FIG. 40. Sindbis replicon vector expressing single chain antibody to OX40. FIG. 40 depicts plasmid map with T7, promoter for *in vitro* transcription; Replicase, SV RNA polymerase; Psg, subgenomic SV promoter; IL12 signal peptide, signal peptide fused to the sequence encoding anti-OX40 single chain antibody; AmpR, ampicillin resistance gene; ColE1, plasmid origin of replication. Numbers show nucleotide positions of genes in the replicon plasmid.

[000164] FIG. 41. Sindbis Replicon Vector expressing NY-ESO-1. FIG. 41 depicts plasmid map with T7, promoter for *in vitro* transcription; Replicase, SV RNA polymerase ; Psg, subgenomic promoter for transcription; Hu NY-ESO-1, coding sequence for human NY-ESO-1

tumor associated antigen, Poly A, poly A tail transcribed onto NY-ESO mRNA; AmpR, ampicillin resistant gene; ColE, plasmid origin of replication. Numbers show nucleotide positions of genes in the replicon plasmid.

[000165] FIG. 42. pT7StuIR1-FcOX40L_T2A_NY-ESO1. Sindbis replicon vector expressing the OX40 Ligand fused with the Fc receptor sequence and NY-ESO-1. FIG. 42 depicts plasmid map with T7, promoter for *in vitro* transcription; Replicase, SV RNA polymerase; Psg, subgenomic SV promoter; FcOX40L coding sequence; T2A, termination peptide sequence; NY-ESO-1, coding sequence; AmpR, ampicillin resistance; ColE1, plasmid origin of replication. Numbers show nucleotide positions of genes in the replicon plasmid.

DETAILED DESCRIPTION OF THE INVENTION

[000166] Oncolytic virus (OV) therapy has become a novel immunotherapeutic approach to treat cancer. A rationale for oncolytic virus is that they can infect and lyse the tumor cell. They have been made to selectively replicate in tumor cells either through the direction of tumor specific promoters or through direct intratumoral administration. Most OVs encounter a number of barriers to systemic administration. Once lysed by OVs, tumor cells release tumor associated antigens (TAAs) that can stimulate cytotoxic T cells. OV infection also induces an inflammatory response that helps to trigger an immune anti-tumor response. Several OV clinical trials are underway and have shown promising results. However, whether OV therapy can effectively treat tumors that they are unable to infect remains an unresolved limitation.

[000167] Sindbis virus (SV) belongs to alphavirus genus and is one type of OV. Although it does not lyse infected tumor cells, it can cause their apoptotic death. It offers several important benefits. SV is known as one of the least virulent alphaviruses with clinical signs and symptoms usually unapparent. It has been estimated that there are 17 times more subclinical than symptomatic SV infections. In general, when symptoms do occur in humans they consist of a self-limiting, mild, febrile disease with vesicular exanthema and arthralgia from which most patients recover within 14 days. The disease is in part self-limiting because SV is an RNA virus that does not integrate in the host genome and hence its presence is transitory. The lack of an integrative step in its replication cycle also avoids insertional mutagenesis risks. In addition, SV vectors of the present disclosure

were generated from the laboratory strain AR339, which is not known to cause disease in humans. These vectors were further attenuated by rendering them replication-defective.

[000168] SV vectors can target tumors systemically and can reach metastatic tumor cells throughout the body. They can target tumors without infecting normal tissues.

However, susceptibility to infection by SV vectors depends on a number of factors including laminin receptor expression and distribution, as well as, defects in IFN signaling in tumors.

The present disclosure demonstrates that SV vectors can effectively help cure tumors that they are unable to infect and further demonstrates that the combination antibodies and SV vectors provide a surprising synergistic therapeutic effect against cancer.

[000169] The present disclosure provides methods for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of (a) an oncolytic viral vector and (b) an antibody directed against a co-stimulatory molecule or a nucleic acid encoding same; or an antibody to an immune system agonist molecule or a nucleic acid encoding same.

[000170] The oncolytic viral vector can be a Sindbis viral vector. The Sindbis viral vector can be replication defective. Sindbis viral vectors were produced as described in US Patent No. 8,093,021 (incorporated herein by reference in its entirety). The Sindbis viral vector can comprise at least one nucleic acid encoding a therapeutic protein. The Sindbis viral vector can comprise at least one nucleic acid encoding an immunostimulatory or an immunomodulatory protein. The immunostimulatory or immunomodulatory protein can be IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-34, IL-35, IL-36, or any combination thereof. In a preferred aspect, the immunostimulatory or immunomodulatory protein is IL-12. The Sindbis viral vector can comprise at least one nucleic acid encoding LacZ, Fluc or GFP.

[000171] The antibody can be an anti-OX40 antibody, an anti-4-1BB antibody, an anti-CD28 antibody, an anti-GITR antibody, an anti-CD137 antibody, an anti-cd37 antibody, an anti-HVEM antibody, or a combination thereof.

[000172] The Sindbis viral vector and the antibody can induce an immune response in a tumor associated antigen (TAA) nonspecific manner. The induced and nonspecific

immune response can be a first immune response. The first immune response can be followed by a secondary immune response. The secondary immune response can be the result of one or more TAAs released from the dead tumor cells. The secondary immune response can comprise memory T cells directed against one or more TAAs released from the dead tumor cells.

[000173] The present disclosure also provides methods for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and (b) an anti-OX40 monoclonal antibody or a nucleic acid encoding same, thereby treating cancer in the subject.

[000174] The Sindbis viral vector can be replication defective. The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 and can further comprise the nucleic acid encoding the anti-OX40 monoclonal antibody. The method can comprise administering a Sindbis viral vector comprising the nucleic acid encoding interleukin-12 and administering a Sindbis viral vector comprising the nucleic acid encoding the anti-OX40 monoclonal antibody.

[000175] The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit (IL-12 α , IL-12, p35 subunit) and interleukin-12 beta subunit (IL-12 β , IL-12, p40 subunit). The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit of GenBank accession no. M86672 and interleukin-12 beta subunit of GenBank accession no. M86671. The nucleic acid encoding interleukin-12 alpha subunit comprises the nucleic acid sequence of SEQ ID NO: 1. The nucleic acid encoding interleukin-12 beta subunit comprises the nucleic acid sequence of SEQ ID NO: 2.

[000176] The nucleic acid encoding interleukin-12 alpha subunit (IL-12 α , IL-12, p35 subunit) comprises the nucleic acid sequence of SEQ ID NO: 1 shown in the following Table.

The nucleic acid encoding interleukin-12 alpha subunit (IL-12 α , IL-12, p35 subunit)						
1	gcaagagaca	cagtcctggg	aaagtctgcc	ggctatccag	acaattataa	aaatgtgtct
61	cccaaggtca	gcgttccaac	agcctcacc	tcggcatcca	gcagctcctc	tcagtgcctg
121	tccagcatgt	gtcaatcacg	ctacctctc	tttttgcca	cccttgccct	cctaaaccac
181	ctcagtttgg	ccaggggtcat	tccagtctct	ggacctgcca	ggtgtcttag	ccagtcccga
241	aacctgctga	agaccacaga	tgacatgggtg	aagacggcca	gagaaaaact	gaaacattat
301	tcctgcactg	ctgaagacat	cgatcatgaa	gacatcacac	gggaccaaac	cagcacattg
361	aagacctgtt	taccactgga	actacacaag	aacgagagtt	gcctggctac	tagagagact
421	tcttccacaa	caagagggag	ctgcctgcc	ccacagaaga	cgtctttgat	gatgacctg
481	tgccttggtg	gcatttatga	ggacttgaag	atgtaccaga	cagagttcca	ggccatcaac
541	gcagcacttc	agaatcacia	ccatcagcag	atcattctag	acaagggcat	gctggtggcc
601	atcgatgagc	tgatgcagtc	tctgaatcat	aatggcgaga	ctctgcgcca	gaaacctcct

661	gtgggagaag	cagaccctta	cagagtgaag	atgaagctct	gcatcctgct	tcaagccttc
721	agcaccgcgc	tcgtgaccat	caacagggtg	atgggctatc	tgagctccgc	ctgaaagctc
781	aaggccctct	gccacagcgc	cctcctcaca	cagataggaa	acaaagaaag	attcataaga
841	gtcagggtgg	cttggcctgg	tgggcttaag	ctccttcagg	aatctgttct	cccatcacat
901	ctcatctccc	caaagggtgc	acagctacct	cagcatgggtg	ccctccatcg	cttctctcat
961	attcactata	caagttgttt	gtaagttttc	atcaaaatat	tgttaagggg	cgaagacgtc
1021	ctccccctcaa	tgtgttagca	gaagagcaag	aactgataag	ctattgtttt	tgtgccaaag
1081	tgtttatgaa	aacactcagt	cacccttat	ttaaaatat	ttattgctat	atattatact
1141	catgaaagta	catgagccta	tttatattta	tttattttct	atattattata	atatttctta
1201	tcagatgaat	ttgaaacatt	ttgaaacata	ccttattttg	tggttcta	aaagtaatgt
1261	tatca	(SEQ ID NO: 1)				

[000177] The nucleic acid encoding interleukin-12 beta subunit (IL-12 β , IL-12, p40 subunit) comprises the nucleic acid sequence of SEQ ID NO: 2 shown in the following Table.

The nucleic acid encoding interleukin-12 beta subunit (IL-12 β , IL-12, p40 subunit)						
1	gcacatcaga	ccaggcagct	cgcagcaaag	caagatgtgt	cctcagaagc	taaccatctc
61	ctggtttgcc	atcgttttgc	tgggtgtctcc	actcatggcc	atgtgggagc	tggagaaaga
121	cgtttatggt	gtagagggtg	actggactcc	cgatgcccct	ggagaaacag	tgaacctcac
181	ctgtgacacg	cctgaagaag	atgacatcac	ctggacctca	gaccagagac	atggagtcac
241	aggctctgga	aagaccctga	ccatcactgt	caaagagttt	ctagatgctg	gccagtacac
301	ctgccacaaa	ggaggcgaga	ctctgagcca	ctcacatctg	ctgctccaca	agaaggaaaa
361	tggaaatttg	tccactgaaa	ttttaaaaaa	tttcaaaaac	aagactttcc	tgaagtgtga
421	agcaccaaat	tactccggac	ggttcacgtg	ctcatggctg	gtgcaaagaa	acatggactt
481	gaagttcaac	atcaagagca	gtagcagttc	ccctgactct	cgggcagtg	catgtggaat
541	ggcgtctctg	tctgcagaga	aggtcacact	ggaccaaaag	gactatgaga	agtattcagt
601	gtcctgccag	gaggatgtca	cctgcccac	tgccgaggag	accctgccc	ttgaactggc
661	gttggaagca	cggcagcaga	ataaatatga	gaactacagc	accagcttct	tcatcaggga
721	catcatcaaa	ccagaccgc	ccaagaactt	gcagatgaag	cctttgaaga	actcacaggt
781	ggaggtcagc	tgggagtacc	ctgactcctg	gagcactccc	cattcctact	tctccctcaa
841	gttctttgtt	cgaatccagc	gcaagaaaga	aaagatgaag	gagacagagg	aggggtgtaa
901	ccagaaaggt	gcgttcctcg	tagagaagac	atctaccgaa	gtccaatgca	aaggcgaggaa
961	tgtctgcgtg	caagctcagg	atcgctatta	caattcctca	tgcagcaagt	gggcatgtgt
1021	tccctgcagg	gtccgatcct	aggatgcaac	gttggaagag	aaagaaaagt	ggaagacatt
1081	aaggaagaaa	aatttaaact	caggatggaa	gagtccccca	aaagctgtct	tctgcttggt
1141	tggctttttc	cagttttcct	aagttcatca	tgacaccttt	gctgatttct	acatgtaaat
1201	gttaaagtcc	cgcagagcca	gggagcta	gtatgcatag	atattctagc	attccacttg
1261	gccttatgct	gttgaaatat	ttaagtaatt	tatgtattta	ttaattttatt	tctgcatttc
1321	acatttgtat	accaagatgt	attgaatatt	tcatgtgctc	gtggcctgat	ccactgggac
1381	caggccctat	tatgcaaatt	gtgagcttgt	tatcttcttc	aacagctctt	caatcagggc
1441	tgcgtaggtg	cattagcttt	tgtgacaacc	aataagaaca	taattattctg	acacaagcag
1501	tgttacatat	ttgtgaccag	ttaagacata	gggtggtattt	ggagacatga	agaagctgta
1561	aagttgactc	tgaagagttt	agcactagtt	tcaacaccaa	gaaagacttt	ttagaagtga
1621	tattgataag	aaaccagggc	cttctttaga	agggtaacct	aattttaaag	aattttgaaa
1681	ggctgggtat	cgggtgtata	tgctttta	tccagcactc	aggagaccaa	ggcaggcaga
1741	tctctgtgag	tttgaggaca	gcctggtgta	cagagggagt	tccagcacag	ccagtgccac
1801	acagaaattc	tgtctcaaaa	acaattaaaa	aaaaaaaaaa	(SEQ ID NO: 2)	

[000178] The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit of amino acid sequence of GenBank accession no. AAA39292.1 and interleukin-12 beta subunit of amino acid sequence of GenBank accession no. AAA39296.1. The amino acid

sequence of the interleukin-12 alpha subunit is of amino acid sequence of SEQ ID NO: 3. The amino acid sequence of the interleukin-12 beta subunit is of amino acid sequence of SEQ ID NO: 4.

[000179] The Sindbis viral vector can comprise the nucleic acid encoding the interleukin-12 alpha subunit of amino acid sequence of GenBank accession no. AAA39292.1 and a nucleic acid encoding the interleukin-12 beta subunit of amino acid sequence of GenBank accession no. AAA39296.1. The amino acid sequence of the interleukin-12 alpha subunit comprises the amino acid sequence of SEQ ID NO: 3 shown in the following Table.

The amino acid sequence of the interleukin-12 alpha subunit						
1	MCQSRYLFL	ATLALLNHLS	LARVIPVSGP	ARCLSQSRNL	LKTTDDMVKT	AREKLKHYS
61	TAEDIDHEDI	TRDQTSTLKT	CLPLELHKNE	SCLATRETSS	TTRGSCLPPO	KTSLMMTLCL
121	GSIIYEDLKM	QTEFQAINAA	LQNNHQQII	LDKGMVLVAID	ELMQSLNHNH	ETLRQKPPVG
181	EADPYRVKMK	LCILLHAFST	RVVTINRVMG	YLSSA	(SEQ ID NO: 3)	

[000180] The amino acid sequence of the interleukin-12 beta subunit comprises the amino acid sequence of SEQ ID NO: 4 shown in the following Table.

The amino acid sequence of the interleukin-12 beta subunit						
1	MCPQKLTISW	FAIVLLVSPL	MAMWELEKDV	YVVEVDWTPD	APGETVNLTC	DTPEEDDITW
61	TSDQRHGVIG	SGKTLTITVK	EFLDAGQYTC	HKGGETLSHS	HLLHKKENG	IWSTEILKNF
121	KNKTFLKCEA	PNYSGRFTCS	WLVQRNMDLK	FNKSSSSSP	DSRAVTCGMA	SLSAEKVTLD
181	QRDYEKYSVS	CQEDVTCPTA	EETLPIELAL	EARQQNKYEN	YSTSFFIRDI	IKPDPPKNLQ
241	MKPLKNSQVE	VSWEYPDSWS	TPHSYFSLKF	FVRIQRKKEK	MKETEEGCNQ	KGAFLVEKTS
301	TEVQCKGGNV	CVQAQDRYYN	SSCSKWACVP	CRVRS	(SEQ ID NO: 4)	

[000181] The Sindbis viral vector can comprise a nucleic acid encoding an interleukin-12 alpha subunit that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 3 and a nucleic acid encoding an interleukin-12 beta subunit that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 4.

[000182] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 5. The nucleic acid sequence encoding the anti-OX40 variable heavy chain is SEQ ID NO: 6. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 7. The nucleic acid sequence encoding the anti-OX40 variable light chain is SEQ ID NO: 8.

[000183] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO:5 shown in the following Table.

anti-OX40 variable heavy chain amino acid (mouse anti-OX40 variable heavy chain)	
1	MAEVQLVESG GGLVQPGGSL RLSCAASGFT FSNYTMNWVR QAPGKGLEWV
51	SAISGSGGST YYADSVKGRF TISRDNKNT LYLQMNSLRA EDTAVYYCAK
101	DRYSQVHYAL DYWGQGTTLVT V (SEQ ID NO: 5)

[000184] The nucleic acid sequence encoding the anti-OX40 variable heavy chain comprises the nucleic acid sequence of SEQ ID NO:6 shown in the following Table.

anti-OX40 variable heavy chain nucleic acid (mouse anti-OX40 variable heavy chain)	
5'	atggccgaggtgcagctggtggagagcgggcgccgctggtgcagcccgccggcagccctgaggctgagctgctgcgc
	cgccagcggttcaccttcagcaactacaccatgaactgggtgagggcagggcccggaagggcctggagtggtg
	agcgccatcagcgccagcgccggcagcacctactacgcccagagcgtgaagggcaggttcaccatcagcagggaca
	acagcaagaacacccctgtacctgcagatgaacagcctgagggccgaggacaccgcccgtgtactactgcccgaagga
	caggtacagccaggtgcactacgccctggactactggggccagggcaccctggtgaccgtg 3' (SEQ ID NO: 6)

[000185] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO:7 shown in the following Table.

anti-OX40 variable light chain amino acid (mouse anti-OX40 variable light chain)	
1	DIQMTQSPDS LPVTPGEPAS ISCRSSQSL LHSNGYNYLDW YLQKAGQSPQ
51	LLIYLGSR (SEQ ID NO: 7)

[000186] The nucleic acid sequence encoding the anti-OX40 variable light chain comprises the nucleic acid sequence of SEQ ID NO:8 shown in the following Table.

anti-OX40 variable light chain nucleic acid (mouse anti-OX40 variable light chain)	
5'	gacatccagatgacccagtcacccgactccctgcccgtgacccccggcgagcccgccctccatctcctgcccgtc
	ctcccagtcacctgctgcactccaacggctacaactacctggactggtacctgcagaagggccggccagtcacccag
	ctgctgatctacctgggtccaacgggcctccggcgtgcccagccggttctccggctccggctccggcaccgact
	tcacctgaagatctccgggtggaggccgaggacgtgggcgtgtactactgccagcagtagtactacaaccacccac
	caccttcggccagggcaccaagctggagatcaagcgg-3' (SEQ ID NO: 8)

[000187] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable heavy chain of amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding an anti-OX40 variable light chain of amino acid sequence of SEQ ID NO: 7. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid

sequence of SEQ ID NO: 5 and a nucleic acid encoding an anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 7.

[000188] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain comprising the amino acid sequence of SEQ ID NO:9 shown in the following Table.

anti-OX40 antibody heavy chain amino acid sequence (mouse anti-OX40 IgG2a antibody heavy chain)
MGQSRYLFLATLALLNHLSLMAEVQLVESGGGLVQPGGSLRLSCAASGFTFSNYTMNWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDRYSQVHYALDYWGQGTLVTVAAKTTAPSV YPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNV AHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISW FVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLP PEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSGSYFMYSKLRVEKKNWVERNSYSCSVVH EGLHNHHTTKSFSRTPGK (SEQ ID NO:9)

[000189] The nucleic acid sequence encoding the anti-OX40 antibody heavy chain comprises the nucleic acid sequence of SEQ ID NO:10 shown in the following Table.

anti-OX40 antibody heavy chain nucleic acid sequence (mouse anti-OX40 IgG2a antibody heavy chain)
5' <u>gccaccatggggccagagcaggtacctgctgttctctggccaccctggccctgctgaaccacctgagcctggccat</u> <u>ggccgaggtgcagctggtggagagcggcgccgctggtgcagcccgccgagcctgaggctgagctgcccgc</u> <u>agcggcttcaccttcagcaactacacatgaactgggtgagggcagggcccggaagggcctggagtggtgagcg</u> <u>ccatcagcggcagcggcgagcacctactacgacagcgtgaagggcaggttcacatcagcagggacaacag</u> <u>caagaacaccctgtacctgcagatgaacagcctgagggccgaggacaccgcccgtgtactactgcgccaaaggacagg</u> <u>tacagccaggtgcactacgcccctggactactggggccagggcaccctggtgacccgtggccgccaagaccgccc</u> <u>ccagcgtgtaccccctggccccctgctgctgcgccgacaccaccggcagcagcgtgacccctgggctgctggtgaaggg</u> <u>ctacttccccgagcccgtgacccctgacctggaacagcggcagcctgagcagcggcgtgcacaccttccccgcccgtg</u> <u>ctgcagagcgacctgtacacccctgagcagcagcgtgacccgtgaccagcagcacctggcccagccagagcatccct</u> <u>gcaacgtggcccaccccgcagcagcaccaggtggacaagaagatc</u><u>gagccaggggcctcaccatcaagccctg</u> <u>ccccccctgcaagtgc</u><u>cccccccccaacctgctggcgcccccagcgtgttcattcttcccccccaagatcaaggac</u> <u>gtgctgatgatcagcctgagccccatcgtgacctgctggtggtggagctgagcgaggagacacccgacgtgcaga</u> <u>tcagctggttcgtgaacaacgtggaggtgcacaccgcccagaccagacccacagggaggactacaacagcacccct</u> <u>gaggtggtgagcgccctgccatccagcaccaggactggatgagcggcaaggagttcaagtgaaggtgaacaac</u> <u>aaggacctgcccgcccccatcgagaggaccatcagcaagcccaagggcagcgtgagggcc</u><u>t</u><u>cccaggtgtacgtgc</u> <u>tgcccccccccgaggaggagatgaccaagaagcaggtgacctgacctgcatggtgaccgacttcatgcccagga</u> <u>catctacgtggagtggaccaacaacggcaagaccgagctgaactacaagaacaccgagcccgtgctggacagcgac</u> <u>ggcagctacttcatgtacagcaagctgaggggtggagaagaagaactgggtggagagggaacagctacagctgcagcg</u> <u>tggtgcagagggcctgcacaaccaccacaccaccaagagcttcagcaggacccccggcaagtaa-3'</u> (SEQ ID NO:10)

[000190] In SEQ ID NOs: 9 and 10, the underlined residues indicate IL-2 signal peptide; the **Bold residues** indicate variable antigen binding region; the non-underlined residues indicate mouse heavy chain IgG2a constant region, GB Accession BC080671; and the **bold and underlined residues** indicate the Hinge and disulfide bond region. The double underlined residue in SEQ ID

[000195] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%,

95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 10 and an anti-OX40 antibody light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 12.

[000196] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO:13 shown in the following Table.

amino acid sequence of the Target antigen (Target Antigen: OX40/CD134, Receptor for TNFSF4/OX40L/GP34)				
1	MCVGARRLGR	GPCAALLLLG	LGLSTVTGLH	CVGDTYPSND
51	GMVSRCSRSQ	NTVCRPCGPG	FYNDVVSSKP	CKPCTWCNLR
101	ATQDTVCRRCR	AGTQPLDSYK	PGVDCAPCPP	GHFSPGDNQA
151	GKHTLQPASN	SSDAICEDRD	PPATQPQETQ	GPPARPITVQ
201	GPSTRPVEVP	GGRAVAAILG	LGLVLGLLGP	LAILLALYLL
251	HKPPGGGSFR	TPIQEEQADA	HSTLAKI	(SEQ ID NO: 13)

[000197] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 antibody variable heavy chain comprising the amino acid sequence of SEQ ID NO: 31 shown in the following Table.

Human anti-OX40 antibody, 1-12Z5 (ATCC No. PTA-7216) Heavy chain variable region amino acid sequence. Leader sequence in bold.	
1	MTMITPSLVP SSDPLVTAAS VLEFALLIRL TIGQAVVSTQ STGGGLVQPG RSLRLSCAAS
61	GFTLDDYGMH WVRQAPGKGL EWVSGISWNS DSIGYVDSVK GRFTISRDNK KNSLYLQMNS
121	LRVEDTALYY CVKDISGWYS FDYWGQGLTV TVSS (SEQ ID NO: 31)

[000198] The nucleic acid sequence encoding the human anti-OX40 antibody heavy chain variable region comprises the nucleic acid sequence of SEQ ID NO: 32 shown in the following Table.

Human anti-OX40 antibody 1-12Z5 (ATCC No. PTA-7216) Heavy chain variable region nucleic acid sequence	
5'	-atgaccatgattacgccaagcttggtaccgagctcggatccactagtaacggccgcccagt
	gtgctggaattcgcccttctaatacagactcactatagggcaagcagtggtatcaacgcag
	agtaggggggaggccttggtacagcctggcaggtccctgagactctcctgtgcagcctct
	ggattcacccttgatgattatggcatgcactgggtccggcaagctccaggggaaggccctg
	gagtggtctcaggtattagttggaatagtgatagtataggctatgtggactctgtgaag
	ggccgattcaccatctccagagacaacgccaagaactccctgtatctgcaaatgaacagt
	ctgagagttg aggacacggccttgattactgtgtaaaagatattagtggtggtacagc

agggccgattcaccatctccagagacaatgccagaactcactgtatctgcaaataaacagcctgagagacgagga cacggctgtgtattactgtgcgagaggagtgtatcacaatggctggctccttctt tgactactggggccaggaaccctactcaccgtctcctca-3' (SEQ ID NO: 36)
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[000203] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 antibody variable light chain comprising the amino acid sequence of SEQ ID NO: 37 shown in the following Table.

Human anti-OX40 antibody 112F32 (ATCC No. PTA-7217) Light chain variable region amino acid sequence.	
1	MDMRVLAQLL GLLLLCFPGA RCDIQMTQSP SLSASVGNR VTITCRASQD ISSWLAWYQQ
061	KPEKAPKSLI YAASSLQSGV PSRFSGSGSG TDFTLTISSL QPEDFATYYC QQYNSYPLTF
121	GQGTRLEIKR (SEQ ID NO: 37)

[000204] The nucleic acid sequence encoding the human anti-OX40 antibody light chain variable region comprises the nucleic acid sequence of SEQ ID NO: 38 shown in the following Table.

Human anti-OX40 antibody 12F32 LV (ATCC No. PTA-7217) Light chain variable region nucleic acid sequence	
5'	-atggacatgagggctcctcgctcagctcctggggctcctgctgctctgtttcccagggtgcc
	agatgtgacatccagatgaccagtcctccatcctcactgtctgcatctgtaggaaacaga
	gtcaccattacttgtcgggagtcaggtatattagcagctggtagcctgggtatcagcag
	aaaccagagaaagcccctaagtccctgatctatgctgcatccagtttgcaaagtggggtc
	ccatcaaggttcagcggcagtggtatctgggacagatttcactctcaccatcagcagcctg
	cagcctgaagattttgcaacttattactgccaacagttataatagttacccctcaccttc
	ggccaaggacacgactggagattaaacga-3' (SEQ ID NO: 38)

[000205] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 antibody variable heavy chain comprising the amino acid sequence of SEQ ID NO: 39 shown in the following Table.

Human anti-OX40 antibody 112Y1 31 (ATCC NO. PTA-7218) Heavy chain variable region amino acid sequence	
1	MDTLCSTLL LTI PSWVLSQ ITLKESGPTL VKPTQTLTLT CTFSGFSLST SGVGVGWIRQ
61	PPGKALEWLA LIYWDDHSPY SPSLKSRLTI TKDTSKNQVV LTMTNMDPVD TATYYCARTR
121	GAFDIWGQGT MVTVSS (SEQ ID NO: 39)

[000206] The nucleic acid sequence encoding the human anti-OX40 antibody heavy chain variable region comprises the nucleic acid sequence of SEQ ID NO: 40 shown in the following Table.

Human anti-OX40 antibody 112Y1 31 (ATCC NO. PTA-7218) Heavy chain variable region nucleic acid sequence	
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5'-atggacactctttgctccacgctcctgctgctgaccatcccttcatgggtcttgtcccag
atcaccttgaaggagtctgtgctcctacgctgggtgaaacccacacagaccctcacgctgacc
tgcaccttctcttgattctcactcagcactagtggagtgggtgtgggctggatccgtcag
ccccaggaa aggccttgagtggttgcactcatttattgggatgatcatagcccctac
agcccatctctgaagagcaggctcaccatcaccaaggacacctccaaaaaccaggtggtc
cttacaatgaccaacatggaccctgtggacacagccacatattactgtgcacgcacccgg
ggggcttttgatatctggggccaagggaacaatggtcaccgtctcttca-3' (SEQ ID NO: 40)

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[000207] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 antibody variable light chain comprising the amino acid sequence of SEQ ID NO: 41 shown in the following Table.

Human anti-OX40 antibody 112Y1 31 (ATCC NO. PTA-7218) Light chain variable region amino acid sequence	
1	MEAPQQLFL LLLWLPDTTG EIVLTQSPAT LSLSPGERAT LSCRASQGVSYLAWYQQKP
61	GQAPRLLIYD ASNRATGIPA RFGSGPGTD FTLTISLEP EDFAVYYCQQ RSNWHPTFGQ
121	GTKVEIK (SEQ ID NO: 41)

[000208] The nucleic acid sequence encoding the human anti-OX40 antibody light chain variable region comprises the nucleic acid sequence of SEQ ID NO: 42 shown in the following Table.

Human anti-OX40 antibody 112Y1 31 (ATCC NO. PTA-7218) Light chain variable region nucleic acid sequence	
5'	-atggaagccccagcgcagcttctcttctcctgctactctggctcccagataaccaccgga
	gaaattgtgttgacacagtctccagccacctgtctttgtctccaggggaaagagccacc
	ctctcctgcagggccagtcaggggtgttagcagctacttagcctggtagcagcagaaacctggccaggtcccaggc
	tcctcatctatgatgcacccaacagggccactggcatcccagccaggttcagtgccagtgggcctgggacagactt
	cactctcaccatcagcagcctagagcctgaagattttgcagtttattactgtcagcagcgtagcaactggcatccg
	acgttcggccaagggaaccaaggtggaaatcaaacgaactgtggctgcaccatc-3' (SEQ ID NO: 42)

[000209] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 antibody variable heavy chain comprising the amino acid sequence of SEQ ID NO: 43 shown in the following Table.

Human anti-OX40 antibody 112V8 (ATCC NO. PTA-7219) Heavy chain variable region amino acid sequence	
1	MDTLCSTLLL LTIPSWVLSQ ITLKESGPTL VKPKQTLTLT CTFSGFSLST SGMGVGWIRQ
61	PPGKALEWLA VIYWDDHQLY SPSLKSRLTI TKDTSKNQVV LTMTNMDPVD TATYYCAHRR
121	GAFAQHWGQGT LTVSSASTK (SEQ ID NO: 43)

[000210] The nucleic acid sequence encoding the human anti-OX40 antibody heavy chain variable region comprises the nucleic acid sequence of SEQ ID NO: 44 shown in the following Table.

Human anti-OX40 antibody 112V8 (ATCC NO. PTA-7219) Heavy chain variable region nucleic acid sequence
5'-atggacacactttgctccacgctcctgctgctgaccatcccttcattgggtcttgtcccag atcaccttgaaggagtctggtcctacgctagtgaagccaaacagaccctcacgctgacc tgcaccttctctggattctcactcagcactagtgggaatgggtgtgggtggatccgtcag ccccaggaaaggccctggagtggcttgagtcatttattgggatgatcatcaactctac agtccatctctgalgagcaggctcaccatcaccaaggacacctccaaaaaccagggtggtc cttacaatgaccaacatggaccctgtggacacagccacatattactgtgcacacagacga ggggccttcagcactggggccagggcaccctggtcaccgtctcctcagcttccaccaagggc-3' (SEQ ID NO: 44)

[000211] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 antibody variable light chain comprising the amino acid sequence of SEQ ID NO: 45 shown in the following Table.

Human anti-OX40 antibody 112V8 (ATCC NO. PTA-7219) Light chain variable region amino acid sequence
1 METPAQLLFL LLLWLPDTTG EIVLTQSPGT LSLSPGERAT LSCRASQSVS SSYLAWYQQK 61 PGQAPRLLIY GASSRATGIP DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYDSSLTFGG 121 GTKVEIKRT (SEQ ID NO: 45)

[000212] The nucleic acid sequence encoding the human anti-OX40 antibody light chain variable region comprises the nucleic acid sequence of SEQ ID NO: 46 shown in the following Table.

Human anti-OX40 antibody 112V8 (ATCC NO. PTA-7219) Light chain variable region nucleic acid sequence
5'-atggagtgggggccgtgctgggttttccttgttgttatttttagaaggtgtccagtggtggg atggaaacccagcgcagcttctcttcctcctgctactctggctcccagataccaccgga gaaattgtgttgacgcagctctccaggcaccctgtctttgtctccaggggaaagagccacc ctctcctgcagggccagtcagagtgttagcagcagctacttagcctggtaccagcagaaa cctggccaggctcccaggctcctcatctatggtgcatccagcagggccactggcatcca gacaggttcagtggcagtggtctgggacagacttcactctcaccatcagcagactggag cctgaagattttgcagtgattactgtcagcagtatgatagctcgctcactttcggcgga gggaccaaggtggagatcaaacgaact-3' (SEQ ID NO: 46)

[000213] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 antibody variable heavy chain comprising the amino acid sequence of SEQ ID NO: 47 shown in the following Table.

Human anti-OX40 antibody 112Y55 (ATCC No. PTA-7220) Heavy chain variable region amino acid sequence
1 MDTLCSLTL LTI PSWVLSQ ITLKESGPTL VKPTQTLTLS CTFSGFSLST SGVGVGWIRQ 61 PPGKALEWLA LIHWDDAERY SPSLKSRLTI TKDTSKNQVV LTMTNMDLVD TATYYCAHTR 121 GAFDIWGQGT MVTVSS (SEQ ID NO: 47)

[000214] The nucleic acid sequence encoding the human anti-OX40 antibody heavy chain variable region comprises the nucleic acid sequence of SEQ ID NO: 48 shown in the following Table.

Human anti-OX40 antibody 112Y55 (ATCC No. PTA-7220) Heavy chain variable region nucleic acid sequence
5'-atggacacactttgctccacgctcctgctgctgaccatcccttcatgggtcttgtcccag atcaccttgaaggagtctggtcctacgctggtgaaacccacacagaccctcacgctgtcc tgcaccttctctgggttctcactcagcactagtggagtgggtgtgggtggtatccgtcag ccccaggaaaggccctggaatggcttgactcattcattgggatgatgctgagcgctac agtccatctctgaagagcaggctcaccatcaccaggacacctccaaaaaccagggtggtc cttacaatgaccaacatggaccttgtggacacagccacatattactgtgcacacaccgg ggggcttttgatatctggggccaagggaatggtcaccgtctcttca-3' (SEQ ID NO: 48)

[000215] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 antibody variable light chain comprising the amino acid sequence of SEQ ID NO: 49 shown in the following Table.

Human anti-OX40 antibody 112Y55 (ATCC No. PTA-7220) Light chain variable region amino acid sequence
1 METPAQLLFL LLLWLPD TTG EIVLTQSPGT LSLSPGERAI LSCRASQSVS SSFLAWYQQK 61 PGQAPRLLIY GAFSRATGIP DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYDSSRTFGQ 121 GTKVEIK (SEQ ID NO: 49)

[000216] The nucleic acid sequence encoding the human anti-OX40 antibody light chain variable region comprises the nucleic acid sequence of SEQ ID NO: 50 shown in the following Table.

Human anti-OX40 antibody 112Y55 (ATCC No. PTA-7220) Light chain variable region nucleic acid sequence
5'-atggaaaccccagcgcagcttctcttctcctcctgctactctgggtcccagataccaccgga gaaattgtgttgacgcagctctccaggcaccctgtctttgtctccaggggaaagagccatc ctctcctgcaggggccagtcagagtgttagcagcagcttcttagcctggtaccaacagaaa cctggccaggctcccaggctcctcatctatggtgcatttagcagggccactggcatccca gacaggttcagtggtgagtggtctgggacagacttcactctcaccatcagcagactggag cctgaagattttgcagtgattactgtcagcagtatgatagctcacggacgttcggccag gggaccaagggtggaaatcaaa-3' (SEQ ID NO: 50)

[000217] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain with an amino acid sequence that binds to a target antigen of the

amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 IgG2a antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 IgG2a antibody light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000218] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain amino acid sequence, and a mouse anti-OX40 antibody variable light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain amino acid sequence, and an anti-OX40 antibody light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000219] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 5. The nucleic acid sequence encoding the anti-OX40 variable heavy chain is SEQ ID NO: 6. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 7. The nucleic acid sequence encoding the anti-OX40 variable light chain is SEQ ID NO: 8.

[000220] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain of amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain of amino acid sequence of SEQ ID NO: 7. The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 7.

[000221] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain comprising the amino acid sequence of SEQ ID NO: 9. The nucleic acid sequence encoding the an anti-OX40 antibody heavy chain is SEQ ID NO: 10.

[000222] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 antibody light chain comprising the amino acid sequence of SEQ ID NO: 11. The nucleic acid sequence encoding the anti-OX40 antibody light chain is SEQ ID NO: 12.

[000223] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence of SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain of SEQ ID NO: 10 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 12.

[000224] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 10 and an anti-OX40 antibody light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 12.

[000225] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 31. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 32. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 33. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 34.

[000226] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 31. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 32. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 33. The nucleic acid sequence encoding the human

anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 34.

[000227] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 35. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 36. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 37. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 38.

[000228] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 35. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 36. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 37. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 38.

[000229] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 39. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 40. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 41. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 42.

[000230] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 39. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 40. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%,

98%, 99% or 100% identical to SEQ ID NO: 41. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 42.

[000231] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 43. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 44. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 45. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 46.

[000232] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 43. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 44. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 45. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 46.

[000233] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 47. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain is SEQ ID NO: 48. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 49. The nucleic acid sequence encoding the human anti-OX40 variable light chain is SEQ ID NO: 50.\

[000234] The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable heavy chain comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 47. The nucleic acid sequence encoding the human anti-OX40 variable heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to is SEQ ID NO: 48. The Sindbis viral vector can comprise a nucleic acid encoding a human anti-OX40 variable light chain

comprising the amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 49. The nucleic acid sequence encoding the human anti-OX40 variable light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 50.

[000235] The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000236] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000237] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain amino acid sequence, and an anti-OX40 antibody variable light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain amino acid sequence, and an anti-OX40 antibody light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000238] The Sindbis viral vector and the anti-OX40 monoclonal antibody can be administered sequentially or concurrently. The Sindbis viral vector can be administered systemically. The anti-OX40 monoclonal antibody can be administered systemically. Both the Sindbis viral vector and the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be administered systemically. The Sindbis viral vector can be administered parenterally. The anti-OX40 monoclonal antibody can be administered parenterally. Both the Sindbis viral vector and the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be administered parenterally. The Sindbis viral vector can be administered intraperitoneally. The anti-OX40 monoclonal antibody can be administered

intraperitoneally. Both the Sindbis viral vector and the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be administered intraperitoneally.

[000239] A “monoclonal antibody” as disclosed herein, can be a full-length antibody or an antigen binding fragment thereof, wherein the “antigen binding fragment” is a fragment of the full length antibody that retains binding to the target antigen of the said monoclonal antibody. For example, a monoclonal antibody against OX-40 or an anti-OX40 monoclonal antibody, as described herein, can be a full length antibody against OX40 antibody or an antigen binding fragment thereof, wherein the fragment retains binding to the OX40 receptor on a cell surface. An “antigen-binding fragment” of an anti-OX-40 antibody, as described herein can include any fragment selected from the group consisting of Fv, Fav, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, scFv-CH₃, scFv-Fc, and diabody fragments.

[000240] The cancer can be a solid cancer or a liquid/hematologic cancer. The cancer can comprise metastatic cancer. The cancer can comprise a solid tumor. The cancer can be a carcinoma, a lymphoma, a blastoma, a sarcoma, a leukemia, a brain cancer, a breast cancer, a blood cancer, a bone cancer, a lung cancer, a skin cancer, a liver cancer, an ovarian cancer, a bladder cancer, a renal cancer, a gastric cancer, a thyroid cancer, a pancreatic cancer, an esophageal cancer, a prostate cancer, a cervical cancer or a colorectal cancer. In one preferred aspect, the cancer is colon cancer. In one preferred aspect, the cancer is prostate cancer. In one preferred aspect, the cancer is ovarian cancer.

[000241] Sindbis Viral Vector and Anti-OX40 Monoclonal Antibody

[000242] The present disclosure further provides a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a nucleic acid encoding an anti-OX40 monoclonal antibody. The present disclosure also provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and (b) an anti-OX40 monoclonal antibody or a nucleic acid encoding same. The present disclosure also provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and (b) a Sindbis viral vector comprising a nucleic acid encoding an anti-OX40 monoclonal antibody.

[000243] The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit (IL-12 α , IL-12, p35 subunit) and interleukin-12 beta subunit (IL-12 β , IL-12, p40 subunit). The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12

alpha subunit of GenBank accession no. M86672 and interleukin-12 beta subunit of GenBank accession no. M86671. The nucleic acid encoding interleukin-12 alpha subunit comprises the nucleic acid sequence of SEQ ID NO: 1. The nucleic acid encoding interleukin-12 beta subunit comprises the nucleic acid sequence of SEQ ID NO: 2.

[000244] The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit of amino acid sequence of GenBank accession no. AAA39292.1 and interleukin-12 beta subunit of amino acid sequence of GenBank accession no. AAA39296.1. The amino acid sequence of the interleukin-12 alpha subunit is of amino acid sequence of SEQ ID NO: 3. The amino acid sequence of the interleukin-12 beta subunit is of amino acid sequence of SEQ ID NO: 4.

[000245] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 5. The nucleic acid sequence encoding the anti-OX40 variable heavy chain is SEQ ID NO: 6. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 7. The nucleic acid sequence encoding the anti-OX40 variable light chain is SEQ ID NO: 8.

[000246] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain of amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain of amino acid sequence of SEQ ID NO: 7. The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 7.

[000247] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain comprising the amino acid sequence of SEQ ID NO: 9. The nucleic acid sequence encoding the an anti-OX40 antibody heavy chain is SEQ ID NO: 10.

[000248] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 antibody light chain comprising the amino acid sequence of SEQ ID NO: 11. The nucleic acid sequence encoding the anti-OX40 antibody light chain is SEQ ID NO: 12.

[000249] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence of SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain of SEQ ID NO: 10 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 12.

[000250] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 10 and an anti-OX40 antibody light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 12.

[000251] The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000252] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000253] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain amino acid sequence, and an anti-OX40 antibody variable light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody

heavy chain amino acid sequence, and an anti-OX40 antibody light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000254] The present disclosure also provides methods for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and (b) an anti-OX40 monoclonal antibody or a nucleic acid encoding same, thereby treating cancer in the subject.

[000255] The Sindbis viral vector can be replication defective. The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 and can further comprise the nucleic acid encoding the anti-OX40 monoclonal antibody. The method can comprise administering a Sindbis viral vector comprising the nucleic acid encoding interleukin-12 and administering a Sindbis viral vector comprising the nucleic acid encoding the anti-OX40 monoclonal antibody.

[000256] The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit (IL-12 α , IL-12, p35 subunit) and interleukin-12 beta subunit (IL-12 β , IL-12, p40 subunit). The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit of GenBank accession no. M86672 and interleukin-12 beta subunit of GenBank accession no. M86671. The nucleic acid encoding interleukin-12 alpha subunit comprises the nucleic acid sequence of SEQ ID NO: 1. The nucleic acid encoding interleukin-12 beta subunit comprises the nucleic acid sequence of SEQ ID NO: 2.

[000257] The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit of amino acid sequence of GenBank accession no. AAA39292.1 and interleukin-12 beta subunit of amino acid sequence of GenBank accession no. AAA39296.1. The amino acid sequence of the interleukin-12 alpha subunit is of amino acid sequence of SEQ ID NO: 3. The amino acid sequence of the interleukin-12 beta subunit is of amino acid sequence of SEQ ID NO: 4.

[000258] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 5. The nucleic acid sequence encoding the anti-OX40 variable heavy chain is SEQ ID NO: 6. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 7. The nucleic acid sequence encoding the anti-OX40 variable light chain is SEQ ID NO: 8.

[000259] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain of amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain of amino acid sequence of SEQ ID NO: 7. The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 7.

[000260] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain comprising the amino acid sequence of SEQ ID NO: 9. The nucleic acid sequence encoding the an anti-OX40 antibody heavy chain is SEQ ID NO: 10.

[000261] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 antibody light chain comprising the amino acid sequence of SEQ ID NO: 11. The nucleic acid sequence encoding the anti-OX40 antibody light chain is SEQ ID NO: 12.

[000262] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence of SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain of SEQ ID NO: 10 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 12.

[000263] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 10 and an anti-OX40 antibody light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 12.

[000264] The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000265] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000266] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain amino acid sequence, and an anti-OX40 antibody variable light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain amino acid sequence, and an anti-OX40 antibody light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000267] A “monoclonal antibody” as disclosed herein, can be a full-length antibody or an antigen binding fragment thereof, wherein the “antigen binding fragment” is a fragment of the full length antibody that retains binding to the target antigen of the said monoclonal antibody. For example, a monoclonal antibody against OX-40 or an anti-OX40 monoclonal antibody, as described herein, can be a full length antibody against OX40 antibody or an antigen binding fragment thereof, wherein the fragment retains binding to the OX40 receptor on a cell surface. An “antigen-binding fragment” of an anti-OX-40 antibody, as described herein can include any fragment selected from the group consisting of Fv, Fav, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, scFv-CH₃, scFv-Fc, and diabody fragments.

[000268] The Sindbis viral vector and the anti-OX40 monoclonal antibody can be administered sequentially or concurrently. The Sindbis viral vector can be administered systemically. The anti-OX40 monoclonal antibody can be administered systemically. Both the Sindbis viral vector and the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be administered systemically. The Sindbis viral vector can be administered parenterally. The anti-OX40 monoclonal antibody can be administered parenterally. Both the Sindbis viral vector and the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be

administered parenterally. The Sindbis viral vector can be administered intraperitoneally. The anti-OX40 monoclonal antibody can be administered intraperitoneally. Both the Sindbis viral vector and the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be administered intraperitoneally.

[000269] The cancer can be a solid cancer or a liquid/hematologic cancer. The cancer can comprise metastatic cancer. The cancer can comprise a solid tumor. The cancer can be a carcinoma, a lymphoma, a blastoma, a sarcoma, a leukemia, a brain cancer, a breast cancer, a blood cancer, a bone cancer, a lung cancer, a skin cancer, a liver cancer, an ovarian cancer, a bladder cancer, a renal cancer, a gastric cancer, a thyroid cancer, a pancreatic cancer, an esophageal cancer, a prostate cancer, a cervical cancer or a colorectal cancer. In one preferred aspect, the cancer is colon cancer. In one preferred aspect, the cancer is prostate cancer. In one preferred aspect, the cancer is ovarian cancer.

[000270] Sindbis virus can be administered at least one time, at least two times, at least three times, at least four times or at least five times per week. Sindbis virus can be administered for at least one week, at least two weeks, at least three weeks, or at least four weeks. Sindbis virus can be administered from $10^6 - 10^9$ TU/mL. Preferably, Sindbis virus can be administered from $10^6 - 10^9$ TU/mL.

[000271] An anti-OX40 monoclonal antibody can be administered at least one time, at least two times, at least three times, at least four times or at least five times per week. An anti-OX40 monoclonal antibody can be administered for at least one week, at least two weeks, at least three weeks, or at least four weeks. An anti-OX40 monoclonal antibody can be administered from 25 μ g – 500 μ g, 25 μ g – 450 μ g, 50 μ g – 400 μ g, from 50 μ g – 350 μ g, from 50 μ g – 300 μ g, from 50 μ g – 250 μ g, from 50 μ g – 200 μ g, from 50 μ g – 150 μ g or from 50 μ g – 100 μ g. An anti-OX40 monoclonal antibody can be administered at 250 μ g. An anti-OX40 monoclonal antibody can be administered at 250 μ g once a week for one week. An anti-OX40 monoclonal antibody can be administered at 250 μ g once a week for two weeks. An anti-OX40 monoclonal antibody can be administered at 250 μ g once a week for three weeks. An anti-OX40 monoclonal antibody can be administered at 250 μ g three times a week for one week. An anti-OX40 monoclonal antibody can be administered at 250 μ g three times a week for two weeks. An anti-OX40 monoclonal antibody can be administered at 250 μ g three times a week for three weeks.

[000272] The results provided in the instant disclosure demonstrate that administration of the a Sindbis virus expressing IL-12 (SV.IL12) markedly increases the expression of OX40 on CD4 T cells and demonstrate that administration of a combination of SV.IL12 and anti-OX40 monoclonal antibody resulted in complete tumor regression in colon cancer, prostate cancer and ovarian cancer *in vivo* models and led to a greater than 60% survival rate (in some instances to a greater than 90% survival rate). This combined therapeutic effect was dramatically more effective when compared to either SV.IL12 or anti-OX40 monoclonal antibody treatment alone. These results also confirm that the oncolytic activity of the Sindbis virus is not required to induce a robust and effective anti-tumor response.

[000273] The results provided in the instant disclosure demonstrate that the combination of SV.IL12 or anti-OX40 monoclonal antibody treatment markedly changes the transcriptome signature of T cells and favors the differentiation of terminal effector T cells (*e.g.*, effector T cells with a Th1 type phenotype). In particular, pathways upregulated by the combination treatment were dominated by DNA replication, chromosomal organization and cell cycle regulation, but also included various metabolic and immunological processes, such as mitochondrial respiration, nucleotide metabolism and adaptive immune responses. Specifically, only T cells from combined therapy expressed the gene signature of terminally differentiated effector T cells, which are characterized by high expression of the killer lectin-like receptor (KLRG1) and low expression of the interleukin 7 receptor (IL-7R). Furthermore, genes encoding products associated with the differentiation and function of effector cells, such as *Batf*, *Id2*, *Tbet*, *Gzmb* and *Ifng*, were also highly expressed in T cells following combination therapy. Furthermore, CD4 T cells also expressed a marked anti-tumor effector phenotype (ICOS⁺Tbet⁺) which was on average 2 to 3-fold higher during combined therapy compared with SV.IL12 or anti-OX40 treatment.

[000274] The tumor microenvironment can be a very challenging milieu for an effector T cell as it is characterized by hypoxia, acidosis and low levels of nutrient sources such as glucose and glutamine. Even if T cell activation and initiation of effector function is allowed, T cells may be unable to generate the bioenergetics intermediates necessary to carry out effector function in the tumor microenvironment. Thus, providing a metabolic support for T cells is crucial for the success of cancer treatments. The results provided in

the instant disclosure demonstrate that the combination of SV.IL12 or anti-OX40 monoclonal antibody promotes metabolic reprogramming of T cells. Specifically, the basal rate of oxygen consumption (OCR) was enhanced and spare respiratory capacity was dramatically increased in T cells following combination treatment. The combination also induced elevated protein expression of c-MYC as well as rate of extracellular acidification (ECAR). Collectively, these results show that SV.IL12 induces enhanced oxidative phosphorylation in CD8 T cells and the combination treatment is required to push CD4 T cells towards glycolysis by increasing the protein expression of c-MYC. Thus, the combination of SV.IL12 or anti-OX40 monoclonal antibody metabolically rewires T cells to an energetic state using both metabolic pathways, oxidative phosphorylation and glycolysis.

[000275] The results provided in the instant disclosure demonstrate that metabolic reprogrammed T cells display enhanced CD4 mediated cytokine production and anti-tumor activity following treatment with the combination of SV.IL12 and anti-OX40 monoclonal antibody. Specifically, genes encoding pro-inflammatory cytokines *ifn γ* and *il2* were upregulated in T cells and the secretion of interferon- γ (IFN γ) by splenocytes was increased following combination treatment. Additionally, the levels of the cytotoxic proteases, granzyme A and B, were upregulated following combination treatment. Further, granzyme B positive cells were detected in CD8 as well as CD4 T cells, indicating the presence of cytotoxic CD4 T cells following combination treatment. In addition, tumor growth was markedly reduced when co-cultured with splenocytes from mice receiving combined therapy. Surprisingly, tumor growth inhibition was mediated by CD4 T cells. Together, these results clearly show that T cells from combined therapy elicit enhanced anti-tumor and functional activity, such as granzyme B and IFN γ production driven by CD4 T cells.

[000276] The results provided in the instant disclosure demonstrate that treatment with the combination of SV.IL12 and anti-OX40 monoclonal antibody results in enhanced T cell migration and intratumoral T cell immunity. Specifically, CXCR3 levels were significantly upregulated on CD4 T cells following combination therapy. In contrast, CXCR3 expression on CD8 T cells only appeared later on in treatment, indicating that CD4 T cells are first recruited to the inflamed site followed by CD8 T cells. Combination therapy also enhanced

the production of CXCL9 and CXCL10 in the tumor microenvironment, indicating that CXCR3 positive T cells migrate to the tumor site. These results clearly show that the combination of SV.IL12 and anti-OX40 monoclonal antibody alter the tumor microenvironment by facilitating T cell infiltration via modulation of the CXCR3/CXCL9-11 axis. Not only did combination therapy increase T cell infiltration but CD4 as well as CD8 T cells also demonstrated enhanced functional activity in the tumor, as judged by the Ki-67 and granzyme B expression. These results indicate that the presence of activated T cells in the tumor microenvironment exert anti-tumor activity which inhibits tumor growth. Enhanced iNOS production was also demonstrated in tumors treated with combination therapy. Interestingly, the amount of iNOS inversely correlated with arginase1 production, indicating a repolarization of tumor associated macrophages from the M2-like (pro-tumor) into M1-like (anti-tumor) phenotype during combination therapy.

[000277] Thus, the data provided herein clearly shows that even in absence of direct Sindbis virus infectivity, SV.IL12 in combination with an anti-OX40 monoclonal antibody alter the tumor microenvironment by enhancing T cell infiltration and intratumoral T cell immunity, especially against low immunogenic tumors. The synergistic therapeutic efficacy of the systemic administration of the combination is driven by T cell modulation and reprogramming of its metabolic state, in order to enhance the anti-tumor response in the periphery and in the tumor microenvironment. Furthermore, the use of Sindbis virus allows these metabolically reprogrammed T cells to better infiltrate the tumor microenvironment, which is crucial for an adequate immunotherapy.

[000278] Sindbis Viral Vector and Anti-4-1BB Monoclonal Antibody

[000279] The present disclosure also provides methods for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount (a) a Sindbis viral vector and (b) an anti-4-1BB monoclonal antibody or a nucleic acid encoding same, thereby treating cancer in the subject. The present disclosure further provides *in vitro* or *ex vivo* methods for treating cancer or assessing the treatment of cancer in a subject comprising contacting a biological sample from the subject with (a) a Sindbis viral vector and (b) an anti-4-1BB monoclonal antibody or a nucleic acid encoding same. Preferably, the Sindbis viral vector does not comprise an endogenous nucleic acid encoding

any protein. Sindbis viral vectors were produced as described in US Patent No. 8,093,021 (incorporated herein by reference in its entirety).

[000280] The Sindbis viral vector is replication defective. The Sindbis viral vector can comprise a nucleic acid sequence encoding a therapeutic protein, an immunostimulatory protein or an immunomodulatory protein. The Sindbis viral vector can comprise the nucleic acid encoding the therapeutic protein, an immunostimulatory protein or an immunomodulatory protein and further comprise the nucleic acid encoding the anti-4-1BB monoclonal antibody. The Sindbis viral vector can comprise a nucleic acid sequence encoding LacZ (lac operon structural gene lacZ encoding β -galactosidase), Flue (firefly luciferase) or GFP (green fluorescent protein). The Sindbis viral vector can comprise the nucleic acid encoding LacZ, Flue or GFP and further comprise the nucleic acid encoding the anti-4-1BB monoclonal antibody.

[000281] The Sindbis viral vector can comprise a nucleic acid encoding an anti-4-1BB antibody heavy chain comprising the heavy chain complementarity determining region 1 (HCDR1), HCDR2 and HCDR3 amino acid sequences as follows: HCDR1: GFIFSFDMA (SEQ ID NO: 16), HCDR2: SISPDGSIPYYRDSVK (SEQ ID NO: 17) and HCDR3: RSYGGYSELDY (SEQ ID NO: 18).

[000282] The Sindbis viral vector can comprise a nucleic acid encoding an anti-4-1BB heavy chain comprising the amino acid sequence of SEQ ID NO:19 shown in the following Table.

anti-4-1BB heavy chain amino acid
DVQLVESGGGLVQPGRSLKLSCAAS GFIFSFDMA WVRQAPTQGLEWVAS SISPDGSIPYYRDSVK GRFTVSRENAK SSLYLQMDSLRSEDATATYYCARR RSYGGYSELDY WGQGVMTVSS (SEQ ID NO: 19).

[000283] The Sindbis viral vector can comprise a nucleic acid encoding an anti-4-1BB antibody light chain comprising the light chain complementarity determining region 1 (LCDR1), LCDR2 and LCDR3 amino acid sequences as follows: LCDR1: QASQDIGNWLA (SEQ ID NO: 20), LCDR2: GSTSLAD (SEQ ID NO: 21) and LCDR3: LQAYGAPW (SEQ ID NO: 22).

[000284] The Sindbis viral vector can comprise a nucleic acid encoding an anti-4-1BB light chain comprising the amino acid sequence of SEQ ID NO:23 shown in the following Table.

anti-4-1BB light chain amino acid
DIQMTQSPASLSASLEEIVTITCQASQDIGNWLAWYHQKPGKSPQLLIYGSTSLADGVPSRFSGSSSGSQYSLKIS RLQVELDIGIYYCLQAYGAPWTFGGGTKLELK (SEQ ID NO: 23)

[000285] The Sindbis viral vector can comprise a nucleic acid encoding anti-4-1BB antibody heavy chain CDR1, CDR2 and CDR3 amino acid sequences that binds to a target antigen comprising the amino acid sequence of SEQ ID NO:24 shown in the following Table.

anti-4-1BB target antigen					
1	MGNNCYNVVV	IVLLLVGCEK	VGAVQNSCDN	CQPGTFCRKY	NPVCKSCPPS
51	TFSSIGGQPN	CNICRVCAGY	FRFKKFCSS	HNAECECIEG	FHCLGPQCTR
101	CEKDCRPGQE	LTQKGCKTCS	LGTENDQNGT	GVCRPWTNCS	LDGRSVLKTG
151	TTEKDVVCGP	PVVSFSPSTT	ISVTPEGGPG	GHSLOVLTFL	LALTSALLLA
201	LIFITLLFSV	LKWIRKKFPH	IFKQPFKTT	GAAQEEDACS	CRCPQEEEGG
251	GGGYEL	(SEQ ID NO: 24)			

[000286] The Sindbis viral vector can comprise a nucleic acid encoding a 4-1BB heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 19 and a nucleic acid encoding a 4-1BB light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 23.

[000287] The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody light chain CDR1, CDR2 and CDR3 amino acid sequences that binds to a target antigen (4-1BB antigen) of the amino acid sequence of (SEQ ID NO: 24). The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody heavy chain amino acid sequence that binds to a target antigen (4-1BB antigen) of the amino acid sequence of (SEQ ID NO: 24). The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody light chain amino acid sequence that binds to a target antigen (4-1BB antigen) of the amino acid sequence of (SEQ ID NO: 24).

[000288] The Sindbis viral vector can comprise a nucleic acid encoding an anti-4-1BB antibody heavy chain comprising the heavy chain complementarity determining region 1 (HCDR1), HCDR2 and HCDR3 amino acid sequences of SEQ ID NOs: 16, 17 and 18, respectively. The Sindbis viral vector can comprise the nucleic acid encoding an anti-4-1BB antibody heavy chain comprising the amino acid sequence of SEQ ID NO: 19. The Sindbis viral vector can comprise a nucleic acid encoding an anti-4-1BB antibody heavy chain comprising the light chain complementarity determining region 1 (LCDR1), LCDR2 and LCDR3 amino acid sequences of SEQ ID NOs: 20, 21 and 22, respectively. The Sindbis viral vector can comprise

the nucleic acid encoding an anti-4-1BB antibody light chain comprising the amino acid sequence of SEQ ID NO: 23.

[000289] The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody heavy chain CDR1, CDR2 and CDR3 amino acid sequences that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24. The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody light chain CDR1, CDR2 and CDR3 amino acid sequences that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24. The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody heavy chain amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24. The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody light chain amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24.

[000290] The immunostimulatory or immunomodulatory protein can be IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-34, IL-35, IL-36, or any combination thereof. In a preferred aspect, the immunostimulatory or immunomodulatory protein is IL-12. The anti-4-1BB antibody can be urelumab, utomilumab or a combination thereof. The anti-4-1BB antibody can be InVivoMAb anti-mouse 4-1BB (BioXCell, Clone: LOB12.3, Cat.No. BE0169).

[000291] The Sindbis viral vector and the anti-4-1BB monoclonal antibody can be administered sequentially or concurrently. The Sindbis viral vector can be administered systemically. The anti-4-1BB monoclonal antibody can be administered systemically. Both the Sindbis viral vector and the anti-4-1BB monoclonal antibody, or a nucleic acid encoding same, can be administered systemically. The Sindbis viral vector can be administered parenterally. The anti-4-1BB monoclonal antibody can be administered parenterally. Both the Sindbis viral vector and the anti-4-1BB monoclonal antibody, or a nucleic acid encoding same, can be administered parenterally. The Sindbis viral vector can be administered intraperitoneally. The anti-4-1BB monoclonal antibody can be administered intraperitoneally. Both the Sindbis viral vector and the anti-4-1BB monoclonal antibody, or a nucleic acid encoding same, can be administered intraperitoneally.

[000292] A “monoclonal antibody” as disclosed herein, can be a full-length antibody or an antigen binding fragment thereof, wherein the “antigen binding fragment” is a fragment of the full length antibody that retains binding to the target antigen of the said monoclonal antibody. For example, a monoclonal antibody against 4-1BB or an anti-4-1BB monoclonal antibody, as described herein, can be a full length antibody against 4-1BB antibody or an antigen binding fragment thereof, wherein the fragment retains binding to the 4-1BB receptor on a cell surface. An “antigen-binding fragment” of an anti-4-1BB antibody, as described herein can include any fragment selected from the group consisting of Fv, Fav, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, scFv-CH₃, scFv-Fc, and diabody fragments.

[000293] The cancer can be a solid cancer or a liquid/hematologic cancer. The cancer can comprise metastatic cancer. The cancer can comprise a solid tumor. The cancer can be a carcinoma, a lymphoma, a blastoma, a sarcoma, a leukemia, a brain cancer, a breast cancer, a blood cancer, a bone cancer, a lung cancer, a skin cancer, a liver cancer, an ovarian cancer, a bladder cancer, a renal cancer, a gastric cancer, a thyroid cancer, a pancreatic cancer, an esophageal cancer, a prostate cancer, a cervical cancer or a colorectal cancer. In one preferred aspect, the cancer is a lymphoma. In one preferred aspect, the cancer is a B cell lymphoma.

[000294] The present disclosure provides a Sindbis viral vector comprising a nucleic acid encoding encoding a therapeutic protein, an immunostimulatory protein or an immunomodulatory protein and a nucleic acid encoding an anti-4-1BB monoclonal antibody. The present disclosure provides a Sindbis viral vector comprising a nucleic acid encoding encoding LacZ, Flue or GFP and a nucleic acid encoding an anti-4-1BB monoclonal antibody. The present disclosure also provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding a therapeutic protein, an immunostimulatory protein or an immunomodulatory protein and (b) a Sindbis viral vector comprising a nucleic acid encoding an anti-4-1BB monoclonal antibody. The present disclosure also provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding LacZ, Flue or GFP and (b) a Sindbis viral vector comprising a nucleic acid encoding an anti-4-1BB monoclonal antibody. The present disclosure further provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding a therapeutic protein, an immunostimulatory protein or an immunomodulatory protein and (b) an anti-4-1BB monoclonal antibody or a nucleic acid encoding same. The

present disclosure provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding encoding LacZ, Flue or GFP and (b) an anti-4-1BB monoclonal antibody or a nucleic acid encoding same.

[000295] The Sindbis viral vector can comprise a nucleic acid encoding an anti-4-1BB antibody heavy chain comprising the heavy chain complementarity determining region 1 (HCDR1), HCDR2 and HCDR3 amino acid sequences of SEQ ID NOs: 16, 17 and 18, respectively. The Sindbis viral vector can comprise the nucleic acid encoding an anti-4-1BB antibody heavy chain comprising the amino acid sequence of SEQ ID NO: 19. The Sindbis viral vector can comprise a nucleic acid encoding an anti-4-1BB antibody heavy chain comprising the light chain complementarity determining region 1 (LCDR1), LCDR2 and LCDR3 amino acid sequences of SEQ ID NOs: 20, 21 and 22, respectively. The Sindbis viral vector can comprise the nucleic acid encoding an anti-4-1BB antibody light chain comprising the amino acid sequence of SEQ ID NO: 23.

[000296] The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody heavy chain CDR1, CDR2 and CDR3 amino acid sequences that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24. The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody light chain CDR1, CDR2 and CDR3 amino acid sequences that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24. The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody heavy chain amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24. The Sindbis viral vector can comprise the nucleic acid encoding anti-4-1BB antibody light chain amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 24.

[000297] The immunostimulatory or immunomodulatory protein can be IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-34, IL-35, IL-36, or any combination thereof. In a preferred aspect, the immunostimulatory or immunomodulatory protein is IL-12. Additional cytokines include IL-18-IL-36. In addition to CCL17, other chemokines can also be used, including, but not limited to, CCL1-CCL27 and other CC chemokines, CXCL1-CXCL13 and other CXC chemokines, C chemokines, and CX3C chemokines. Cytokine or chemokine receptors

and soluble receptors can also be used. Additional immune modulators that can be used include TGF- β and TNF α . In addition, different combinations of the above-mentioned (or alternative) cytokines can be used.

[000298] A “monoclonal antibody” as disclosed herein, can be a full-length antibody or an antigen binding fragment thereof, wherein the “antigen binding fragment” is a fragment of the full length antibody that retains binding to the target antigen of the said monoclonal antibody. For example, a monoclonal antibody against 4-1BB or an anti-4-1BB monoclonal antibody, as described herein, can be a full length antibody against 4-1BB antibody or an antigen binding fragment thereof, wherein the fragment retains binding to the 4-1BB receptor on a cell surface. An “antigen-binding fragment” of an anti-4-1BB antibody, as described herein can include any fragment selected from the group consisting of Fv, Fav, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, scFv-CH₃, scFv-Fc, and diabody fragments.

[000299] Sindbis virus can be administered at least one time, at least two times, at least three times, at least four times or at least five times per week. Sindbis virus can be administered for at least one week, at least two weeks, at least three weeks, or at least four weeks. Sindbis virus can be administered from $10^6 - 10^9$ TU/mL. Preferably, Sindbis virus can be administered from $10^6 - 10^9$ TU/mL.

[000300] An anti-4-1BB monoclonal antibody can be administered at least one time, at least two times, at least three times, at least four times or at least five times per week. An anti-4-1BB monoclonal antibody can be administered for at least one week, at least two weeks, at least three weeks, or at least four weeks. An anti-4-1BB monoclonal antibody can be administered from 25 μ g – 500 μ g, 25 μ g – 450 μ g, 50 μ g – 400 μ g, from 50 μ g – 350 μ g, from 50 μ g – 300 μ g, from 50 μ g – 250 μ g, from 50 μ g – 200 μ g, from 50 μ g – 150 μ g or from 50 μ g – 100 μ g. An anti-4-1BB monoclonal antibody can be administered at 50 μ g. An anti-4-1BB monoclonal antibody can be administered at 50 μ g once a week for three weeks. An anti-4-1BB monoclonal antibody can be administered at 250 μ g. An anti-4-1BB monoclonal antibody can be administered at 250 μ g three times week for two weeks. An anti-4-1BB monoclonal antibody can be administered at 350 μ g. An anti-4-1BB monoclonal antibody can be administered at 350 μ g three times week for two weeks.

[000301] The results provided in the instant disclosure demonstrate that administration of the combination of Sindbis virus and anti-4-1BB monoclonal antibody resulted in

complete tumor regression in an lymphoma *in vivo* model and that this therapeutic effect was dramatically more effective when compared to either Sindbis virus or anti-4-1BB monoclonal antibody treatment alone. Tumor elimination involves a synergistic effect of the combination that significantly boosts T cell cytotoxicity, IFN γ production, T cell proliferation, migration, and glycolysis. As described in more detail below, the data identified the molecular pathways, including upregulated cytokines, chemokines and metabolic pathways in T cells that are triggered by the combined therapy and help to achieve a highly effective anti-tumor response.

[000302] The results provided in the instant disclosure demonstrate that administration of the combination of Sindbis virus and anti-4-1BB monoclonal antibody resulted in increased T cell cell cycle progression, cytokine production and activation. T cell proliferation is critical for an effective anti-tumor response.

[000303] The results provided in the instant disclosure demonstrate that administration of the combination of Sindbis virus and anti-4-1BB monoclonal antibody resulted in increased cytotoxicity (e.g., increased cytotoxic T cell function). Specifically, genes such as *Gzmb* (granzyme B), *Prfl* (perforin) and *Klrl* (NKG2D) are significantly upregulated in T cells (particularly CD8 T cells) following administration of Sindbis virus and anti-4-1BB monoclonal antibody.

[000304] The results provided in the instant disclosure demonstrate that administration of the combination of Sindbis virus and anti-4-1BB monoclonal antibody resulted in increased IFN γ production from T cells and Th1 differentiation. The combination of Sindbis virus and anti-4-1BB monoclonal antibody upregulated the expression of *STAT4*, *Ccr5*, *Cxcr3*, *Havcr2* (Tim3), *IL12rb1* and *Klrl* in T cells, which are required for the development of Th1 cells from naïve CD4⁺ T cells and IFN γ production. This increase was independent of the presence or absence of TAA. The combination of Sindbis virus and anti-4-1BB monoclonal antibody increased IFN γ production from both CD4 and CD8 T cells (with a larger portion CD4 T cells producing IFN γ) and demonstrated that antigen presenting cells (APCs) are essential for helping T cells produce IFN γ . The combination of Sindbis virus and anti-4-1BB monoclonal antibody also increased T-bet in T cells. T-bet is a key transcription factor which is essential for type I immune response (IFN γ production, T cell cytotoxicity) and memory T cell differentiation. Thus, this

indicates that the combination of Sindbis virus and anti-4-1BB monoclonal antibody boosts the type I immune response, which is critical for controlling tumor growth. The combination of Sindbis virus and anti-4-1BB monoclonal antibody also increased Eomesodermin (EOMES) in T cells. EOMES, another important transcription factor, is upregulated in activated T cells and is essential for memory CD8 T cell development.

[000305] The results provided in the instant disclosure demonstrate that administration of the combination of Sindbis virus and anti-4-1BB monoclonal antibody resulted in increased chemotaxis, adhesion and enhanced T cell infiltration and activation in tumors. Specifically, the combination significantly upregulates CD11a and ICAM-1(CD54) in both CD4 and CD8 T cells, which are two adhesion molecules expressed on activated T cells and are essential for the formation of immune synapses between T cells and APCs and are also required for T cell/T cell homotypic aggregation and activation. The combination of Sindbis virus and anti-4-1BB monoclonal antibody also significantly upregulated OX40 and ICOS in T cells. OX40 engagement promotes effector T cell function and survival and ICOS is another key CD4 T cell costimulatory molecule. Tumor infiltrating lymphocytes play a critical anti-tumor role and are an important marker for prognosis. The percentage of CD3 and CD8 T cells increased about two-fold following combination treatment. Thus, these results demonstrate that combination treatment enhanced T cell infiltration, division, activation, cytotoxicity and downregulated the inhibitory Treg population.

[000306] The results provided in the instant disclosure demonstrate that administration of the combination of Sindbis virus and anti-4-1BB monoclonal antibody resulted in enhanced T cell glycolysis and oxidative phosphorylation. T cell activation requires a quick consumption of energy through both enhanced glycolysis and oxidative phosphorylation. Metabolic switch is a major feature of T cell activation and memory T cell development. Upregulation of glycolysis genes quickly produce ATP and supports T cell migration and cytotoxicity in hypoxic or acidific microenvironments (such as in and around a tumor). The instant results demonstrate that combination treatment significantly increased both oxygen consumption rate (OCR, represents oxidative phosphorylation) and extracellular acidification rate (ECAR, represents glycolysis). This indicates that both glycolysis and oxidative phosphorylation are activated in combination treated T cells.

[000307] The results provided in the instant disclosure demonstrate that mice cured by the administration of the combination of Sindbis virus and anti-4-1BB monoclonal antibody are completely protected from cancer rechallenge demonstrating that these mice acquired long lasting antitumor immunity.

[000308] The conventional view of oncolytic virus therapy against tumors is that it requires selective infection of cancer cells resulting in the induction of cancer cell lysis and apoptosis. Tumor specific antigens (TAAs), released from dead tumor cells, attract and further stimulate an antitumor immune response. The data presented herein demonstrates that encoding a TAA is not necessary for the combination of Sindbis virus and anti-4-1BB monoclonal antibody to be fully successful in eradicating growing tumors.

[000309] The quick inhibition of tumor growth is critical for cancer therapy because tumor cells undergo exponentially rapid division. However, the induction of adaptive immunity and establishment of tumor specific immunity takes a long time. An ideal therapy requires an early, quick reduction of tumor burden, and a later induction of anti-tumor specificity that prevents relapse. The data presented herein demonstrates that the combination of Sindbis virus and anti-4-1BB monoclonal antibody treatment induced massive T cell activation due to viral induced immune response. This massive activation helps to control the tumor in a TAA nonspecific manner.

[000310] It was shown herein that both NKG2D (KLRKI) and granzyme B are highly expressed under combination treatment. This massive nonspecific activation is critical for controlling tumor growth at an early time point (day 7). This step is also important for inducing anti-tumor specificity that is mediated by TAAs released from dead tumor cells due to nonspecific killing. After tumor regression, T cells from treated animals were able maintain the ability to produce IFN γ and acquired immunological memory to rapidly reject tumor rechallenges. IFN γ production from purified T cells of cured mice was significantly enhanced after encountering tumor cells. This demonstrates that anti-tumor specificity is fully established in cured mice.

[000311] The data also shows that Sindbis viral infection of tumor cells, inclusion of dendritic cells and lymphodepletion are not necessary for successful cancer treatment. The omission of these additional features decreases costs, any risks related to toxicity and infection.

[000312] Thus, the data provided herein demonstrates that the combination of Sindbis virus and anti-4-1BB monoclonal antibody completely eradicated a B-cell lymphoma in a preclinical mouse model, a result that could not be achieved with either treatment alone. Tumor elimination involves a synergistic effect of the combination that significantly boosts T cell cytotoxicity, IFN- γ production, migration, tumor infiltration and oxidative phosphorylation. In addition, all mice that survived after treatment developed long lasting antitumor immunity.

[000313] Sindbis Viral Vector and Sindbis Viral Vector NY-ESO-1

[000314] The present disclosure also provides methods for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1, thereby treating cancer in the subject. The present disclosure also provides methods for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a nucleic acid encoding NY-ESO-1, thereby treating cancer in the subject.

[000315] The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit (IL-12 α , IL-12, p35 subunit) and interleukin-12 beta subunit (IL-12 β , IL-12, p40 subunit). The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit of GenBank accession no. M86672 and interleukin-12 beta subunit of GenBank accession no. M86671. The nucleic acid encoding interleukin-12 alpha subunit comprises the nucleic acid sequence of SEQ ID NO: 1. The nucleic acid encoding interleukin-12 beta subunit comprises the nucleic acid sequence of SEQ ID NO: 2.

[000316] The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit of amino acid sequence of GenBank accession no. AAA39292.1 and interleukin-12 beta subunit of amino acid sequence of GenBank accession no. AAA39296.1. The amino acid sequence of the interleukin-12 alpha subunit is of amino acid sequence of SEQ ID NO: 3. The amino acid sequence of the interleukin-12 beta subunit is of amino acid sequence of SEQ ID NO: 4.

[000317] The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of NCBI Reference accession no. NM_001327.1. The nucleic acid encoding NY-ESO-1 comprises the nucleic acid sequence of SEQ ID NO:14 shown in the following Table.

Nucleic acid sequence encoding NY-ESO-1 (NM_001327.1)					
1	agcagggggc	gctgtgtgta	ccgagaatac	gagaatacct	cgtggggcct
61	ctgagagccg	ggcagaggct	ccggagccat	gcaggccgaa	ggccggggca
121	gacggggcga	gctgatggcc	caggaggccc	tggcattcct	gatggcccag
181	tggcggccca	ggagaggcgg	gtgccacggg	cggcagaggt	ccccggggcg
241	aagggcctcg	gggcccggag	gaggcgcccc	gcgggggtccg	catggcgggc
301	gctgaatgga	tgctgcagat	gcggggccag	ggggccggag	agccgcctgc
361	cctcgccatg	ccttttcgca	cacccatgga	agcagagctg	gcccgcagga
421	ggatgccccca	ccgcttcccg	tgccaggggt	gcttctgaag	gagttcactg
481	catactgact	atccgactga	ctgctgcaga	ccaccgcca	ctgcagctct
541	ctgtctccag	cagctttccc	tgttgatgtg	gatcacgcag	tgctttctgc
601	ggctcagcct	ccctcagggc	agaggcgcta	agcccagcct	ggcgcccctt
661	gcctcctccc	ctaggggaatg	gtcccagcac	gagtggccag	ttcattgtgg
721	gtttgtcgct	ggaggaggac	ggcttacatg	tttgtttctg	tagaaaataa
781	cgaaaaaaaa	aaaaaaaaaa	aaaaaa	(SEQ ID NO: 14)	

[000318] The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of amino acid sequence of NCBI Reference accession no. NP_001318.1. The amino acid sequence of the NY-ESO-1 comprises the amino acid sequence of SEQ ID NO:15 shown in the following Table.

Amino acid sequence of NY-ESO-1 (NP_001318.1)					
1	mqaegrgtgg	stgdadgpgg	pgipdgpggn	agggpgeagat	ggrgprgaga
61	prgphggaas	glngccrcga	rgpesrllef	ylampfatpm	eaelarrsla
121	vllkeftvsg	niltirltaa	dhrlqlslis	sclqqlslm	witqcflpvf
(SEQ ID NO: 15)					

[000319] The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of NCBI Reference accession no. NM_001327.1. The nucleic acid encoding NY-ESO-1 comprises the nucleic acid sequence of SEQ ID NO: 14. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of amino acid sequence of NCBI Reference accession no. NP_001318.1. The amino acid sequence of the NY-ESO-1 comprises the amino acid sequence of SEQ ID NO: 15.

[000320] A replication defective Sindbis viral vector as described herein can be any replication defective Sindbis viral vector including a replication defective viral vector described, for example, in US Patent Nos. 7,303,898, 7,306,792, and 8,093,021. Replication defective vectors are preferred for use in the present invention in order to prevent infection of healthy tissues.

[000321] The Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and the Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 can be administered sequentially or concurrently. The Sindbis viral vector comprising a nucleic acid encoding interleukin-12 can be administered systemically. The Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 can be administered systemically. Both the Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and the Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 can be administered systemically. The Sindbis viral vector comprising a nucleic acid encoding interleukin-12 can be administered parenterally. The Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 can be administered parenterally. Both the Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and the Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 can be administered parenterally. The Sindbis viral vector comprising a nucleic acid encoding interleukin-12 can be administered intraperitoneally. The Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 can be administered intraperitoneally. Both the Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and the Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 can be administered intraperitoneally.

[000322] The cancer can be a solid cancer or a liquid/hematologic cancer. The cancer can comprise metastatic cancer. The cancer can comprise a solid tumor. The cancer can be a carcinoma, a lymphoma, a blastoma, a sarcoma, a leukemia, a brain cancer, a breast cancer, a blood cancer, a bone cancer, a lung cancer, a skin cancer, a liver cancer, an ovarian cancer, a bladder cancer, a renal cancer, a gastric cancer, a thyroid cancer, a pancreatic cancer, an esophageal cancer, a prostate cancer, a cervical cancer or a colorectal cancer. In one preferred aspect, the cancer is colon cancer. In one preferred aspect, the cancer is prostate cancer. In one preferred aspect, the cancer is ovarian cancer.

[000323] The present disclosure further provides a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a nucleic acid encoding NY-ESO-1. The present disclosure also provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and (b) a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1.

[000324] The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit (IL-12 α , IL-12, p35 subunit) and interleukin-12 beta subunit (IL-12 β , IL-12,

p40 subunit). The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit of GenBank accession no. M86672 and interleukin-12 beta subunit of GenBank accession no. M86671. The nucleic acid encoding interleukin-12 alpha subunit comprises the nucleic acid sequence of SEQ ID NO: 1. The nucleic acid encoding interleukin-12 beta subunit comprises the nucleic acid sequence of SEQ ID NO: 2.

[000325] The Sindbis viral vector can comprise the nucleic acid encoding interleukin-12 alpha subunit of amino acid sequence of GenBank accession no. AAA39292.1 and interleukin-12 beta subunit of amino acid sequence of GenBank accession no. AAA39296.1. The amino acid sequence of the interleukin-12 alpha subunit is of amino acid sequence of SEQ ID NO: 3. The amino acid sequence of the interleukin-12 beta subunit is of amino acid sequence of SEQ ID NO: 4.

[000326] The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of NCBI Reference accession no. NM_001327.1. The nucleic acid encoding NY-ESO-1 comprises the nucleic acid sequence of SEQ ID NO: 14. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of amino acid sequence of NCBI Reference accession no. NP_001318.1. The amino acid sequence of the NY-ESO-1 comprises the amino acid sequence of SEQ ID NO: 15.

[000327] The present disclosure provides methods for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of (a) a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 and (b) an anti-OX40 monoclonal antibody or a nucleic acid encoding same, thereby treating cancer in the subject.

[000328] The Sindbis viral vector can be replication defective. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 and can further comprise the nucleic acid encoding the anti-OX40 monoclonal antibody. The method can comprise administering a Sindbis viral vector comprising the nucleic acid encoding NY-ESO-1 and administering a Sindbis viral vector comprising the nucleic acid encoding the anti-OX40 monoclonal antibody. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of NCBI Reference accession no. NM_001327.1. The nucleic acid encoding NY-ESO-1 comprises the nucleic acid sequence of SEQ ID NO: 14. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of amino acid sequence of NCBI Reference accession no. NP_001318.1. The amino acid sequence of the NY-ESO-1 comprises the amino acid sequence of SEQ ID NO: 15.

[000329] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 5. The nucleic acid sequence encoding the anti-OX40 variable heavy chain is SEQ ID NO: 6. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 7. The nucleic acid sequence encoding the anti-OX40 variable light chain is SEQ ID NO: 8.

[000330] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain of amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain of amino acid sequence of SEQ ID NO: 7. The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 7.

[000331] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain comprising the amino acid sequence of SEQ ID NO: 9. The nucleic acid sequence encoding the an anti-OX40 antibody heavy chain is SEQ ID NO: 10.

[000332] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 antibody light chain comprising the amino acid sequence of SEQ ID NO: 11. The nucleic acid sequence encoding the anti-OX40 antibody light chain is SEQ ID NO: 12.

[000333] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence of SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain of SEQ ID NO: 10 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 12.

[000334] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain that is at least 70%, 75%, 80%, 85%,

90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 10 and an anti-OX40 antibody light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 12.

[000335] The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000336] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000337] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain amino acid sequence, and an anti-OX40 antibody variable light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain amino acid sequence, and an anti-OX40 antibody light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000338] The Sindbis viral vector and the anti-OX40 monoclonal antibody can be administered sequentially or concurrently. The Sindbis viral vector can be administered systemically. The anti-OX40 monoclonal antibody can be administered systemically. Both the Sindbis viral vector and the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be administered systemically. The Sindbis viral vector can be administered parenterally. The anti-OX40 monoclonal antibody can be administered parenterally. Both the Sindbis viral vector and the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be administered parenterally. The Sindbis viral vector can be administered intraperitoneally. The anti-OX40 monoclonal antibody can be administered intraperitoneally. Both the Sindbis viral vector and

the anti-OX40 monoclonal antibody, or a nucleic acid encoding same, can be administered intraperitoneally.

[000339] A “monoclonal antibody” as disclosed herein, can be a full-length antibody or an antigen binding fragment thereof, wherein the “antigen binding fragment” is a fragment of the full length antibody that retains binding to the target antigen of the said monoclonal antibody. For example, a monoclonal antibody against OX-40 or an anti-OX40 monoclonal antibody, as described herein, can be a full length antibody against OX40 antibody or an antigen binding fragment thereof, wherein the fragment retains binding to the OX40 receptor on a cell surface. An “antigen-binding fragment” of an anti-OX-40 antibody, as described herein can include any fragment selected from the group consisting of Fv, Fav, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, scFv-CH₃, scFv-Fc, and diabody fragments.

[000340] The cancer can be a solid cancer or a liquid/hematologic cancer. The cancer can comprise metastatic cancer. The cancer can comprise a solid tumor. The cancer can be a carcinoma, a lymphoma, a blastoma, a sarcoma, a leukemia, a brain cancer, a breast cancer, a blood cancer, a bone cancer, a lung cancer, a skin cancer, a liver cancer, an ovarian cancer, a bladder cancer, a renal cancer, a gastric cancer, a thyroid cancer, a pancreatic cancer, an esophageal cancer, a prostate cancer, a cervical cancer or a colorectal cancer. In one preferred aspect, the cancer is colon cancer. In one preferred aspect, the cancer is prostate cancer. In one preferred aspect, the cancer is ovarian cancer.

[000341] The present disclosure further provides a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 and a nucleic acid encoding an anti-OX40 monoclonal antibody. The present disclosure also provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 and (b) an anti-OX40 monoclonal antibody or a nucleic acid encoding same. The present disclosure also provides a composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 and (b) a Sindbis viral vector comprising a nucleic acid encoding an anti-OX40 monoclonal antibody. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of NCBI Reference accession no. NM_001327.1. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of NCBI Reference accession no. NM_001327.1. The nucleic acid encoding NY-ESO-1 comprises the nucleic acid sequence of SEQ ID NO: 14. The Sindbis viral vector can comprise the nucleic acid encoding NY-ESO-1 of amino acid sequence

of NCBI Reference accession no. NP_001318.1. The amino acid sequence of the NY-ESO-1 comprises the amino acid sequence of SEQ ID NO: 15.

[000342] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable heavy chain comprising the amino acid sequence of SEQ ID NO: 5. The nucleic acid sequence encoding the anti-OX40 variable heavy chain is SEQ ID NO: 6. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 variable light chain comprising the amino acid sequence of SEQ ID NO: 7. The nucleic acid sequence encoding the anti-OX40 variable light chain is SEQ ID NO: 8.

[000343] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain of amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain of amino acid sequence of SEQ ID NO: 7. The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 5 and a nucleic acid encoding a mouse anti-OX40 light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to amino acid sequence of SEQ ID NO: 7.

[000344] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain comprising the amino acid sequence of SEQ ID NO: 9. The nucleic acid sequence encoding the an anti-OX40 antibody heavy chain is SEQ ID NO: 10.

[000345] The Sindbis viral vector can comprise a nucleic acid encoding a mouse anti-OX40 antibody light chain comprising the amino acid sequence of SEQ ID NO: 11. The nucleic acid sequence encoding the anti-OX40 antibody light chain is SEQ ID NO: 12.

[000346] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence of SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain of SEQ ID NO: 10 and an anti-OX40 antibody light chain with an amino acid sequence of SEQ ID NO: 12.

[000347] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 9 and an anti-OX40 antibody light chain with an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%,

97%, 98%, 99% or 100% identical to SEQ ID NO: 11. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 10 and an anti-OX40 antibody light chain that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 12.

[000348] The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000349] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise the nucleic acid encoding an anti-OX40 antibody light chain with an amino acid sequence that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000350] The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody variable heavy chain amino acid sequence, and an anti-OX40 antibody variable light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13. The Sindbis viral vector can comprise a nucleic acid encoding an anti-OX40 antibody heavy chain amino acid sequence, and an anti-OX40 antibody light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13.

[000351] A “monoclonal antibody” as disclosed herein, can be a full-length antibody or an antigen binding fragment thereof, wherein the “antigen binding fragment” is a fragment of the full length antibody that retains binding to the target antigen of the said monoclonal antibody. For example, a monoclonal antibody against OX-40 or an anti-OX40 monoclonal antibody, as described herein, can be a full length antibody against OX40 antibody or an antigen binding fragment thereof, wherein the fragment retains binding to the OX40 receptor on a cell surface. An “antigen-binding fragment” of an anti-OX-40 antibody, as described herein can include any

fragment selected from the group consisting of Fv, Fav, F(ab')₂, Fab', dsFv, scFv, sc(Fv)₂, scFv-CH₃, scFv-Fc, and diabody fragments.

[000352] The results provided in the instant disclosure demonstrate that administration of a combination of IL-12 and NY-ESO-1, expressed by separate Sindbis virus vector synergistically enhances the survival rate of a subject bearing an established tumor. The results described herein show that mice transplanted with Alm5-2Fluc-17 ovarian cancer cells by re-injection to establish tumor as depicted in FIG. 19, when treated post-tumor establishment with a SV vector expressing NY-ESO-1 (SVNYESO) showed no enhancement of survival, with a percentage survival rate similar to untreated tumor bearing mice, thereby showing that some tumors are resistant to treatment with SV expressing a TAA, like NY-ESO-1. The results described herein show that treatment of the tumor bearing mice, with a SV expressing NYESO (SV-NYESO_SV-IL12) showed improvement in survival rate. The results show that surprisingly treatment with a 50% mix in one injection of a SV expressing IL-12 (SV-IL-12) and a SV expressing NYESO (SV-NYESO_SV-IL12), demonstrated synergistically enhanced survival as compared to mice treated with the SV-IL-12 or SV-NYESO. The results described herein clearly show the possibility of using a combination of SV vectors expressing IL-12 and NY-ESO-1, for treatment of cancers that may be resistant to treatment with a SV expressing a tumor associated antigen.

[000353] The results provided in the instant disclosure demonstrate that administration of a combination of IL-12 and NY-ESO-1, expressed by the same Sindbis virus vector synergistically enhances the survival rate of a subject bearing an established tumor. The results show that mice bearing established tumors of Alm5-2Fluc-17 ovarian cancer cells, when treated with a Sindbis viral vector that expresses both IL-12 and NYESO (SV-NYESO_SGP2_IL12), demonstrated synergistically enhanced survival as compared to mice treated with the SV-IL-12 or SV-NYESO. The results described herein clearly show the possibility of using a single SV vectors expressing both IL-12 and NY-ESO-1, for treatment of cancers that may be resistant to treatment with a SV expressing a tumor associated antigen.

[000354] Treating cancer means treating at least one symptom of cancer. Treating at least one symptom of cancer can include any of the following, or any combination thereof: inhibiting tumor growth, reducing tumor size, reducing tumor number, reducing tumor burden, preventing cancer recurrence, preventing metastasis of a primary tumor.

[000355] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Included in this definition are benign and malignant cancers. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, leukemia and germ cell tumors. More particular examples of such cancers include adrenocortical carcinoma, bladder urothelial carcinoma, breast invasive carcinoma, cervical squamous cell carcinoma, endocervical adenocarcinoma, cholangiocarcinoma, colon adenocarcinoma, lymphoid neoplasm diffuse large B-cell lymphoma, esophageal carcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, kidney chromophobe, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, acute myeloid leukemia, brain lower grade glioma, liver hepatocellular carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, mesothelioma, ovarian serous cystadenocarcinoma, pancreatic adenocarcinoma, pheochromocytoma, paraganglioma, prostate adenocarcinoma, rectum adenocarcinoma, sarcoma, skin cutaneous melanoma, stomach adenocarcinoma, testicular germ cell tumors, thyroid carcinoma, thymoma, uterine carcinosarcoma, uveal melanoma. Other examples include breast cancer, lung cancer, lymphoma, melanoma, liver cancer, colorectal cancer, ovarian cancer, bladder cancer, renal cancer or gastric cancer. Further examples of cancer include neuroendocrine cancer, non-small cell lung cancer (NSCLC), small cell lung cancer, thyroid cancer, endometrial cancer, biliary cancer, esophageal cancer, anal cancer, salivary, cancer, vulvar cancer, cervical cancer, Acute lymphoblastic leukemia (ALL), Acute myeloid leukemia (AML), Adrenal gland tumors, Anal cancer, Bile duct cancer, Bladder cancer, Bone cancer, Bowel cancer, Brain tumors, Breast cancer, Cancer of unknown primary (CUP), Cancer spread to bone, Cancer spread to brain, Cancer spread to liver, Cancer spread to lung, Carcinoid, Cervical cancer, Children's cancers, Chronic lymphocytic leukemia (CLL), Chrome myeloid leukemia (CML), Colorectal cancer, Ear cancer, Endometrial cancer, Eye cancer, Follicular dendritic cell sarcoma, Gallbladder cancer, Gastric cancer, Gastro esophageal junction cancers, Germ cell tumors, Gestational trophoblastic disease (GIT)), Hairy cell leukemia, Head and neck cancer, Hodgkin lymphoma, Kaposi's sarcoma, Kidney cancer, Laryngeal cancer, Leukemia, Gastric linitis plastica, Liver cancer, Lung cancer, Lymphoma, Malignant schwannoma, Mediastinal germ cell tumors, Melanoma skin cancer, Men's cancer, Merkel cell skin cancer, Mesothelioma, Molar pregnancy, Mouth and oropharyngeal cancer, Myeloma, Nasal and paranasal sinus cancer, Nasopharyngeal

cancer, Neuroblastoma, Neuroendocrine tumors, Non-Hodgkin lymphoma (NHL), Esophageal cancer, Ovarian cancer, Pancreatic cancer, Penile cancer, Persistent trophoblastic disease and choriocarcinoma, Pheochromocytoma, Prostate cancer, Pseudomyxoma peritonei, Rectal cancer, Retinoblastoma, Salivary gland cancer, Secondary' cancer, Signet cell cancer, Skin cancer, Small bowel cancer, Soft tissue sarcoma, Stomach cancer, T cell childhood non Hodgkin lymphoma (NHL), Testicular cancer, Thymus gland cancer, Thyroid cancer, Tongue cancer, Tonsil cancer, Tumors of the adrenal gland, Uterine cancer, Vaginal cancer, Vulval cancer, Wilms' tumor, Womb cancer and Gynaecological cancer. Examples of cancer also include, but are not limited to, Hematologic malignancies, Lymphoma, Cutaneous T cell lymphoma, Peripheral T cell lymphoma, Hodgkin's lymphoma, Non-Hodgkin's lymphoma, Multiple myeloma, Chrome lymphocytic leukemia, chronic myeloid leukemia, acute myeloid leukemia, Myelodysplastic syndromes, Myelofibrosis, Biliary tract cancer, Hepatocellular cancer, Colorectal cancer, Breast cancer, Lung cancer, Non-small cell lung cancer, Ovarian cancer, Thyroid Carcinoma, Renal Cell Carcinoma, Pancreatic cancer, Bladder cancer, skin cancer, malignant melanoma, merkel cell carcinoma, Uveal Melanoma or Glioblastoma multiforme.

[000356] The nucleotide sequences encoding the TAAs to be expressed by a Sindbis viral vector as described herein are well known in the art and can be easily obtained from the literature. For example, the sequence of NY-ES0-1, a testicular antigen aberrantly expressed in human cancers was published in 1997 (<http://www.pnas.org/content/94/5/1914.full> , Yao-Tseng Chen, Matthew J. Scanlant, Ugur Sahin, Ozlem Tiireci, Ali O. Guret, Solam Tsangt, Barbara Williamsont, Elisabeth Stockertt, Michael Pfreundschuh, and Lloyd J. Old, PNAS 1997.), whereas the Carcinoembryonic antigen sequence was published in 1987 (<http://mcb.asm.org/content/7/9/3221.short> Isolation and characterization of full-length functional cDNA clones for human carcinoembryonic antigen. N Beauchemin, .S. Benchimol, D Cournoyer, A Fuks and C P Stanners, Molecular and Cellular Biology.

[000357] Although in mice a single i.p. injection of the SV/TAA as described herein, is sufficient to elicit a detectable CD8⁺ mediated immune response directed against the tumor, other regimens may be necessary for achieving a maximal response. For example, between 1 and about 8 i.p. injections over a time period of between 1 week and many

weeks, with the possibility of injecting one or more booster injections 1 or more years later, may be preferably administered for a maximum effect.

EXAMPLES

[000358] Example 1: Sindbis with anti-OX40 enable immune responses to cold tumors

[000359] This study, investigates the therapeutic efficacy of a replication-deficient oncolytic viral vector called Sindbis Virus. Because Sindbis Virus (SV) is a blood-borne pathogen, vectors from this virus can be administered in the bloodstream via the intravenous (i.v.) and intraperitoneal (i.p.) routes, which greatly facilitates their delivery [Tseng, JC, et al., Nature Biotech., 2004]. Furthermore, SV was genetically modified to be replication-defective by splitting its genome and deleting the packaging signal to block viral assembly after viral replication [Bredenbeek PJ, et al., J. Virol. 1993]. This study shows that SV expressing the pro-inflammatory cytokine IL-12 (SV.IL12) activates T cells as well as enhances the expression of OX40 on CD4 T effector cells and, therefore, potentiates efficacy of the agonistic anti-OX40 antibody therapy. The data indicates that combination of SV.IL12 and anti-OX40 activates tumor immunity against low immunogenic tumors through the metabolic rewiring of T cells into highly activated effector cells. Furthermore, SV.IL12 in combination with anti-OX40 induces a marked immune cell infiltration into the tumor microenvironment. Considering that tumors tend to quickly escape the immune response by mutating or losing the expression of drug targets or tumor antigens targeted by the immune response, the treatment approach disclosed herein reduces the risk of developing tumor resistances and offers an attractive and safe strategy to change the immunogenic phenotype of various cancers without prior knowledge of tumor antigens.

[000360] The studies presented herein describe several, non-limiting examples of anti-OX-40 antibody, Sindbis viral vector (SV), Sindbis viral vector expressing IL-12 (SV.IL-12), Sindbis viral vector expressing an anti-OX-40 antibody and Sindbis viral vector expressing both IL-12 and an anti-OX-40 antibody. These examples are provided below to further illustrate different features of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not and are not intended to limit the claimed invention.

[000361] Materials and Methods

[000362] Cell lines

[000363] Baby hamster kidney (BHK), BALB/c colon carcinoma [CT26.WT (ATCC® CRL-2638™)] and FVB prostate carcinoma [MyC-CaP (ATCC® CRL-3255™)] cell lines were obtained from the American Type Culture Collection (ATCC). Firefly luciferase (Fluc)-expressing CT26 and MyC-CaP cells (CT26.Fluc and MyC-CaP.Fluc) were generated by stable transfection of pGL4.20_Fluc plasmid.

[000364] BHK cells were maintained in minimum essential α -modified media (α -MEM) (Corning CellGro) with 5% fetal bovine serum (FCS, Gibco) and 100 mg/ml penicillin-streptomycin (Corning CellGro). CT26.Fluc and MyC-CaP.Fluc cells were maintained in Dulbecco's modified Eagles medium containing 4.5 g/l Glucose (DMEM, Corning CellGro) supplemented with 10% FCS, 100 mg/ml penicillin-streptomycin, 7.5 μ g/ml Puromycin or 400 μ g/ml Gentamycin, respectively. All cell lines were cultured at 37 °C and 5% CO₂.

[000365] SV Production

[000366] SV-LacZ production and titering were done the same as previously described [Scherwitzl I, Mal Ther Oncolytics. 2018]. SV.IL12 and SY.Lacz vectors were produced as previously described [Subramanian A et al., Proc Natl Acad Sci US A. 2005; Leonard WJ et al., F1000Res. 2016; Rowell JF et al., J. Immunol. 1999; Metcalf TU et al., J. Virol. 2013]. All SV viral vectors used in these studies are replication-defective. Vectors were produced as previously described. SV.IL12 plasmid used in this study has been published in 2002 [Tseng JC et al., J Natl Cancer Inst. 2002]. To construct a Sindbis viral vector containing genes for interleukin 12 (IL-12), the Sindbis viral vector SinRep/2PSG was first constructed, which contains a secondary subgenomic promoter that is responsive to the Sindbis replicase. Two DNA oligonucleotide primers (sequence 5' CGCGTAAAGCATCTCTACGGTGGTCCTAATAGTGCATG-3'; SEQ ID NO: 29) and its complementary strand 5'CACTATTAGGACCACCGTCGAGATGCTTTA-3'; SEQ ID NO: 30) containing the subgenomic promoter sequence were annealed and ligated into the MluI and SphI sites of the SinRep plasmid. The murine IL-12 α subunit gene (mp35; ATCC 87596) and the IL-12 β subunit gene (mp40; ATCC 87595) were subcloned into the MluI and the StuI sites of SinRep/2PSG, respectively, to produce the Sin-Rep/IL12 plasmid.

[000367] SV empty is the same plasmid without an additional gene of interest (e.g.IL12). SV.Luc was generated as described [Tseng, JC et al., Nature Biotech., 2004]. SV.GFP was generated as published in 2012 [Suzme R et al., Cancer Gene Ther., 2012]. Briefly, plasmids carrying the replicon (e.g. SinRep-IL12 or SinRep-IL-12) or DHBB helper RNAs were

linearized with XhoI. In vitro transcription was performed using the mMessage mMachine RNA transcription kit (Ambion). Helper and replicon RNAs were then electroporated into BHK cells and incubated at 37°C in α MEM supplemented with 10% FCS. After 12 hours, the media was replaced with OPTI-MEM (GIBCO-BRL) supplemented with CaCl₂ (100 mg/l) and cells were incubated at 37°C. After 24 hours, the supernatant was collected, centrifuged to remove cellular debris, and frozen at -80°C. Vectors were titrated as previously described [Tseng JC et al., J. Natl. Can. Inst., 2002].

[000368] In vivo experiments and tumor models

[000369] All experiments were performed in accordance with the Institutional Animal Care and Use Committee of New York University Health. Six to 12-week old female BALB/c mice were purchased from Taconic (Germantown, NY) and age matched male FVB/NJ mice were purchased from Jackson Laboratory.

[000370] Tumor inoculation and animal studies

[000371] Treatment started on day 4 after i.p. inoculation of 7×10^4 CT26.Fluc cells or 105 cells of MyC-CaP.Fluc in 500 μ l OPTI-MEM. For treatments, mice were randomized and SV (10^7 TU/ml), in a total volume of 500 μ l, was injected i.p. into the left side of the animal once for CT26.Fluc and 4 days a week (days 1, 2, 3, 4) for a total of 4 weeks for MyC-CaP.Fluc inoculated mice. The immune checkpoint inhibitor anti-OX40 (clone OX-86, BioXCell) was injected i.p. into the left side of the animal at a dose of 250 μ g per injection (1x/week for the CT26.Fluc and 3x/week for MyC-CaP.Fluc tumor bearing mice). Therapeutic efficacy of the treatment was monitored in two ways: tumor luminescence and survival. Noninvasive bioluminescent imaging was performed using the IVIS Spectrum imaging system (Caliper Life Science) at the indicated time points and tumor growth was quantified using the Living Image 3.0 software (Caliper Life Science) as previously described 86. Relative tumor growth for each mouse was calculated dividing total body counts of a given day by total body counts of the first IVIS image. Survival was monitored and recorded daily.

[000372] Flow cytometry

[000373] For flow cytometry analysis, spleens and tumors were harvested from mice and processed as previously described [Scherwitzl I, et al., Mol. Ther. Oncol, 2018]. The extracted tumors were chopped into small pieces and incubated with a digestive mix containing RPMI with

collagenase IV (50 µg/ml) and DNase I (20 U/ml) for 1 hour at 37 °C. Tumor samples had additional hyaluronidase V (50 µg/ml) in the digestive mix.

[000374] Splens and digested tumors were mashed through a 70-µm strainer before red blood cells were lysed using ammonium-chloride-potassium (ACK) lysis (Gibco). Cells were washed with PBS containing 1% FCS and surface receptors were stained using various antibodies. Fluorochrome-conjugated antibodies against mouse CD3, CD4, CD44, ICOS, OX40, CD69, Foxp3, Granzyme B and Tbet, were purchased from Biolegend. Fluorochrome-conjugated antibodies against mouse CD8a were purchased from BD Biosciences. Mitotracker Deep Red FM, Mitotracker Green and Fluorochrome-conjugated antibodies against CXCR3 and Ki67 were purchased from Thermofisher. Stained cells were fixed with PBS containing 4% Formaldehyde. For intracellular staining, the forkhead box P3 (FOXP3) staining buffer set was used (eBioscience). Flow cytometry analysis was performed on a LSR II machine (BD Bioscience) and data were analyzed using FlowJo (Tree Star).

[000375] T cell isolation

[000376] Total T cells were freshly isolated with the EasySep™ mouse T Cell Isolation Kit. Freshly isolated lymphocytes were depleted of either CD4 or CD8 specific T cells using EasySep™ mouse CD4 and CD8 Positive Selection Kits II. Isolation of T cells and depletions were performed according to the manufacturer's protocols (Stemcell Technologies).

[000377] Enzyme-Linked Immunospot (ELISPOT)

[000378] Enzyme-linked immunospot was performed as previously described [Scherwitzl I, et al., Mol. Ther. Oncol, 2018]. Splenocytes and T cells were prepared as described for flow cytometry. Mouse IFN γ ELISPOT was performed according to the manufacturer's protocol (BD Bioscience). Lymphocytes (4 x 10⁵ cells) and isolated (8 x 10⁴) T cells were plated per well overnight in RPMI supplemented with 10% FCS. No additional stimulus was used in the ELISPOT. As positive control, cells were stimulated with 5ng/ml PMA+1µg/ml Ionomycin.

[000379] Ex vivo cytotoxic assay

[000380] T cells were isolated on day 7 and day 14 during treatment. 8 x 10⁵/ml T cells were co-cultured with CT26.Fluc cells (2 x 10⁴/ml) or MyC-CaP.Fluc cells (2 x 10⁴/ml) in a 24 well plate for 2 days in 1 ml RPMI supplemented with 10% FCS. Cells were washed with PBS and lysed with 100 µl of M-PER Mammalian Protein Extraction Reagent (Promega) per well. Cytotoxicity was assessed based on the viability of CT26 cells, which was determined by

measuring the luciferase activity in each well. Luciferase activity was measured by adding 100 µl of Steady-Glo reagent (Promega) to each cell lysate and measuring the luminescence using a GLOMAX portable luminometer (Promega).

[000381] CD8+ and CD4+ T-cell depletion in vivo

[000382] CD8+ T cells were depleted using anti-CD8 antibody (clone 2.43) (Bio X cell, Lebanon, NH). 0.1 mg antibody in 0.2ml PBS was injected into each mouse, starting 1 day before the first SV treatment, and then every 4 days for 2 weeks. CD4+ T cells were depleted using anti-CD4 antibody (clone GK 1.5) (Bio X cell, Lebanon, NH). 0.4 mg were injected into each mouse, starting day 1 before the first treatment. Control mice were injected with PBS and isotype controls.

[000383] Quantitative real-time PCR

[000384] RNA was extracted from tumor samples using RNeasy Kit (Qiagen), followed by cDNA synthesis with the iScript II Kit (Bio-Rad). qRT-PCR was performed using iQTM SYBR Green Supermix (Biorad) and an StepOne™ Real-Time PCR Detection System (Applied Biosystems). PCR conditions were as follows: 95 °C for 10 min, followed by 40 cycles (94 °C for 30 s, 58 °C for 30 s) of amplification. For quantitation, CT values were normalized to GAPDH and expression was analysed using the 2^{-ΔΔCT} method. Primers for CXCL9, CXCL10 and GAPDH were used. CXCL9 (Forward: GAAGTCCGCTGTTCTTTTCC; SEQ ID NO: 25 Reverse: TTGACTTCCGTTCTTCAGTG; SEQ ID NO: 26), CXCL10 (Forward: GCTGCAACTGCATCCATATC; SEQ ID NO: 27; Reverse: AGGAGCCCTTTTAGACCTTT; SEQ ID NO: 28).

[000385] Transcriptome analysis of T cells

[000386] Total RNA was extracted from freshly isolated T cells on day 7 of treatment from spleens using RNeasy Kit (Qiagen). For each group, 3 BALB/C mice or 3 FVB/J mice were used for biological repeats. RNA-seq was done by NYUMC Genome Center. RNA quality and quantity was analyzed. RNAseq libraries were prepared and loaded on the automated HiSeq 4000 Sequencing System (Illumina) and run as single 50 nucleotide reads.

[000387] Alignment and Differential Expression Analysis

[000388] Sequences were aligned to the mm10 mouse genome using Bowtie software, Version 1.0.0⁸⁷ [Langmead R et al., Genome Biol. 2009] with two mismatches allowed. Uniquely mapped reads were further processed by removing PCR duplicates with Picard

(“Picard Tools.” Broad Institute, GitHub repository. <http://broadinstitute.github.io/picard/>) MarkDuplicates and transcripts were counted using HTSeq⁸⁸ and differential gene expression was performed between all groups using DESeq [Anders S et al., Genome Biol. 2010]. Differences in gene expression were considered significant if $\text{padj} < 0.05$.

[000389] GSEA and Enrichment Map Analysis

[000390] The network-based method enrichment map 90 was used for gene-set enrichment visualization and interpretation of data. As a follow up analysis of Gene-Set Enrichment Analysis2 (GSEA) [Mootha VK et al., Nat. Genet., 2003] it reduces redundancy and helps in the interpretation of large gene sets and helps to quickly identify major enriched functional themes in the gene expression data. To perform this analysis, we first assigned a unique row identifier for each transcript and obtained differentially expressed genes through DESeq [Anders S et al., Genome Biol. 2010]. These genes were then ranked and GSEA was performed in Gene Pattern 92 server using GSEA pre-ranked module. We then obtained the gene identifiers corresponding to the gene names using the Bioconductor package ‘org.Mm.eg.db’ and the resulting positively and negatively regulated gene identifiers were used to generate enrichment maps in Cytoscape [Shannon P et al., Genome Res. 2003]. Expression heatmap is drawn by Morpheus (<https://software.broadinstitute.org/morpheus/>). Highest and lowest expression for each gene (row min. and row max.) were displayed as red or blue color, respectively.

[000391] Measurement of Oxygen Consumption and Extracellular Acidification Rates of T cells

[000392] T cell metabolic output was measured by Seahorse technology as previously described [Scharping NE et al., Cancer Immunol. Res., 2017]. Purified T cells were plated at 6×10^5 cells/well in a Seahorse XF24 cell culture microplate. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using an Agilent Seahorse XFe24 metabolic analyzer following the procedure recommended by the manufacturer (Agilent). For the mitochondrial stress test, 1) oligomycin (1 μM), 2) FCCP (1.5 μM) and 3) rotenone (100 nM) and antimycin A (1 μM) were injected sequentially through ports A, B and C.

[000393] Immunoblot analysis

[000394] Cells were lysed in M-PER[®] Mammalian Protein Extraction Reagent according to the manufacturer’s protocol. Lysates were separated by SDS-PAGE on 4-12% Bio-Rad gels, transferred to polyvinylidene difluoride (PVDF) membranes, blocked in 5% Milk in TBS buffer

with 0.1% Tween-20 (TBST). Primary antibodies to c-Myc (Santa Cruz Biotechnology) and GAPDH (Thermofisher) were added at room temperature or overnight at 4 °C. Secondary fluorescent antibodies (IRDye®, LI-COR) were added in 5% Milk in TBST for 1 h at room temperature. Odyssey® Classic Infrared Imaging System was used for visualization.

[000395] Histochemistry and Multiplex immunofluorescence (MIF)

[000396] Tumors of mice were collected, fixed in 4 % PFA for 2 days and embedded in paraffin, sectioned and H&E stained. For Multiplex immunofluorescence staining and imaging, five micron paraffin sections were stained with Akoya Biosciences® Opal™ multiplex automation kit on a Leica BondRX® autostainer, according to the manufacturers' instructions. Prior to incubation with the first primary antibody, sections underwent heat retrieval with Bond Epitope Retrieval Buffer 2 (Leica ER2, AR9640) and blocking. Primary antibodies in Panel 1 were against CD3 (1:200, Biorad, MCA1477T), CD8 (1:2000, Cell Signaling, 98941S), Ki67 (1:200, Abcam, AB16667). Primary antibodies in Panel 2 recognized iNos (1:1000, Genetex, GTX130246), Arg1 (1:750, Genetex, GTX109242), Granzyme B (1:1000, Abcam, AB4059), CD11b (1:10,000, Abcam, AB133357), F480 (1:250, Cell Signaling, 70076S). Each primary antibody was followed by a cocktail of horse radish peroxidase-conjugated secondary antibodies against mouse and rabbit IgG (RTU, Akoya/Perkin Elmer, Cat# ARH1001) and then tyramide mediated signal amplification (TSA) with covalent linkage of the individual Opal fluorophor (each 1:250, Opal 520 (FP1496001KT), 540 (FP1487001KT), 570 (FP1494001KT), 620 (FP1488001KT), 650 (FP1495001KT) or 690 (FP1497001KT), Akoya/Perkin Elmer Cat#'s) to the tissue antigen. Antibodies were subsequently stripped using either ER1 (Leica, AR9961) or ER2 (Leica, AR9640) heat retrieval buffer and the next round of immunostaining initiated. After completion of the sequential incubations and stripping, slides were counterstained with spectral DAPI (Akoya/PerkinElmer, FP1490). Monoplex controls were used to confirm appropriate staining for antibodies integrated into the multiplex panels. Multispectral imaging was performed on a Vectra3 imaging system (Akoya/PerkinElmer) at 20x. The fluorophore emission signatures were captured by a multispectral camera and then unmixed with InForm software (Akoya/PerkinElmer). Autofluorescence, obtained from an unstained slide, was removed from the composites and pseudo-colored images exported as tif files.

[000397] Statistical analysis

[000398] Statistical analysis was performed using GraphPad Prism 7.0 as described in Figure legends. All data are shown as mean $\pm$ s.e.m. Figures were prepared using GraphPad Prism 7, Adobe Photoshop and ImageJ Software. Treated groups were compared, using a one-way analysis using Prism7 (GraphPad Software), to naïve mice. Differences with a P value of <0.05 were considered significant: * $P<0.05$; ** $P<0.005$; *** $P<0.0001$.

[000399] SV expressing IL-12 enhances the expression of OX40 on CD4 T cells

[000400] The study described herein investigated the therapeutic effect of SV.IL12 in immune-competent tumor bearing mice (colon cancer; CT26). To exploit SV.IL12 for cancer therapy, tumor cells were i.p. implanted and after tumor establishment (4 days after tumor cell injection [day 0]), SV, SV.IL12 or IL-12 were i.p. injected on 4 consecutive days (day 1, 2, 3 and 4) for a total of 4 weeks (FIGs. 1A and B). While untreated (control), SV and IL-12 treated mice succumb to cancer after 3 weeks, treatment with SV.IL12 slightly prolonged survival time with an overall long-term survival rate of 7.1%. These data suggest that SV expressing IL-12 is needed to induce the observed therapeutic efficacy. Shortly after i.p. injection, SV infects macrophages in mediastinal lymph nodes where T cells get subsequently activated (FIG. 2). Even though SV.IL12 infected cells secrete significant amounts of IL-12 as observed in in vitro experiments (FIG. 3A), i.p. injection of SV.IL12 did not significantly change levels of plasma IL-12 in mice (FIG. 3B). Thus, suggesting that IL-12 produced by SV acts locally and stimulates transduced macrophages (FIG. 2) that present tumor antigens to corresponding T cells and activates them further. That shapes the subsequent anti-tumor immune response, such as promoting the differentiation into Th1 cells as well as increasing IFN γ production (FIGs. 3C and 3D)^{27, 28, 29, 30}. After one week of treatment, we analyzed the T cell response for their expression of inhibitory and activation markers. OX40 was markedly upregulated on CD4 T cells during SV.IL12 treatment, which was mostly among the effector CD4 T cells and less on the regulatory T cells (FIGs. 1C and 1D). Interestingly, SV treatment also induced OX40 upregulation on CD4 T cells but to a lesser extent (FIGs. 1C and 1D). On the basis of the results above and previous studies, reporting a beneficial effect of anti-OX40 in cancer treatment [Aspeslagh S et al., Eur. J Cancer, 2016], it was hypothesized that the agonistic anti-OX40 antibody could augment the therapeutic efficacy of SV.IL12 and help induce anti-tumor immune responses without to require knowledge of the tumor antigens.

[000401] Intraperitoneal delivery of SV.IL12 and anti-OX40 antibody cures established cancers

[000402] To investigate whether the oncolytic activity of SV.IL12 in combination with anti-OX40 is required for successful anti-cancer therapy, SV non-susceptible (colon cancer; CT26) and susceptible (prostate cancer; MyC-CaP) tumor cell lines were used in this study (FIG. 4) [Granot T et al., Mol. Ther., 2014; Huang PY et al., Mol. Ther., 2012] Immuno-competent female BALB/c and male FVB/NJ mice were implanted with either CT26 or MyC-CaP tumor cell lines, which expressed the firefly luciferase (Fluc) protein, respectively. This allowed monitoring tumor growth in vivo using noninvasive bioluminescent imaging. Once tumors became established (day 0), mice were treated with SV.IL12 in combination with anti-OX40. SV.IL12 was i.p. injected on 4 consecutive days (day 1, 2, 3 and 4) for a total of 4 weeks (FIG. 5A). Anti-OX40 was injected 3 times a week (day 0, 2 and 4) for a total of 2 weeks. In both tumor models, all untreated animals experienced progressive tumor growth and succumbed to cancer on week 3 (FIGs. 5 and 6). Mice bearing CT26.Fluc or MyC-CaP.Fluc tumors showed some delay in tumor growth when treated with i.p. injected SV.IL12 or anti-OX40 alone but with only a moderate effect on long-term survival (FIGs. 5 and 6). However, the combination of SV.IL12 with anti-OX40 resulted in complete regression of tumors in both tumor models (FIGs. 5 and 6). Tumors occasionally did recur in mice treated with combination therapy after treatment was completed, resulting in a long-term survival rate of 91.6% and 50% in the CT26 and MyC-CaP tumor model, respectively. In conclusion, combination of SV.IL12 with anti-OX40 elicits a strong therapeutic efficacy against two distinct solid tumors. Furthermore, these findings confirm that the oncolytic activity of SV is not required to induce a robust and effective anti-tumor response. Due to the fact that anti-OX40 monotherapy already resulted in a 20-50% survival rate, whether the addition of SV.IL12 would allow reduction of treatment frequencies while still maintaining the strong therapeutic efficacy of combination therapy, was investigated. This is especially important for lowering risks of adverse events as well as being more convenient for patients in clinics. Interestingly, therapeutic efficacy in the CT26.Fluc tumor model was maintained with only one injection per week of SV.IL12 and anti-OX40 (Fig. 7). This is in contrast with MyC-CaP.Fluc tumor bearing mice for which the full treatment regimen was required (data not shown). Thus, in the following experiments mice bearing CT26 tumors were

treated with one injection a week whereas MyC-CaP tumor bearing mice received the full treatment regimen.

[000403] Combination therapy markedly changes the transcriptome signature of T cells

[000404] The requirement of T cells during SV.IL12 with anti-OX40 treatment was assessed. The presence of both CD4 and CD8 T cells was required for eliciting the observed therapeutic efficacy as mice treated with the corresponding depleting antibodies were unable to control tumor growth (FIG. 8). To better understand the impact of the combination therapy on T cells, RNA sequencing was performed on isolated T cells from spleens derived from naïve, control as well as anti-OX40 and SV with or without anti-OX40 treated mice on day 7. Principal component analysis (PCA) of normalized reads showed a distinct segregation between combined therapy and all other groups in both tumor models (FIGs. 9A and 9B). These data suggest that combined therapy induces a distinct T cell response in the periphery independently of tumor model and mouse strain suggesting an indirect and immunity driven role of SV vectors with a negligible direct effect of the vector in tumors (FIG. 9C). Indeed, gene expression profiles of control versus anti-OX40, SV or SV in combination with anti-OX40 were clearly distinct, with the most differentially expressed genes (DEG) in the latter one (503, 49 and 2100 DEG, respectively) (FIG. 9C). Of 2100 DEG in control versus combination therapy, more genes were downregulated than upregulated (1637 vs. 463) > two-fold. Similarly, in control versus anti-OX40 as well as control versus SV more DEG were downregulated than upregulated (393 versus 110 and 30 versus 19, respectively).

[000405] Unbiased pathway enrichment and network analyses of DEG from control versus combination therapy was performed to determine biological processes in T cells that are influenced by this treatment (FIG. 9D). Although both upregulated and downregulated DEG were included in the analysis, the vast majority of pathways were upregulated in T cells treated with combination therapy with the exception of four clusters (TGFbeta signaling, ribosomal biogenesis, translation and chromatin modification). The upregulated pathways were dominated by DNA replication, chromosomal organization and cell cycle regulation, but also included various metabolic and immunological processes, such as mitochondrial respiration, nucleotide metabolism, and adaptive immune responses.

[000406] Combination therapy enhances systemic T cell responses, favoring Th1 like ICOS CD4 T cells

[000407] As T cells from combination therapy express a marked change in their transcriptome signature compared with all other groups, markers for T cell differentiation and activation (e.g., PD-1, ICOS, OX40, TIM3, KLRG1, IL7R) as well as T cell lineage transcription factors (e.g., EOMES, TBET, GATA3, BCL6, RORC, FOXP3) were analyzed (FIG. 9E). Only T cells from combined therapy expressed the gene signature of terminally differentiated effector T cells, which are characterized by high expression of the killer lectin-like receptor (KLRG1) and low expression of the interleukin 7 receptor (IL-7R) [Joshi NS et al., Immunity, 2007]. Furthermore, genes encoding products associated with the differentiation and function of effector cells, such as *Batf*, *Id2*, *Tbet*, *Gzmb* and *Ifng*, were also highly expressed in T cells isolated from mice treated with combined therapy compared with all other groups. The enhancement of effector T cells in combined therapy was confirmed by flow cytometry in both tumor models, as judged by the increased expression of the activation and proliferation markers CD44 and Ki-67, respectively (FIGs. 9F, 9G and 10). Interestingly, CD4 T cells also expressed a marked anti-tumor effector phenotype (ICOS⁺Tbet⁺) which was on average 2 to 3-fold higher during combined therapy compared with SV.IL12 or anti-OX40 treatment (FIGs. 9H and 9I). Previous studies reported a correlation between expansion of ICOS⁺Tbet⁺ CD4 T cells and clinical benefit in cancer patients who received anti-CTLA4 therapy [Wei SC et al., Cell, 2017; Ng Tang G et al., Cancer. Immunol. Res. 2003; Carthon BC et al., Clin. Cancer Res., 2010]. In summary, SV.IL12 in combination with anti-OX40 induces a marked systemic T cell response and favors the differentiation of terminal effector T cells. Furthermore, combined therapy induces a sustained increase in the frequency of ICOS⁺Tbet⁺ CD4 T cells which has also been reported to be elevated during successful anti-CTLA4 cancer therapy.

[000408] CD4 and CD8 T cells are metabolically reprogrammed in mice treated with SV.IL12 and anti-OX40

[000409] The tumor microenvironment can be a very challenging milieu for an effector T cell as it is characterized by hypoxia, acidosis and low levels of nutrient sources such as glucose and glutamine [Delgoffe, GM et al, Cancer Immunol. Res., 2016; Scharping, N.E, Vaccines, 2016; Chang, CH et al., Cell, 2015]. Even if T cell activation and initiation of effector function is

allowed, T cells may be unable to generate the bioenergetic intermediates necessary to carry out effector function in the tumor microenvironment. Thus, providing a metabolic support for T cells is crucial for the success of cancer treatments as previously reported [Scharping, N.E. et al., *Immunity*, 2016; Ho, PC et al., *Cell*, 2015; Siska PJ., et al, *Trends immunol.*, 2015; Zhao et al., *Nat. Immunol.*, 2016]. To test if SV.IL12 in combination with anti-OX40 influences the metabolic state of T cells, Gene Set Enrichment Analysis (GSEA) of the RNA sequencing data was performed between T cells from combined therapy and control. GSEA analysis showed significantly higher expression of genes involved in oxidative phosphorylation and glycolysis pathways during combination therapy (FIG. 11). To confirm GSEA analysis, peripheral T cells from both tumor models were metabolically profiled using Seahorse analysis on day 7 (FIGs. 11B, 12A and 12B). Oxidative phosphorylation and glycolytic profiles in T cells from naïve, control and mice treated with SV.IL12 and/or anti-OX40 were determined by measuring the rate of oxygen consumption (OCR) and the rate of extracellular acidification (ECAR), respectively. Basal OCR was enhanced in T cells from combined therapy and SV.IL12 treatment, but only the former harbored a dramatic increase in spare respiratory capacity in the CT26 model (FIGs. 11B and 12A). This was in contrast to T cells from combined therapy in the MyC-CaP.Fluc tumor model which expressed 3.75-fold higher basal OCR with no spare respiratory capacity (Fig. 12B). The reason for this discrepancy between the two models might be the differences in the number of treatments as MyC-CaP.Fluc bearing mice receive 3 and 4 times more injections of anti-OX40 and SV.IL12, respectively.

[000410] Analysis of mitochondrial mass (FIGs. 11C and 12C) and activity (FIGs. 11D and Fig. 12C), using flow cytometry with the mitochondrial stain Mitotracker Green and DeepRed respectively, revealed that SV.IL12 with or without anti-OX40 induced higher mitochondrial mass and activity in CD8 T cells but not in CD4 T cells. These data suggest that the observed increase in basal OCR was mainly driven by CD8 T cells. Interestingly, a slight decrease of active mitochondria occurred in CD4 T cells from mice treated with combined therapy, which might explain the increase in spare respiratory capacity in this group. To test if the reduction of active mitochondria in CD4 T cells is associated with a switch towards glycolysis, the master regulator for glycolysis c-MYC and basal ECAR were measured in T cells from all groups. Indeed, the addition of anti-OX40 to SV.IL12 induced elevated protein expression of c-MYC as well as basal ECAR (FIG. 11E and 11F). T cells

from naïve and control as well as SV.IL12 or anti-OX40 treated mice showed no signs of elevations. Collectively, these findings reveal that SV.IL12 induces enhanced oxidative phosphorylation in CD8 T cells, whereas the addition of anti-OX40 to SV.IL12 is needed to push CD4 T cells towards glycolysis by increasing the protein expression of c-MYC.

[000411] To determine the kinetics of peripheral T cell metabolism over the course of treatment with SV.IL12 and anti-OX40, OCR and ECAR were measured on day 7, 14 and 40 in CT26.Fluc bearing mice (FIG. 11G). As shown above, T cells on day 7 shifted towards a glycolytic state which is associated with the initial effector phase. Two weeks into the treatment, T cells switched to a highly energetic state utilizing both metabolic pathways, oxidative phosphorylation and glycolysis, as reported for highly activated T cells [Buck MD et al., J. Exp. Med., 2015] Once tumors were fully rejected and mice were tumor-free for a month, T cells returned to a more quiescent state, such as naïve cells. Interestingly, T cells from MyC-CaP.Fluc bearing mice switched to a highly energetic state early on during treatment (day 7) and remained in this metabolic phenotype 2 weeks after treatment has stopped (FIG. 12D). The reason for this discrepancy might be the differences in the number of treatments applied in both tumor models as MyC-CaP.Fluc bearing mice receive 3 and 4 times more anti-OX40 and SV.IL12, respectively. T cells from control as well as anti-OX40 or SV.IL12 treated mice in both tumor models remained in a quiescent state over the course of treatment (FIG. 12E). In summary, SV.IL12 in combination with anti-OX40 metabolically rewires T cells to an energetic state using both metabolic pathways, oxidative phosphorylation and glycolysis. This phenotype does not occur in SV.IL12 or anti-OX40 treated mice, which succumb to cancer. Thus, the changed metabolic state of T cells correlate with an efficient anti-tumor response and better survival rate.

[000412] Metabolic reprogrammed T cells in SV.IL12 with anti-OX40 treated mice display enhanced CD4 mediated cytokine production and anti-tumor activity

[000413] To test if metabolic reprogrammed T cells in combined therapy possess enhanced effector functions, cytokine production and cytotoxicity were analyzed in T cells isolated from spleens on day 7. Genes encoding pro-inflammatory cytokines *ifnγ* and *il2* were upregulated in T cells from mice treated with SV in combination with anti-OX40 (FIG. 13A). ELISPOT analysis of interferon-γ (IFNγ) by splenocytes confirmed RNA sequencing data, showing the strongest IFNγ secretion in mice treated with combined therapy in both tumor models (FIGs. 13B and 13C). Splenocytes from SV.IL12 treated mice also produced IFNγ but to a lesser extent.

Interestingly, the main producer of IFN γ were CD4 T cells as depletion of CD4 T cells but not CD8 T cells abolished IFN γ secretion in splenocytes from mice treated with combined therapy.

[000414] In addition, RNA levels of the cytotoxic proteases, granzyme A and B, were upregulated in mice treated with combination therapy compared with all other groups (FIGs. 13A). Protein expression of granzyme B correlated with RNA levels as measured by flow cytometry in both tumor models (FIGs. 13D, 13E, and 14A-14D). Further, granzyme B positive cells were detected in CD8 as well as CD4 T cells, suggesting the presence of cytotoxic CD4 T cells in mice treated with combined therapy [Brown DM et al., Cell Immunol, 2010; Mucida D. et al., Nat Immunol, 2013; Reis BS et al., Nat Immunol 2013] Upregulation of granzyme B was associated with downregulation of the transcription factor Foxo1 which is known to control granzyme transcription through repression of the transcription factor T-bet (FIG. 13A) [Rao RR et al., Immunity, 2012]. Last, the enhanced cytotoxic potential of T cells from combined therapy was also supported by elevated expression of the NKG2D receptor which has been shown to trigger TCR-independent cytotoxicity in activated T cells (FIG. 13A, 14E and 14F) [Verneris MR et al., Blood 2004].

[000415] Having observed upregulation of granzymes and cytotoxic receptors in combination therapy, the function of T cells was investigated using an *ex vivo* tumor growth assay. Splenocytes obtained from all groups were co-cultured at an effector-to-target cell ratio of 10:1 with either CT26.FLUC (FIG. 13F) or MyC-CaP.Fluc (FIG. 13G) tumor cell lines. The anti-tumor activity of splenocytes was determined by measuring the luciferase activity of cell lines, which correlates with tumor growth. Tumor growth was markedly reduced when co-cultured with splenocytes from mice receiving combined therapy compared with splenocytes from naive, control and mice treated with anti-OX40 in both tumor models. The anti-tumor activity of splenocytes from mice treated with SV.IL12 alone was weaker than that from combined therapy. Surprisingly, tumor growth inhibition was mediated by CD4 T cells as depletion of CD4 T cells but not CD8 T cells abolished the inhibitory effect on tumor cells. Together, these results clearly show that T cells from combined therapy elicit enhanced anti-tumor and functional activity, such as granzyme B and IFN γ production driven by CD4 T cells.

[000416] Mice treated with SV.IL12 in combination with anti-OX40 display enhanced T cell migration and intratumoral T cell immunity

[000417] Only a minority of the total of treated patients respond to current immunotherapy and the presence of TILs has been shown to be one of the main factors that influence the responsiveness towards various therapies in multiple cancers [Galon, J et al., Science 2006; Hwang WT et al., Gynecol Oncol 2012]. Due to the fact that SV elicited anti-tumor responses do not necessarily require direct infection of the tumor or intratumoral injection, whether SV.IL12 therapy in combination with anti-OX40 could nevertheless alter the local tumor microenvironment and favor intratumoral immunity, was investigated. To assess whether SV.IL12 in combination with anti-OX40 induces T cell infiltration into the tumor, the chemokine receptor CXCR3 on peripheral T cells was analyzed after one week of treatment. In the CT26.Fluc model CXCR3 levels were significantly upregulated on CD4 T cells during combination therapy compared with all other groups and CXCR3 levels remained elevated over the course of treatment (FIG. 15A, 15B and 16A). In contrast, CXCR3 expression on CD8 T cells only appeared later on in treatment, suggesting that CD4 T cells are first recruited to the inflamed site followed by CD8 T cells (FIG. 16A). MyC-CaP.Fluc tumor bearing mice showed elevated levels of CXCR3 on CD4 and CD8 T cells after one week of combination treatment (FIGs. 16B and 16C). Furthermore, SV.IL12 treatment also increased CXCR3 expression on T cells but to lesser extent. The reason for this discrepancy between the two models might be the differences in the number of treatments as MyC-CaP.Fluc bearing mice receive 3 and 4 times more injections of anti-OX40 and SV.IL12, respectively. Cells expressing CXCR3 follow the gradient of their ligands CXCL9, CXCL10 and CXCL11 [Groom, J.R. & Luster, A.D. Exp. Cell Res. 2011]. Indeed, combination therapy also enhanced the production of CXCL9 and CXCL10 in the tumor microenvironment, as judged by real-time PCR, suggesting that CXCR3 positive T cells migrate to the tumor site (FIG. 15C). Treatment of SV.IL12 or anti-OX40 alone did not alter the expression of these ligands. In line with these observations, an overall increase in T cells was observed in CT26.Fluc and MyC-CaP.Fluc peritoneally disseminated tumors from mice treated with combined therapy compared with control and anti-OX40 treated mice (FIGs. 15E and 15F). SV.IL12 treated mice also showed enhanced T cell infiltration but to a lesser extent. Furthermore, dissecting CD4 and CD8 T cells by flow cytometry revealed that combination therapy increases the proportion of CD4 T cells in CT26.Fluc tumors, which is consistent with the elevated CXCR3 expression on peripheral CD4 T cells (FIGs. 15D). These results clearly show that SV.IL12 in combination with anti-OX40 alter the tumor microenvironment by

facilitating T cell infiltration via modulation of the CXCR3/CXCL9-11 axis. Not only did combination therapy increase T cell infiltration in both tumor models but CD4 as well as CD8 T cells also demonstrated enhanced functional activity in the tumor, as judged by the Ki-67 and granzyme B expression (Figs. 15D, 16D and 17). In line with these results, a decrease in proliferation, as judged by the expression of Ki-67 in tumor cells, was observed in CT26.Fluc and MyC-CaP.Fluc tumor cells when treated with combined therapy compared with all other treatments (FIGs. 15E and 15F). These results suggest that the presence of activated T cells in the tumor microenvironment exert anti-tumor activity which inhibits tumor growth. Besides from T cell activation, we also observed enhanced iNOS production in MyC-CaP.Fluc tumors treated with combination therapy compared with control or anti-OX40 treatment (FIG. 18). SV.IL12 treatment alone also induced iNOS production but to a lesser extent. Interestingly, the amount of iNOS inversely correlated with arginase1 production, suggesting a repolarization of tumor associated macrophages from the M2-like (pro-tumor) into M1-like (anti-tumor) phenotype during combination therapy. These trends were only observed in MyC-CaP.Fluc and not in CT26.Fluc tumors, which might be a consequence of SV directly infecting MyC-CaP cells.

[000418] The study described herein provides a practical strategy for cancer immunotherapy using an OV and anti-OX40. This strategy takes advantage of the preexisting T cell immune repertoire in vivo, removing the need to know about present tumor antigens. The study described herein shows that the combination of replication-deficient SV.IL12 and anti-OX40 amplifies these antitumor T cells and induces their action throughout the body against two distinct solid tumors, reversing effectively local tumor-mediated immune suppression. This effect was specific for combination therapy and was not observed during SV.IL12 or anti-OX40 treatment alone.

[000419] The high metabolic activity of cancer cells together with the poor vasculature blood supply in the tumor microenvironment can induce nutrient deprivation [Delgoffe, GM et al., Cancer Immunol Res. 2016; Scharping, NE & Delgoffe, GM, Vaccines, 2016; Chang, CH et al., Cell, 2015]. These conditions can impair TCR signaling, glycolytic and mitochondrial metabolism, as well as decrease mitochondrial mass, all hallmarks of T effector cells, resulting in impaired anti-tumor effector functions of tumor-specific T cells. 39–42 Scharping, N.E. et al., Immunity 2016; Ho, P.C. et al., Cell 2015; Siska, PJ & Rathmell, JC, Trends Immunol., 2015; Zhao, E. et al., Nat Immunol, 2016]. The data in two distinct models of cancer immunotherapy

disclosed in the study described herein, shows that SV.IL12 in combination with OX40 signaling provides the necessary metabolic support to T cells to generate an efficient antitumor response. This metabolic support is characterized most prominently by elevated mitochondrial function and mass in CD8 T cells as well as a switch to aerobic glycolysis in CD4 T cells. T cells from mice treated with SV.IL12 in combination with anti-OX40 demonstrated enhanced protein expression of c-Myc compared with all other groups. Thus, the study described herein clearly shows that T cells are metabolically reprogrammed in the periphery during combination therapy.

[000420] The study described herein strongly shows that the therapeutic efficacy of SV.IL12 with anti-OX40 is driven by T cell modulation and reprogramming of its metabolic state, in order to enhance the anti-tumor response in the periphery and in the tumor microenvironment. Furthermore, the use of SV allows these metabolically reprogrammed T cells to better infiltrate the tumor microenvironment, which is crucial for an adequate immunotherapy. Anti-OX40 antibody is currently being studied in phase 1 and 2 clinical trials. SV will be tested as a single agent in its first clinical trial in the third quarter of 2020. The results from our current preclinical studies provide a strong rationale for combining SV.IL12 with agonistic anti-OX40 antibodies in a therapeutic format in patients with solid tumors. In summary, the studies described herein clearly show that even in absence of direct SV tumor targeting, SV.IL12 in combination with anti-OX40, or SV vector encoding IL-12 and anti-OX40, can alter the tumor microenvironment in distinct solid tumors through an indirect and immunity driven mechanism that enhances T cell infiltration and intratumoral T cell immunity.

[000421] Example 2: Combination of IL-12 and anti-OX40 expressed by Sindbis viral vectors synergistically enhances survival of subjects with established tumors.

[000422] The study described herein investigates the effect of administering IL-12 and anti-OX40 antibody, both expressed by Sindbis viral vectors, on established tumors. This strategy is particularly advantageous for treatment of cancers like ovarian cancer, wherein the combination of SV.IL-12 and anti-OX40 antibody is not found to be as effective, as observed in colon and prostate cancers. The administration of SV/IL-12 and an anti-OX40 antibody enhanced clearance of established tumor of colon and prostate cancer cell lines, CT26 and MyC-Cap respectively. C57/Bl6 albino (female) mice re-injected with Alm5-2Fluc-17 ovarian cancer cells to establish a tumor (FIG. 19), and treated with: a) a SV vector expressing IL-12; b) a combination of SV vectors expressing IL-12 and an anti-OX-40 antibody (aOX40_REP-IL12),

c) a 50% mixture of either a fragmented SV expressing OX-40 IgG plus a fragmented SV expressing IL-12 (RepOX40IgG_Rep-IL12) or d) a 50% mix of a fragmented SV expressing OX-40 IgG plus a full length SV expressing IL-12 (RepOX40IgG_SV-IL12). The percentage survival rate of the treatment groups RepOX40IgG_SV-IL12 and aOX40_Rep-IL12 were comparable, and higher than the SV-IL-12 and RepOX40IgG_Rep-IL12 treatment group. However, the results showed that, the RepOX40IgG_SV-IL12 treatment group showed the highest enhancement of survival rate (FIG. 20).

[000423] The study described herein, provides plasmid constructs for expressing IL-12, and anti-OX40 in a SV vector. The study described herein, provides plasmid constructs encoding IL-12 a and b subunits (FIG. 38), anti-OX40 IgG2a heavy and light chains (FIG. 39) and a single chain antibody to OX40 (FIG. 40).

[000424] SV-IL12 plasmid used in this study has been published in 2002 [Tseng JC et al., J Natl Cancer Inst. 2002]. To construct a Sindbis viral vector containing genes for interleukin 12 (IL-12), the Sindbis viral vector SinRep/2PSG was first constructed, which contains a secondary subgenomic promoter that is responsive to the Sindbis replicase. Two DNA oligonucleotide primers (sequence 5' CGCGTAAAGCATCTCTACGGTGGTCCTAATAGTGCATG-3'; SEQ ID NO: 29) and its complementary strand 5'CACTATTAGGACCACCGTCGAGATGCTTTA-3'; SEQ ID NO: 30) containing the subgenomic promoter sequence were annealed and ligated into the MluI and SphI sites of the SinRep plasmid. The murine IL-12 α subunit gene (mp35; ATCC 87596) and the IL-12 β subunit gene (mp40; ATCC 87595) were subcloned into the MluI and the StuI sites of SinRep/2PSG, respectively, to produce the Sin-Rep/IL12 plasmid.

[000425] The H and L chains of the OX40 Ab are expressed from a single SV using two subgenomic promoters. The synthesized sequences were designed to encode an IL-12 secretory signal peptide upstream of both H and L polypeptide sequences preceded by a ribosome binding site and the start codon. The variable Ab binding sequences that functionally bind to activate the OX40 Receptor contain complementarity determining regions that are not unique. The variable chain is linked to the respective L (GenBank accession BAR42292) and H chain (GenBank accession CAC20702) constant region sequences of mouse IgG2a; the murine IgG2a isotype is comparable to the BioXcell OX40 Ab used in parallel *in vivo* experiments.

[000426] In summary results described herein clearly show the possibility of using a combination of SV vectors expressing IL-12 and anti-OX40 antibody or a SV vector expressing

both expressing IL-12 and anti-OX40 antibody, for treatment of cancers that may be resistant to treatment with anti-OX40 antibody administered directly.

[000427] Example 3. Molecular and metabolic pathways mediating curative treatment of a non-Hodgkin B cell lymphoma by Sindbis viral vectors and anti-4-1BB monoclonal antibody.

[000428] The studies described herein use an antibody directed at 4-1BB (CD137, TNFRSF9), a T cell costimulatory molecule. 4-1BB agonist stimulation greatly enhances NK and cytotoxic T cell activity. There are preclinical studies showing that α 4-1BB effectively treats lymphoma and that depletion of Treg cells enhances the therapeutic effect of α 4-1BB [Houot R et al., Blood, 2009]. The A20 tumor cells used in the study described herein were derived from a spontaneously arising reticulum cell sarcoma (a non-Hodgkin lymphoma) in a BALB/c mouse.

[000429] Previously, SV carrying NYESO-1 was used, which encodes the cancer testis TAA, NYESO-1, to cure CT26 tumors expressing NYESO-1 [Scherwitzl I et al., Mol. Ther. Oncolytics, 2018]. The studies described herein show that systemically disseminated A20 lymphoma can be completely cured by SV plus α 4-1BB mAb combination therapy without the need to produce a SV that encodes a TAA known to be present in the A20 lymphoma cells. Further, neither intratumoral injection of the SV vectors nor infection of the tumors is required as the A20 B lymphoma cells used in the current model are resistant to SV infection.

[000430] One difference in the current study, compared with those previously published, is the use of SV vector combination therapy that involves an agonistic mAb for a costimulatory receptor versus targeting checkpoint blockade molecules such as CTLA4 and PD-1. The studies described herein show that agonistic mAbs in combination with SV vectors trigger a cascade of events that results in curative results. The findings disclosed herein reveal the potential of SV combination therapy to cure tumors for which TAAs are completely unknown.

[000431] Materials and Methods

[000432] Firefly luciferase (Fluc)-expressing A20 cells generation

[000433] A20 cells were transfected with pGL4-neo_Fluc plasmid (Promega) by electroporation via Nucleofector™ kit V (Lonza). Fluc-A20 cell clones were selected and maintained in RPMI1640 (Cellgro) + 10% FBS (Gibco) + 250 μ g/ml G418 (Gibco). One A20 clone stably expressed fLuc and was used for tumor inoculation and consecutive experiments.

[000434] SV production

[000435] SV-LacZ production and titering were done the same as previously described [Scherwitzl I et al., Mol. Ther. Oncolytics, 2018].

[000436] SV-GFP infection

[000437] A20 cells and control BHK cells were infected by SV carrying GFP for 1 h. The GFP expression was observed the next day by fluorescence microscopy.

[000438] A20 tumor inoculation and In Vivo Imaging System (IVIS) Imaging

[000439] 3×10^6 fLuc-A20 cells were inoculated to BALB/C mice by i.p injection. Tumor growth was monitored as previously described [Scherwitzl I et al., Mol. Ther. Oncolytics, 2018].

[000440] SV and α 4-1BB Ab treatment

[000441] Treatment was started after successful tumor inoculation (4 days after tumor cell injection, confirmed by IVIS imaging). Tumor growth was measured every week by noninvasive bioluminescent imaging. SVLacZ was injected 4 times per week, for totally 3 weeks. The virus (10^7 – 10^8 TU/mL) in a total volume of 500 μ L was i.p. injected. For 2 groups (4-1BB and SV plus 4-1BB), 350 μ g/mouse 41BB Ab was injected 3 times/week for 2 weeks. InVivo MAb anti-mouse 4-1BB was ordered from BioXCell (Clone: LOB12.3, Cat.No. BE0169). In low dose treatment protocol, SVLacZ was injected i.p. 3 times per week, for totally 3 weeks. 41BB Ab (50 μ g/mouse) was injected once a week for 3 weeks.

[000442] Elispot

[000443] Mouse IFN γ ELISPOT was performed according to the manufacturer's protocol (BD Biosciences). 2×10^5 splenocytes or 1×10^5 T cells were plated per well O/N in RPMI supplemented with 10% FBS. For a positive control, splenocytes were stimulated with 5 ng/ml PMA + 1 μ g/ml Ionomycin.

[000444] Flow cytometry

[000445] Fluorochrome-conjugated antibodies against mouse CD3, CD4, CD8, CD25, CD44, CD62L, ICOS, CD11a, ICAM-1 were purchased from Biolegend (San Diego, CA). Fluorochrome-conjugated antibodies against mouse Foxp3, EOMES and CCR5 were purchased from Thermofisher. BUV395 conjugated antibody against mouse CD8a was purchased from BD Biosciences. For surface staining, cells were washed and stained with anti-mouse direct conjugated antibodies. Cells were analyzed using the LSRII flow cytometer (BD Biosciences) and data were analyzed using Flowjo software (Treestar, Ashland, OR). For intracellular cytokines staining, stimulated cells were fixed with cytofix/cytoperm solution (BD Biosciences),

permeabilized with perm/wash buffer (BD Biosciences) and stained with anti-mouse IFN γ antibodies. For nuclear antigen, cells were fixed and permeabilized by Foxp3 fixation/permeabilization buffer (eBioscience) and stained with anti-Foxp3, T-bet, Ki67 and EOMES antibody.

[000446] RNA isolation and transcriptome analysis

[000447] Total RNA was harvested by RNAeasy isolation kit (Qiagen, Valencia, CA). For each group, 3 BALB/C mice were used as biological repeats. RNA-seq was performed by NYUMC Genome Technology Center (GTC). To identify significant differences in expression between any pair of groups, differential expression analysis was performed using Deseq2 and an adjusted p value cutoff of 0.05 was applied [19, Love MI et al, Genome Biol. 2014] ($q < 0.05$). To increase stringency, only genes with a Log2 fold change ≥ 1 (upregulated) or ≤ -1 (downregulated) were selected for further analysis. Gene cluster analysis was performed by DAVID analysis using the selected differentially expressed genes [Huang da W et al., Nucleic Acids Res 2009, Huang da W et al., Nat Protoc. 2009]. RNA-seq results (normalized counts) were used as input to perform with Gene Set Enrichment Analysis (GSEA) [Subramanian A, et al., Proc Natl Acad Sci U S A. 2005]. Molecular Signatures Database (MSigDB)v4.0 were used as screening database. For each gene, the gene expression value is normalized by the relative log2 fold change compared to the median value of this gene. Expression heatmap is drawn by Morpheus (<https://software.broadinstitute.org/morpheus/>). Canonical pathway and disease and biological functional analysis were generated by ingenuity pathway analysis (IPA; Ingenuity Systems, Redwood City, CA) using the statistical differential expressed genes list. To increase the sample representativeness, for IPA, we choose nominal $p < 0.05$ as cutoff value.

[000448] Tumor infiltrating lymphocyte (TIL) harvest

[000449] To investigate the phenotype of TIL, all treatments were started 11 days after tumor inoculation, After 7 days treatment, tumor mass was harvested and the phenotype of TIL were analyzed as previously described [18, Scherwitzl I, Mol Ther Oncolytics. 2018].

[000450] T cell Seahorse assay

[000451] T cells were isolated from spleen by using pan T cell isolation kit (Stemcells). T cells were plated at 6×10^5 cells/well in 24 well plate. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured by Agilent Seahorse XFe24.

[000452] Statistical analysis

[000453] For the two group comparison, statistical difference was determined by unpaired two tail Student t-test. The multiple sample comparison was analyzed by one way ANOVA. $P < 0.05$ was determined to be significant for all experiments. All values were calculated with Excel (Microsoft) and Prism software (GraphPad).

[000454] SV and $\alpha 4$ -1BB mAb combination completely cured A20 lymphoma

[000455] To explore if SV has therapeutic effect on tumors not targeted or infected by SV vectors, the A20 B cell lymphoma was used, which is highly resistant to SV infection (FIG. 21). To monitor tumor growth in vivo, a firefly luciferase (f-Luc) expression vector was transfected into the A20 lymphoma cell line by electroporation. A stable f-Luc expressing A20 clone was isolated through G418 selection. 3×10^6 /mouse f-Luc A20 tumor cells were inoculated by intraperitoneal (i.p.) injection. Tumor growth was monitored by IVIS imaging once per week. Tumors were successfully established after 4 days inoculation (FIG. 22A). After tumors were established, SV and $\alpha 4$ -1BB mAb treatment started (designated as day 0). A therapeutic protocol similar to that previously described [Scherwitzl I et al., Mol Ther Oncolytics. 2018], was used. SV plus $\alpha 4$ -1BB mAb combination achieved the best therapeutic effect (FIG. 22B). All mice in that group showed complete tumor regression in 2 weeks. Although, both SV or $\alpha 4$ -1BB treatments alone achieved obvious therapeutic effects compared with untreated mice, they were not as effective as the combination and a fraction of mice in these two groups eventually succumbed to tumor (FIG. 22C).

[000456] SV alone and SV plus $\alpha 4$ -1BB mAb stimulated cell cycle progression, cytokine production, and activation

In the study described herein, SV significantly inhibited tumor growth by day 7 (FIG. 22A). T cells play a critical role in SV induced anti-tumor immunity. T cell response reaches a peaked on day 7 after infection [Scherwitzl I et al., Mol Ther Oncolytics. 2018]. To explore how SV induced T cell responses that help to eradicate A20 lymphoma, RNA-Seq was performed using purified splenic T cells from all groups on day 7. Compared with untreated samples, 271 genes upregulated ($q < 0.05$ and $\text{Log}_2 \text{Fold Change} \geq 1$) and 28 genes downregulated ($q < 0.05$ and $\text{Log}_2 \text{Fold Change} \leq -1$) were identified in the SV infected group through Deseq2 analysis (FIG. 23A, Table 1).

Table 1: The SD expressed genes list for SV vs. untreated group by RNA-Seq ($q < 0.05$, $\text{Log}_2 \text{FC} \geq 1$ and $\text{Log}_2 \text{FC} \leq -1$).

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Gm2762	38.5	3.15	4.04	0	0
Il21	229	2.57	3.66	1.81E-13	2.63E-11
Ascl2	24.4	2.55	3.83	2.83E-12	3.8E-10
Akr1c18	25.3	2.4	4.32	6.37E-11	6.98E-09
Angptl2	741.2	2.35	2.59	0	0
E2f8	535.1	2.33	2.61	0	0
Vat1l	14.9	2.33	4.01	1.65E-10	1.68E-08
Aurkb	806.6	2.3	2.46	0	0
Gzmb	4113.9	2.26	2.51	0	0
Cdc25c	143.9	2.08	2.51	3E-15	5.19E-13
Prr11	288	2.04	2.36	0	3.3E-14
Ighg2c	873	2.04	4.64	6.37E-08	4.29E-06
Tpx2	1113.7	2.03	2.19	0	0
Bub1	362.3	2.02	2.22	0	0
Bub1b	1344.3	2.01	2.14	0	0
Nusap1	813.1	2	2.12	0	0
Kntc1	402.4	2	2.22	0	0
Kif18b	606.6	1.99	2.14	0	0
Gzmk	1785.2	1.99	2.28	0	4E-15
Nuf2	279.6	1.97	2.25	0	1.8E-14
Mcm10	477.4	1.96	2.11	0	0
Mki67	5899.1	1.96	2.12	0	0
Ccnb2	1255.4	1.95	2.1	0	0
Rad54l	350.2	1.95	2.16	0	0
Hmmr	311.7	1.94	2.19	0	1E-15
Chn1	24	1.94	2.52	2.4E-09	2.01E-07
Ccna2	1680.2	1.93	2.04	0	0
Pclaf	522.9	1.93	2.17	0	5E-15
Cenpf	495.6	1.92	2.07	0	0
Kif2c	341.3	1.92	2.1	0	0
Cdkn3	129.2	1.92	2.16	0	0
Vdr	250.7	1.92	2.2	1.02E-13	1.57E-11
Figl1	145.7	1.91	2.15	0	0
Ttk	211.5	1.9	2.14	0	5E-15
Diaph3	356.3	1.89	2.03	0	0
Oip5	50.6	1.88	2.49	1.92E-10	1.94E-08
Kif4	650.2	1.87	1.98	0	0
Rrm2	1718.2	1.87	2.01	0	0
Sccpdh	123.3	1.86	2.05	0	3.6E-14
Clspn	637.1	1.83	1.98	0	0
Padi4	34.4	1.83	2.41	6.18E-08	4.18E-06
Shcbp1	274.8	1.82	1.98	0	0

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Rad51	401.4	1.82	1.99	0	0
Tox2	889.5	1.82	2.01	0	1.5E-14
Spc24	506	1.81	1.92	0	0
Sgo1	162.8	1.81	2.01	0	5E-15
Depdc1a	29.6	1.81	2.69	1.72E-08	1.26E-06
Lrr1	59	1.8	2.37	9.15E-10	8.14E-08
Cdca8	1251.5	1.79	1.91	0	0
Kif20a	898.5	1.78	1.9	0	0
Espl1	809.6	1.78	1.9	0	0
Cdk1	1228.8	1.78	1.92	0	0
Sapcd2	275.5	1.78	1.98	0	5E-14
Pbk	107.5	1.78	2.08	1.08E-13	1.63E-11
Gfra4	95	1.78	2.1	9.39E-11	9.94E-09
Fcer1a	69.7	1.77	2.87	1.43E-06	6.68E-05
Lag3	2916.7	1.76	1.84	0	0
Rad51ap1	205.6	1.76	1.92	0	0
Ube2c	1391	1.75	1.95	5E-15	9.54E-13
Kn11	203.3	1.75	1.98	2.7E-14	4.39E-12
Kif15	595.3	1.74	1.87	0	0
Cep55	503.9	1.74	1.88	0	0
Ticrr	558.4	1.74	1.88	0	0
Neil3	228.1	1.72	1.89	0	7E-15
Spc25	211.3	1.72	1.94	1.08E-13	1.63E-11
Lpar3	17.1	1.72	2.4	4.07E-07	2.23E-05
Ncapg	383.1	1.71	1.87	0	4E-15
Iqgap3	284.2	1.71	1.86	0	4E-15
Nek2	554.5	1.71	1.87	0	2.9E-14
1500009L16Rik	143.2	1.69	2.2	2.79E-08	1.97E-06
Ckap2l	455.4	1.68	1.86	3E-15	6.37E-13
Ncaph	822.4	1.67	1.76	0	0
Uhrf1	1603.6	1.67	1.77	0	0
Pimreg	230	1.67	1.88	1.32E-13	1.96E-11
Top2a	4726.3	1.66	1.73	0	0
Spag5	1183.2	1.66	1.77	0	0
Kif14	299.1	1.66	1.84	3E-15	6.22E-13
Ii10	1005.5	1.66	1.83	3.1E-14	4.98E-12
Pif1	204	1.66	1.94	1.16E-11	1.41E-09
Parpbp	117.3	1.66	1.94	1.52E-11	1.79E-09
Ly6a	2208.2	1.65	2.56	2.41E-06	0.000106
Melk	329.8	1.64	1.75	0	0
Ccnb1	160.8	1.63	1.88	1.91E-11	2.2E-09
Cit	784.3	1.62	1.74	0	4E-15

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Esco2	149.5	1.61	1.83	4.58E-12	5.99E-10
CT030166.6	24.2	1.61	2.49	2.45E-06	0.000107
Trip13	233.7	1.6	1.74	0	5E-14
Cenpe	685.5	1.59	1.71	0	1E-15
Fads2	70.5	1.57	1.98	1.07E-07	6.9E-06
Cdc45	628.7	1.56	1.62	0	0
Cdca5	791.4	1.56	1.63	0	0
Cdca3	1038.6	1.56	1.64	0	0
Coro2b	349.5	1.56	1.68	1.3E-14	2.31E-12
Cenph	201.9	1.55	1.73	2.02E-12	2.73E-10
Troap	236.4	1.54	1.73	6.69E-12	8.53E-10
Birc5	1540.7	1.54	1.73	3.18E-11	3.56E-09
Ifi271l2a	8730.4	1.54	1.88	1.34E-07	8.38E-06
Mcpt1	510.2	1.54	3.77	4.63E-05	0.001264
Plk1	794.8	1.53	1.65	1E-15	1.43E-13
Ankle1	238.9	1.53	1.72	1.64E-11	1.92E-09
Cd109	30.7	1.53	2.47	3.8E-05	0.001099
Cdca2	444.5	1.52	1.64	0	2.9E-14
Siglec1	622.7	1.52	1.69	1.49E-11	1.77E-09
Ska1	210.9	1.52	1.73	2.11E-10	2.12E-08
Igkv14-111	309.6	1.51	2.66	7.06E-05	0.001789
Alox5	104.2	1.5	2.08	6.41E-06	0.000241
Stmn1	257.1	1.49	1.61	1.1E-14	2E-12
Ect2	255.1	1.49	1.71	1.15E-09	9.97E-08
Asf1b	1276.4	1.48	1.55	0	0
Ncapg2	526.2	1.48	1.58	0	1.5E-14
Cenpm	289.6	1.48	1.64	8.64E-12	1.07E-09
Cenpp	81.2	1.48	1.69	2.19E-10	2.19E-08
F13a1	227.4	1.48	2.13	1.59E-05	0.000526
Oas1a	498.3	1.47	1.73	2.33E-08	1.66E-06
Oas2	1573.5	1.47	2.24	3.32E-05	0.000987
Cenpi	136.8	1.46	1.61	1.49E-12	2.03E-10
Tex15	58.4	1.46	1.88	2.46E-07	1.43E-05
Lif	439.6	1.45	1.5	0	0
Tk1	1004.9	1.45	1.54	0	1E-15
Kif22	1022.2	1.45	1.56	1.09E-13	1.63E-11
BC030867	129.6	1.45	1.65	7E-10	6.44E-08
Tnp2	17.6	1.45	2.43	6.42E-05	0.001655
Tph1	43	1.45	2.97	0.000121	0.0028
Ighv1-39	28.6	1.45	2.67	0.000133	0.003011
Tyms	809.6	1.44	1.51	0	0
Kif11	1109.9	1.44	1.51	0	0

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Ighv2-6	141.1	1.44	1.89	9.88E-06	0.000349
Gng3	34.8	1.44	2.26	1.32E-05	0.000449
Spdl1	204.5	1.43	1.54	1E-14	1.71E-12
Cdh23	89.8	1.43	1.55	8.05E-12	1.01E-09
Aspm	319.4	1.43	1.59	9.94E-11	1.04E-08
Dsp	79.2	1.43	1.78	2.44E-06	0.000107
Ighv1-19	10.2	1.43	2.88	0.000154	0.003401
Depdc1b	350.8	1.42	1.54	3.09E-13	4.45E-11
Ctsg	151.2	1.42	2.54	0.000128	0.002935
Mxd3	280.1	1.41	1.5	2E-15	3.2E-13
Chaf1a	1027.2	1.4	1.49	2E-15	4.68E-13
Aunip	24.6	1.4	2.68	8.31E-05	0.00205
Mt3	18.3	1.4	2.93	0.000162	0.003547
Tcf19	1158.6	1.39	1.45	0	0
Gpm6b	315	1.39	1.48	1.5E-13	2.21E-11
Cd5l	2824.6	1.39	1.52	7.15E-11	7.78E-09
Msr1	48.9	1.39	1.89	1.13E-05	0.000392
Ighg1	2604.8	1.39	3.8	0.00023	0.004805
Ncapd2	3741.3	1.38	1.42	0	0
Oas3	1474.3	1.38	1.71	2.37E-06	0.000105
Necab3	27.4	1.38	1.88	1.94E-05	0.000622
Tpsab1	262.4	1.37	1.66	1.78E-06	8.17E-05
Gm4951	44.3	1.37	1.8	3.61E-06	0.000148
Exo1	187.1	1.36	1.54	1.17E-08	8.79E-07
Adam33	60.3	1.36	1.62	1.37E-06	6.46E-05
Klrb1a	36.2	1.36	2	7.82E-05	0.001956
Ndc80	345.5	1.35	1.44	2.6E-14	4.36E-12
Slc16a2	105.6	1.35	1.56	1.32E-07	8.29E-06
Klhl23	20.6	1.35	2.46	0.000156	0.003429
Ckap2	394.5	1.32	1.39	0	2.3E-14
Cdc6	570.6	1.32	1.4	2.3E-14	3.85E-12
E2f7	275.3	1.32	1.48	3.98E-09	3.21E-07
Mpo	1003.3	1.32	2.2	0.000333	0.006459
Mastl	108.5	1.31	1.51	7.78E-08	5.19E-06
Cenpn	307	1.3	1.38	6.9E-14	1.09E-11
Rad51b	65.9	1.3	1.52	2.32E-07	1.37E-05
Pgam2	13	1.3	2.75	0.000571	0.009954
Smtn	271.7	1.29	1.4	2.59E-10	2.57E-08
Car5b	136	1.29	1.45	6.02E-10	5.61E-08
Wfdc17	99.9	1.29	1.47	2.18E-07	1.3E-05
Xkr5	39.4	1.29	1.62	5.06E-06	0.000197
Ifit1bl1	137.8	1.29	1.67	2.19E-05	0.000693

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Dhfr	503.3	1.28	1.35	2.1E-14	3.58E-12
Ccne1	503.1	1.28	1.37	8.53E-12	1.06E-09
Il4	152.5	1.28	1.52	3.69E-06	0.000151
Ms4a3	31.1	1.28	2.52	0.000653	0.011034
Fancd2	392.9	1.27	1.33	0	3.4E-14
Syce2	175.1	1.27	1.39	1.01E-09	8.79E-08
Slc43a3	380.4	1.26	1.36	1.77E-11	2.05E-09
Mcpt2	69.1	1.26	3.47	0.000769	0.012455
Dna2	343.7	1.25	1.36	3.64E-10	3.52E-08
Stil	422.9	1.25	1.39	3.95E-08	2.72E-06
Ms4a4a	77.2	1.24	1.41	1.03E-07	6.69E-06
Ifit3	421.9	1.24	2	0.000649	0.010983
Ighv1-9	232.8	1.24	2.33	0.001087	0.016261
Brca1	443.2	1.23	1.32	6.31E-12	8.12E-10
Cks1b	772.5	1.22	1.32	1.23E-09	1.05E-07
Scin	117.4	1.22	1.59	0.000102	0.002435
Fanca	375.8	1.21	1.29	3.34E-12	4.44E-10
Sostdc1	55.7	1.2	1.42	2.05E-05	0.000654
Tmprss4	19.7	1.2	1.67	0.000585	0.010122
Pcdhgc4	19.5	1.2	1.98	0.000598	0.010298
C3	1368.2	1.19	1.36	9.68E-07	4.74E-05
Fbn2	27	1.19	1.43	6.33E-05	0.001639
Igf2bp2	71.9	1.19	1.64	0.000461	0.008431
Pask	202.9	1.18	1.28	1.46E-09	1.24E-07
Atp6v1g3	12.9	1.18	2.18	0.001663	0.022323
Tmem121	19.5	1.17	1.86	0.001247	0.017975
E2f1	876.4	1.16	1.19	0	0
Zan	929.3	1.16	1.27	3.35E-08	2.33E-06
Plac8	2583	1.16	1.28	1.19E-07	7.61E-06
Rad54b	114.1	1.16	1.29	1.79E-07	1.09E-05
Lig1	3384.1	1.15	1.2	7E-15	1.29E-12
Gins2	413.5	1.15	1.22	1.04E-11	1.28E-09
Gpsm2	436.7	1.15	1.23	2.7E-11	3.06E-09
Arhgap11a	847.4	1.15	1.22	7.3E-11	7.83E-09
Gbp11	355.1	1.15	1.23	6.55E-10	6.06E-08
Gm15987	44.4	1.15	1.51	0.000198	0.004191
Efcab11	31.5	1.15	1.62	0.000442	0.008194
Snca	15	1.15	2.01	0.001303	0.018592
Il13	60.8	1.15	1.81	0.001306	0.01862
Smpd13b	185.8	1.14	1.28	1.47E-07	9.11E-06
Ispd	31.4	1.14	1.42	0.000161	0.003543
Igf2bp3	26.6	1.14	1.91	0.001053	0.015932

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Platr11	10.2	1.14	2.09	0.00249	0.029873
Cpa3	467.2	1.14	2.12	0.002534	0.03018
Tacc3	1922.3	1.13	1.16	0	1E-15
Rmi2	184.3	1.13	1.21	5.58E-10	5.23E-08
Nsl1	195.3	1.13	1.21	1.45E-09	1.23E-07
AC151730.1	71.6	1.13	1.55	0.000357	0.006861
Myh3	15.8	1.13	1.64	0.001352	0.019086
Ighg2b	522.3	1.13	2.65	0.002343	0.028708
Klrg1	746.6	1.12	1.21	3.27E-10	3.18E-08
Ociad2	184.7	1.12	1.27	1.87E-08	1.35E-06
Mis18bp1	180.6	1.12	1.24	1.95E-07	1.17E-05
Ccl1	31	1.12	1.77	0.001407	0.019614
Prtn3	290.9	1.12	2.13	0.002845	0.032835
Cdc20	1268.9	1.1	1.14	7.2E-14	1.12E-11
Tfec	71.7	1.1	1.32	6.43E-05	0.001657
Isg15	787.9	1.1	1.38	0.000249	0.005119
Klra7	38.4	1.1	1.58	0.00111	0.016512
Hist1h2bj	17.7	1.1	1.75	0.001687	0.022479
Kbtbd6	12	1.1	2.04	0.002527	0.030129
Stx11	1153.5	1.09	1.14	3.55E-12	4.68E-10
Lgals1	11014.9	1.09	1.15	9.3E-10	8.22E-08
Ccl8	29.5	1.09	4.6	0.003929	0.041586
Nt5dc2	188.1	1.08	1.22	4.38E-06	0.000174
Dnph1	136.4	1.08	1.29	6.66E-05	0.001706
Bcat1	363.8	1.07	1.14	8.94E-10	8.07E-08
Kifc1	310.5	1.07	1.17	8.33E-08	5.52E-06
Mybl2	481.2	1.07	1.17	5.93E-07	3.09E-05
Mx1	719.3	1.07	1.66	0.002705	0.031699
Mcm5	5776.8	1.06	1.1	0	9.6E-14
Cdkn2c	421.3	1.06	1.11	1.25E-11	1.49E-09
Ccl2	77.5	1.06	1.47	0.001637	0.022077
Ifit1	298.3	1.06	1.61	0.002709	0.031699
Ighv5-2	17.8	1.06	2.05	0.004935	0.049159
Ccr5	1338.8	1.05	1.1	1.43E-12	1.97E-10
Serpinb6b	1199	1.05	1.12	3.96E-09	3.2E-07
Smc2	1176.2	1.05	1.12	4.67E-09	3.71E-07
Isg20	861.4	1.05	1.15	1.91E-06	8.67E-05
Klrc3	41.9	1.05	1.37	0.000456	0.008347
Rtp4	1189.3	1.05	1.43	0.001656	0.022272
Nrg1	17.4	1.05	1.91	0.00423	0.043995
Gins1	191.5	1.04	1.17	3.75E-06	0.000152
Knstrn	855.1	1.03	1.06	5.4E-14	8.61E-12

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Phf19	475.4	1.03	1.08	7.44E-11	7.92E-09
Art2a-ps	407.6	1.03	1.17	4.28E-05	0.001207
Rfc4	533.3	1.02	1.04	0	0
Tnfsf11	468.5	1.02	1.11	4.92E-07	2.61E-05
Fn1	453.7	1.02	1.33	0.001188	0.01738
Gna14	24.8	1.02	1.42	0.002372	0.028943
Bard1	316.8	1.01	1.07	2.26E-09	1.9E-07
Pole	1124.1	1.01	1.07	2.85E-08	2.01E-06
Pdcd1	880.8	1.01	1.08	6.75E-08	4.53E-06
Prc1	830.9	1.01	1.1	1.43E-06	6.68E-05
Art2b	542.6	1.01	1.19	0.000104	0.002467
Mcm8	150.3	1	1.09	8.42E-07	4.25E-05
Xaf1	1257.6	1	1.1	1.6E-06	7.42E-05
Tnfrsf8	167.1	1	1.1	4.36E-06	0.000173
Apitd1	305.6	1	1.14	2.51E-05	0.000779
Gstt3	58.6	1	1.15	5.96E-05	0.001564
Hist1h1b	56.5	1	1.32	0.001234	0.01782
Cfap77	26	1	1.31	0.001439	0.019968
Myo1d	68	1	1.42	0.002852	0.032892
4930438A08Rik	145.8	-1.01	-1.17	0.000312	0.006134
Gm7860	120.6	-1.02	-1.09	1.76E-07	1.08E-05
Gm38405	61.2	-1.03	-1.22	0.000285	0.005674
Gm5608	44.9	-1.03	-1.27	0.000717	0.011861
Fjx1	45.8	-1.03	-1.38	0.002866	0.033002
Gm37510	31.1	-1.03	-1.48	0.004083	0.042776
9230114K14Rik	64.2	-1.04	-1.19	6.16E-05	0.001606
Cd164l2	60	-1.04	-1.24	0.000281	0.005618
Podn	23.2	-1.04	-1.32	0.001135	0.016833
Mfrp	55.6	-1.04	-1.33	0.0016	0.021746
Timm8a2	27.2	-1.05	-1.28	0.000484	0.00875
Gm11210	24.9	-1.06	-1.38	0.000895	0.014062
Trim72	99	-1.08	-1.33	0.000468	0.008514
Rbm44	28.3	-1.09	-1.5	0.001468	0.020306
Pygm	1170.9	-1.12	-1.19	1.17E-10	1.2E-08
Prr15	32.2	-1.12	-1.31	5.32E-05	0.001422
Prdm14	18.6	-1.13	-1.53	0.001494	0.020587
Tm4sf1	20.6	-1.14	-1.56	0.001205	0.017549
Rpl31-ps6	17.6	-1.15	-1.6	0.000885	0.013952
Six4	17.7	-1.17	-1.57	0.000705	0.011726
Tex45	736.4	-1.19	-1.29	6.92E-09	5.36E-07
Apol8	1161.9	-1.2	-1.25	3E-15	4.88E-13
AC158622.5	86.8	-1.23	-1.44	9.3E-06	0.00033

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Fgf17	23	-1.27	-1.72	0.000249	0.005119
AC166361.2	101	-1.3	-1.42	2.71E-09	2.24E-07
Gsdmc4	19.2	-1.33	-1.63	1.68E-05	0.00055
Ap3s1-ps2	15.1	-1.34	-1.96	0.000176	0.003785
Pmel	62.3	-1.73	-4.35	1.15E-06	5.52E-05

[000457] NIH DAVID cluster analysis was performed using the upregulated gene list. Enriched clusters were ranked based on enrichment score. Cell cycle gene cluster achieved the highest enrichment score (FIGs. 23B and 24A). This result was confirmed by KEGG gene set enrichment analysis (GSEA) (FIG. 24B). Cell cycle gene set ranks as the highest (enrichment score = 0.64, FDR q value = 0.1, nominal p value = 0). These results indicate that SV infection enhances T cell cycle progression. SV induced upregulation of a series of cytokine and chemokine/chemokine receptors (FIG. 23C, left). To identify cytokines/chemokines that are upregulated by the administration of SV vectors, we compared SV plus α 4-1BB mAb versus α 4-1BB mAb (FIG. 23C, right). CCL8, IL-4, IL-13 and IL-21 were among those RNAs whose expression was upregulated by SV treatment. IL-21 anti-tumor effect is dependent on the activation of T, B and NK cells [Leonard WJ et al., F1000Res. 2016]. IL-4, IL-10, IL-21 upregulation is consistent with previous reports [Rowell JF et al., J Immunol. 1999, Metcalf TU et al., J Virol. 2013]. In addition, Ingenuity Pathway Analysis (IPA) indicates that SV treatment enhances T cell movement by altering the expression of a number of molecules involved migration (Table 2, FIG. 24C), including a number of chemokines and chemokine receptors.

[000458] **Table 2:** The upregulated cell movement pathway for SV vs. untreated group by IPA. SV induced SD upregulated gene sets are clustered by DAVID analysis (SV vs. Untreated). Gene clusters are ranked by enrichment score.

ID	Genes in dataset	Prediction (based on measurement direction)	Expr Log Ratio	Findings
Il21	IL21	Increased	2.57	Increases (2)
Lag3	LAG3	Decreased	1.76	Decreases (3)
Il10	IL10	Decreased	1.66	Decreases (2)
Ccl1	CCL1	Increased	1.12	Increases (1)

ID	Genes in dataset	Prediction (based on measurement direction)	Expr Log Ratio	Findings
Ccr5	CCR5	Increased	1.05	Increases (3)
Pdcd1	PDCD1	Decreased	1.01	Decreases (3)
Tnfrsf8	TNFRSF8	Increased	1	Increases (1)
Cxcr5	CXCR5	Increased	0.98	Increases (1)
Ccr2	CCR2	Increased	0.9	Increases (9)
Cxcr3	CXCR3	Increased	0.77	Increases (1)
Sh2d1a	SH2D1A	Increased	0.68	Increases (1)
Batf	BATF	Increased	0.67	Increases (7)
Pycard	PYCARD	Increased	0.64	Increases (3)
Ccr4	CCR4	Increased	0.62	Increases (4)
Ccl5	CCL5	Increased	0.62	Increases (2)
Itgb1	ITGB1	Increased	0.51	Increases (1)
S1pr2	S1PR2	Increased	0.5	Increases (1)
Lcp1	LCP1	Affected	0.5	Affects (1)
Tnfsf14	TNFSF14	Increased	0.5	Increases (1)
Hspd1	HSPD1	Decreased	0.41	Decreases (1)
Cbfb	CBFB	Decreased	0.4	Decreases (1)
Jak3	JAK3	Increased	0.36	Increases (1)
Rap1a	RAP1A	Decreased	0.31	Decreases (3)
Was	WAS	Increased	0.29	Increases (2)
Etv6	ETV6	Increased	0.26	Increases (3)
Rac2	RAC2	Increased	0.25	Increases (36)
Apbb1ip	APBB1IP	Increased	-0.21	Decreases (2)
Pecam1	PECAM1	Decreased	-0.34	Increases (10)
Ldlr	LDLR	Decreased	-0.41	Increases (1)
Bach2	BACH2	Increased	-0.46	Decreases (1)

ID	Genes in dataset	Prediction (based on measurement direction)	Expr Log Ratio	Findings
S1pr1	S1PR1	Decreased	-0.58	Increases (6)
Gpr132	GPR132	Increased	-0.6	Decreases (1)

[000459] To understand why SV plus $\alpha 4$ -1BB mAb achieves the best therapeutic effect, Deseq2 analysis was run for SV plus $\alpha 4$ -1BB mAb vs. untreated samples. 1046 upregulated genes ($q < 0.05$ and $\text{Log}_2 \text{Fold Change} \geq 1$) and 877 downregulated genes ($q < 0.05$ and $\text{Log}_2 \text{Fold Change} \leq -1$) in the SV plus $\alpha 4$ -1BB mAb group were identified (FIG. 23A, Table 3). T cells from animals treated with SV + $\alpha 4$ -1BB mAb vs. treated were also compared with SV only and 316 upregulated genes ($p < 0.05$ and $\text{Log}_2 \text{Fold Change} \geq 1$) and 439 downregulated genes ($p < 0.05$ and $\text{Log}_2 \text{Fold Change} \leq -1$) in the SV + $\alpha 4$ -1BB mAb treated group were found (FIG. 23A, Table 4).

[000460] **Table 3:** The SD expressed genes list for SV + $\alpha 4$ -1BB vs. untreated group by RNA-Seq ($q < 0.05$, $\text{Log}_2 \text{FC} \geq 1$ and $\text{Log}_2 \text{FC} \leq -1$).

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Gzmk	1785.2	4.75	5.32	0	0
Gzmb	4113.9	4.37	4.81	0	0
Ccl8	29.5	3.64	8.24	0	0
Ociad2	184.7	3.53	3.76	0	0
Aurkb	806.6	3.51	3.73	0	0
Ret	794.7	3.51	4.41	0	0
E2f8	535.1	3.5	3.88	0	0
Tpx2	1113.7	3.34	3.58	0	0
Kif2c	341.3	3.33	3.58	0	0
Kif18b	606.6	3.29	3.51	0	0
Hmmr	311.7	3.26	3.61	0	0
Cdc25c	143.9	3.25	3.81	0	0
Pbk	107.5	3.24	3.63	0	0
Cenpf	495.6	3.23	3.46	0	0
Prr11	288	3.23	3.68	0	0
Ccna2	1680.2	3.18	3.34	0	0
Ccnb2	1255.4	3.16	3.38	0	0
Bub1	362.3	3.16	3.44	0	0
Pif1	204	3.16	3.57	0	0
Klrg1	746.6	3.15	3.34	0	0

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Nusap1	813.1	3.14	3.31	0	0
Bub1b	1344.3	3.13	3.32	0	0
Rad54l	350.2	3.13	3.43	0	0
Cdk1	1228.8	3.11	3.32	0	0
Mki67	5899.1	3.11	3.36	0	0
Rad51	401.4	3.08	3.34	0	0
Ttk	211.5	3.08	3.4	0	0
Nuf2	279.6	3.08	3.46	0	0
Figl1	145.7	3.07	3.37	0	0
Shcbp1	274.8	3.05	3.26	0	0
Cdkn3	129.2	3.05	3.35	0	0
Rrm2	1718.2	3.04	3.26	0	0
Ube2c	1391	3.03	3.35	0	0
Pclaf	522.9	3.03	3.36	0	0
Lrr1	59	3.03	3.74	0	0
Cdca8	1251.5	3.02	3.21	0	0
Cep55	503.9	3.02	3.24	0	0
Mcm10	477.4	3.01	3.22	0	0
Sapcd2	275.5	2.99	3.28	0	0
Pimreg	230	2.99	3.3	0	0
Kif4	650.2	2.97	3.13	0	0
Kntc1	402.4	2.96	3.25	0	0
Ccr5	1338.8	2.95	3.07	0	0
Kif14	299.1	2.94	3.2	0	0
Sgo1	162.8	2.94	3.21	0	0
Spc24	506	2.93	3.08	0	0
Espl1	809.6	2.93	3.12	0	0
Clspn	637.1	2.93	3.14	0	0
Wdr95	226	2.88	3.09	0	0
Nek2	554.5	2.88	3.13	0	0
Ccnb1	160.8	2.88	3.25	0	0
Ncapg	383.1	2.86	3.09	0	0
Top2a	4726.3	2.85	2.96	0	0
Ncaph	822.4	2.85	3	0	0
Kif20a	898.5	2.85	3.03	0	0
Diaph3	356.3	2.85	3.04	0	0
Akr1c18	25.3	2.85	4.88	6E-15	1.84E-13
Ckap2l	455.4	2.83	3.1	0	0
Oip5	50.6	2.83	3.55	0	0
Ermn	17.7	2.82	5.41	3.2E-14	8.82E-13
Spag5	1183.2	2.81	2.97	0	0
Birc5	1540.7	2.81	3.13	0	0

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Dpysl5	94.9	2.78	3.57	0	0
Cdca5	791.4	2.77	2.88	0	0
Iqgap3	284.2	2.77	2.98	0	0
Kif15	595.3	2.76	2.95	0	0
Plk1	794.8	2.75	2.94	0	0
Troap	236.4	2.75	3.04	0	0
Cenpe	685.5	2.73	2.91	0	0
Esco2	149.5	2.73	3.04	0	0
Gng3	34.8	2.72	3.74	0	1E-15
Cit	784.3	2.71	2.9	0	0
Kif22	1022.2	2.71	2.91	0	0
Car5b	136	2.71	2.94	0	0
Ncald	297.2	2.71	2.95	0	0
Cdca2	444.5	2.7	2.87	0	0
Kn1l	203.3	2.7	3.01	0	0
Hist1h1b	56.5	2.7	3.28	0	0
Aunip	24.6	2.69	4.29	1.5E-14	4.26E-13
1500009L16Rik	143.2	2.68	3.37	0	0
Osr2	54.7	2.68	4.06	8E-15	2.43E-13
Depdc1b	350.8	2.67	2.86	0	0
Ticrr	558.4	2.67	2.87	0	0
Melk	329.8	2.66	2.82	0	0
Tpsab1	262.4	2.66	3.16	0	0
Smpdl3b	185.8	2.65	2.9	0	0
Spc25	211.3	2.65	2.95	0	0
Cdc45	628.7	2.64	2.73	0	0
Ccr2	1142.1	2.64	2.83	0	0
Il21	229	2.64	3.76	3.3E-14	9.01E-13
BC030867	129.6	2.63	2.93	0	0
Uhrfl	1603.6	2.61	2.76	0	0
Mxd3	280.1	2.61	2.76	0	0
Tyms	809.6	2.6	2.7	0	0
Neil3	228.1	2.6	2.82	0	0
Trip13	233.7	2.59	2.78	0	0
Rad51ap1	205.6	2.58	2.78	0	0
Asf1b	1276.4	2.57	2.69	0	0
Kif11	1109.9	2.55	2.67	0	0
Ska1	210.9	2.55	2.86	0	0
Stmn1	257.1	2.53	2.71	0	0
Ccl5	22142.8	2.53	2.75	0	0
Ly6a	2208.2	2.53	3.76	5.03E-13	1.2E-11
Igf2bp3	26.6	2.52	3.61	1.47E-13	3.72E-12

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Aspm	319.4	2.51	2.77	0	0
Parpbp	117.3	2.51	2.86	0	0
Ect2	255.1	2.49	2.82	0	0
Cdca3	1038.6	2.48	2.6	0	0
E2f7	275.3	2.48	2.74	0	0
Depdc1a	29.6	2.48	3.46	2E-15	7.6E-14
Gm33460	48.9	2.47	2.96	0	0
Tex15	58.4	2.47	3	0	0
Tk1	1004.9	2.46	2.59	0	0
Lag3	2916.7	2.45	2.56	0	0
Spdl1	204.5	2.45	2.61	0	0
Gm15056	28.9	2.45	4.48	4.74E-11	8.5E-10
Ckap2	394.5	2.44	2.55	0	0
Ankle1	238.9	2.44	2.71	0	0
Mt3	18.3	2.42	4.28	3.9E-11	7.15E-10
Cenph	201.9	2.41	2.66	0	0
Arsb	1795.9	2.4	2.55	0	0
Cenpi	136.8	2.4	2.6	0	0
Gm6637	196.3	2.4	2.69	0	0
Ndc80	345.5	2.39	2.53	0	0
Chaf1a	1027.2	2.39	2.53	0	0
Cenpm	289.6	2.39	2.61	0	0
CT030166.6	24.2	2.37	3.39	1.66E-12	3.74E-11
Ms4a3	31.1	2.37	4.07	2E-10	3.29E-09
Il13	60.8	2.36	3.42	3.84E-11	7.06E-10
Ctsg	151.2	2.36	3.9	2.01E-10	3.3E-09
Col6a5	17.7	2.36	6.13	4.48E-10	6.83E-09
Ncapd2	3741.3	2.33	2.39	0	0
Tcf19	1158.6	2.32	2.41	0	0
Gbp11	355.1	2.31	2.46	0	0
Fhl2	217.6	2.3	2.44	0	0
Ncapg2	526.2	2.29	2.43	0	0
Cks1b	772.5	2.28	2.46	0	0
Gzma	3261.2	2.28	3.76	8.07E-10	1.18E-08
Nkg7	8456.1	2.27	2.34	0	0
Pask	202.9	2.27	2.43	0	0
Il10	1005.5	2.27	2.5	0	0
Prc1	830.9	2.26	2.46	0	0
Wipf3	37.6	2.26	3.33	3.67E-12	7.88E-11
Mpo	1003.3	2.26	3.54	7.46E-10	1.09E-08
Osbp13	1358.5	2.25	2.33	0	0
Mastl	108.5	2.25	2.54	0	0

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Serpinb6b	1199	2.24	2.38	0	0
Adgrg1	449.6	2.24	2.4	0	0
Slc16a2	105.6	2.24	2.53	0	0
Ccl1	31	2.24	3.2	7.42E-11	1.29E-09
Cdc6	570.6	2.22	2.34	0	0
Bspry	174.2	2.22	2.64	1E-15	3.6E-14
Ccne1	503.1	2.21	2.36	0	0
Cenpp	81.2	2.21	2.47	0	0
Lxn	79	2.21	2.45	0	0
Lgals1	11014.9	2.2	2.34	0	0
Adap1	1257.2	2.18	2.3	0	0
F13a1	227.4	2.17	3.04	1.97E-10	3.24E-09
Cdc20	1268.9	2.16	2.24	0	0
Tigit	2942.9	2.16	2.38	0	0
Cdkn2a	59.4	2.15	2.56	3.4E-14	9.34E-13
Exo1	187.1	2.14	2.4	0	0
Clip4	39.8	2.14	2.75	7.69E-12	1.58E-10
F2rl2	42.8	2.13	2.58	1.57E-13	3.95E-12
Hist1h2bj	17.7	2.13	2.99	4.05E-10	6.22E-09
Glde	15.3	2.13	3.09	1.37E-09	1.92E-08
Dhfr	503.3	2.12	2.24	0	0
Lig1	3384.1	2.11	2.19	0	0
Cenpn	307	2.11	2.23	0	0
Tox2	889.5	2.11	2.32	0	0
Cdkn2c	421.3	2.1	2.2	0	0
Kifc1	310.5	2.1	2.26	0	0
Slc22a3	85.3	2.1	2.57	1.47E-12	3.33E-11
Msr1	48.9	2.1	2.73	1.56E-11	3.04E-10
Trp73	22	2.1	3.54	2.07E-08	2.37E-07
Prtn3	290.9	2.1	3.62	2.37E-08	2.68E-07
Csf2	72.9	2.09	2.47	8E-14	2.1E-12
Fancd2	392.9	2.08	2.16	0	0
Gm2788	29.7	2.08	2.77	5.16E-11	9.2E-10
Nfe2	91.3	2.08	2.85	9.6E-10	1.38E-08
Rnase2a	5.3	2.08	6.48	1.58E-08	1.84E-07
Mis18bp1	180.6	2.07	2.26	0	0
Samd14	203.2	2.07	2.39	3E-15	8.5E-14
Oas1a	498.3	2.07	2.42	4E-15	1.1E-13
Hist1h3c	11.8	2.07	3.72	2.61E-08	2.93E-07
Tacc3	1922.3	2.06	2.12	0	0
Wdr31	15.1	2.06	3.44	1.23E-08	1.46E-07
Knstrn	855.1	2.05	2.12	0	0

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Anxa2	5777.8	2.05	2.21	0	0
Tpbp	19.3	2.04	3.63	2.53E-08	2.85E-07
Cst7	1893.9	2.02	2.09	0	0
Slc43a3	380.4	2.02	2.16	0	0
Muc13	227	2.02	2.24	0	0
Rad51b	65.9	2.02	2.3	0	5E-15
Xkr5	39.4	2.02	2.42	2.9E-13	7.07E-12
Chil3	42.4	2.02	3.21	4.25E-08	4.61E-07
Bard1	316.8	2.01	2.11	0	0
Acot7	2318.6	2.01	2.12	0	0
Arhgap11a	847.4	2.01	2.13	0	0
Rad54b	114.1	2.01	2.21	0	0
Pcdhgc4	19.5	2.01	2.97	5.51E-09	6.96E-08
Mcm5	5776.8	2	2.06	0	0
Rmi2	184.3	2	2.12	0	0
Dna2	343.7	2	2.16	0	0
S100a6	3227.2	2	2.17	0	0
Mybl2	481.2	2	2.19	0	0
Brca1	443.2	1.99	2.11	0	0
Esm1	680.1	1.99	2.16	0	0
E2f1	876.4	1.98	2.04	0	0
F10	33.6	1.98	3.1	4.08E-08	4.44E-07
Lif	439.6	1.97	2.05	0	0
Pglyrp1	1046.5	1.97	2.15	0	0
Fam19a3	168.7	1.97	2.22	1E-15	1.9E-14
Spp1	51	1.97	2.41	2.47E-11	4.68E-10
Faxc	12.4	1.97	3.44	1.3E-07	1.3E-06
Stil	422.9	1.96	2.17	0	0
Dapk2	315.5	1.95	2.04	0	0
Serpib9	1224.7	1.95	2.06	0	0
Fanca	375.8	1.95	2.06	0	0
Bcat1	363.8	1.95	2.06	0	0
Neurl1b	49.9	1.95	2.46	2.74E-10	4.38E-09
Gins1	191.5	1.94	2.14	0	0
Tg	98.4	1.94	2.15	0	1E-15
Dnph1	136.4	1.94	2.27	5.77E-13	1.36E-11
Tmem40	47.5	1.94	2.52	2.2E-09	2.98E-08
Hist1h3g	9.3	1.94	3.19	1.43E-07	1.41E-06
Ighg1	2604.8	1.94	4.76	2.48E-07	2.33E-06
Pscl1	122.1	1.93	2.07	0	0
Serpina3f	452	1.93	2.12	0	0
Robo3	27.3	1.93	2.44	7.7E-10	1.13E-08

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Gcg	62.2	1.93	5.91	2.19E-07	2.08E-06
Smc2	1176.2	1.92	2.03	0	0
Sgo2a	143.4	1.92	2.11	0	0
Nt5dc2	188.1	1.92	2.15	0	5E-15
Klrc2	53.2	1.92	2.27	5.7E-13	1.35E-11
Prss57	9.8	1.92	3.88	2.9E-07	2.7E-06
Gpsm2	436.7	1.91	2.02	0	0
Nsl1	195.3	1.9	2.03	0	0
C3	1368.2	1.9	2.15	6E-15	1.81E-13
Angpt1	63.2	1.9	2.28	1.89E-11	3.65E-10
Popdc2	54.7	1.89	2.36	3.65E-10	5.66E-09
Kbtbd6	12	1.89	3.03	1.35E-07	1.34E-06
Stk32c	341	1.87	1.99	0	0
Syce2	175.1	1.87	2.03	0	0
Gp9	24.8	1.87	2.53	3.19E-08	3.56E-07
Pocla	388.3	1.86	1.95	0	0
2610318N02Rik	193.2	1.86	2.02	0	0
Ifng	1413.7	1.86	2.11	5.7E-14	1.51E-12
Hcn2	36.3	1.86	2.42	6.4E-09	7.97E-08
Klhl23	20.6	1.86	3.13	1.4E-07	1.38E-06
Nrg1	17.4	1.86	3	3.31E-07	3.06E-06
Insrr	64.7	1.85	2.05	3E-15	1.03E-13
Cldnd2	53.5	1.85	2.12	3.45E-13	8.31E-12
Ifit1bl1	137.8	1.85	2.34	1.18E-09	1.67E-08
Scn11a	5.9	1.85	5.36	7.48E-07	6.42E-06
Pdzph1	10.7	1.85	4.11	9.95E-07	8.34E-06
Gins2	413.5	1.84	1.94	0	0
Gm12250	177.3	1.84	2.07	6E-15	1.73E-13
Dach1	18.3	1.84	2.8	3.05E-07	2.83E-06
Rasgef1a	270.7	1.83	1.94	0	0
Pcytlb	106.8	1.83	2.01	0	0
Oas3	1474.3	1.83	2.25	3.83E-10	5.9E-09
Cd70	12.5	1.83	2.73	3.29E-07	3.05E-06
Tuba8	42.2	1.83	2.76	4.67E-07	4.21E-06
Tph1	43	1.83	3.53	1.35E-06	1.11E-05
Zbtb32	667.1	1.82	1.9	0	0
Tfr2	139.5	1.82	2.41	2.31E-08	2.62E-07
Mmrn1	13.3	1.82	2.99	7.75E-07	6.64E-06
Gm24289	6.9	1.82	5.21	1.22E-06	1.01E-05
Dlgap5	705.3	1.81	1.9	0	0
Ska3	252.1	1.81	1.91	0	0
Fam81a	55.4	1.81	2.16	1.92E-10	3.16E-09

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Plppr3	42.4	1.81	2.74	7.18E-07	6.2E-06
Mpl	45.7	1.81	2.69	7.96E-07	6.8E-06
Hist1h2ag	6.4	1.81	3.59	1.52E-06	1.23E-05
Fbxo41	15.3	1.8	2.59	1.51E-07	1.48E-06
Mcm3	5809.4	1.79	1.84	0	0
Ska2	254.2	1.79	1.91	0	0
Art2b	542.6	1.79	2.08	6.27E-12	1.3E-10
Necab3	27.4	1.79	2.36	1.87E-08	2.16E-07
Lilr4b	1553.9	1.78	1.91	0	0
AA467197	30.3	1.78	2.21	9E-09	1.09E-07
Maats1	7.8	1.78	3.55	2.36E-06	1.84E-05
Col6a2	16.9	1.78	3.82	2.57E-06	1.99E-05
Cdca7	631.9	1.77	1.89	0	0
Serpina3g	2916.5	1.77	1.92	0	0
Dyrk3	249.1	1.77	1.95	2E-15	5.8E-14
Apitd1	305.6	1.77	1.98	9.1E-14	2.37E-12
AC153938.2	40.5	1.77	2.19	2.58E-09	3.47E-08
Eomes	4109.7	1.75	1.8	0	0
Pola1	686.1	1.75	1.83	0	0
Pole	1124.1	1.75	1.86	0	0
Eme1	219.4	1.75	1.88	0	0
Map6	151	1.75	1.88	0	0
Gm4951	44.3	1.75	2.24	1.88E-09	2.57E-08
Tff3	16.7	1.75	2.36	1.47E-07	1.45E-06
Nmral1	561.7	1.74	1.86	0	0
Plac8	2583	1.74	1.92	2E-15	6.3E-14
Klrc1	236.6	1.74	1.95	5E-14	1.34E-12
S100a4	1170.3	1.74	1.96	7.04E-13	1.66E-11
Mns1	129.3	1.74	2.05	2.42E-10	3.91E-09
Miat	31.2	1.74	2.07	5.13E-10	7.74E-09
Efcab11	31.5	1.74	2.33	6.62E-08	6.98E-07
Erfe	15.4	1.74	2.77	9.89E-07	8.3E-06
Kcnk5	399.5	1.73	1.81	0	0
Stx11	1153.5	1.73	1.81	0	0
Smtn	271.7	1.73	1.87	0	0
Anln	217.2	1.73	1.89	1E-15	3.1E-14
Gm17745	84	1.73	1.92	1.9E-14	5.44E-13
Krt18	26.4	1.73	2.17	1.75E-08	2.03E-07
2900011O08Rik	14.8	1.73	2.39	9.4E-07	7.92E-06
Tpi1	2445.6	1.72	1.78	0	0
Chsy1	2251.6	1.72	1.8	0	0
Gtse1	554	1.72	1.81	0	0

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Pdcd1	880.8	1.72	1.84	0	0
Vdr	250.7	1.72	1.97	2.66E-11	5E-10
Ltb4r1	44.5	1.72	2.06	1.19E-09	1.68E-08
2010110K18Rik	30.1	1.72	2.11	9.25E-09	1.12E-07
Alox5	104.2	1.72	2.35	2.33E-07	2.2E-06
Pde10a	17	1.72	2.48	7.24E-07	6.24E-06
Dscc1	150.3	1.71	1.83	0	0
Mcm6	5226.1	1.7	1.74	0	0
Tfdp1	1161.4	1.7	1.76	0	0
E2f2	2088.1	1.7	1.78	0	0
1700011L03Rik	6.7	1.7	5.77	5.28E-06	3.81E-05
Col6a1	15.6	1.7	4.62	6.57E-06	4.64E-05
Chst11	1091.1	1.69	1.75	0	0
Il12rb1	1086.8	1.69	1.76	0	0
Gm4841	29.9	1.69	2.35	3.46E-07	3.19E-06
Trem1	31.4	1.69	2.36	1.75E-06	1.4E-05
Fcer1a	69.7	1.69	2.77	3.83E-06	2.85E-05
Rfc4	533.3	1.68	1.72	0	0
Rgs16	7742.7	1.68	1.75	0	0
Ezh2	1820.5	1.68	1.76	0	0
Lockd	222.2	1.68	1.85	3.1E-14	8.5E-13
Fam57b	23.3	1.68	5.08	4.18E-07	3.8E-06
Dixdc1	18.6	1.68	2.66	2.93E-06	2.25E-05
Trpc6	7.4	1.68	4.05	5.76E-06	4.12E-05
Adra1b	14.9	1.68	2.83	6.8E-06	4.78E-05
Ttc39c	656.7	1.67	1.72	0	0
Wee1	206.8	1.67	1.75	0	0
Neb	354.6	1.67	1.9	2.03E-11	3.9E-10
Prdm1	912.1	1.66	1.74	0	0
Gimap7	2437.5	1.66	1.76	0	0
Mcm8	150.3	1.66	1.79	0	4E-15
Tnfrsf8	167.1	1.66	1.82	1E-14	3.02E-13
Lgals1	189.5	1.66	1.82	2.5E-14	7.06E-13
Ptgr1	201.9	1.66	1.84	1.25E-13	3.19E-12
Fads2	70.5	1.66	2.07	1.89E-08	2.18E-07
Usp18	491.6	1.66	2.34	1.26E-06	1.03E-05
Serpinb9b	63.4	1.66	2.45	1.55E-06	1.26E-05
Vwa2	4.2	1.66	5.89	3.9E-06	2.9E-05
Saa3	14.8	1.66	4.96	6.94E-06	4.87E-05
Mad2l1	857.6	1.65	1.69	0	0
Hells	609.3	1.65	1.71	0	0
Cisd1	468.5	1.65	1.73	0	0

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Slc35d3	110.5	1.65	1.77	0	5E-15
Sncb	15	1.65	2.65	2.82E-06	2.17E-05
Ppbp	30.6	1.65	2.38	3.36E-06	2.54E-05
Tff1	8.6	1.65	3.22	1.24E-05	8.24E-05
Nsd2	2242.8	1.64	1.68	0	0
Phf19	475.4	1.64	1.71	0	0
Ccdc34	308.1	1.64	1.73	0	0
1700001O22Rik	140.3	1.64	1.78	4E-15	1.36E-13
Gm20667	5.2	1.64	4.98	1.13E-05	7.61E-05
Ppp1r3g	7.3	1.64	3.91	1.3E-05	8.58E-05
4930519L02Rik	9.9	1.64	4.33	1.41E-05	9.23E-05
Racgap1	3392.2	1.63	1.67	0	0
Lmnbl	6788.3	1.63	1.69	0	0
Kif23	1414.3	1.63	1.69	0	0
Ryk	207.2	1.63	1.76	1E-15	2.6E-14
Heatr9	204.6	1.63	1.85	1.58E-11	3.07E-10
Ifi2712a	8730.4	1.63	1.99	2.36E-08	2.67E-07
Tnip3	36.1	1.63	2.1	2.07E-07	1.97E-06
Vat1l	14.9	1.62	3.1	9.48E-06	6.47E-05
Tpsb2	486.9	1.62	2.93	2.12E-05	0.000133
AW112010	5981.3	1.61	1.69	0	0
9630013D21Rik	122.8	1.61	1.8	4.6E-12	9.71E-11
Mtfp1	42.1	1.61	1.98	4.08E-08	4.44E-07
Col1a2	95	1.61	2.11	6.19E-07	5.41E-06
Ascl2	24.4	1.61	2.62	1.12E-05	7.53E-05
Ccdc18	45.6	1.6	1.91	1.15E-08	1.37E-07
Myct1	34.7	1.6	2.16	1.88E-06	1.49E-05
Hist1h2bm	8.5	1.6	3.51	2.45E-05	0.000151
Elane	132	1.6	3.14	2.69E-05	0.000165
Serping1	49.6	1.59	1.9	6.03E-09	7.54E-08
Rab44	136.4	1.59	2.08	8.37E-07	7.11E-06
Dcn	49	1.59	2.48	3.24E-06	2.45E-05
6530402F18Rik	553.7	1.58	1.63	0	0
Klrk1	793.9	1.58	1.68	0	0
Nup37	207.1	1.58	1.74	3.05E-13	7.43E-12
Bst1	74.2	1.58	1.75	1.64E-12	3.71E-11
Il1rl1	328.6	1.58	1.74	1.73E-12	3.88E-11
Klrc3	41.9	1.58	1.99	7.66E-08	7.98E-07
Adgrg7	5.3	1.58	5.46	9.43E-06	6.44E-05
Gp1bb	21.6	1.58	2.44	2.04E-05	0.000128
Sh2d1a	1356.7	1.57	1.64	0	0
Areg	192.8	1.57	1.78	1.04E-10	1.78E-09

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Mlkl	114.8	1.57	1.78	1.33E-10	2.24E-09
D630039A03Rik	66.5	1.57	1.86	1.8E-08	2.09E-07
Sdsl	60	1.57	2.27	7.96E-06	5.53E-05
Oas2	1573.5	1.57	2.38	8.81E-06	6.05E-05
Tjp2	411.7	1.56	1.67	1E-15	3E-14
Fut7	69.8	1.56	1.8	1.61E-09	2.23E-08
Ces2g	190	1.56	2.29	8.88E-06	6.1E-05
Tmem59l	15.2	1.56	2.3	1.24E-05	8.24E-05
Kcnj5	5.3	1.56	5.41	1.25E-05	8.31E-05
Maob	9.3	1.56	3.57	3.89E-05	0.000229
Dut	2331.7	1.55	1.6	0	0
4933404O12Rik	326.5	1.55	1.66	0	1.4E-14
Alox8	57.9	1.55	1.79	1.05E-09	1.5E-08
Runx2os1	41.2	1.55	2.01	1.56E-06	1.26E-05
Etv4	18.3	1.55	2.25	1.04E-05	7E-05
Ccnf	676.9	1.54	1.62	0	0
Spns2	183.2	1.54	1.86	1.1E-07	1.11E-06
H1fx	65.9	1.54	1.97	1.34E-06	1.1E-05
Chtf18	707.9	1.53	1.58	0	0
Gbp2b	1682.8	1.53	1.59	0	0
Cmtm7	2372.8	1.53	1.59	0	0
Gstt3	58.6	1.53	1.73	3.64E-10	5.65E-09
Chek2	113.8	1.53	1.77	3.66E-09	4.79E-08
Rab39b	35.1	1.53	1.86	7.94E-08	8.25E-07
Vash1	14.6	1.53	2.36	2.63E-05	0.000162
Incenp	3803.5	1.52	1.56	0	0
Zwilch	309.4	1.52	1.59	0	0
Brip1	231.5	1.52	1.6	0	0
Gm14005	181.4	1.52	1.61	0	2E-15
Foxm1	1814.7	1.52	1.62	0	5E-15
1700020L24Rik	90.2	1.52	1.72	8.22E-10	1.2E-08
Il4	152.5	1.52	1.8	3.53E-08	3.9E-07
Mx1	719.3	1.52	2.3	2.01E-05	0.000127
Gna15	580.1	1.51	1.6	0	1E-15
Dctpp1	771.7	1.51	1.64	2.43E-13	5.96E-12
Angptl2	741.2	1.51	1.67	1.1E-11	2.2E-10
Mgarp	30.5	1.51	1.87	4.92E-07	4.41E-06
Gm19585	364.2	1.5	1.56	0	0
Zfp367	650.8	1.5	1.58	0	0
S100a10	7676.1	1.5	1.58	0	0
C330027C09Rik	421.9	1.5	1.63	1.44E-13	3.66E-12
Cenpk	43.5	1.5	1.73	9.19E-09	1.11E-07

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Ly6e	13759	1.5	1.83	2.5E-07	2.35E-06
Ccl4	2257.9	1.5	1.9	1.59E-06	1.28E-05
Casp7	625.6	1.49	1.53	0	0
Zfand4	84.9	1.49	1.61	1.84E-13	4.58E-12
Tstd3	269.1	1.49	1.65	1.75E-11	3.39E-10
Lmtk3	107.1	1.49	1.75	4.88E-08	5.24E-07
AC153498.1	62.1	1.49	1.77	7.06E-08	7.4E-07
Grtp1	36.2	1.49	1.96	3.97E-06	2.94E-05
Gm2762	38.5	1.49	2.09	6.91E-06	4.85E-05
D130058E05Rik	5.1	1.49	5.39	2.27E-05	0.000142
Npy	11.8	1.49	2.45	6.48E-05	0.00036
Serpina3h	8.8	1.49	2.77	7.52E-05	0.000411
Ighg2c	873	1.49	3.73	7.66E-05	0.000418
Nkain1	8	1.49	3.23	7.74E-05	0.000421
Podnl1	2382.6	1.48	1.53	0	0
Gzmm	496.8	1.48	1.56	0	0
Haspin	214.2	1.48	1.6	1.76E-13	4.4E-12
Csfl	1178.9	1.48	1.64	5.94E-11	1.05E-09
Lamc2	28.8	1.48	1.89	2.97E-06	2.28E-05
Gm11454	22.4	1.48	1.93	3.25E-06	2.46E-05
Tal1	90.9	1.48	2.14	2.23E-05	0.000139
Ybx3	2394.9	1.47	1.56	0	0
Rln3	181.3	1.47	1.61	1.29E-11	2.56E-10
Rai14	77.8	1.47	1.68	9.24E-09	1.11E-07
Gmpr	24.8	1.47	2.3	5.94E-05	0.000334
Drp2	31.1	1.47	2.94	9.79E-05	0.000518
Cma1	339	1.47	2.71	0.00011	0.000572
Gbp5	1110.5	1.46	1.49	0	0
Alad	1397.6	1.46	1.63	2.48E-10	4E-09
Tnfsf4	81.6	1.46	1.73	2.63E-07	2.47E-06
Rtkn2	30.4	1.46	1.85	1.64E-06	1.32E-05
Klra7	38.4	1.46	2.03	1.43E-05	9.35E-05
Pf4	162.2	1.46	2.25	9.08E-05	0.000485
Gbp2	3001.3	1.45	1.5	0	0
Ercc6l	189.4	1.45	1.55	2.2E-14	6.19E-13
Iigp1	872.5	1.45	1.55	2.8E-14	7.75E-13
Gemin6	89.9	1.45	1.55	8.5E-14	2.2E-12
Paqr4	188.3	1.45	1.58	4.17E-12	8.88E-11
Cd160	483.3	1.45	1.63	1.06E-09	1.51E-08
Ttc16	66.2	1.45	1.72	1.54E-07	1.51E-06
Ulk4	24.5	1.45	1.83	2.23E-06	1.75E-05
Ccnblip1	40.8	1.45	1.87	6.02E-06	4.29E-05

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Tubb1	17.5	1.45	2.25	5.51E-05	0.000312
Vax2	12.7	1.45	2.38	0.000106	0.000558
Slc16a11	9.1	1.45	2.57	0.000108	0.000563
Col3a1	82.1	1.45	2.78	0.000124	0.000638
Sdf2l1	994.9	1.44	1.51	0	0
Psmc3ip	149.8	1.44	1.53	1E-15	3.8E-14
Tmem107	70.8	1.44	1.63	4.82E-09	6.16E-08
Tmem121	19.5	1.44	2.22	6.17E-05	0.000344
Platr11	10.2	1.44	2.5	0.00012	0.000617
9230102O04Rik	5.8	1.44	3.53	0.000127	0.000649
Ms4a4b	9942.9	1.43	1.46	0	0
Gmnn	974.1	1.43	1.47	0	0
Bak1	2591.4	1.43	1.48	0	0
Casp3	2922.8	1.43	1.48	0	0
Sass6	197.1	1.43	1.51	0	4E-15
2310031A07Rik	64.6	1.43	1.81	4.21E-06	3.1E-05
Gda	44.3	1.43	2.06	3.9E-05	0.00023
Ckm	8	1.43	3.17	0.000143	0.000725
Mfsd13a	222.3	1.42	1.53	1.9E-13	4.74E-12
Gna14	24.8	1.42	1.92	1.63E-05	0.000106
Retnla	7.6	1.42	5.6	8.17E-05	0.000441
4930579G24Rik	292.8	1.41	1.44	0	0
Alcam	430	1.41	1.5	0	1.7E-14
Ak6	275.7	1.41	1.56	3.31E-10	5.2E-09
Ical	85.1	1.41	1.64	1.66E-07	1.62E-06
Smo	59	1.41	1.66	3.83E-07	3.5E-06
A930002I21Rik	49.6	1.41	1.91	2.49E-05	0.000154
Colla1	89.2	1.41	1.94	2.5E-05	0.000154
Cd34	96.8	1.41	2.01	5.84E-05	0.000329
Lhx2	15.9	1.41	2.11	6.05E-05	0.000339
Ptger3	20.8	1.41	2.29	0.000156	0.000779
Xdh	1588.9	1.4	1.45	0	0
Ttn	292.1	1.4	1.52	3.22E-11	5.97E-10
Asns	221.7	1.4	1.57	2.68E-09	3.59E-08
Cmc2	422.7	1.4	1.6	2.68E-08	3.01E-07
Cks2	105.1	1.4	1.64	3.29E-07	3.05E-06
Gzmc	56.6	1.4	1.74	4.29E-06	3.16E-05
Hist1h2bb	17.7	1.4	2	7.72E-05	0.00042
Epx	8	1.4	6.63	8.75E-05	0.00047
Gata2	341.6	1.4	2.14	0.000102	0.000536
Hp	143.1	1.4	2.47	0.000179	0.000879
Gm11843	7.5	1.4	4.37	0.000181	0.000888

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Batf	1910.4	1.39	1.44	0	0
Hip1	1867.7	1.39	1.46	0	0
Havcr2	486.2	1.39	1.5	1.88E-12	4.18E-11
Acod1	232.1	1.39	1.69	1.35E-06	1.11E-05
Grb10	61	1.39	1.77	5.98E-06	4.26E-05
Reg2	5.6	1.39	5.03	7.66E-06	5.33E-05
N4bp1	3266.2	1.38	1.4	0	0
Prim1	1043.4	1.38	1.41	0	0
Mtfr2	115.7	1.38	1.47	2.56E-13	6.28E-12
Hmgb2	1572.1	1.38	1.49	1.93E-12	4.28E-11
Isg20	861.4	1.38	1.52	2.64E-10	4.24E-09
Cmpk2	311.4	1.38	1.66	1E-06	8.41E-06
Isg15	787.9	1.38	1.72	4.32E-06	3.17E-05
Il1rn	44.9	1.38	1.79	1.05E-05	7.06E-05
Fkbp1b	22.4	1.38	1.9	3.16E-05	0.00019
Nwd1	24.3	1.38	2.01	7.94E-05	0.000431
Tnp2	17.6	1.38	2.34	0.000128	0.000654
Ifit3	421.9	1.38	2.21	0.000147	0.00074
Hist1h2ai	9.8	1.38	2.17	0.000184	0.000901
Cdc20b	14.4	1.38	2.55	0.00019	0.000927
a	12.6	1.38	2.36	0.000204	0.000988
Tubb5	27245.9	1.37	1.4	0	0
Myo1f	6082	1.37	1.41	0	0
Cdc25b	5230.3	1.37	1.42	0	0
E2f3	421.5	1.37	1.42	0	0
Impa1	900.5	1.37	1.42	0	0
Kif24	76.5	1.37	1.56	2.19E-08	2.49E-07
Art2a-ps	407.6	1.37	1.56	4.59E-08	4.94E-07
Gp5	24.7	1.37	1.84	5.7E-05	0.000321
Dio2	18.9	1.37	2.13	0.000105	0.000551
Cdk6	2038	1.36	1.38	0	0
Ppil1	932	1.36	1.4	0	0
Shmt1	546.5	1.36	1.41	0	0
Plxdc1	231.9	1.36	1.46	6.89E-12	1.42E-10
Hopx	270.1	1.36	1.48	2.27E-11	4.33E-10
Serp2	160.9	1.36	1.5	5.14E-10	7.76E-09
Dusp14	437.4	1.36	1.5	1.35E-09	1.89E-08
Lgals7	154	1.36	1.59	1.04E-06	8.72E-06
Nqo1	35.8	1.36	1.7	5.6E-06	4.01E-05
Aldh1a1	40.1	1.36	1.98	0.000105	0.000552
Ifit1	298.3	1.36	2.02	0.000125	0.000642
Psat1	1715.9	1.35	1.37	0	0

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Gins3	181.3	1.35	1.46	6.05E-11	1.07E-09
Slc16a3	161.5	1.35	1.49	3.5E-10	5.45E-09
Gbp10	432.2	1.35	1.51	4.13E-09	5.34E-08
Ube2l6	515.6	1.35	1.54	4.68E-08	5.03E-07
Slfn3	67.7	1.35	1.6	6.85E-07	5.93E-06
Nccrp1	65	1.35	1.64	4.48E-06	3.27E-05
Rgs8	18.7	1.35	1.85	9.21E-05	0.000492
Trim58	10.1	1.35	2.9	0.000362	0.001642
Cpa3	467.2	1.35	2.45	0.000376	0.001695
Fkbp5	2388.9	1.34	1.37	0	0
Tmpo	4623.5	1.34	1.39	0	0
Cxcr3	2032	1.34	1.39	0	0
Mrps25	464.1	1.34	1.41	0	7E-15
Zdhhc2	533.9	1.34	1.42	3E-15	8.8E-14
Slc25a13	161.1	1.34	1.42	1.21E-13	3.1E-12
Nudcd1	238.9	1.34	1.43	1.69E-13	4.24E-12
Phf11b	840.6	1.34	1.45	4.58E-12	9.71E-11
Galr3	5.7	1.34	4.53	0.000359	0.001631
Kctd17	526.7	1.33	1.39	0	0
Klre1	117.7	1.33	1.66	8.94E-06	6.13E-05
Gm14130	18.9	1.33	1.81	4.17E-05	0.000244
Myo1d	68	1.33	1.84	7.41E-05	0.000405
Ica11	24.1	1.33	1.82	0.000114	0.00059
Coa6	201.6	1.32	1.4	5.2E-14	1.39E-12
Mettl7a1	240.2	1.32	1.42	4.43E-12	9.41E-11
Syt13	850.3	1.32	1.44	5.64E-10	8.46E-09
Lrrc75a	59.6	1.32	1.6	5.23E-06	3.78E-05
Hspa1b	148.2	1.32	1.66	1.9E-05	0.000121
Serpina3i	24.8	1.32	2.37	0.000411	0.00183
Mmp8	16.8	1.32	2.57	0.000494	0.002149
Mcm2	4604.3	1.31	1.34	0	0
Wdr62	861.5	1.31	1.35	0	0
Cdk5r1	454.4	1.31	1.35	0	0
Cenpu	141.6	1.31	1.39	1.83E-13	4.57E-12
Myl6b	55.6	1.31	1.55	2.08E-06	1.64E-05
Nmur1	2.7	1.31	4.59	9.87E-05	0.000522
Prim2	843.4	1.3	1.34	0	0
Rrm1	4168.2	1.3	1.34	0	0
Dnajc15	778.1	1.3	1.35	0	0
Brip1os	583.1	1.3	1.37	1E-15	3.8E-14
Perp	113.8	1.3	1.41	4.33E-10	6.63E-09
Msantd3	9.2	1.3	2.05	0.000408	0.00182

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Col6a6	6.3	1.3	4.16	0.000432	0.001913
Gnaz	9.7	1.3	2.35	0.000531	0.002295
Syt11	1025	1.29	1.31	0	0
Rab27a	1564.8	1.29	1.32	0	0
Stip1	4349.6	1.29	1.32	0	0
Cep19	280.3	1.29	1.34	0	0
Selenoh	1560.4	1.29	1.34	0	1E-15
Clec1b	45.4	1.29	1.59	1.54E-05	0.0001
Il5	3.6	1.29	5.79	0.000125	0.000643
Klrb1a	36.2	1.29	1.9	0.000175	0.000861
Serpina3n	16.7	1.29	2.04	0.000428	0.001901
Ms4a2	35.1	1.29	2.29	0.000563	0.002414
Tmem171	12.4	1.29	2.26	0.000567	0.002428
Snai3	164.9	1.28	1.39	2.53E-10	4.06E-09
CT030173.1	47.8	1.28	1.54	5.74E-06	4.11E-05
Iqcg	28.8	1.28	1.6	1.76E-05	0.000113
Mst1r	38.6	1.28	1.61	2.34E-05	0.000146
Serpine2	31.1	1.28	1.66	0.000104	0.000547
Pawr	19.1	1.28	1.82	0.00015	0.000752
D630045J12Rik	11.4	1.28	2.12	0.000411	0.001831
Oasl2	98.6	1.28	3	0.000737	0.003051
Rab19	1074.2	1.27	1.31	0	0
Rap2a	566	1.27	1.31	0	0
Tm6sf1	1262.4	1.27	1.32	0	0
Caskin2	253.4	1.27	1.33	6E-15	1.65E-13
Gm7901	153.5	1.27	1.38	7.96E-10	1.16E-08
Ly6c1	2390.4	1.27	1.41	2.71E-08	3.04E-07
Pxmp2	56.9	1.27	1.44	4.38E-07	3.98E-06
Il3	4	1.27	5.13	0.000123	0.000633
C1ql1	3.8	1.27	5.38	0.000393	0.001762
Car1	559.7	1.27	1.97	0.000423	0.001879
Sla	9431	1.26	1.29	0	0
Runx2	2056.2	1.26	1.3	0	0
Impa2	575	1.26	1.3	0	0
Atp2b4	1723.8	1.26	1.3	0	0
Cpsf2	1192.5	1.26	1.32	2.6E-14	7.37E-13
Bcl2l1	1866.8	1.26	1.33	3.2E-14	8.76E-13
2810006K23Rik	128.8	1.26	1.41	3.26E-08	3.63E-07
Sostdc1	55.7	1.26	1.49	6.65E-06	4.69E-05
Gm15987	44.4	1.26	1.64	4.28E-05	0.00025
Mest	21.1	1.26	1.73	0.000157	0.000783
Fkbp10	29.4	1.26	1.74	0.000252	0.001191

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Hnf4a	4.7	1.26	3.99	0.000517	0.002238
Gp1ba	18.9	1.26	2.02	0.000659	0.002769
AC153369.2	8.4	1.26	2.51	0.000813	0.003321
Prss34	356.8	1.26	2.96	0.000841	0.003422
Tarm1	9.2	1.26	3.21	0.000847	0.003442
Nudt5	931.4	1.25	1.28	0	0
Aurka	633.5	1.25	1.29	0	0
C1qtnf6	340.9	1.25	1.3	0	0
Nrm	1120.2	1.25	1.3	0	0
Kifc5b	272.7	1.25	1.31	1E-15	3.3E-14
H2afx	3402.7	1.25	1.31	2E-15	5.8E-14
Gyg	764.1	1.25	1.31	8E-15	2.44E-13
Lsm2	779.8	1.25	1.34	8.52E-12	1.74E-10
Xaf1	1257.6	1.25	1.36	2.65E-09	3.56E-08
Kndc1	53.5	1.25	1.48	2.11E-06	1.66E-05
Xkr8	51.5	1.25	1.48	3.91E-06	2.91E-05
Gm37004	26	1.25	1.56	3.67E-05	0.000218
Scin	117.4	1.25	1.62	6.9E-05	0.000381
Sgms2	21.4	1.25	1.8	0.00038	0.00171
Vcan	11.1	1.25	2.23	0.000894	0.003604
Lrp3	7.7	1.25	2.52	0.000956	0.003818
Nde1	1235.2	1.24	1.26	0	0
Hn1l	449.3	1.24	1.3	6E-15	1.74E-13
Lamc1	923.9	1.24	1.31	1.71E-13	4.3E-12
Alg6	94.2	1.24	1.39	1.32E-07	1.31E-06
AC126459.2	179	1.24	1.4	3.61E-07	3.32E-06
Dsp	79.2	1.24	1.55	4.45E-05	0.000258
Gm14148	21.4	1.24	1.54	5.86E-05	0.00033
AC151730.1	71.6	1.24	1.69	8.59E-05	0.000463
Gm13461	16.5	1.24	1.82	0.000465	0.002039
Fhl1	19.9	1.24	1.74	0.000492	0.002143
Smpx	6.2	1.24	4.22	0.000628	0.002658
Tom1l1	20.3	1.24	1.95	0.00073	0.003028
Hist1h2ab	3.6	1.24	3.05	0.000791	0.003245
Il33	4.5	1.24	3.09	0.000871	0.003531
Epha3	8.5	1.24	2.62	0.000892	0.003598
Hist1h2ae	10.9	1.24	2.06	0.000918	0.003688
Gm13031	8	1.24	2.51	0.000941	0.003764
Gm14569	11.7	1.24	3.38	0.000999	0.003964
Slamf7	804.9	1.23	1.27	0	0
Cxcr6	818	1.23	1.27	0	0
Cpox	574.5	1.23	1.28	0	8E-15

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Adam19	5999.2	1.23	1.29	2E-15	5.2E-14
Ms4a4c	692.6	1.23	1.3	4.5E-14	1.22E-12
Penk	1018	1.23	1.3	2.51E-12	5.5E-11
Klrb1f	190	1.23	1.32	3.65E-10	5.66E-09
Gm6166	141.1	1.23	1.32	1.18E-09	1.67E-08
Fam184a	33.5	1.23	1.5	1.8E-05	0.000116
Meis1	68.8	1.23	1.55	8.54E-05	0.00046
Erg	37.2	1.23	1.55	0.000101	0.000531
Enpp6	21.8	1.23	1.82	0.00032	0.001474
Gm6982	3.3	1.23	4.32	0.000669	0.002804
Gm7456	10.4	1.23	2.12	0.000809	0.003311
Spon1	28.9	1.23	2.16	0.000894	0.003604
Unc80	11	1.23	2.08	0.001129	0.004421
Ran	2657.7	1.22	1.24	0	0
Zranb3	259.4	1.22	1.27	0	0
Cbx5	1657.3	1.22	1.27	0	1E-15
Ms4a6d	734.6	1.22	1.28	3E-15	1.07E-13
Fbxo5	492.7	1.22	1.28	1.3E-14	3.61E-13
Hsph1	3580.6	1.22	1.28	4.8E-14	1.29E-12
Pttg1	1600.8	1.22	1.28	1.21E-13	3.11E-12
Cysltr2	169.1	1.22	1.37	3.3E-07	3.06E-06
Phf11a	221.4	1.22	1.4	9.86E-07	8.28E-06
Rgs18	52.3	1.22	1.54	9.22E-05	0.000492
Wdr54	19.6	1.22	1.61	0.000186	0.000909
Clstn3	34.6	1.22	1.6	0.000219	0.001054
Aqp1	400.8	1.22	1.93	0.00081	0.003312
Il20ra	9.2	1.22	1.97	0.001035	0.00409
Hist1h2bk	8.4	1.22	2.66	0.001176	0.004581
Chaf1b	1135	1.21	1.23	0	0
Rnaseh2b	648.9	1.21	1.24	0	0
Tnfrsf9	1547.1	1.21	1.29	1.28E-10	2.17E-09
Smoc2	90.6	1.21	1.34	6.72E-08	7.07E-07
Fancf	96.5	1.21	1.34	9.78E-08	1E-06
Tex13c2	4.1	1.21	3.28	0.000884	0.003573
Mcpt1	510.2	1.21	3.19	0.001329	0.005105
Chit1	6.9	1.21	2.51	0.00139	0.005311
Psmal	2415.5	1.2	1.22	0	0
Zcchc18	377.7	1.2	1.23	0	0
Ppal	1390.3	1.2	1.25	3E-15	9.8E-14
Alms1	195.5	1.2	1.33	1.17E-07	1.18E-06
Ms4a4a	77.2	1.2	1.36	2.06E-07	1.97E-06
Farp1	144.7	1.2	1.34	4.58E-07	4.13E-06

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Lrrc49	50.8	1.2	1.38	1.78E-06	1.42E-05
Tfec	71.7	1.2	1.43	1.18E-05	7.88E-05
Gm15232	43.8	1.2	1.46	1.89E-05	0.00012
Fgd1	24	1.2	1.52	0.000114	0.00059
C78197	19.1	1.2	1.74	0.000569	0.002437
Slc18a2	46.4	1.2	1.9	0.000856	0.003474
Clgn	5.1	1.2	3.51	0.001392	0.005316
Calm3	5962.7	1.19	1.21	0	0
Dtl	1077.7	1.19	1.26	4.67E-11	8.43E-10
Ptpn13	1107.8	1.19	1.3	6.78E-09	8.4E-08
Mrpl34	516.5	1.19	1.33	2.57E-07	2.41E-06
Nphp4	99.7	1.19	1.33	3.18E-07	2.95E-06
Trim46	87.2	1.19	1.34	6.83E-07	5.91E-06
Fn1	453.7	1.19	1.54	0.000169	0.000836
I830127L07Rik	6.6	1.19	5.81	0.00103	0.004071
Reps2	11	1.19	2.16	0.001383	0.005292
Mcm7	3981.2	1.18	1.21	0	0
Trim37	677.9	1.18	1.21	0	0
BC055324	200.3	1.18	1.22	0	7E-15
Gm4737	198.1	1.18	1.25	3.91E-11	7.17E-10
Pycard	1522.9	1.18	1.25	8.74E-11	1.51E-09
Fam185a	192.8	1.18	1.28	2.12E-09	2.88E-08
Bag2	63	1.18	1.31	4.71E-07	4.24E-06
Ifitm1	214.1	1.18	1.44	6.52E-05	0.000362
Alox12	39.7	1.18	1.46	0.00013	0.000665
Cebpe	5.7	1.18	3.45	0.001328	0.005104
Ppp2r2c	6.5	1.18	4.48	0.00155	0.005841
Mcpt2	69.1	1.18	3.32	0.001613	0.006049
Fam46c	5669.2	1.17	1.2	0	0
Plscr1	472.1	1.17	1.22	7.7E-14	2.01E-12
Grb7	380.9	1.17	1.3	7.86E-07	6.72E-06
Phlda3	37.7	1.17	1.38	2.49E-05	0.000154
Rtkn	29.5	1.17	1.44	0.000126	0.000646
Cym	46.2	1.17	1.51	0.000165	0.000821
Cfap77	26	1.17	1.51	0.000167	0.000826
Gm10286	2.8	1.17	4.98	0.000868	0.00352
AC158990.2	7.6	1.17	2.88	0.001943	0.007104
Entpd1	1241.1	1.16	1.2	0	0
Dbi	1291.8	1.16	1.21	2.3E-14	6.44E-13
Hmgal-rs1	312.3	1.16	1.21	1.05E-12	2.43E-11
Tmem237	165.9	1.16	1.22	1.29E-12	2.96E-11
Raph1	355.4	1.16	1.22	5.71E-12	1.19E-10

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Echdc1	172.2	1.16	1.23	3.63E-11	6.71E-10
Tuba1b	1989.9	1.16	1.26	1.8E-08	2.09E-07
Ifi214	383.7	1.16	1.31	9.86E-07	8.28E-06
Pvrig	126.3	1.16	1.31	1.56E-06	1.26E-05
Steap3	134.6	1.16	1.4	5.47E-05	0.00031
Fam89a	34.7	1.16	1.45	5.54E-05	0.000313
Gm8719	29.4	1.16	1.47	0.000149	0.000748
Epas1	1633	1.16	1.5	0.000353	0.001608
Adamts3	19.6	1.16	1.69	0.000885	0.003576
Opn3	13.4	1.16	1.74	0.000965	0.003851
Syng4	9.5	1.16	2.04	0.001733	0.006439
Tmem98	10.9	1.16	1.94	0.002001	0.007282
Rfc5	1166.1	1.15	1.17	0	0
Pdk3	769.1	1.15	1.17	0	0
Samd3	769	1.15	1.2	5E-15	1.56E-13
Psmb9	7187.6	1.15	1.22	2.5E-11	4.73E-10
Slc9a5	265.3	1.15	1.22	7.94E-11	1.38E-09
Fbxl8	274.2	1.15	1.23	6.27E-10	9.33E-09
Myh10	131.2	1.15	1.37	3.82E-05	0.000226
Ighv1-81	14	1.15	3.56	0.00155	0.005841
Palm3	10.2	1.15	1.86	0.001827	0.006731
Ifit3b	107.9	1.15	2.04	0.002247	0.008038
Pla2g16	2185.4	1.14	1.15	0	0
Ptpcap	9906.9	1.14	1.18	0	1E-15
Zbp1	5544.8	1.14	1.22	1.03E-09	1.47E-08
Eif2ak2	397.5	1.14	1.23	5.43E-09	6.87E-08
Ifi209	837.9	1.14	1.24	2.48E-08	2.79E-07
Prkar2b	166.3	1.14	1.28	1.79E-06	1.43E-05
Gm5620	33.2	1.14	1.38	0.000109	0.00057
Tspo2	25.6	1.14	2.05	0.002092	0.007567
Gm12641	4.5	1.14	2.63	0.002216	0.007947
Gm5391	5.3	1.14	2.44	0.002442	0.008648
Chga	5.7	1.14	2.51	0.002652	0.00928
Bhlhe40	29564.9	1.13	1.15	0	0
Scd2	2011	1.13	1.16	0	0
Arl6ip1	6248	1.13	1.17	0	1E-15
Pycrl	578	1.13	1.17	1E-15	1.8E-14
Erg28	683.6	1.13	1.18	3E-15	8.3E-14
Pgk1	407	1.13	1.18	3.15E-13	7.63E-12
Rom1	500.9	1.13	1.2	4.67E-11	8.43E-10
Eef1akmt1	350.7	1.13	1.22	1.39E-08	1.64E-07
Cenpv	293.7	1.13	1.23	6.57E-08	6.93E-07

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Xk	84.8	1.13	1.25	2.68E-07	2.51E-06
Clec7a	192.7	1.13	1.26	6.18E-07	5.4E-06
Nudt1	390.3	1.13	1.26	6.42E-07	5.59E-06
Ltbp1	63.3	1.13	1.33	3.43E-05	0.000205
9430037O13Rik	48.3	1.13	1.37	0.000118	0.000609
Dgkg	28.8	1.13	1.49	0.000398	0.001781
Bex4	6.5	1.13	2.52	0.002771	0.009627
Prfl	1704.5	1.12	1.14	0	0
Dpagt1	515.2	1.12	1.18	7.25E-12	1.49E-10
Enkd1	215	1.12	1.2	5.04E-09	6.42E-08
Klra3	69.3	1.12	1.33	3.42E-05	0.000204
G0s2	77.4	1.12	1.38	0.000194	0.000946
4930520O04Rik	19.9	1.12	1.53	0.00072	0.002993
Gm24507	10.1	1.12	1.9	0.002546	0.008969
Irx3	8.6	1.12	1.79	0.002652	0.00928
Zfp575	7.4	1.12	2.11	0.002982	0.010267
4930427A07Rik	1017.7	1.11	1.13	0	0
Fen1	1518.2	1.11	1.13	0	0
Gng2	1586.5	1.11	1.15	0	0
Polq	483.2	1.11	1.17	4.68E-11	8.45E-10
Aplf	178.2	1.11	1.19	2.84E-09	3.78E-08
Optn	199.7	1.11	1.22	3.7E-07	3.4E-06
Dhx58	1168.4	1.11	1.24	8.05E-07	6.86E-06
Nudt7	57.3	1.11	1.34	6.92E-05	0.000382
Prr18	25.2	1.11	1.42	0.000467	0.002048
Fam109b	49.4	1.11	1.49	0.000642	0.002708
Thbs1	150.8	1.11	1.48	0.00069	0.002882
2210011C24Rik	23	1.11	1.46	0.000846	0.003439
Rtp4	1189.3	1.11	1.5	0.000884	0.003572
Myom2	3.3	1.11	2.84	0.002323	0.00828
Ncr1	264.6	1.11	1.76	0.002392	0.008499
Adgrl4	27.6	1.11	1.74	0.002603	0.009132
Gm15941	4.3	1.11	2.54	0.003237	0.011024
Hoxa9	9	1.11	2.09	0.003272	0.011122
Myrf	11.2	1.11	2.56	0.00334	0.011303
Endod1	867.3	1.1	1.12	0	0
Ruvbl2	1746.2	1.1	1.14	0	2E-15
Mrps6	469.4	1.1	1.16	1.21E-11	2.4E-10
Naa38	642.5	1.1	1.18	1.39E-08	1.64E-07
Gcat	256.4	1.1	1.23	2.8E-06	2.16E-05
Tmem163	137	1.1	1.32	0.000126	0.000646
Ccdc80	29.4	1.1	1.48	0.000747	0.003085

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Epdr1	29.9	1.1	1.56	0.001471	0.005584
Naaladl1	17.4	1.1	1.55	0.001554	0.005857
Gm5541	13.5	1.1	1.97	0.003169	0.010832
Cfb	9.7	1.1	2.3	0.003841	0.012715
Tipin	1045.9	1.09	1.13	1E-15	4.3E-14
Hikeshi	408	1.09	1.15	6.66E-12	1.38E-10
Epsti1	2970.1	1.09	1.14	2.87E-11	5.39E-10
Mfsd2b	366.8	1.09	1.19	1.24E-07	1.24E-06
Zan	929.3	1.09	1.19	2.42E-07	2.29E-06
1190007107Rik	77.8	1.09	1.22	2.98E-06	2.28E-05
Kcnip3	48.9	1.09	1.29	3.51E-05	0.000209
Ryr1	67.2	1.09	1.33	0.000206	0.000995
Rsph1	29	1.09	1.35	0.000451	0.001985
Slfn4	78.7	1.09	1.51	0.000918	0.003688
Gm42517	3	1.09	4.44	0.00121	0.004704
Gm31597	30.9	1.09	1.54	0.001558	0.005869
Col8a2	11.9	1.09	2.08	0.003591	0.012023
Gm5787	4.5	1.09	2.84	0.003715	0.012368
Glp1r	11.2	1.09	2.1	0.004071	0.013338
Taf6	1182.3	1.08	1.1	0	0
Plp2	432.8	1.08	1.12	1E-15	2.3E-14
Atcay	258.4	1.08	1.12	3E-15	1.06E-13
Casp4	449.3	1.08	1.13	8.76E-12	1.79E-10
Syt12	1359.2	1.08	1.14	6.48E-11	1.14E-09
Txn1	4856.9	1.08	1.14	1.86E-09	2.55E-08
Banfl	2759.2	1.08	1.17	1.7E-07	1.65E-06
Tubd1	123.6	1.08	1.19	5.64E-07	4.98E-06
Apol10b	57.6	1.08	1.37	0.000331	0.001516
9130604C24Rik	12.8	1.08	1.68	0.002537	0.008944
AC133083.4	8.9	1.08	2.13	0.003676	0.012261
Mcpt8	355.9	1.08	2.25	0.004527	0.01461
Bub3	2635.7	1.07	1.09	0	0
Sept11	2112.3	1.07	1.11	5.1E-14	1.36E-12
5830432E09Rik	221.9	1.07	1.12	1.77E-12	3.97E-11
Tbc1d31	347.8	1.07	1.11	2.85E-12	6.2E-11
Sema7a	543.5	1.07	1.12	7.39E-12	1.52E-10
Pgp	482.7	1.07	1.12	9.9E-11	1.69E-09
Mrpl51	433.6	1.07	1.13	1.05E-09	1.5E-08
AC123720.1	301.8	1.07	1.14	5.56E-09	7.01E-08
Slc29a4	63.5	1.07	1.17	9.44E-07	7.95E-06
Dctd	147.9	1.07	1.19	2.49E-06	1.93E-05
Csrp2	91.9	1.07	1.19	3.25E-06	2.46E-05

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Pthr1	154.5	1.07	1.2	6.71E-06	4.72E-05
Unc5b	14.9	1.07	1.45	0.001958	0.00715
A430018G15Rik	17	1.07	1.55	0.001969	0.007187
Upk1b	3.1	1.07	3.96	0.003029	0.010405
Gm11223	13	1.07	1.72	0.003491	0.011736
Scrn1	9.5	1.07	1.96	0.004421	0.014317
G6b	5.8	1.07	2.42	0.004604	0.014825
Rangap1	5185.4	1.06	1.07	0	0
Cd8b1	8616.8	1.06	1.08	0	0
Mms22l	883	1.06	1.08	0	0
Cchcr1	724.1	1.06	1.09	0	0
Rnpep	2406	1.06	1.1	0	1E-15
Cntln	217.1	1.06	1.09	0	7E-15
Clic1	10203.8	1.06	1.09	1E-15	2.1E-14
Pkmyt1	1872.8	1.06	1.1	6E-15	1.73E-13
Phgdh	591.6	1.06	1.1	6.8E-14	1.79E-12
Lcp1	29534.7	1.06	1.1	9.5E-14	2.46E-12
Trbv3	327	1.06	1.1	1.27E-13	3.23E-12
Gm13394	2531.6	1.06	1.11	1.77E-11	3.42E-10
Gm5855	163.2	1.06	1.12	3.97E-10	6.11E-09
Rad51c	174.3	1.06	1.13	6.37E-09	7.94E-08
Fgl2	387.2	1.06	1.15	4.93E-07	4.42E-06
Fam69b	58.7	1.06	1.17	1.34E-06	1.1E-05
Ddah2	90	1.06	1.21	3.01E-05	0.000182
Mid2	43.1	1.06	1.37	0.000679	0.002842
C430042M11Rik	20.4	1.06	1.44	0.001415	0.00539
1700006J14Rik	34.9	1.06	1.52	0.002226	0.007977
Kifc5c-ps	3.2	1.06	5.01	0.002273	0.008113
Gm4316	14.1	1.06	1.62	0.002956	0.010189
Ighv1-77	5.6	1.06	2.91	0.004291	0.013953
Syng3	14.2	1.06	1.89	0.004613	0.014847
Garem1	10.4	1.06	2.16	0.004725	0.015161
Gm5732	8.2	1.06	2.1	0.004985	0.01591
9230110C19Rik	6.9	1.06	2.22	0.005156	0.016393
Cdk4	5123.3	1.05	1.07	0	0
Psm8	13142.6	1.05	1.09	5.3E-14	1.42E-12
Cdc25a	933.6	1.05	1.09	5.4E-14	1.43E-12
Pmfl	1789.9	1.05	1.09	1.87E-12	4.17E-11
Galm	344.1	1.05	1.1	1.09E-11	2.19E-10
Tpgs1	515.9	1.05	1.11	2.78E-09	3.71E-08
2610524H06Rik	134.2	1.05	1.12	3.58E-08	3.95E-07
St14	207.6	1.05	1.16	2.31E-06	1.8E-05

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Ifi206	467.7	1.05	1.18	6.08E-06	4.32E-05
F2rl3	91.5	1.05	1.18	1.08E-05	7.26E-05
Ctnnbip1	164	1.05	1.18	1.81E-05	0.000116
Ccl12	4.4	1.05	3.63	0.003485	0.011718
Slc17a6	4.8	1.05	3.66	0.003716	0.012368
Gm867	11.8	1.05	1.74	0.00416	0.013584
Srd5a1	9.7	1.05	1.76	0.004432	0.014342
Bad	457.8	1.04	1.08	1.55E-11	3.03E-10
Pafah2	209.2	1.04	1.11	2.07E-08	2.37E-07
Dzip3	213.7	1.04	1.12	4.28E-08	4.63E-07
Casp1	695.8	1.04	1.13	1.06E-07	1.08E-06
Nrgn	587.4	1.04	1.2	5.55E-05	0.000314
Gm28942	25.8	1.04	1.35	0.000795	0.003259
Gm13361	26.6	1.04	1.39	0.00157	0.005905
AC151730.3	14.5	1.04	1.64	0.003469	0.011674
Cyp4f39	4	1.04	3.04	0.003903	0.012858
Ranbp17	12.9	1.04	1.93	0.005338	0.01689
Gm6419	7.9	1.04	2.1	0.006041	0.0188
Unc119b	2622.3	1.03	1.05	0	0
Ak3	1405.9	1.03	1.05	0	0
Brca2	378	1.03	1.06	0	0
Prelid2	433.7	1.03	1.06	0	1E-15
Hmbs	853.4	1.03	1.07	2.67E-12	5.82E-11
Cobll1	564	1.03	1.07	1.31E-11	2.6E-10
Ttf2	773.8	1.03	1.08	1.77E-10	2.94E-09
Ap1s2	357.9	1.03	1.09	6.21E-10	9.25E-09
Arhgap33	461.4	1.03	1.1	1.1E-07	1.11E-06
Cxcr5	1400.5	1.03	1.11	1.62E-07	1.58E-06
Zfp324	80.8	1.03	1.16	1.23E-05	8.15E-05
1700008J07Rik	46	1.03	1.2	8.13E-05	0.00044
Pla2g1b	3.1	1.03	5.68	0.000671	0.002812
Rasd2	26.4	1.03	1.31	0.001055	0.004161
Gm19412	24.7	1.03	1.45	0.002222	0.007968
Gm21850	5.8	1.03	5.15	0.003849	0.012735
Ninj2	22.5	1.03	1.57	0.003881	0.012815
Cacna1b	12.9	1.03	1.59	0.003981	0.013088
Hspd1	2211.8	1.02	1.03	0	0
Gins4	994.2	1.02	1.03	0	0
Hirip3	1284.6	1.02	1.04	0	1E-15
Dtx3l	2072.2	1.02	1.05	1E-15	3.7E-14
Atad5	649	1.02	1.05	4E-15	1.19E-13
Slc22a15	789	1.02	1.05	2.1E-13	5.2E-12

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Nefh	428.6	1.02	1.08	2.24E-09	3.03E-08
Gemin8	131.1	1.02	1.14	1.99E-05	0.000126
Coa3	751.4	1.02	1.17	3.92E-05	0.000231
AL591582.1	67.5	1.02	1.2	0.000137	0.000694
Rabl2	66.4	1.02	1.21	0.000142	0.000719
BC147527	314.7	1.02	1.21	0.000232	0.001111
F5	26.3	1.02	1.32	0.001751	0.006497
Cysltrl	12.2	1.02	1.53	0.003789	0.012571
Ankrd29	17.2	1.02	1.59	0.003942	0.01298
Serpinb1b	5.6	1.02	3.25	0.00527	0.016699
Cxcl11	12.9	1.02	1.71	0.005596	0.017603
A730089K16Rik	8.9	1.02	3.51	0.006351	0.019658
Gm12669	5.9	1.02	2.72	0.006491	0.020036
Draxin	7.5	1.02	2.51	0.006903	0.021116
Lrrn4	10.2	1.02	2.39	0.00739	0.022374
Fbxw8	1446.7	1.01	1.03	0	0
Mis18a	835	1.01	1.03	0	0
Adprh	1898	1.01	1.03	0	1E-15
Gmids	345.1	1.01	1.05	2.03E-12	4.49E-11
Hat1	1129	1.01	1.06	7.79E-10	1.14E-08
Psmbl10	4675.3	1.01	1.07	1.49E-09	2.07E-08
F730043M19Rik	151.6	1.01	1.1	5.92E-07	5.2E-06
Map1a	43.3	1.01	1.14	5.25E-05	0.000299
Gata1	221	1.01	1.18	0.000138	0.0007
4930524J08Rik	30.3	1.01	1.2	0.00043	0.001909
Ly6c2	4431.1	1.01	1.23	0.000513	0.002224
Gm30211	96.4	1.01	1.34	0.001432	0.005448
Slc30a2	29	1.01	1.33	0.002911	0.010056
Lpar3	17.1	1.01	1.53	0.003294	0.011174
Gm35037	11.6	1.01	1.65	0.005347	0.016913
Pcdh7	8.7	1.01	1.64	0.006542	0.020151
Ntf5	8.2	1.01	1.67	0.006819	0.020886
Olfr414	5.8	1.01	3.46	0.007252	0.022
AC153562.2	4.6	1.01	2.14	0.007428	0.022477
Cd248	7.6	1.01	2.32	0.007647	0.023068
Ccdc120	9.6	1.01	1.83	0.007745	0.023309
Ahsa1	2898.5	1	1.02	0	0
Cdca4	1330.7	1	1.03	0	0
Ebp	1270.8	1	1.03	8E-15	2.22E-13
Thop1	736	1	1.03	6.1E-14	1.6E-12
Jdp2	335.6	1	1.03	2.76E-13	6.73E-12
Rpa3	304.5	1	1.04	3E-10	4.76E-09

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Ece2	225.8	1	1.06	1.04E-08	1.25E-07
Ctnn	156.2	1	1.07	2.39E-08	2.7E-07
Ydjc	187.1	1	1.07	1.09E-07	1.1E-06
5033430I15Rik	174.1	1	1.07	4.67E-07	4.21E-06
Orc1	394.8	1	1.09	8.9E-07	7.52E-06
Cx3cr1	141.8	1	1.09	3.2E-06	2.43E-05
Ltbp3	64	1	1.12	2.83E-05	0.000173
Zfp239	60.8	1	1.15	5.9E-05	0.000332
Mansc1	76.8	1	1.17	0.000128	0.000654
Bahcc1	65.2	1	1.17	0.000156	0.00078
Rpl39-ps	163.8	1	1.2	0.000445	0.001964
Ccl2	77.5	1	1.39	0.002955	0.010188
Col5a1	37.5	1	1.48	0.004515	0.014573
Wisp1	171.4	1	1.46	0.004818	0.015407
Hist1h2bl	3.2	1	2.02	0.00672	0.020634
Gm11658	7.3	1	1.65	0.007621	0.023001
Gm44101	5.1	1	2.58	0.007779	0.02339
Atp6v1g3	12.9	1	1.89	0.007957	0.023852
Bbs2	593.4	-1	-1.02	0	0
Cdc42bpb	487.3	-1	-1.06	4.36E-09	5.61E-08
Dapk1	459.6	-1	-1.06	1.22E-08	1.45E-07
Lonrf1	133.9	-1	-1.06	2.72E-08	3.04E-07
Vps37b	99356.7	-1	-1.07	1.11E-07	1.12E-06
C130050O18Rik	95.9	-1	-1.14	1.81E-05	0.000116
Thrb	56.4	-1	-1.13	3.27E-05	0.000196
Gm45743	60.6	-1	-1.14	4.4E-05	0.000256
Gm42743	41.9	-1	-1.13	4.43E-05	0.000257
Nectin1	128.6	-1	-1.15	7.02E-05	0.000387
Proscos	49.9	-1	-1.15	0.000156	0.00078
Snx24	100.2	-1	-1.2	0.000273	0.00128
Gm43919	34.9	-1	-1.19	0.000325	0.001493
Fbxl12os	26.5	-1	-1.25	0.001077	0.004236
Robo4	55.1	-1	-1.28	0.001574	0.00592
Fam149a	48.8	-1	-1.32	0.001849	0.006801
Gm17021	42.4	-1	-1.35	0.00255	0.008981
Gm15918	28.4	-1	-1.36	0.003178	0.010856
Dcst2	12.5	-1	-1.43	0.004038	0.013242
Traj23	11.2	-1	-1.45	0.004281	0.013929
Cyp1a1	14.7	-1	-1.53	0.005398	0.017057
Siglecg	751.8	-1	-1.64	0.005784	0.018124
Gm5538	4.9	-1	-3.78	0.006528	0.020119
2310026I22Rik	9.2	-1	-1.66	0.006733	0.020671

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Zfp536	9.7	-1	-1.65	0.006926	0.02116
Slc8a2	9.9	-1	-1.82	0.007393	0.022379
C130074G19Rik	7.9	-1	-1.78	0.008117	0.024268
Gm37078	5.3	-1	-2.53	0.008127	0.024294
Enthd1	11.5	-1	-1.83	0.008208	0.024502
Oprl1	7.9	-1	-1.99	0.008234	0.024563
Gm16069	513.8	-1.01	-1.03	0	1.4E-14
Slc12a7	12768.1	-1.01	-1.05	2.7E-14	7.4E-13
Gm1043	603.2	-1.01	-1.06	2.48E-10	4E-09
Gm16638	160.1	-1.01	-1.07	7.79E-10	1.14E-08
Skil	15256	-1.01	-1.08	3.99E-08	4.35E-07
Gm6085	80.3	-1.01	-1.16	0.000113	0.000586
Arhgap20	94.6	-1.01	-1.18	0.000163	0.00081
AC158605.3	38.2	-1.01	-1.27	0.000516	0.002237
She	55.5	-1.01	-1.25	0.000684	0.002858
Abca9	130.3	-1.01	-1.29	0.000993	0.003946
Chrna9	16	-1.01	-1.35	0.002576	0.009052
Cplx1	15.4	-1.01	-1.49	0.004189	0.013661
Gm2694	5.2	-1.01	-3.48	0.006165	0.019142
Fxyd1	9.9	-1.01	-1.64	0.006183	0.01919
Gm31479	6.5	-1.01	-2.85	0.007441	0.022507
Clec2h	5.3	-1.01	-2.58	0.007558	0.022832
Hba-a2	368.3	-1.01	-2.17	0.007639	0.023049
G730013B05Rik	7.5	-1.01	-1.88	0.00769	0.02318
A430072C10Rik	4.4	-1.01	-2.03	0.007723	0.023258
Slc6a19os	5.5	-1.01	-1.86	0.008023	0.024027
Sema3b	11.3	-1.01	-1.97	0.008087	0.024202
Ldhal6b	9.7	-1.01	-1.95	0.008111	0.024255
Dip2c	434.7	-1.02	-1.08	1.85E-09	2.54E-08
Trio	1619.6	-1.02	-1.08	2.11E-08	2.41E-07
Il13ra1	83.3	-1.02	-1.13	3.14E-06	2.39E-05
Gm45059	77.9	-1.02	-1.12	5.67E-06	4.06E-05
Lrrc25	483.1	-1.02	-1.15	9.03E-06	6.18E-05
Ptpro	88.1	-1.02	-1.16	1.63E-05	0.000106
Susd4	89.1	-1.02	-1.16	2.64E-05	0.000162
AC152827.1	55.1	-1.02	-1.19	0.000137	0.000695
Epb41l1	134.6	-1.02	-1.21	0.000176	0.000867
Cd177	45.2	-1.02	-1.33	0.001186	0.004619
AC104880.1	22.4	-1.02	-1.36	0.00212	0.007654
Gm16060	18	-1.02	-1.37	0.002489	0.008799
Pmepalos	12.1	-1.02	-1.46	0.003899	0.012852
Hoga1	13.2	-1.02	-1.61	0.004419	0.014313

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Gm37109	3.1	-1.02	-2.45	0.005212	0.016548
Plxna2	19.9	-1.02	-1.61	0.005262	0.016685
A430027C01Rik	13.4	-1.02	-1.85	0.006086	0.018924
Gm21984	9.4	-1.02	-1.85	0.006525	0.020112
Gm44860	11.3	-1.02	-2.21	0.006672	0.020505
Gramd2	8.5	-1.02	-2.62	0.006793	0.020823
Mcf2	7.3	-1.02	-3.16	0.006935	0.02118
Ablim3	13.3	-1.02	-2.32	0.006988	0.021312
4922502D21Rik	7.8	-1.02	-1.93	0.007227	0.021936
Cyp4f13	1429.7	-1.03	-1.04	0	0
Gm37494	567.7	-1.03	-1.07	1.06E-11	2.12E-10
Maff	11634.4	-1.03	-1.08	1.68E-11	3.25E-10
Tdrp	4170.6	-1.03	-1.08	1.84E-10	3.04E-09
Dennd2c	139.5	-1.03	-1.1	2.13E-08	2.43E-07
Pla2g7	1374.2	-1.03	-1.11	9.79E-08	1E-06
Apoe	8629.4	-1.03	-1.11	1.62E-07	1.58E-06
AC140264.2	106	-1.03	-1.13	1.08E-06	8.95E-06
Alpk1	263.7	-1.03	-1.12	1.08E-06	8.96E-06
Jmy	4073.6	-1.03	-1.13	2.17E-06	1.7E-05
Fdx1l	80.3	-1.03	-1.13	2.58E-06	1.99E-05
Pnck	68.8	-1.03	-1.15	9.22E-06	6.3E-05
Ttc12	74.5	-1.03	-1.25	0.000243	0.001157
Gm12966	32.5	-1.03	-1.23	0.000274	0.001283
AC117769.3	40.9	-1.03	-1.27	0.000618	0.002621
Foxq1	65.3	-1.03	-1.28	0.0007	0.002921
Gm12000	21.3	-1.03	-1.42	0.002794	0.009693
Mir7046	10.4	-1.03	-1.64	0.005264	0.016687
Gm31532	8.9	-1.03	-1.73	0.006325	0.019582
L3mbtl1	7.3	-1.03	-2.07	0.006502	0.020058
Bnip3l-ps	6.6	-1.03	-1.96	0.006735	0.020672
Sfmbt2	429.2	-1.04	-1.09	2.21E-11	4.22E-10
Cd86	1049.2	-1.04	-1.09	3.35E-10	5.26E-09
Cdc14b	1062	-1.04	-1.1	2.04E-09	2.79E-08
Gm42595	333.4	-1.04	-1.1	2.24E-09	3.02E-08
Tlr13	195.6	-1.04	-1.13	5.29E-08	5.62E-07
Egf	186.8	-1.04	-1.12	1.95E-07	1.87E-06
Fgfr1	240.7	-1.04	-1.13	6.63E-07	5.75E-06
Cap2	83.3	-1.04	-1.14	8.33E-07	7.09E-06
C1qb	6167.6	-1.04	-1.15	4.45E-06	3.26E-05
Fam167a	155	-1.04	-1.16	5.75E-06	4.11E-05
Cspg5	62.6	-1.04	-1.17	2.27E-05	0.000142
Tnnt2	65.1	-1.04	-1.2	2.53E-05	0.000156

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Cxcl16	314.6	-1.04	-1.2	5.52E-05	0.000313
A430057M04Rik	48.4	-1.04	-1.27	0.000387	0.001739
Oscpl	44.4	-1.04	-1.29	0.000555	0.002385
H2-Ea-ps	7790.9	-1.04	-1.31	0.000565	0.002422
Il12b	54.2	-1.04	-1.45	0.001388	0.005304
Pde8b	30.7	-1.04	-1.64	0.003238	0.011026
Ptch2	15.2	-1.04	-1.56	0.003758	0.01249
Tmtcl	26.5	-1.04	-1.66	0.004404	0.01428
1810011O10Rik	24.2	-1.04	-1.65	0.004405	0.014281
Gm12167	10.2	-1.04	-1.7	0.005323	0.016848
Gm12089	3.8	-1.04	-2.79	0.005526	0.017413
Gm28035	6	-1.04	-2.57	0.006042	0.0188
C230037L18Rik	243.6	-1.05	-1.1	7.77E-13	1.82E-11
Dyx1c1	106	-1.05	-1.13	3.09E-07	2.87E-06
Hebp1	934.9	-1.05	-1.18	5.55E-06	3.99E-05
Tmem51	428.5	-1.05	-1.17	6E-06	4.27E-05
Naip5	181.7	-1.05	-1.19	1.01E-05	6.87E-05
Cd300c2	673.6	-1.05	-1.2	2.32E-05	0.000144
Card10	54.5	-1.05	-1.19	2.47E-05	0.000153
Coro6	40.8	-1.05	-1.22	8.79E-05	0.000471
Bhlhe41	99.6	-1.05	-1.24	9.66E-05	0.000512
Fhit	63.9	-1.05	-1.24	0.000136	0.000692
Gm18194	29.5	-1.05	-1.28	0.000246	0.001168
Lin7b	27.7	-1.05	-1.28	0.000301	0.001393
Gm6012	40.5	-1.05	-1.29	0.000404	0.001806
Sox5	27.1	-1.05	-1.31	0.000448	0.001973
P2ry2	61.4	-1.05	-1.36	0.000804	0.003293
Gm44214	21.7	-1.05	-1.35	0.000952	0.003805
Blnk	622	-1.05	-1.41	0.001157	0.004518
Acot4	15.6	-1.05	-1.67	0.003129	0.010702
AC160562.1	14.3	-1.05	-1.6	0.003251	0.011059
Myo15	15.2	-1.05	-1.66	0.003332	0.011284
Extl1	17.9	-1.05	-1.78	0.005054	0.0161
Gm15929	8.8	-1.05	-1.92	0.005178	0.016449
Gm43777	8.3	-1.05	-1.79	0.005291	0.016755
Gm2814	9.6	-1.05	-2.68	0.005673	0.017801
Rassf3	6479.1	-1.06	-1.09	0	8E-15
2810021J22Rik	1264.6	-1.06	-1.12	9.26E-11	1.59E-09
Ovgp1	326.4	-1.06	-1.12	1.35E-10	2.27E-09
Mapk12	152.1	-1.06	-1.15	5.43E-08	5.76E-07
AC125071.1	106	-1.06	-1.17	6.39E-07	5.57E-06
Bbs1	97.2	-1.06	-1.17	1.57E-06	1.27E-05

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Gm10167	58.6	-1.06	-1.22	2.61E-05	0.00016
Rassf8	83.1	-1.06	-1.23	4.05E-05	0.000238
Mapk15	40.7	-1.06	-1.27	0.000162	0.000807
Ghrl	55	-1.06	-1.33	0.000426	0.001892
Slc4a11	40.7	-1.06	-1.33	0.000485	0.002114
Cybrd1	29.8	-1.06	-1.35	0.000586	0.002499
Gm8251	24.9	-1.06	-1.4	0.000924	0.003706
Tceal1	18.7	-1.06	-1.52	0.002435	0.00863
Pifo	13.3	-1.06	-1.59	0.003076	0.010546
Bicdl2	10.3	-1.06	-1.77	0.003577	0.011987
CT009757.4	3.8	-1.06	-3.2	0.004176	0.013625
Tmem30b	16	-1.06	-1.86	0.004968	0.015862
Gm826	7.6	-1.06	-2.41	0.0051	0.01623
Tmem236	8.3	-1.06	-1.94	0.005165	0.016415
Gm13201	8.2	-1.06	-2.49	0.00522	0.01657
Zbtb20	1429.4	-1.07	-1.1	0	1E-15
Dstyk	697.7	-1.07	-1.1	0	8E-15
I830077J02Rik	399	-1.07	-1.15	9.22E-09	1.11E-07
Stac3	119.4	-1.07	-1.15	1.12E-07	1.13E-06
Xkrx	235.4	-1.07	-1.18	5.59E-07	4.94E-06
Aph1c	92.6	-1.07	-1.18	1.71E-06	1.37E-05
Ly86	762.3	-1.07	-1.21	8.49E-06	5.85E-05
Stab2	1255.1	-1.07	-1.21	8.58E-06	5.91E-05
4921507P07Rik	57.2	-1.07	-1.21	8.97E-06	6.15E-05
Lst1	1097.1	-1.07	-1.21	1.14E-05	7.63E-05
Erbp2	135	-1.07	-1.26	4.73E-05	0.000273
Syde2	248.2	-1.07	-1.24	4.75E-05	0.000274
Hspg2	41.3	-1.07	-1.3	0.00025	0.001187
Cfap74	36.6	-1.07	-1.35	0.000347	0.001581
Rusc2	49.2	-1.07	-1.34	0.000354	0.001609
Cpe	36.2	-1.07	-1.35	0.000616	0.002616
1700061G19Rik	22.8	-1.07	-1.36	0.000645	0.002719
Rab30	116	-1.07	-1.57	0.001884	0.006917
Gm15537	21	-1.07	-1.58	0.002688	0.009387
Dlk1	20.9	-1.07	-1.54	0.002932	0.010115
Akp-ps1	12.6	-1.07	-1.63	0.003213	0.010955
Gm23346	11.3	-1.07	-2.42	0.003589	0.012019
Col2a1	13.8	-1.07	-1.69	0.003605	0.012063
Muc3	12.6	-1.07	-1.82	0.004403	0.014278
Rgs11	4.5	-1.07	-2.66	0.004427	0.014331
Gm42655	7.7	-1.07	-2.37	0.004556	0.014693
Hbb-bs	1829.7	-1.07	-2.23	0.004758	0.015252

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Crip3	8.6	-1.07	-2.83	0.004816	0.015403
4732440D04Rik	113	-1.08	-1.14	2.24E-10	3.65E-09
Mmp15	73.3	-1.08	-1.16	2.28E-08	2.6E-07
Rgl1	1317.4	-1.08	-1.18	1.34E-07	1.33E-06
Gm15706	118.9	-1.08	-1.18	1.66E-07	1.62E-06
Cdk14	85	-1.08	-1.28	5.71E-05	0.000322
Gm18310	30.9	-1.08	-1.37	0.000572	0.002447
4933433G15Rik	17.6	-1.08	-1.52	0.001341	0.005145
Blk	523.7	-1.08	-1.89	0.0037	0.012329
Hrh1	9.5	-1.08	-1.97	0.003762	0.012499
Dusp10	33817.9	-1.09	-1.12	0	1E-15
Gm42829	278.5	-1.09	-1.12	0	1E-15
Klf7	2672.7	-1.09	-1.12	0	1E-15
Gpr157	197.5	-1.09	-1.12	7E-15	2.04E-13
Sez6l2	335	-1.09	-1.13	1.4E-14	3.99E-13
1700109H08Rik	571.4	-1.09	-1.15	4.74E-11	8.5E-10
Arsg	171.4	-1.09	-1.17	4.69E-09	6E-08
Lypd6b	490.3	-1.09	-1.17	1.28E-08	1.51E-07
Gm14168	317.9	-1.09	-1.17	3.99E-08	4.35E-07
Rnd2	50.2	-1.09	-1.21	3.16E-06	2.4E-05
1700003F12Rik	31.2	-1.09	-1.35	0.000266	0.001254
Cd300c	47.9	-1.09	-1.42	0.000366	0.001658
1700028N14Rik	48.1	-1.09	-1.37	0.000451	0.001986
Gm11210	24.9	-1.09	-1.41	0.000578	0.002472
Stac2	76.6	-1.09	-1.44	0.000652	0.002746
Gm13199	23	-1.09	-1.44	0.000847	0.003442
Wfdc3	16.1	-1.09	-1.49	0.00138	0.005282
D630033O11Rik	38.5	-1.09	-1.71	0.002251	0.00805
Pcdhga12	13.8	-1.09	-1.85	0.003166	0.010824
Mcm2dc2	8.5	-1.09	-2.47	0.004266	0.013892
Atf3	5537.4	-1.1	-1.17	2.92E-10	4.66E-09
Cage1	267.2	-1.1	-1.17	4.95E-09	6.32E-08
Plce1	62.8	-1.1	-1.22	1.85E-06	1.47E-05
Wdfy3	451.6	-1.1	-1.27	1.85E-05	0.000118
Gm13054	37.4	-1.1	-1.34	0.000134	0.000681
Snord72	35.8	-1.1	-1.34	0.000207	0.000998
Tdrd9	21.8	-1.1	-1.44	0.00065	0.00274
Fam229a	25.2	-1.1	-1.44	0.000757	0.003124
Gm38192	22.6	-1.1	-1.57	0.001304	0.005024
A930038B10Rik	14.3	-1.1	-1.62	0.002057	0.007455
AL607131.1	38.2	-1.1	-1.7	0.002202	0.007906
Prox1	12.4	-1.1	-1.72	0.002422	0.008591

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Alas2	140.4	-1.1	-1.81	0.002845	0.009851
Gm15696	19.1	-1.1	-1.81	0.00296	0.010201
Arhgap42	15.2	-1.1	-1.96	0.002998	0.010315
Fam13a	8.3	-1.1	-2.15	0.003567	0.011962
Rgcc	877.6	-1.11	-1.14	0	0
Thbs3	181.1	-1.11	-1.16	2.09E-12	4.62E-11
Tnfrsf12a	1193.9	-1.11	-1.18	2.33E-10	3.77E-09
Wnt5b	363.9	-1.11	-1.17	2.97E-10	4.72E-09
D7Bwg0826e	309.2	-1.11	-1.18	6.4E-10	9.5E-09
Cdo1	154.9	-1.11	-1.18	1.84E-09	2.52E-08
Ing4	286.6	-1.11	-1.2	1.16E-08	1.38E-07
Dock4	301.1	-1.11	-1.21	3.54E-08	3.91E-07
Sh2d4b	213.3	-1.11	-1.21	1.23E-07	1.23E-06
Nr4a3	16228.2	-1.11	-1.22	6.69E-07	5.8E-06
Fam213b	477.2	-1.11	-1.23	6.75E-07	5.85E-06
Gk5	113.2	-1.11	-1.23	8.68E-07	7.36E-06
Hey1	1015.7	-1.11	-1.23	1.04E-06	8.68E-06
Bach2it1	141.1	-1.11	-1.23	1.16E-06	9.58E-06
Sorcs2	70.7	-1.11	-1.26	5.81E-06	4.15E-05
Bmp2	169.3	-1.11	-1.27	7.01E-06	4.92E-05
Snta1	208.6	-1.11	-1.27	1.06E-05	7.16E-05
Gm13710	186.2	-1.11	-1.29	1.71E-05	0.00011
Cascl	50.4	-1.11	-1.3	2.08E-05	0.000131
Nr3c2	42.8	-1.11	-1.35	7.47E-05	0.000408
Myo5c	12.5	-1.11	-1.65	0.001671	0.006233
Gm45140	14.1	-1.11	-1.7	0.001727	0.006423
Scx	11.1	-1.11	-1.65	0.002003	0.007288
AC156952.1	12.6	-1.11	-1.89	0.002764	0.009612
D6Ert474e	4.7	-1.11	-3.38	0.003004	0.010332
Gm9873	11.3	-1.11	-1.97	0.003018	0.010375
Smim6	7.7	-1.11	-2.44	0.003338	0.011301
Gm20513	6.4	-1.11	-2.77	0.003468	0.011673
Hap1	446.1	-1.12	-1.18	2.38E-10	3.85E-09
Epb41l3	649.5	-1.12	-1.22	1.19E-07	1.19E-06
Gm9403	86.5	-1.12	-1.24	2.13E-07	2.03E-06
Ccpg1	685.5	-1.12	-1.24	7.55E-07	6.47E-06
Clec4a1	389.4	-1.12	-1.28	6.12E-06	4.35E-05
CAAA01194877.1	40.7	-1.12	-1.37	0.000103	0.000544
Padi1	28.6	-1.12	-1.39	0.000201	0.000973
Cd36	136.3	-1.12	-1.45	0.000339	0.001549
Bcar1	37.6	-1.12	-1.43	0.00035	0.001594
Snx22	65.3	-1.12	-1.61	0.001167	0.004552

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Trim7	268.2	-1.12	-1.78	0.001673	0.006239
Actl7b	11.5	-1.12	-1.66	0.001764	0.006538
Cacna1f	12.7	-1.12	-1.62	0.001813	0.006692
Gm10425	5.3	-1.12	-3.54	0.002191	0.007874
Prph	18.6	-1.12	-1.79	0.002429	0.00861
Mfap2	4.7	-1.12	-2.58	0.00275	0.009577
Gm16083	11.2	-1.12	-2.24	0.002964	0.010212
Peg13	3375.2	-1.13	-1.17	0	0
Basp1	514.9	-1.13	-1.21	5.81E-10	8.69E-09
Gm42659	103.8	-1.13	-1.21	4.02E-09	5.22E-08
Tagln	142.5	-1.13	-1.21	1.06E-08	1.27E-07
Syk	3325.1	-1.13	-1.23	5.23E-08	5.56E-07
Gm28731	120.2	-1.13	-1.23	6.72E-08	7.06E-07
Trim36	327.4	-1.13	-1.23	7.28E-08	7.61E-07
Smagp	295.1	-1.13	-1.24	1.66E-07	1.62E-06
Wdfy4	2179.7	-1.13	-1.39	9.1E-05	0.000486
Tenm3	7.8	-1.13	-3.31	0.000878	0.003554
Srms	29.4	-1.13	-1.59	0.000938	0.003755
Ccr10	27.6	-1.13	-1.67	0.001242	0.00481
Scn3a	17.9	-1.13	-1.76	0.001259	0.00487
Six1	18.8	-1.13	-1.61	0.00127	0.004905
Mab21l3	31.1	-1.13	-1.89	0.001644	0.006154
Tcf21	11.3	-1.13	-1.75	0.002034	0.007381
Gm20506	6.3	-1.13	-3.45	0.00239	0.008493
Hbb-bt	232	-1.13	-2.38	0.002847	0.009853
Chka	3724.8	-1.14	-1.18	0	7E-15
Ttc28	2577.9	-1.14	-1.22	6.08E-10	9.07E-09
C330013E15Rik	164.4	-1.14	-1.25	2.81E-07	2.62E-06
E230029C05Rik	167.6	-1.14	-1.28	7.77E-07	6.65E-06
Gm22596	38.9	-1.14	-1.32	1.85E-05	0.000118
Gm37856	32.5	-1.14	-1.38	5.67E-05	0.00032
Acot1	60.2	-1.14	-1.39	6.79E-05	0.000375
Zfp811	50.1	-1.14	-1.41	0.000133	0.000678
Ctnnd2	131.9	-1.14	-1.44	0.000139	0.000703
5430402O13Rik	22.1	-1.14	-1.55	0.000666	0.002793
Btnl4	51.4	-1.14	-1.72	0.000896	0.00361
Tex26	22.2	-1.14	-1.65	0.000907	0.003649
Cecr2	73.9	-1.14	-1.95	0.001666	0.00622
Cd209c	9.8	-1.14	-2.49	0.002577	0.009055
Msi1	220.6	-1.15	-1.2	8.41E-13	1.97E-11
P2ry13	230.8	-1.15	-1.26	1.71E-08	1.99E-07
Sgip1	455.7	-1.15	-1.28	3.33E-07	3.08E-06

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
C1qc	6828	-1.15	-1.29	6.45E-07	5.61E-06
1700047K16Rik	77	-1.15	-1.31	6.01E-06	4.28E-05
Fam83e	25.7	-1.15	-1.41	8.3E-05	0.000448
Mxra7	39.3	-1.15	-1.43	0.00011	0.000574
Cspg4	3.3	-1.15	-5.62	0.000117	0.000603
Car3	34.9	-1.15	-1.84	0.001092	0.00429
Ephx3	23.3	-1.15	-1.75	0.001523	0.005749
Des	17.8	-1.15	-1.87	0.00184	0.006771
Neurl1a	7.4	-1.15	-2.41	0.002443	0.008651
Stox2	6.7	-1.15	-2.3	0.00249	0.008799
Lepr	5.9	-1.15	-2.45	0.002517	0.008882
Gm45212	5.8	-1.15	-2.56	0.002531	0.008927
Cregl	2831	-1.16	-1.22	4.72E-12	9.94E-11
Txnrd3	195.6	-1.16	-1.25	7.32E-10	1.08E-08
Cd163	1248.1	-1.16	-1.31	6.08E-07	5.33E-06
Rab34	100.4	-1.16	-1.31	1.51E-06	1.23E-05
Fgf17	23	-1.16	-1.55	0.000756	0.00312
Prlr	10.5	-1.16	-2.27	0.001924	0.007048
Il1bos	10.4	-1.16	-2.22	0.002031	0.007373
Gm38299	5.4	-1.16	-3.44	0.002059	0.007461
Gm35584	9	-1.16	-2.58	0.002101	0.007598
Ras10b	6.3	-1.16	-2.53	0.002111	0.007625
Il22ra2	7.5	-1.16	-2.84	0.002262	0.008085
Tgfb3	2708.7	-1.17	-1.2	0	0
Bach2	5671.9	-1.17	-1.21	0	6E-15
Gm43352	344.3	-1.17	-1.24	3.79E-11	6.97E-10
Tnfrsf21	641.6	-1.17	-1.25	8.28E-11	1.43E-09
Mpeg1	6302.5	-1.17	-1.25	7.72E-10	1.13E-08
Tbxas1	561.4	-1.17	-1.27	7.87E-09	9.61E-08
Thsd1	89.3	-1.17	-1.3	2.62E-07	2.46E-06
Il1b	670.8	-1.17	-1.31	7.38E-07	6.36E-06
Tbx2	144.3	-1.17	-1.34	3.67E-06	2.74E-05
March1	226	-1.17	-1.41	1.83E-05	0.000117
Serpine1	218.8	-1.17	-1.42	5.48E-05	0.00031
Gm37509	25.6	-1.17	-1.44	6.32E-05	0.000352
Ptgis	82.5	-1.17	-1.47	9.03E-05	0.000483
Crnde	21.9	-1.17	-1.47	0.000116	0.000599
Gm15448	29.6	-1.17	-1.71	0.000537	0.002315
Mir5107	156.7	-1.17	-1.83	0.001041	0.004111
1700001J03Rik	13.9	-1.17	-2.01	0.001786	0.006611
Gm17999	9.8	-1.17	-2.54	0.0018	0.006652
Gm37621	8.8	-1.17	-2.16	0.001873	0.006882

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Myzap	9.4	-1.17	-2.33	0.001875	0.006886
Gm15156	8.3	-1.17	-2.23	0.001925	0.00705
4930455G09Rik	13.9	-1.17	-2.58	0.002094	0.007572
Mtssl	2502.5	-1.18	-1.19	0	0
1700056E22Rik	810.3	-1.18	-1.24	3E-14	8.31E-13
Plekha6	574.9	-1.18	-1.24	3.19E-13	7.72E-12
Gnal	314.2	-1.18	-1.26	4.93E-10	7.47E-09
Wnk4	72.2	-1.18	-1.29	1.22E-08	1.45E-07
Clec12a	552.3	-1.18	-1.35	2.93E-06	2.25E-05
9330102E08Rik	20.4	-1.18	-1.59	0.000312	0.001441
Adgrl3	33.2	-1.18	-1.74	0.00049	0.002135
AC152065.1	24.8	-1.18	-1.71	0.000632	0.002673
Gm26685	15.5	-1.18	-1.8	0.000915	0.003677
Gm5466	17.5	-1.18	-1.99	0.001304	0.005023
Reln	18.1	-1.18	-2.28	0.001614	0.006051
Gucy2c	7.2	-1.18	-2.88	0.001714	0.006382
Gm45572	4.9	-1.18	-3.42	0.001752	0.0065
Gm2058	5.4	-1.18	-2.64	0.001753	0.006503
Fcnaos	7.6	-1.18	-2.37	0.001762	0.006532
Gm13868	10	-1.18	-2.61	0.001817	0.006701
Ldlr	1329.5	-1.19	-1.23	0	1E-15
Slprl	21169	-1.19	-1.24	3.9E-14	1.05E-12
Ksr2	333.8	-1.19	-1.27	7.47E-11	1.3E-09
Lrrc75b	342.7	-1.19	-1.3	5.18E-09	6.59E-08
Dnah17	303	-1.19	-1.3	4.01E-08	4.37E-07
Tmem141	255.2	-1.19	-1.37	1.75E-06	1.4E-05
Gpat3	224.7	-1.19	-1.35	1.96E-06	1.55E-05
Adhfe1	58	-1.19	-1.4	5.68E-06	4.06E-05
Poln	40.7	-1.19	-1.45	5.15E-05	0.000294
Klra17	46.4	-1.19	-1.57	0.000193	0.000938
Sycell	12.2	-1.19	-1.87	0.000875	0.003544
Gm15523	17.3	-1.19	-1.93	0.001035	0.00409
0610040J01Rik	15.3	-1.19	-2.08	0.001054	0.004156
Gm11525	16.1	-1.19	-2.03	0.001213	0.004715
6330403L08Rik	20.6	-1.19	-2.07	0.001227	0.00476
Gm15848	16.2	-1.19	-3.07	0.001697	0.006322
Gm26799	792.8	-1.2	-1.26	7.8E-14	2.04E-12
Nr1d2	2875.9	-1.2	-1.26	3.15E-13	7.65E-12
Tcf4	708.9	-1.2	-1.3	6.96E-09	8.59E-08
Cd300a	1109.4	-1.2	-1.33	5.7E-08	6.03E-07
Ccnd1	258	-1.2	-1.35	1.29E-07	1.29E-06
Gm12474	77.6	-1.2	-1.34	2.3E-07	2.18E-06

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Kcnj9	35.1	-1.2	-1.78	0.000455	0.002001
Baiap2l1	10.8	-1.2	-2.01	0.001135	0.00444
Slc6a1	17.6	-1.2	-2.23	0.001191	0.004634
Gpr152	13.5	-1.2	-2.12	0.001386	0.0053
Gm44735	8.1	-1.2	-2.18	0.001412	0.005383
Gm15675	565.2	-1.21	-1.28	2.06E-13	5.1E-12
Plxna1	900.2	-1.21	-1.3	3.45E-10	5.38E-09
Slc7a7	700.4	-1.21	-1.31	1.07E-09	1.52E-08
P4ha2	71.8	-1.21	-1.35	9.41E-08	9.65E-07
Dbnnd2	61.2	-1.21	-1.35	1.45E-07	1.43E-06
Angptl7	118.5	-1.21	-1.38	5.17E-07	4.61E-06
Sirpb1a	105.6	-1.21	-1.39	5.82E-07	5.13E-06
Kcnk13	70	-1.21	-1.41	2.14E-06	1.68E-05
Gm2238	53.2	-1.21	-1.43	9.18E-06	6.28E-05
AC139941.2	39.3	-1.21	-1.54	8.74E-05	0.000469
Plekhg1	29	-1.21	-1.66	0.000292	0.001357
AC154548.2	22	-1.21	-1.72	0.000515	0.002231
Gm36937	12.9	-1.21	-2.31	0.001265	0.004889
Gm36159	8.5	-1.21	-2.36	0.001348	0.005169
Rgs7bp	8.5	-1.21	-2.61	0.001437	0.005464
Amigo2	1152.3	-1.22	-1.27	1E-15	3.2E-14
Pde5a	641	-1.22	-1.29	4E-14	1.08E-12
Myo9a	1226.4	-1.22	-1.29	1.92E-13	4.77E-12
Ptpdc1	185.8	-1.22	-1.3	3.71E-12	7.94E-11
Rab7b	324.8	-1.22	-1.32	6.47E-10	9.59E-09
Ptgs1	1227.5	-1.22	-1.31	6.72E-10	9.94E-09
Siglech	840.4	-1.22	-1.36	9.21E-08	9.46E-07
Gm13340	40.7	-1.22	-1.42	3.06E-06	2.34E-05
Slpi	1443.3	-1.22	-1.51	3.7E-05	0.00022
Crabp2	41.6	-1.22	-1.55	0.000115	0.000594
Cpne9	20.6	-1.22	-1.59	0.000175	0.000861
Wtip	28.7	-1.22	-1.67	0.00019	0.000927
Nox1	20	-1.22	-1.77	0.000288	0.00134
Kcnj2	25	-1.22	-2.13	0.000891	0.003595
Gm38160	15.5	-1.22	-2.33	0.001201	0.004671
Tulp1	4.4	-1.22	-2.59	0.001242	0.00481
Tbx4	5.5	-1.22	-2.3	0.001274	0.004917
Rapgef4	2629.2	-1.23	-1.28	0	1E-15
Zfyve28	1185	-1.23	-1.32	2.46E-11	4.68E-10
Tns3	884	-1.23	-1.34	1.44E-09	2.01E-08
Alpk3	97.8	-1.23	-1.35	3.57E-09	4.69E-08
Ucp3	43.4	-1.23	-1.42	1.96E-06	1.55E-05

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Slc26a8	47	-1.23	-1.43	3.29E-06	2.48E-05
Slc16a7	76.5	-1.23	-1.46	6.11E-06	4.34E-05
Ssc5d	41.7	-1.23	-1.5	2.14E-05	0.000134
AC159649.1	24.5	-1.23	-1.54	6.02E-05	0.000337
Clec9a	155.9	-1.23	-1.61	8.74E-05	0.00047
Has3	31.1	-1.23	-1.68	0.000212	0.001023
Muc2	15.7	-1.23	-1.78	0.000371	0.001676
Lsamp	11.3	-1.23	-1.86	0.000524	0.002268
Gm44185	16.5	-1.23	-1.85	0.000526	0.002273
Gm12263	11.8	-1.23	-2.06	0.000838	0.003411
Adam11	386.6	-1.24	-1.3	0	1.4E-14
9230114K14Rik	64.2	-1.24	-1.43	1.77E-06	1.42E-05
AC158605.2	23.8	-1.24	-1.72	0.000182	0.000892
Ccser1	25.6	-1.24	-1.92	0.000376	0.001694
Kncn	8.8	-1.24	-1.98	0.000829	0.00338
5930403N24Rik	16	-1.24	-2.23	0.000843	0.003427
Sbsn	107.9	-1.25	-1.36	1.8E-09	2.47E-08
Adgrl2	148.4	-1.25	-1.38	1.1E-08	1.31E-07
Slc16a9	181.6	-1.25	-1.43	4.53E-07	4.09E-06
Gm17116	29.3	-1.25	-1.58	3.4E-05	0.000203
Cxcl2	1196	-1.25	-1.58	4.79E-05	0.000276
Tpm2	26.7	-1.25	-1.65	9.83E-05	0.00052
AC158622.3	22.2	-1.25	-1.68	0.00016	0.0008
Gm37755	11.4	-1.25	-2.1	0.000797	0.003266
Tmem132b	9.3	-1.25	-3.04	0.001003	0.003981
Gtf2ird1	693.3	-1.26	-1.37	1.66E-10	2.76E-09
Rhbdf1	115.9	-1.26	-1.4	2.97E-08	3.32E-07
Slc23a3	71.8	-1.26	-1.41	1.12E-07	1.13E-06
Spon2	49.3	-1.26	-1.42	1.54E-07	1.51E-06
Gm11342	46.6	-1.26	-1.46	9.11E-07	7.69E-06
Gm15728	46.1	-1.26	-1.49	4.42E-06	3.24E-05
Skor1	38.4	-1.26	-1.48	5.95E-06	4.25E-05
Olfr1033	26.2	-1.26	-1.71	0.000174	0.000858
Gm15327	25.1	-1.26	-1.81	0.000356	0.001619
Gm32999	14.5	-1.26	-2.13	0.000663	0.002785
4833411C07Rik	12.7	-1.26	-2.47	0.000791	0.003247
Gm12290	7.9	-1.26	-2.93	0.000865	0.003511
Slc28a1	7.4	-1.26	-2.34	0.000891	0.003595
Cd55	2669.5	-1.27	-1.32	0	0
Adra2a	136.7	-1.27	-1.34	4.7E-14	1.26E-12
Dnhd1	272.9	-1.27	-1.35	6.78E-13	1.6E-11
Pirb	1339.8	-1.27	-1.41	1.54E-08	1.8E-07

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Cd300lf	581.8	-1.27	-1.41	1.64E-08	1.92E-07
Zfhx3	113.3	-1.27	-1.44	1.52E-07	1.49E-06
Amn	69.8	-1.27	-1.5	2.81E-06	2.16E-05
Gm44401	34.9	-1.27	-1.51	3.5E-06	2.62E-05
Cecr6	48.6	-1.27	-1.52	4.28E-06	3.15E-05
Sirpb1b	32.1	-1.27	-1.69	2.66E-05	0.000163
Zfp618	38	-1.27	-1.64	4.75E-05	0.000274
Apoc1	69.6	-1.27	-1.69	7.23E-05	0.000397
Ltbp2	21	-1.27	-1.83	0.000197	0.000955
Gm17455	30.9	-1.27	-1.88	0.000295	0.001371
St18	3.6	-1.27	-4.89	0.000421	0.001874
Gpbar1	9.8	-1.27	-2.27	0.000836	0.003403
Ptk2	617.3	-1.28	-1.32	0	0
Sbf2	450.1	-1.28	-1.33	0	0
AC135019.1	300.9	-1.28	-1.34	0	1E-15
Ctsf	383.8	-1.28	-1.37	4.71E-12	9.94E-11
Npm2	80.8	-1.28	-1.37	9.51E-11	1.63E-09
Rin1	193.4	-1.28	-1.38	5.16E-10	7.79E-09
Adgre1	1926.6	-1.28	-1.4	8.52E-10	1.24E-08
Hpgd	485.4	-1.28	-1.4	2.16E-09	2.93E-08
Gm18407	52.8	-1.28	-1.47	3.97E-07	3.62E-06
AC164573.1	54.6	-1.28	-1.47	7.45E-07	6.4E-06
Arg2	120.5	-1.28	-1.49	7.92E-07	6.76E-06
Flt3	441.7	-1.28	-1.5	8.85E-07	7.48E-06
Gm26615	44.6	-1.28	-1.65	2.71E-05	0.000166
A530064D06Rik	30	-1.28	-1.72	3.42E-05	0.000204
Gm33280	43.4	-1.28	-1.76	9.08E-05	0.000485
Gm15930	22	-1.28	-1.93	0.000219	0.001054
Hoxaas3	15.8	-1.28	-1.9	0.000251	0.001189
Gm11980	17.1	-1.28	-1.92	0.000307	0.001421
Gm43857	14.2	-1.28	-2.08	0.000361	0.001637
Gm37510	31.1	-1.28	-1.87	0.000376	0.001697
Gm12972	20.1	-1.28	-2.06	0.000381	0.001713
Cyp26b1	13.2	-1.28	-1.96	0.000381	0.001714
P4ha3	10.9	-1.28	-1.95	0.000471	0.002065
Prex2	8.7	-1.28	-2.59	0.000692	0.00289
Usp2	832.7	-1.29	-1.39	4.34E-11	7.86E-10
Tlr2	527.6	-1.29	-1.46	7.25E-08	7.59E-07
Matn2	73.1	-1.29	-1.57	2.24E-06	1.75E-05
AC159196.3	73.6	-1.29	-1.53	2.47E-06	1.92E-05
Fam135a	30.1	-1.29	-1.79	7.1E-05	0.000391
Gm4247	22.2	-1.29	-1.72	7.22E-05	0.000397

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Fam83c	5.2	-1.29	-3.81	0.000414	0.001843
Otud1	1927.6	-1.3	-1.33	0	0
Fam209	161.5	-1.3	-1.41	5.91E-10	8.83E-09
Spred3	56.8	-1.3	-1.44	8.54E-09	1.04E-07
Lman1l	148.2	-1.3	-1.46	2.39E-08	2.7E-07
C1qa	7016.9	-1.3	-1.46	2.44E-08	2.75E-07
Msantdl	67.1	-1.3	-1.47	1.68E-07	1.64E-06
Nlgn3	46.3	-1.3	-1.5	3.68E-07	3.38E-06
Samd4	57.2	-1.3	-1.64	1.07E-05	7.19E-05
Cyp2d40	3.9	-1.3	-3.38	0.000438	0.001938
Usp28	3778	-1.31	-1.34	0	0
Ntn4	238.3	-1.31	-1.42	1.89E-10	3.13E-09
Gm19325	31.3	-1.31	-1.59	6.63E-06	4.67E-05
Ccdc148	86.4	-1.31	-1.62	6.67E-06	4.7E-05
Matn1	29.8	-1.31	-1.6	7.41E-06	5.18E-05
Art5	20.4	-1.31	-1.74	4.51E-05	0.000261
Slc6a7	16	-1.31	-1.9	0.000175	0.000862
Paqr6	11.7	-1.31	-2.35	0.000444	0.001958
6430548M08Rik	386.7	-1.32	-1.41	4.35E-13	1.04E-11
Jup	1184.5	-1.32	-1.41	4.36E-13	1.04E-11
Lima1	405.9	-1.32	-1.43	4.11E-11	7.48E-10
Tgm2	2908.8	-1.32	-1.44	1.38E-10	2.32E-09
Cfp	5197	-1.32	-1.44	4.73E-10	7.18E-09
Igf1	580.6	-1.32	-1.5	3.7E-08	4.07E-07
Gm44053	47.1	-1.32	-1.6	1.61E-06	1.3E-05
Gpr4	64.4	-1.32	-1.62	4.44E-06	3.25E-05
Mc1r	8.3	-1.32	-2.63	0.000507	0.002201
Ampd1	414.5	-1.33	-1.39	0	0
C6	1221.3	-1.33	-1.46	3.07E-10	4.85E-09
Gfra2	1060.6	-1.33	-1.46	5.81E-10	8.69E-09
Clec4n	902.8	-1.33	-1.49	4.25E-09	5.48E-08
Krt83	243.6	-1.33	-1.49	2.68E-08	3.01E-07
Clec4b1	78.8	-1.33	-1.72	1.43E-05	9.36E-05
Clec4a4	10.7	-1.33	-2.9	0.000393	0.001763
Nr2e3	6.8	-1.33	-4.34	0.000443	0.001955
Irs2	11401.3	-1.34	-1.4	0	1E-15
Fbxl22	362.3	-1.34	-1.41	6E-15	1.88E-13
Csflr	9890.8	-1.34	-1.45	2.94E-12	6.38E-11
Zfp608	304	-1.34	-1.47	7.59E-10	1.11E-08
Gm42636	106.9	-1.34	-1.48	1.27E-09	1.79E-08
Fgd4	96.4	-1.34	-1.66	3.01E-06	2.3E-05
Gm9530	13.7	-1.34	-2.18	0.00018	0.000884

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Gli1	13	-1.34	-2.06	0.00019	0.000928
Epha4	19.9	-1.34	-2.3	0.000222	0.001066
Tmem132c	5.6	-1.34	-3.55	0.000311	0.001435
Vipr1	6553.5	-1.35	-1.4	0	0
Adamdec1	659.5	-1.35	-1.47	7.08E-11	1.24E-09
Fmn12	329.1	-1.35	-1.5	1.5E-09	2.08E-08
Guca2a	39.9	-1.35	-1.58	4.21E-07	3.82E-06
Tgm4	40.9	-1.35	-1.6	7.48E-07	6.42E-06
Gm12259	65.1	-1.35	-1.67	4.31E-06	3.17E-05
Trf	681.7	-1.35	-1.77	1.94E-05	0.000123
Podn	23.2	-1.35	-1.75	2.38E-05	0.000148
Aldh6a1	522.8	-1.36	-1.47	2.47E-11	4.68E-10
Adh1	149.8	-1.36	-1.49	4.27E-10	6.55E-09
Gm20544	121	-1.36	-1.51	9.16E-10	1.32E-08
Lpcat2	297.8	-1.36	-1.51	1.63E-09	2.25E-08
Rufy4	34.6	-1.36	-1.67	2.98E-06	2.28E-05
Syt1	3.7	-1.36	-5.79	0.000188	0.000918
Ankrd63	174.2	-1.37	-1.48	8.29E-12	1.7E-10
Slco2b1	818.9	-1.37	-1.49	9.09E-12	1.84E-10
Gm44907	211	-1.37	-1.48	2.4E-11	4.58E-10
Gm26888	163	-1.37	-1.5	8.25E-11	1.43E-09
Bcl2a1a	59.4	-1.37	-1.77	7.74E-06	5.39E-05
AC153955.5	18.5	-1.37	-2.1	9.26E-05	0.000494
Lrrc3	23.5	-1.37	-2.17	0.000121	0.000625
Armex4	774.4	-1.37	-2.13	0.000162	0.000806
Gm12689	10.9	-1.37	-2.37	0.000236	0.001124
Slc40a1	4625.9	-1.38	-1.49	3.67E-12	7.88E-11
Tmem8b	139.4	-1.38	-1.5	8.99E-12	1.83E-10
Gm14027	93.7	-1.38	-1.5	3.48E-11	6.44E-10
Gm15503	167.6	-1.38	-1.51	8.32E-11	1.44E-09
Cd302	387	-1.38	-1.53	1.75E-10	2.9E-09
Mafb	1846.3	-1.38	-1.52	2.27E-10	3.69E-09
Gm15931	220.2	-1.38	-1.57	6.29E-09	7.84E-08
Apol9b	51.1	-1.38	-1.56	1.78E-08	2.06E-07
Pid1	185.8	-1.38	-1.68	8.53E-07	7.24E-06
Hes1	309.1	-1.38	-1.68	1.9E-06	1.51E-05
Jag1	135.9	-1.38	-1.7	4.46E-06	3.26E-05
A530099J19Rik	38	-1.38	-2.21	9.87E-05	0.000522
4930471C04Rik	10.8	-1.38	-2.24	0.000147	0.000741
Slc13a2	13.1	-1.38	-2.43	0.000153	0.000766
Gm15880	16.2	-1.38	-2.53	0.000207	0.000999
H2-Ob	1767	-1.39	-1.47	2E-15	6.2E-14

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
St8sia6	1584.6	-1.39	-1.47	4E-15	1.36E-13
Kcnip2	299.8	-1.39	-1.49	9.54E-13	2.22E-11
Agap1	495.9	-1.39	-1.54	3.02E-10	4.8E-09
Cyp27a1	464.9	-1.39	-1.57	5.08E-09	6.47E-08
Glis3	183.7	-1.39	-1.6	3.44E-08	3.81E-07
Ttc36	55	-1.39	-1.62	1.95E-07	1.88E-06
Slpr3	58.4	-1.39	-1.66	2.67E-07	2.5E-06
Shisa4	42.4	-1.39	-1.69	1.93E-06	1.53E-05
Notch4	137.5	-1.39	-1.8	8.56E-06	5.9E-05
Unc5a	20.3	-1.39	-2.82	0.000189	0.000922
Gm43364	7	-1.39	-2.54	0.000253	0.001196
Myo10	1090.4	-1.4	-1.49	8E-15	2.44E-13
Ifi207	440.3	-1.4	-1.55	2.58E-10	4.15E-09
Ppfibp2	358.3	-1.4	-1.57	9.23E-10	1.33E-08
Kcnh4	54	-1.4	-1.8	8.38E-06	5.79E-05
Ppp1r14a	48.4	-1.4	-1.89	1.95E-05	0.000124
AC160403.2	22.2	-1.4	-1.93	2.84E-05	0.000173
Gm37900	17.4	-1.4	-1.95	3.9E-05	0.00023
Tal2	7.8	-1.4	-3.32	0.000204	0.000985
Gper1	10.3	-1.4	-2.81	0.000226	0.001083
Gm45053	497.4	-1.41	-1.46	0	0
Erp27	298.4	-1.41	-1.48	0	0
Rims3	1029.1	-1.41	-1.5	2E-15	5.8E-14
Epha2	215.5	-1.41	-1.55	4.1E-11	7.48E-10
Dll4	78	-1.41	-1.6	4.69E-09	6E-08
Cacnale	284.9	-1.41	-1.69	2.23E-07	2.12E-06
AC159502.1	24.7	-1.41	-1.84	1.34E-05	8.79E-05
Gm14221	27.8	-1.41	-2.18	5.98E-05	0.000335
Gm24371	8.6	-1.41	-2.69	0.000193	0.000938
Haglr	8.2	-1.41	-2.62	0.000212	0.001022
Tnfrsf10b	1091.3	-1.42	-1.48	0	0
Lhfp13	194	-1.42	-1.5	0	1E-15
Ppp1r32	136.8	-1.42	-1.54	1E-12	2.32E-11
Cystm1	143.7	-1.42	-1.62	6.84E-09	8.46E-08
Bank1	715.4	-1.42	-1.65	3.75E-08	4.12E-07
Trim47	215.2	-1.42	-1.64	3.98E-08	4.35E-07
Gm14548	229.3	-1.42	-1.68	5.07E-08	5.42E-07
A930036K24Rik	102.7	-1.42	-1.69	4.41E-07	4E-06
Bmf	141.4	-1.42	-1.87	8.04E-06	5.59E-05
Tnnt3	267.5	-1.43	-1.49	0	0
Abcg3	1117.1	-1.43	-1.52	0	3E-15
Lilra6	252.1	-1.43	-1.59	5.27E-11	9.37E-10

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Kirrel3	96.4	-1.43	-1.63	2.29E-09	3.09E-08
Nlrp3	1148.6	-1.43	-1.64	6.8E-09	8.42E-08
Tspan15	134	-1.43	-1.63	6.99E-09	8.63E-08
Pdzd2	59.3	-1.43	-1.68	4.54E-08	4.89E-07
Adap2	260.1	-1.43	-1.7	7.96E-08	8.26E-07
E230013L22Rik	56	-1.43	-1.81	1.9E-06	1.5E-05
Kcnj10	1374.3	-1.44	-1.55	1.4E-14	4.01E-13
Plbd1	1881.3	-1.44	-1.58	7.79E-12	1.6E-10
Nphp3	103.3	-1.44	-1.58	3.52E-11	6.5E-10
Trim72	99	-1.44	-1.79	3.19E-06	2.42E-05
9330179D12Rik	27.2	-1.44	-1.85	4E-06	2.96E-05
Gm44889	45.5	-1.44	-1.82	4.43E-06	3.25E-05
Mcc	21.2	-1.44	-2.36	4.81E-05	0.000277
Gm14137	13.8	-1.44	-3.59	0.000153	0.000766
Ifngr2	7439.3	-1.45	-1.52	0	0
Ripor3	228.4	-1.45	-1.53	0	0
Atp2a1	251.9	-1.45	-1.54	0	1.7E-14
Lrrc23	180.5	-1.45	-1.54	1E-15	2.6E-14
Map3k9	162.8	-1.45	-1.56	9E-15	2.57E-13
Ccr3	902.9	-1.45	-1.57	1.22E-12	2.78E-11
Gm13919	14.4	-1.45	-2.29	6.69E-05	0.00037
Gm43263	4.5	-1.45	-4.23	0.000109	0.00057
Gm16378	7.4	-1.45	-3.04	0.00012	0.000616
Hpgds	565.3	-1.46	-1.58	3.5E-14	9.57E-13
Grk3	1230.7	-1.46	-1.58	6.3E-14	1.66E-12
Itgb5	955.2	-1.46	-1.58	2.04E-13	5.07E-12
Slc45a3	1350.4	-1.46	-1.61	1.37E-11	2.69E-10
St6galnac2	280.3	-1.46	-1.66	1.57E-09	2.17E-08
B230398E01Rik	42.2	-1.46	-1.79	5.23E-07	4.65E-06
Tm4sf1	20.6	-1.46	-2.07	3.08E-05	0.000186
Elfn2	10.1	-1.46	-2.85	0.000117	0.000607
4930426D05Rik	77.3	-1.46	-3.31	0.000118	0.000608
Neurl3	9998.4	-1.47	-1.51	0	0
Gas6	210.8	-1.47	-1.69	7.28E-09	8.96E-08
Arhgap32	163.9	-1.47	-1.72	1.72E-08	2E-07
Slc8a1	117.6	-1.47	-1.87	8.73E-07	7.39E-06
Riad1	6.9	-1.47	-3.36	0.000112	0.000581
Gm5150	271.8	-1.48	-1.61	1.69E-13	4.24E-12
Ctsk	47.3	-1.48	-1.67	1.41E-09	1.96E-08
Vwa3b	95.8	-1.48	-1.69	2.72E-09	3.64E-08
Gm42778	66.3	-1.48	-1.69	2.76E-09	3.68E-08
Apol9a	29.5	-1.48	-1.72	3.39E-08	3.76E-07

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Capn9	80.5	-1.48	-1.77	3.53E-08	3.9E-07
Cd164l2	60	-1.48	-1.78	2.67E-07	2.5E-06
Jhy	47.7	-1.48	-2.71	6.28E-05	0.00035
Gm26583	155.4	-1.49	-1.61	1E-13	2.59E-12
Axl	7411.5	-1.5	-1.62	4.7E-14	1.27E-12
Cd209b	9.6	-1.5	-3.41	7.28E-05	0.0004
Mpzl1	469.8	-1.51	-1.65	1.12E-12	2.58E-11
Stra6l	77.8	-1.51	-1.78	3.26E-09	4.3E-08
Slc1a3	115.6	-1.51	-1.87	2.05E-07	1.96E-06
Plaur	2412.8	-1.53	-1.62	0	0
Tmem26	319.6	-1.53	-1.69	2.21E-12	4.88E-11
Islr2	51.9	-1.53	-1.85	1.27E-07	1.27E-06
Serinc4	39.2	-1.53	-1.89	2.14E-07	2.04E-06
Gm28496	23.9	-1.53	-1.91	7E-07	6.05E-06
Adam22	142.7	-1.53	-2.01	7.91E-07	6.76E-06
Gm39059	17	-1.53	-2.24	8.57E-06	5.9E-05
Rbm44	28.3	-1.53	-2.17	8.64E-06	5.94E-05
Gm45767	59.3	-1.54	-1.71	2.82E-11	5.29E-10
Rgl3	91.8	-1.54	-1.8	1.32E-09	1.86E-08
Rasal2	84.5	-1.54	-1.79	2.62E-09	3.51E-08
Gpc4	55.1	-1.54	-1.88	6.39E-08	6.74E-07
Mybpc3	45.4	-1.54	-1.95	3.3E-07	3.06E-06
Timm8a2	27.2	-1.54	-1.94	3.96E-07	3.61E-06
Cryaa	25.4	-1.54	-2.02	1.86E-06	1.48E-05
Gm32914	19	-1.54	-2.22	9.67E-06	6.58E-05
Cd207	65.2	-1.54	-3.46	4.96E-05	0.000285
Kcnj16	221.3	-1.55	-1.75	2.52E-11	4.76E-10
Sult6b2	52.2	-1.55	-1.74	3.35E-10	5.26E-09
9430038I01Rik	518.8	-1.55	-1.75	3.56E-10	5.54E-09
Dmpk	201.4	-1.55	-1.77	3.83E-10	5.9E-09
Ace	93.4	-1.55	-2.34	4.87E-06	3.54E-05
Maml3	448.5	-1.56	-1.64	0	0
Vcam1	9147.6	-1.56	-1.73	1.93E-12	4.28E-11
AC160405.2	68.7	-1.56	-1.74	2.56E-11	4.83E-10
Marcks	880	-1.56	-1.77	9.63E-11	1.65E-09
Gm16486	59.7	-1.56	-1.75	1.02E-10	1.74E-09
Myo18b	126.8	-1.56	-1.88	6.78E-09	8.4E-08
Adgrg6	43.3	-1.56	-2.1	9.01E-07	7.61E-06
Kif26a	36.7	-1.56	-2.4	9E-06	6.17E-05
Carns1	4046.3	-1.57	-1.6	0	0
Hcar2	808.2	-1.57	-1.79	2.26E-10	3.67E-09
Pyroxd2	242.8	-1.58	-1.75	1.52E-12	3.44E-11

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Lhfp	173.9	-1.58	-1.82	4.67E-10	7.1E-09
Sned1	230.5	-1.58	-1.89	5.36E-09	6.79E-08
Adam30	27.5	-1.59	-2.32	5.57E-06	4E-05
Rasgef1b	1868	-1.61	-1.68	0	0
Shd	168	-1.61	-1.78	9.04E-13	2.11E-11
Ear2	200.6	-1.61	-1.83	1.1E-11	2.2E-10
Dgki	117.3	-1.61	-1.86	5.63E-11	9.99E-10
Mrc1	3441.3	-1.62	-1.79	4.62E-13	1.1E-11
Plpp3	349.2	-1.62	-1.82	3.16E-12	6.83E-11
Armxc1	41.6	-1.62	-2.1	4.7E-07	4.23E-06
AC152979.5	38.7	-1.62	-2.27	1.36E-06	1.11E-05
Cfap61	26	-1.62	-2.46	3.27E-06	2.48E-05
Gm44577	14.8	-1.62	-2.61	5.02E-06	3.64E-05
Gm17040	9.2	-1.62	-2.56	9.58E-06	6.53E-05
Paqr9	427.9	-1.63	-1.84	1.58E-12	3.57E-11
Gm20692	32.4	-1.63	-2.15	3.32E-07	3.07E-06
Trem14	1601.2	-1.64	-1.75	0	0
Pcolce2	67.5	-1.64	-2.02	1.22E-08	1.45E-07
Slc11a1	1813.6	-1.65	-1.79	0	1.3E-14
1700029H14Rik	34.9	-1.65	-2	1.69E-08	1.97E-07
Prrt3	12.9	-1.65	-2.43	3.28E-06	2.48E-05
Hepacam2	34.3	-1.65	-3	7.92E-06	5.51E-05
Ucp1	7.3	-1.65	-4.17	1.21E-05	8.04E-05
Lrp4	160.7	-1.66	-1.93	6.9E-11	1.2E-09
Etv1	13.7	-1.66	-2.72	2.68E-06	2.07E-05
Prtg	9.9	-1.66	-2.49	5.13E-06	3.72E-05
Zbtb10	1450.8	-1.67	-1.75	0	0
Nr1d1	927.5	-1.67	-1.77	0	0
Prkcg	96.9	-1.67	-1.9	5.06E-12	1.06E-10
Gpr137b	186	-1.67	-1.92	8.97E-12	1.82E-10
Tnnc2	16.7	-1.67	-2.35	6.15E-07	5.38E-06
Fstl4	14.3	-1.68	-3.16	3.97E-06	2.95E-05
Hfe	584	-1.69	-1.89	1.61E-13	4.06E-12
Prkar1b	244.4	-1.7	-1.87	2E-15	6.5E-14
Scnn1a	79.5	-1.7	-2.03	3.02E-10	4.79E-09
Fndc7	27.5	-1.7	-2.39	2.09E-07	1.99E-06
Mfrp	55.6	-1.7	-2.23	2.88E-07	2.68E-06
Cd209d	96.9	-1.7	-2.46	7.68E-07	6.58E-06
Cd14	1110.8	-1.71	-1.92	9.3E-14	2.42E-12
Slc15a2	279.3	-1.71	-1.97	5.11E-12	1.07E-10
Gm43063	40.1	-1.71	-2.17	8.64E-09	1.05E-07
Gm26575	11.1	-1.71	-3.24	5.02E-06	3.64E-05

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Gm8463	8.7	-1.71	-4.85	6.5E-06	4.6E-05
Gm20219	486.3	-1.72	-1.84	0	0
Aatk	130.6	-1.72	-1.94	9.9E-14	2.57E-12
Postn	510.9	-1.72	-1.93	1.24E-13	3.17E-12
Gm43786	17.6	-1.72	-2.36	5.5E-07	4.88E-06
Amz1	508.4	-1.73	-1.88	0	1E-15
Nuak1	190.3	-1.73	-1.88	0	1E-15
Pmel	62.3	-1.73	-4.31	1.27E-06	1.04E-05
Itga9	882.5	-1.74	-1.91	0	1.1E-14
Prr15	32.2	-1.74	-2.1	8.59E-10	1.25E-08
AC125351.1	47.4	-1.74	-2.21	3.88E-09	5.05E-08
Mmp19	385.3	-1.74	-2.31	5.08E-08	5.43E-07
Prdm14	18.6	-1.74	-2.51	1.15E-06	9.57E-06
Gm4258	54.5	-1.75	-1.99	2.33E-12	5.13E-11
Nav1	825.1	-1.76	-1.96	4E-15	1.36E-13
Nptxr	82	-1.76	-2.03	2.98E-12	6.45E-11
AC158622.5	86.8	-1.76	-2.1	2.83E-10	4.52E-09
Gsdmc4	19.2	-1.76	-2.22	1.78E-08	2.07E-07
AC153955.2	13.5	-1.76	-3.93	1.96E-06	1.55E-05
Ppp1r9a	128.9	-1.77	-2.16	2.22E-10	3.61E-09
Gm28050	11.2	-1.77	-2.76	8.49E-07	7.21E-06
Pilra	854.5	-1.78	-1.94	0	0
Trpm2	1260.6	-1.78	-1.96	0	6E-15
Lilra5	286.1	-1.78	-2.02	7.9E-14	2.05E-12
Ap3s1-ps2	15.1	-1.78	-2.77	7.4E-07	6.37E-06
Slc22a23	202.4	-1.79	-1.98	0	2E-15
4930438A08Rik	145.8	-1.79	-2.11	2.09E-10	3.43E-09
A930030B08Rik	34.4	-1.79	-2.41	3.65E-08	4.02E-07
Ptgds	5.4	-1.79	-5.45	1.85E-07	1.79E-06
Vstm4	221	-1.8	-2.08	3.94E-13	9.46E-12
Gm9889	639.6	-1.81	-1.87	0	0
Pira2	53.5	-1.81	-2.39	3.64E-09	4.77E-08
Six4	17.7	-1.81	-2.59	2.33E-07	2.2E-06
Gm7860	120.6	-1.82	-1.97	0	0
B230303O12Rik	93.4	-1.82	-1.99	0	0
Cadm1	1067.9	-1.82	-1.99	0	0
Mertk	1388.9	-1.82	-2.24	2.24E-10	3.64E-09
Mmp12	54	-1.82	-2.56	4.76E-08	5.11E-07
Npc1l1	18.3	-1.82	-2.72	2E-07	1.92E-06
Sema6a	113.4	-1.83	-2.2	1.34E-11	2.65E-10
Trpv4	50.5	-1.83	-2.26	1.61E-10	2.68E-09
Snx31	36.9	-1.85	-2.5	4.1E-09	5.31E-08

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Plch2	20.7	-1.85	-2.61	3.52E-08	3.9E-07
Jag2	544.2	-1.86	-1.97	0	0
Fst	29.8	-1.86	-2.48	1.16E-08	1.38E-07
Apol8	1161.9	-1.87	-1.95	0	0
Siglece	886.5	-1.88	-2.03	0	0
Tbc1d9	512.6	-1.88	-2.05	0	0
Tcf7l2	320.1	-1.88	-2.17	7.7E-14	2.03E-12
Pygm	1170.9	-1.89	-2	0	0
Gm5608	44.9	-1.89	-2.41	1.16E-09	1.65E-08
Gm38405	61.2	-1.9	-2.33	5.15E-11	9.19E-10
Gm13830	33	-1.9	-2.36	3.07E-10	4.85E-09
Sash1	878.3	-1.91	-2.13	0	0
Dnah2	262.4	-1.91	-2.26	3.83E-13	9.2E-12
Gm6377	147.2	-1.91	-2.41	6.81E-11	1.19E-09
Cbln1	33.6	-1.92	-2.75	2.21E-09	3E-08
Zfp819	24.1	-1.93	-2.62	5.98E-09	7.49E-08
AC160028.2	11.6	-1.94	-3.91	2.08E-07	1.99E-06
Tenm4	307.5	-1.95	-2.18	0	1E-15
Dlc1	173.9	-1.95	-2.23	1E-15	4.2E-14
Cd209a	127.2	-1.95	-2.5	5.42E-11	9.63E-10
Pilrb1	346.4	-1.96	-2.22	0	3E-15
Aspa	38.6	-1.97	-2.81	9.1E-10	1.31E-08
Abcc3	2372	-1.98	-2.14	0	0
Tex45	736.4	-1.98	-2.15	0	0
Rnf150	123.1	-1.98	-2.22	0	0
Cd300e	272.8	-1.98	-2.43	1.21E-12	2.77E-11
2900052N01Rik	111.1	-2.01	-2.36	7E-15	1.96E-13
Lrp1	4560.8	-2.04	-2.28	0	0
Gpd1	933.9	-2.05	-2.29	0	0
Fam71a	57.8	-2.05	-2.6	1.94E-11	3.73E-10
Mybpc2	29.1	-2.06	-3.63	9.03E-09	1.09E-07
Itgad	5960.5	-2.07	-2.28	0	0
Cxcl1	159.6	-2.08	-2.59	1.52E-12	3.44E-11
Prss29	62.2	-2.09	-2.5	4E-15	1.24E-13
Pilrb2	349.2	-2.1	-2.38	0	0
Gm10605	75.3	-2.1	-2.44	1E-15	4.2E-14
Tecta	37.6	-2.1	-2.63	7.07E-12	1.46E-10
Adam23	301.5	-2.13	-2.37	0	0
Cbr2	76.5	-2.15	-3.07	5.98E-11	1.06E-09
Spic	2050.5	-2.16	-2.37	0	0
Sema6d	939.7	-2.17	-2.48	0	0
Hmox1	16492.8	-2.19	-2.65	4E-15	1.36E-13

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Tgfb2	26.2	-2.19	-2.92	4.6E-12	9.71E-11
Fcna	5828.3	-2.21	-2.4	0	0
Col14a1	699.7	-2.37	-2.68	0	0
Wisp2	41.1	-2.37	-3.69	2.46E-12	5.41E-11
Ptprm	389.7	-2.42	-2.7	0	0
Hs3st2	297.9	-2.45	-2.9	0	0
Adgre4	851.8	-2.47	-2.72	0	0
AC166361.2	101	-2.48	-2.78	0	0
Akr1b7	148.5	-2.51	-2.99	0	0
Gdf15	168.7	-2.59	-3.42	0	5E-15
Nfasc	204.4	-2.61	-3.25	0	0
Il1a	348.3	-2.63	-3.24	0	0
Fjx1	45.8	-2.75	-4.7	7E-15	2.03E-13

[000461] Table 4: The SD expressed genes list for SV + α 4-1BB vs. SV group by RNA-Seq ($q < 0.05$, $\text{Log2FC} \geq 1$ and $\text{Log2FC} \leq -1$).

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Ret	794.7	3.23	4.01	0	0
Gzmk	1785.2	2.77	3.04	0	0
Ermn	17.7	2.73	4.67	1.64E-13	2.4E-11
Ccl8	29.5	2.55	3.64	1.07E-11	9.75E-10
Wdr95	226	2.44	2.58	0	0
Ociad2	184.7	2.4	2.5	0	0
Col6a5	17.7	2.39	5.83	3.12E-10	1.88E-08
Trp73	22	2.15	3.6	9.67E-09	4.02E-07
Ncald	297.2	2.11	2.28	0	0
Gzmb	4113.9	2.11	2.3	0	0
Wipf3	37.6	2.1	2.84	2.82E-11	2.25E-09
2900011O08Rik	14.8	2.04	2.95	8.03E-09	3.4E-07
Klrg1	746.6	2.03	2.13	0	0
Clip4	39.8	1.98	2.46	1.07E-10	7.2E-09
Cd70	12.5	1.98	3.01	2.87E-08	1.05E-06
Adgrg1	449.6	1.93	2.06	0	0
Ccl5	22142.8	1.91	2.08	0	0
Ccr5	1338.8	1.89	1.96	0	0
Faxc	12.4	1.88	3.08	3.97E-07	1.08E-05
Dpysl5	94.9	1.84	2.16	2.13E-11	1.76E-09
Gm6637	196.3	1.83	2.02	3E-15	7.75E-13
Mpl	45.7	1.8	2.67	8.7E-07	2.14E-05
Cdkn2a	59.4	1.79	2.09	6.97E-11	5.07E-09

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
1700011L03Rik	6.7	1.77	6.04	2.28E-06	4.92E-05
Trem1	31.4	1.75	2.44	6.78E-07	1.73E-05
Ccr2	1142.1	1.74	1.86	0	0
Saa3	14.8	1.71	5.22	3.99E-06	7.95E-05
Hist1h1b	56.5	1.7	1.96	2.2E-09	1.07E-07
Osr2	54.7	1.69	2.19	3.53E-07	9.87E-06
Col6a1	15.6	1.69	4.32	7.89E-06	0.000144
Tff1	8.6	1.69	3.25	8.36E-06	0.000151
Fam57b	23.3	1.68	5.02	4.51E-07	1.21E-05
Col3a1	82.1	1.68	3.39	9.31E-06	0.000166
Cldnd2	53.5	1.67	1.88	1.05E-11	9.69E-10
Col6a2	16.9	1.67	3.3	1.11E-05	0.000192
Insrr	64.7	1.62	1.77	7.83E-13	9.25E-11
Dixdc1	18.6	1.62	2.45	5.33E-06	0.000102
Gm33460	48.9	1.61	1.8	6.88E-11	5.04E-09
Csf2	72.9	1.61	1.86	3.27E-09	1.52E-07
Gcg	62.2	1.61	3.73	1.52E-05	0.000252
Nkg7	8456.1	1.57	1.62	0	0
Tmem40	47.5	1.57	1.98	8.68E-07	2.14E-05
Stk32c	341	1.55	1.64	0	1E-15
Serpine2	31.1	1.55	2.06	2.38E-06	5.09E-05
Wdr31	15.1	1.55	2.2	1.3E-05	0.00022
S100a4	1170.3	1.54	1.73	2.18E-10	1.37E-08
AA467197	30.3	1.54	1.86	3.68E-07	1.02E-05
Dcn	49	1.54	2.29	5.15E-06	9.93E-05
Gp1bb	21.6	1.54	2.35	3.11E-05	0.000458
Osbpl3	1358.5	1.52	1.57	0	0
Smpdl3b	185.8	1.52	1.62	6E-15	1.27E-12
Gldc	15.3	1.51	1.94	9.21E-06	0.000165
Arsb	1795.9	1.5	1.59	0	2E-15
Bspry	174.2	1.5	1.74	3.88E-08	1.38E-06
Gm15056	28.9	1.5	2.19	4.69E-05	0.000651
Esm1	680.1	1.49	1.62	2.19E-13	3.05E-11
Pif1	204	1.49	1.64	3.87E-11	2.98E-09
2310031A07Rik	64.6	1.49	1.87	1.65E-06	3.7E-05
Pglyrp1	1046.5	1.48	1.61	9.35E-13	1.08E-10
Slc35d3	110.5	1.46	1.56	3.8E-14	6.51E-12
Pbk	107.5	1.46	1.55	8.9E-14	1.4E-11
Gm2788	29.7	1.46	1.78	1.1E-06	2.61E-05
Slc16a11	9.1	1.46	2.52	9.04E-05	0.001144
Miat	31.2	1.45	1.67	5.48E-08	1.88E-06
Samd14	203.2	1.44	1.65	2.06E-08	8.04E-07

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Tnfsf4	81.6	1.44	1.71	2.52E-07	7.28E-06
Gp5	24.7	1.44	1.94	1.94E-05	0.000307
Kcnj5	5.3	1.44	3.82	6.73E-05	0.000888
Vax2	12.7	1.43	2.3	0.00012	0.001443
Car5b	136	1.42	1.49	0	7.2E-14
Reg2	5.6	1.42	5.34	5.5E-06	0.000105
Tpbp	19.3	1.42	2.07	7.65E-05	0.00099
Pf4	162.2	1.42	2.19	0.000132	0.001566
Trpc6	7.4	1.42	2.85	0.000145	0.001696
Pbbp	30.6	1.41	1.97	6.29E-05	0.000839
Adap1	1257.2	1.4	1.48	1E-15	2.09E-13
Kif2c	341.3	1.4	1.48	4E-15	8.96E-13
Tff3	16.7	1.4	1.76	1.49E-05	0.000248
Muc13	227	1.39	1.53	8.75E-10	4.68E-08
Serpina3h	8.8	1.39	2.39	0.000211	0.002313
Rgs16	7742.7	1.38	1.44	0	0
Fhl2	217.6	1.38	1.45	0	2.7E-14
Serping1	49.6	1.38	1.61	2.11E-07	6.25E-06
Slc22a3	85.3	1.38	1.65	1.66E-06	3.71E-05
Nfe2	91.3	1.38	1.81	4.07E-05	0.000577
Igf2bp3	26.6	1.37	1.7	2.05E-05	0.000323
Ppp1r3g	7.3	1.37	2.61	0.000309	0.003167
Map6	151	1.36	1.45	4.41E-13	5.67E-11
Fkbp10	29.4	1.36	1.89	6.66E-05	0.00088
Tuba8	42.2	1.36	1.95	0.000162	0.00185
Mmrn1	13.3	1.36	1.98	0.000187	0.002084
Hist1h2ag	6.4	1.36	2.16	0.000327	0.003318
Rasgef1a	270.7	1.34	1.42	4E-15	8.29E-13
Fam81a	55.4	1.34	1.55	1.08E-06	2.55E-05
Col1a2	95	1.34	1.73	2.79E-05	0.000418
Fhl1	19.9	1.34	1.9	0.000148	0.001721
Fam19a3	168.7	1.33	1.48	2.06E-08	8.04E-07
Cdk1	1228.8	1.32	1.41	5.17E-13	6.5E-11
1700001O22Rik	140.3	1.32	1.42	7.29E-11	5.24E-09
Pimreg	230	1.32	1.42	1.77E-10	1.14E-08
F2rl2	42.8	1.32	1.52	1.19E-06	2.78E-05
Dach1	18.3	1.32	1.82	0.000178	0.002004
Cenpf	495.6	1.31	1.39	6.5E-14	1.05E-11
Tpx2	1113.7	1.31	1.39	1.56E-12	1.7E-10
Hmmr	311.7	1.31	1.42	2.97E-10	1.8E-08
Tubb1	17.5	1.31	1.94	0.000239	0.002557
Upk3b	15.3	1.31	2.92	0.000536	0.005053

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Kif18b	606.6	1.3	1.37	1.37E-13	2.06E-11
Adgrg7	5.3	1.3	3.04	0.00033	0.003346
Cep55	503.9	1.29	1.36	1.3E-12	1.42E-10
Tpsab1	262.4	1.29	1.5	4E-06	7.97E-05
Unc5b	14.9	1.29	1.8	0.000177	0.001988
Syt12	1359.2	1.28	1.34	1.2E-14	2.26E-12
Kif14	299.1	1.28	1.36	6.35E-11	4.68E-09
Tg	98.4	1.28	1.39	2.03E-09	9.93E-08
Ube2c	1391	1.28	1.4	6.71E-09	2.91E-07
Ifng	1413.7	1.28	1.45	2.03E-07	6.03E-06
Angpt1	63.2	1.28	1.48	3.02E-06	6.25E-05
Aunip	24.6	1.28	1.61	9.97E-05	0.00124
Birc5	1540.7	1.27	1.41	2.84E-08	1.05E-06
AC153938.2	40.5	1.27	1.51	9.04E-06	0.000162
Gng3	34.8	1.27	1.49	1.41E-05	0.000237
Robo3	27.3	1.27	1.51	2.28E-05	0.000357
Myct1	34.7	1.27	1.65	0.000137	0.001615
Kif22	1022.2	1.26	1.35	3.03E-11	2.39E-09
Rad51	401.4	1.26	1.34	5.95E-11	4.4E-09
Havcr2	486.2	1.26	1.36	1.36E-10	9.01E-09
S100a6	3227.2	1.26	1.37	1.24E-09	6.42E-08
Neurl1b	49.9	1.26	1.52	2.54E-05	0.000389
Irx3	8.6	1.26	2.06	0.000738	0.006563
Scrn1	9.5	1.26	2.43	0.000777	0.006836
Depdc1b	350.8	1.25	1.32	7.29E-12	6.94E-10
Prc1	830.9	1.25	1.36	9.49E-10	5E-08
Lxn	79	1.25	1.35	3.32E-09	1.53E-07
Ccnb1	160.8	1.25	1.37	1.44E-08	5.81E-07
Ccna2	1680.2	1.24	1.3	7E-15	1.41E-12
Anxa2	5777.8	1.24	1.34	3.39E-10	2.01E-08
Gzma	3261.2	1.24	1.86	0.000818	0.007121
Shcbp1	274.8	1.23	1.29	2.78E-13	3.78E-11
Cdca8	1251.5	1.23	1.3	2.54E-12	2.68E-10
2610318N02Rik	193.2	1.23	1.32	8.3E-10	4.48E-08
Bst1	74.2	1.23	1.33	7.18E-09	3.08E-07
Sgo2a	143.4	1.23	1.33	9.33E-09	3.88E-07
Lrr1	59	1.23	1.37	8.05E-07	1.99E-05
Epas1	1633	1.23	1.59	0.000164	0.001873
Plppr3	42.4	1.23	1.76	0.000686	0.006205
Dapk2	315.5	1.22	1.27	0	7.7E-14
Aurkb	806.6	1.22	1.28	4.93E-13	6.25E-11
Plk1	794.8	1.22	1.3	3.01E-11	2.38E-09

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Heatr9	204.6	1.22	1.36	3.37E-07	9.5E-06
Npy	11.8	1.22	1.86	0.001046	0.008713
Myrf	11.2	1.22	2.86	0.001338	0.010572
Chsy1	2251.6	1.21	1.27	3E-15	6.66E-13
Mxd3	280.1	1.21	1.26	2.7E-14	4.71E-12
Sapcd2	275.5	1.21	1.3	1.06E-09	5.54E-08
Troap	236.4	1.21	1.31	6.8E-09	2.94E-07
Tigit	2942.9	1.21	1.33	3.76E-08	1.35E-06
Rtkn	29.5	1.21	1.48	5.85E-05	0.000791
Alox12	39.7	1.21	1.5	7.16E-05	0.000937
Il13	60.8	1.21	1.61	0.000618	0.005699
Hist1h3g	9.3	1.21	1.63	0.000849	0.007357
Cdca5	791.4	1.2	1.25	0	1.5E-14
Gimap7	2437.5	1.2	1.27	1.58E-11	1.35E-09
Ccnb2	1255.4	1.2	1.28	8.31E-11	5.8E-09
Fam78b	52.7	1.2	1.45	1.68E-05	0.000274
Gm9531	16	1.2	1.66	0.000496	0.004738
Tmem98	10.9	1.2	2.01	0.001307	0.010371
Eomes	4109.7	1.19	1.22	0	0
Penk	1018	1.19	1.26	1.11E-11	9.94E-10
Serpinb6b	1199	1.19	1.26	1.24E-11	1.08E-09
Prr11	288	1.19	1.32	2.76E-07	7.89E-06
Ica1	85.1	1.19	1.38	6.49E-06	0.000121
Gm4841	29.9	1.19	1.54	0.000218	0.002367
Fbxo41	15.3	1.19	1.51	0.00028	0.002912
Col6a6	6.3	1.19	3.11	0.001409	0.011028
Cst7	1893.9	1.18	1.22	0	1.5E-14
Top2a	4726.3	1.18	1.23	0	1.3E-13
Ncaph	822.4	1.18	1.24	3.2E-14	5.46E-12
Cdca2	444.5	1.18	1.24	1.09E-11	9.85E-10
Rrm2	1718.2	1.18	1.26	3.64E-10	2.15E-08
Rad54l	350.2	1.18	1.27	2.64E-09	1.27E-07
Ttk	211.5	1.18	1.27	4.53E-09	2.04E-07
BC030867	129.6	1.18	1.27	2.3E-08	8.82E-07
Cdc25c	143.9	1.18	1.3	5.8E-07	1.52E-05
Rai14	77.8	1.18	1.34	2.2E-06	4.79E-05
Hist1h3c	11.8	1.18	1.67	0.001203	0.009747
Gbp11	355.1	1.17	1.23	2.77E-11	2.23E-09
Foxd2os	95	1.17	1.24	8.95E-11	6.2E-09
Figl1	145.7	1.17	1.23	1.17E-10	7.79E-09
Nek2	554.5	1.17	1.26	3.39E-09	1.56E-07
E2f8	535.1	1.17	1.27	3.38E-08	1.23E-06

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
C78197	19.1	1.17	1.65	0.00074	0.006571
Mki67	5899.1	1.16	1.24	4.42E-09	1.99E-07
E2f7	275.3	1.16	1.26	5.55E-08	1.9E-06
Spp1	51	1.16	1.36	4.36E-05	0.000612
Gp9	24.8	1.16	1.46	0.000374	0.003703
Vash1	14.6	1.16	1.66	0.001212	0.009798
Gm867	11.8	1.16	1.91	0.001595	0.012127
Nlrp6	5.5	1.16	3.11	0.002039	0.014755
Tyms	809.6	1.15	1.19	0	7.4E-14
Espl1	809.6	1.15	1.22	2.11E-11	1.75E-09
Ncapg	383.1	1.15	1.22	8.97E-10	4.77E-08
Ckap2l	455.4	1.15	1.24	2.19E-08	8.5E-07
Gm12250	177.3	1.15	1.28	5.22E-07	1.38E-05
Spns2	183.2	1.15	1.38	6.6E-05	0.000874
Il20ra	9.2	1.15	1.8	0.001826	0.01357
Nusap1	813.1	1.14	1.19	1.06E-12	1.21E-10
Serpinb9	1224.7	1.14	1.21	1.43E-11	1.24E-09
Spag5	1183.2	1.14	1.2	3.25E-11	2.54E-09
Cenpe	685.5	1.14	1.21	1.07E-10	7.2E-09
Bub1	362.3	1.14	1.22	3.72E-09	1.7E-07
Serpina3f	452	1.14	1.24	7.57E-08	2.52E-06
Ccl4	2257.9	1.14	1.43	0.000269	0.002817
Ckap2	394.5	1.13	1.17	9E-15	1.82E-12
Cdkn3	129.2	1.13	1.18	1.04E-10	7.09E-09
Sgo1	162.8	1.13	1.19	6.38E-10	3.57E-08
Serpina3g	2916.5	1.13	1.23	3.48E-08	1.25E-06
Ccl1	31	1.13	1.43	0.000689	0.006224
Prf1	1704.5	1.12	1.14	0	0
Spc24	506	1.12	1.16	1.96E-13	2.79E-11
Kif11	1109.9	1.12	1.17	2.93E-13	3.94E-11
Bub1b	1344.3	1.12	1.18	7.17E-11	5.17E-09
Lgals1	11014.9	1.12	1.18	2.68E-10	1.64E-08
Esco2	149.5	1.12	1.21	1.11E-07	3.55E-06
Tek	24.8	1.12	1.43	0.000437	0.004239
Msantd3	9.2	1.12	1.65	0.002189	0.015686
Acot7	2318.6	1.11	1.17	2.16E-11	1.77E-09
Ttn	292.1	1.11	1.21	8E-08	2.66E-06
Nuf2	279.6	1.11	1.21	5.67E-07	1.49E-05
Rgs18	52.3	1.11	1.38	0.00036	0.003592
Dio2	18.9	1.11	1.6	0.001465	0.011345
Pcdh7	8.7	1.11	1.82	0.002625	0.018256
Kif4	650.2	1.1	1.15	1.06E-11	9.7E-10

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Clspn	637.1	1.1	1.17	1.4E-09	7.11E-08
Dyrk3	249.1	1.1	1.21	4.14E-07	1.12E-05
1700020L24Rik	90.2	1.1	1.23	4.95E-06	9.58E-05
Lmtk3	107.1	1.1	1.28	4.1E-05	0.00058
Ms4a3	31.1	1.1	1.55	0.002955	0.019997
Asf1b	1276.4	1.09	1.14	1.09E-12	1.23E-10
Pask	202.9	1.09	1.15	7.34E-10	4.02E-08
Cit	784.3	1.09	1.16	2.83E-09	1.35E-07
Pclaf	522.9	1.09	1.19	6.53E-07	1.68E-05
Klrc1	236.6	1.09	1.2	1.62E-06	3.64E-05
Rnase2a	5.3	1.09	1.76	0.003732	0.024243
6530402F18Rik	553.7	1.08	1.1	0	0
Cdc45	628.7	1.08	1.11	0	2E-15
Podn1	2382.6	1.08	1.12	4.8E-14	7.96E-12
Lilr4b	1553.9	1.08	1.16	4.21E-08	1.49E-06
Perp	113.8	1.08	1.17	9.07E-08	2.95E-06
Aspm	319.4	1.08	1.17	2.91E-07	8.3E-06
Gzmc	56.6	1.08	1.32	0.000285	0.002956
Clstn3	34.6	1.08	1.39	0.000965	0.008168
Traj35	15.6	1.08	1.44	0.001132	0.009302
Pcp4l1	21	1.08	1.56	0.002232	0.015944
Serpina3n	16.7	1.08	1.62	0.002935	0.01989
Chil3	42.4	1.08	1.54	0.003165	0.021138
AC131739.1	7.2	1.08	1.94	0.003928	0.025182
Kif20a	898.5	1.07	1.13	3.27E-10	1.95E-08
Tjp2	411.7	1.07	1.14	2.03E-08	7.97E-07
Gm5391	5.3	1.07	2.14	0.004733	0.02915
Cdc20	1268.9	1.06	1.1	1.22E-13	1.87E-11
Kcnk5	399.5	1.06	1.1	6.1E-13	7.51E-11
Psrc1	122.1	1.06	1.12	2.48E-09	1.19E-07
Iqgap3	284.2	1.06	1.13	7.9E-09	3.37E-07
Cks1b	772.5	1.06	1.14	6.32E-08	2.16E-06
Grb10	61	1.06	1.32	0.000464	0.004477
Ptger3	20.8	1.06	1.62	0.004232	0.026687
Cdkn2c	421.3	1.05	1.08	8.39E-13	9.78E-11
Ndc80	345.5	1.05	1.1	1.74E-10	1.12E-08
S100a10	7676.1	1.05	1.1	5.19E-10	2.98E-08
Mcm10	477.4	1.05	1.11	1.26E-09	6.54E-08
Fut7	69.8	1.05	1.18	2.47E-05	0.000382
Tfr2	139.5	1.05	1.34	0.001188	0.009649
Hist1h2bm	8.5	1.05	1.8	0.005816	0.034129
Stmn1	257.1	1.04	1.1	2.99E-09	1.41E-07

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Ska1	210.9	1.04	1.13	4.21E-06	8.29E-05
Klrc2	53.2	1.04	1.17	2.62E-05	0.000399
Cym	46.2	1.04	1.31	0.000743	0.006595
Hist1h2ai	9.8	1.04	1.51	0.004383	0.027452
Phlda3	37.7	1.03	1.2	0.000147	0.00171
Gm14148	21.4	1.03	1.25	0.000572	0.005313
Hist1h2bj	17.7	1.03	1.24	0.001234	0.009915
a	12.6	1.03	1.58	0.004957	0.030315
4930519L02Rik	9.9	1.03	1.87	0.006787	0.038499
Knstrn	855.1	1.02	1.05	5E-15	1.13E-12
Chst11	1091.1	1.02	1.05	1.6E-14	2.94E-12
Spdl1	204.5	1.02	1.07	3.91E-10	2.29E-08
Melk	329.8	1.02	1.07	7E-10	3.88E-08
Kifc1	310.5	1.02	1.09	8.1E-08	2.69E-06
Anln	217.2	1.02	1.1	1.27E-06	2.94E-05
Gm17745	84	1.02	1.11	1.34E-06	3.07E-05
Runx2os1	41.2	1.02	1.28	0.001157	0.009439
H1fx	65.9	1.02	1.27	0.001224	0.00986
Col1a1	89.2	1.02	1.37	0.002114	0.015218
Slc30a2	29	1.02	1.34	0.002562	0.017915
Mt3	18.3	1.02	1.35	0.003871	0.024928
Cxcr6	818	1.01	1.04	7.2E-14	1.15E-11
Kif15	595.3	1.01	1.07	1.32E-08	5.37E-07
Ect2	255.1	1.01	1.12	1.58E-05	0.00026
Tex15	58.4	1.01	1.12	4.2E-05	0.000591
Ddah2	90	1.01	1.15	6.54E-05	0.000868
G0s2	77.4	1.01	1.24	0.000666	0.006046
Hist1h3a	32.2	1.01	1.25	0.001415	0.011057
Pde10a	17	1.01	1.3	0.002501	0.017557
Morc1	8.4	1.01	2.01	0.007372	0.040918
Lmnbl	6788.3	1	1.03	5.3E-14	8.63E-12
Bard1	316.8	1	1.05	1.61E-10	1.06E-08
Ska3	252.1	1	1.05	4.68E-10	2.72E-08
Tk1	1004.9	1	1.05	5.56E-10	3.14E-08
Syl3	850.3	1	1.09	2.44E-06	5.19E-05
Bag2	63	1	1.1	1.06E-05	0.000185
F10	33.6	1	1.38	0.004704	0.029055
C1s1	21.8	1	1.52	0.005149	0.031153
Usp50	16.7	1	1.55	0.006828	0.038667
Dlk2	11.4	1	1.71	0.0082	0.044224
Efna5	6.6	1	1.98	0.00842	0.045107
Ptk2	617.3	-1	-1.03	0	1.3E-13

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Myo10	1090.4	-1	-1.06	3.72E-08	1.34E-06
Tmem8b	139.4	-1	-1.09	8.06E-07	1.99E-05
Tgm2	2908.8	-1	-1.09	1.15E-06	2.7E-05
Dysf	302	-1	-1.1	3.86E-06	7.73E-05
Mreg	104.8	-1	-1.11	7.36E-06	0.000136
Fam167a	155	-1	-1.11	1.28E-05	0.000218
Adam33	60.3	-1	-1.17	0.00027	0.002823
Eps8	43.8	-1	-1.19	0.000346	0.003473
Syt3	18.7	-1	-1.32	0.001772	0.013249
Rubcnl	142.1	-1	-1.34	0.001911	0.014015
Napb	20.4	-1	-1.57	0.004628	0.02868
Sat2	9	-1	-1.91	0.008166	0.044102
Tsix	8.2	-1	-2.12	0.008513	0.045495
Gm42870	22.1	-1	-2.22	0.008535	0.045552
Myo9a	1226.4	-1.01	-1.06	1.33E-09	6.81E-08
Tns3	884	-1.01	-1.1	6.53E-07	1.68E-05
Nat8l	123.4	-1.01	-1.11	2.77E-06	5.81E-05
Col17a1	97	-1.01	-1.13	1.15E-05	0.000198
Adrb1	207	-1.01	-1.15	3.23E-05	0.00047
Sh3tc1	174.9	-1.01	-1.16	6.02E-05	0.000809
Abca9	130.3	-1.01	-1.29	0.000973	0.008226
Igkv6-25	29.5	-1.01	-1.73	0.006776	0.038448
Gm36159	8.5	-1.01	-2.04	0.007309	0.040646
Gm8463	8.7	-1.01	-3.66	0.00768	0.042258
Ebf1	216.1	-1.01	-2.27	0.007802	0.042823
Tbc1d8	696.1	-1.02	-1.12	1.77E-06	3.92E-05
Adgrl2	148.4	-1.02	-1.13	3.22E-06	6.58E-05
Ppp1r14a	48.4	-1.02	-1.4	0.00191	0.014015
Padi4	34.4	-1.02	-1.28	0.002072	0.014949
Gm30292	19.5	-1.02	-1.37	0.00212	0.01525
Slc13a2	13.1	-1.02	-1.9	0.005131	0.031092
Epha4	19.9	-1.02	-1.81	0.005323	0.031904
Stfa3	14.7	-1.02	-1.71	0.005735	0.03378
Gm25776	9.5	-1.02	-1.77	0.00584	0.034194
Crisp3	7.4	-1.02	-4.01	0.00631	0.03631
Igha	3248.3	-1.02	-1.95	0.006417	0.036825
Igkv1-135	261.8	-1.02	-1.85	0.006595	0.037626
Pax5	1019.4	-1.02	-2.07	0.007319	0.040689
Plxna1	900.2	-1.03	-1.11	8.86E-08	2.89E-06
Cpq	349.9	-1.03	-1.12	6.17E-07	1.61E-05
Hck	1125	-1.03	-1.12	6.33E-07	1.64E-05
Pnck	68.8	-1.03	-1.16	6.44E-06	0.000121

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Nectin4	97.5	-1.03	-1.18	5.48E-05	0.000746
Cd180	364.5	-1.03	-1.2	6.2E-05	0.000829
Btnl2	69.3	-1.03	-1.22	0.000154	0.001777
Zfyve9	64.7	-1.03	-1.3	0.000587	0.00543
Krt80	33	-1.03	-1.33	0.000948	0.00806
Cmtm8	27.8	-1.03	-1.34	0.000991	0.008347
Adgra2	49.3	-1.03	-1.44	0.001853	0.013683
Tnnc2	16.7	-1.03	-1.54	0.002356	0.016673
Klhl14	29.4	-1.03	-1.95	0.005716	0.033707
Rasgrp3	260.5	-1.03	-1.87	0.006222	0.035888
Tcn2	1368.6	-1.04	-1.08	1E-15	1.65E-13
Ppp1r32	136.8	-1.04	-1.13	2.11E-07	6.25E-06
Fcgrt	2555.6	-1.04	-1.13	5.87E-07	1.54E-05
Nphp3	103.3	-1.04	-1.16	1.69E-06	3.77E-05
Cd300lf	581.8	-1.04	-1.16	3.88E-06	7.77E-05
Ly86	762.3	-1.04	-1.17	1.71E-05	0.000278
Clec12a	552.3	-1.04	-1.2	3.22E-05	0.00047
Arhgap32	163.9	-1.04	-1.22	6.6E-05	0.000874
Eva1a	33	-1.04	-1.25	0.000199	0.002202
Wtip	28.7	-1.04	-1.44	0.00145	0.011259
B3gnt7	121.7	-1.04	-1.43	0.001841	0.013654
Npc1l1	18.3	-1.04	-1.68	0.003219	0.021411
Prex2	8.7	-1.04	-2.21	0.005599	0.03317
Cd209c	9.8	-1.04	-2.31	0.005938	0.034665
Zbtb10	1450.8	-1.05	-1.09	3.33E-12	3.42E-10
Pla2g7	1374.2	-1.05	-1.13	6.38E-08	2.18E-06
Fcgr1	319.2	-1.05	-1.15	3.57E-07	9.94E-06
Pla2g15	1269.3	-1.05	-1.15	5.99E-07	1.57E-05
B230303O12Rik	93.4	-1.05	-1.16	7.3E-07	1.84E-05
Nr3c2	42.8	-1.05	-1.28	0.000159	0.001828
Gpr4	64.4	-1.05	-1.29	0.00028	0.002912
Chn1	24	-1.05	-1.26	0.000645	0.005891
Bmf	141.4	-1.05	-1.4	0.000933	0.007945
Gm44860	11.3	-1.05	-2.25	0.005162	0.031194
Mcf2	7.3	-1.05	-3.2	0.005808	0.034089
Hebp1	934.9	-1.06	-1.19	4.49E-06	8.77E-05
Susd4	89.1	-1.06	-1.2	1.03E-05	0.000181
Tspan15	134	-1.06	-1.22	1.65E-05	0.00027
Fpr1	89.9	-1.06	-1.32	0.000293	0.003019
Ighv2-3	64.5	-1.06	-1.36	0.000905	0.007792
Alas2	140.4	-1.06	-1.75	0.004005	0.025599
Paqr6	11.7	-1.06	-1.97	0.004373	0.02741

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Gm15929	8.8	-1.06	-1.92	0.004709	0.029072
Gm37829	7	-1.06	-2.2	0.004866	0.02984
Gm38160	15.5	-1.06	-2.07	0.004891	0.029962
Gm26575	11.1	-1.06	-2.25	0.004985	0.03041
Gm2814	9.6	-1.06	-2.68	0.005305	0.031827
Sort1	1028.3	-1.07	-1.19	9.44E-07	2.29E-05
Cdk14	85	-1.07	-1.26	6.08E-05	0.000815
Shank1	125.8	-1.07	-1.41	0.000743	0.006595
Ighv1-43	24.1	-1.07	-1.92	0.004221	0.026637
Ctsf	383.8	-1.08	-1.16	4.68E-09	2.1E-07
Tbxas1	561.4	-1.08	-1.17	1.11E-07	3.55E-06
Alpk1	263.7	-1.08	-1.18	2.53E-07	7.29E-06
Sccpdh	123.3	-1.08	-1.17	6.21E-07	1.61E-05
Lpl	1230.2	-1.08	-1.21	3.08E-06	6.34E-05
Stab2	1255.1	-1.08	-1.22	7.89E-06	0.000144
Apol9b	51.1	-1.08	-1.23	9.87E-06	0.000174
Gm4258	54.5	-1.08	-1.25	1.92E-05	0.000305
H2-Ea-ps	7790.9	-1.08	-1.36	0.000366	0.003633
Ciita	568.4	-1.08	-1.39	0.000641	0.005863
Igkv4-70	65.4	-1.08	-1.47	0.001124	0.009265
Ighv1-42	5.8	-1.08	-3.23	0.002259	0.016093
Igkv4-50	54.1	-1.08	-1.76	0.003011	0.020314
Cd86	1049.2	-1.09	-1.15	3.61E-11	2.81E-09
Fam43a	1414.6	-1.09	-1.18	3.94E-08	1.39E-06
Smagp	295.1	-1.09	-1.2	3.82E-07	1.05E-05
Zfhx3	113.3	-1.09	-1.24	6.09E-06	0.000115
Trim30b	52.4	-1.09	-1.36	0.000154	0.001778
Fst	29.8	-1.09	-1.52	0.000952	0.008075
Pigr	17.1	-1.09	-1.7	0.002701	0.018704
2010007H06Rik	20.7	-1.09	-1.99	0.003403	0.022397
Rgl1	1317.4	-1.1	-1.2	8.26E-08	2.73E-06
Clec4a2	262.2	-1.1	-1.26	1.09E-05	0.00019
Adhfe1	58	-1.1	-1.3	2.42E-05	0.000374
Iglv3	74.4	-1.1	-1.68	0.001978	0.014396
C130050O18Rik	95.9	-1.11	-1.26	1.45E-06	3.32E-05
Slpi	1443.3	-1.11	-1.38	0.00016	0.001838
Mybpc3	45.4	-1.11	-1.44	0.000231	0.002478
Adgrl3	33.2	-1.11	-1.64	0.001005	0.008438
Gm9530	13.7	-1.11	-1.85	0.001935	0.014156
Igkv17-127	57.8	-1.11	-2.86	0.003056	0.020527
4930426D05Rik	77.3	-1.11	-2.7	0.003313	0.021946
Fcer2a	783	-1.11	-2.3	0.003321	0.021992

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Nfam1	713.9	-1.12	-1.19	1.76E-10	1.14E-08
Epb41l3	649.5	-1.12	-1.23	9.17E-08	2.97E-06
Naip5	181.7	-1.12	-1.27	2.06E-06	4.5E-05
Gas6	210.8	-1.12	-1.29	1.06E-05	0.000185
A530099J19Rik	38	-1.12	-1.84	0.001581	0.012052
0610040J01Rik	15.3	-1.12	-1.97	0.001998	0.014505
Gm17999	9.8	-1.12	-2.45	0.00274	0.018921
Gm14963	10.5	-1.12	-1.98	0.002748	0.018963
Tal2	7.8	-1.12	-2.87	0.002908	0.019726
Gm10605	75.3	-1.13	-1.35	2.32E-05	0.000361
Abcd2	85.6	-1.13	-1.35	3.96E-05	0.000563
Pcolce2	67.5	-1.13	-1.42	9.12E-05	0.001153
Pigz	58.4	-1.13	-1.4	0.000141	0.001652
Trf	681.7	-1.13	-1.49	0.000353	0.003532
A930030B08Rik	34.4	-1.13	-1.59	0.000525	0.004961
Mir5107	156.7	-1.13	-1.77	0.001511	0.011598
Kcnj2	25	-1.13	-2	0.001945	0.014221
Tlr7	305.4	-1.14	-1.22	3.03E-09	1.42E-07
Igsf6	661.8	-1.14	-1.24	3.88E-08	1.38E-06
Lrrc25	483.1	-1.14	-1.28	6.57E-07	1.69E-05
Ptpro	88.1	-1.14	-1.29	1.06E-06	2.52E-05
Tppp	109.8	-1.14	-1.29	1.47E-06	3.35E-05
Dll4	78	-1.14	-1.29	2.41E-06	5.14E-05
Snn	825.3	-1.14	-1.31	3.98E-06	7.95E-05
Pdzd2	59.3	-1.14	-1.35	1.39E-05	0.000234
Erbb2	135	-1.14	-1.33	1.55E-05	0.000255
Ctnnd2	131.9	-1.14	-1.44	0.000131	0.001557
Gm15930	22	-1.14	-1.73	0.001034	0.008627
Igkv8-30	318.2	-1.14	-1.89	0.002044	0.014786
Blk	523.7	-1.14	-1.98	0.002156	0.015487
Cyp4f37	6.6	-1.14	-2.97	0.002672	0.018535
Tcf4	708.9	-1.15	-1.26	2.31E-08	8.84E-07
C1qb	6167.6	-1.15	-1.28	3.36E-07	9.48E-06
Ccnd1	258	-1.15	-1.29	3.86E-07	1.05E-05
Cfap61	26	-1.15	-1.82	0.001029	0.008606
Hepacam2	34.3	-1.15	-2.23	0.00188	0.013838
Gm15880	16.2	-1.15	-2.16	0.002139	0.015378
Clec4a4	10.7	-1.15	-2.6	0.002248	0.01604
Fmnl2	329.1	-1.16	-1.29	1.74E-07	5.28E-06
Pdgfc	56.2	-1.16	-1.45	8.78E-05	0.001115
Pde8b	30.7	-1.16	-1.81	0.000914	0.007828
Gm5466	17.5	-1.16	-1.97	0.001427	0.011109

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Lima1	405.9	-1.17	-1.27	4.02E-09	1.82E-07
Slc16a7	76.5	-1.17	-1.39	1.46E-05	0.000243
Snx24	100.2	-1.17	-1.4	1.79E-05	0.000288
Fcrl1	520.9	-1.17	-1.52	0.000191	0.002126
Plxnb3	46.1	-1.17	-1.66	0.000571	0.00531
Gm36937	12.9	-1.17	-2.24	0.001847	0.013668
Igkv5-39	123.1	-1.17	-2.24	0.00205	0.014817
Tnfrsf21	641.6	-1.18	-1.26	5.23E-11	3.91E-09
Syk	3325.1	-1.18	-1.29	1.05E-08	4.34E-07
Cmb1	316.4	-1.18	-1.31	3.55E-07	9.91E-06
AC166361.2	101	-1.18	-1.36	5.35E-07	1.42E-05
Stac2	76.6	-1.18	-1.55	0.000206	0.002258
Ighv2-6	141.1	-1.18	-1.53	0.000266	0.002791
Igkv4-63	73.9	-1.18	-1.74	0.000748	0.006628
Igkv9-124	50.6	-1.18	-2.27	0.001681	0.012661
Mpeg1	6302.5	-1.19	-1.28	3.51E-10	2.08E-08
Siglech	840.4	-1.19	-1.32	1.89E-07	5.64E-06
Ccdc148	86.4	-1.19	-1.48	4.04E-05	0.000573
Blnk	622	-1.19	-1.59	0.000221	0.002396
Ighv1-4	158	-1.19	-1.8	0.000906	0.007795
Siglecg	751.8	-1.19	-1.93	0.001034	0.008627
Slc6a1	17.6	-1.19	-2.21	0.001218	0.009819
Ighv9-2	13.7	-1.19	-2.05	0.001368	0.010761
Igkv4-58	47	-1.19	-2.02	0.001407	0.011025
Fam213b	477.2	-1.2	-1.33	6.6E-08	2.24E-06
Gm13710	186.2	-1.2	-1.39	3.19E-06	6.52E-05
S1pr3	58.4	-1.2	-1.43	8.88E-06	0.00016
Tecta	37.6	-1.2	-1.56	0.000104	0.001281
Fam135a	30.1	-1.2	-1.66	0.000222	0.002413
Gbgt1	24	-1.2	-1.71	0.000334	0.003374
AC153955.5	18.5	-1.2	-1.86	0.000622	0.005722
Trim7	268.2	-1.2	-1.88	0.000812	0.007083
Igkv6-23	42.9	-1.2	-1.99	0.001136	0.009317
Map3k9	162.8	-1.21	-1.3	9.73E-11	6.66E-09
Fndc7	27.5	-1.21	-1.78	0.00023	0.002473
Apoe	8629.4	-1.22	-1.32	4.19E-10	2.45E-08
Sirpa	4992.1	-1.22	-1.35	2.63E-08	9.78E-07
Cd300a	1109.4	-1.22	-1.35	3.42E-08	1.24E-06
Cystm1	143.7	-1.22	-1.4	5.65E-07	1.49E-05
Snta1	208.6	-1.22	-1.4	1.01E-06	2.43E-05
Trpv4	50.5	-1.22	-1.54	2.56E-05	0.000392
Lifr	153.4	-1.22	-1.49	2.67E-05	0.000404

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Pcdhgb4	14.2	-1.22	-1.79	0.00053	0.005005
Sdc3	7230.4	-1.23	-1.34	1.71E-09	8.52E-08
Marcks	880	-1.23	-1.39	3.81E-07	1.04E-05
Flt3	441.7	-1.23	-1.43	2.6E-06	5.48E-05
Wdfy4	2179.7	-1.23	-1.51	1.94E-05	0.000307
Igkv5-45	19.6	-1.23	-2.23	0.001131	0.009302
H2-Ob	1767	-1.24	-1.32	1.21E-12	1.33E-10
Fgd2	1084.5	-1.24	-1.37	3.13E-08	1.14E-06
Dmpk	201.4	-1.24	-1.43	5.01E-07	1.34E-05
Ttc12	74.5	-1.24	-1.49	8.57E-06	0.000154
AC153955.2	13.5	-1.24	-3.15	0.000817	0.00712
Dennd5b	158.4	-1.24	-2.45	0.001058	0.008786
Ppfibp2	358.3	-1.25	-1.4	4.67E-08	1.62E-06
Gm15922	138.1	-1.25	-1.47	2E-06	4.38E-05
Slc1a3	115.6	-1.25	-1.56	1.64E-05	0.000269
Fam71a	57.8	-1.25	-1.63	5E-05	0.000691
Btnl4	51.4	-1.25	-1.87	0.000251	0.002666
Plxnb2	2017.4	-1.26	-1.36	2.1E-10	1.33E-08
Mafb	1846.3	-1.26	-1.39	6.8E-09	2.94E-07
Scamp5	313.9	-1.26	-1.38	8.04E-09	3.4E-07
Lpcat2	297.8	-1.26	-1.41	1.94E-08	7.66E-07
Prkcg	96.9	-1.26	-1.45	1.88E-07	5.63E-06
Cd300c2	673.6	-1.26	-1.44	3.62E-07	1E-05
Cecr6	48.6	-1.26	-1.51	3.93E-06	7.85E-05
Scn3a	17.9	-1.26	-1.92	0.000287	0.002968
A4galt	38.5	-1.26	-1.89	0.000339	0.003419
Unc5a	20.3	-1.26	-2.62	0.000665	0.006038
Dock4	301.1	-1.27	-1.39	2.01E-10	1.28E-08
C1qc	6828	-1.27	-1.42	4.33E-08	1.52E-06
Zfp366	189.3	-1.27	-1.44	6.67E-08	2.26E-06
Samd4	57.2	-1.27	-1.61	1.4E-05	0.000235
Cd209d	96.9	-1.27	-1.89	0.000206	0.002258
Ifi207	440.3	-1.28	-1.42	6.19E-09	2.71E-07
Glis3	183.7	-1.28	-1.48	3.46E-07	9.69E-06
Gpc4	55.1	-1.28	-1.58	6.38E-06	0.00012
Tgfb2	26.2	-1.28	-1.82	7.16E-05	0.000937
Ffar1	97.9	-1.28	-1.83	0.000194	0.002157
Epha2	215.5	-1.29	-1.42	1.19E-09	6.2E-08
Cd5l	2824.6	-1.29	-1.41	1.31E-09	6.73E-08
Nxpe5	131.7	-1.29	-1.49	3.09E-07	8.77E-06
Gm33280	43.4	-1.29	-1.78	7E-05	0.000918
Gm15448	29.6	-1.29	-1.86	0.000127	0.00152

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Tlr13	195.6	-1.3	-1.4	5.26E-12	5.13E-10
Kcnk13	70	-1.3	-1.51	2.63E-07	7.54E-06
Rasal2	84.5	-1.3	-1.53	4.32E-07	1.17E-05
Cd300c	47.9	-1.3	-1.67	1.67E-05	0.000273
Gm14137	13.8	-1.3	-3.35	0.000624	0.005734
6430548M08Rik	386.7	-1.31	-1.4	5.27E-13	6.58E-11
C2	119.2	-1.31	-1.41	2.4E-11	1.95E-09
Fgd4	96.4	-1.31	-1.63	4.58E-06	8.9E-05
Gm14221	27.8	-1.31	-2.05	0.00018	0.002021
Gm28050	11.2	-1.31	-2.15	0.000277	0.002883
Il22ra2	7.5	-1.31	-3.06	0.000546	0.005125
Rims3	1029.1	-1.32	-1.41	7.3E-14	1.16E-11
I830077J02Rik	399	-1.32	-1.41	1.46E-12	1.59E-10
Cfp	5197	-1.32	-1.44	4.78E-10	2.76E-08
Sirpb1c	21.5	-1.32	-1.75	2.9E-05	0.000432
Adam11	386.6	-1.33	-1.39	0	1E-15
Hpgds	565.3	-1.33	-1.43	5.79E-12	5.61E-10
Hdac9	184.1	-1.33	-1.49	8.39E-09	3.53E-07
Clec4a1	389.4	-1.33	-1.52	6.73E-08	2.27E-06
Gm10552	10.1	-1.33	-2.86	0.000376	0.003714
Abcg3	1117.1	-1.34	-1.42	6E-15	1.18E-12
Slc7a7	700.4	-1.34	-1.45	1.16E-11	1.03E-09
Amz1	508.4	-1.34	-1.46	4.31E-11	3.29E-09
Pirb	1339.8	-1.34	-1.49	2.08E-09	1.01E-07
Cd14	1110.8	-1.34	-1.51	5.3E-09	2.36E-07
Cyp27a1	464.9	-1.34	-1.52	1.71E-08	6.82E-07
Clec4b1	78.8	-1.34	-1.73	1.22E-05	0.000208
Rasgef1b	1868	-1.35	-1.41	0	1E-15
Jup	1184.5	-1.35	-1.44	1.31E-13	1.98E-11
Grk3	1230.7	-1.35	-1.46	3.6E-12	3.63E-10
C1qa	7016.9	-1.35	-1.51	8.15E-09	3.43E-07
Dgki	117.3	-1.35	-1.56	3.91E-08	1.38E-06
Agap1	495.9	-1.36	-1.51	6.16E-10	3.46E-08
Timd4	240.3	-1.36	-1.53	7.81E-09	3.33E-07
Ighv1-7	114.3	-1.36	-1.76	1.71E-05	0.000278
Tlr8	86.3	-1.37	-1.62	2.21E-07	6.48E-06
Zfp608	304	-1.38	-1.52	1.86E-10	1.19E-08
Kif26a	36.7	-1.38	-2.16	8.08E-05	0.001037
Reln	18.1	-1.38	-2.59	0.000204	0.002246
Dhtkd1	7	-1.38	-3.09	0.000253	0.002684
Gm15848	16.2	-1.38	-3.38	0.00027	0.002823
Prkar1b	244.4	-1.39	-1.54	8.05E-11	5.67E-09

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Slc16a9	181.6	-1.39	-1.59	1.74E-08	6.93E-07
Gfra4	95	-1.39	-1.61	2.35E-07	6.87E-06
Pid1	185.8	-1.39	-1.68	7.4E-07	1.86E-05
Apoc1	69.6	-1.39	-1.83	1.3E-05	0.00022
Scn4a	77.9	-1.39	-2.02	7.44E-05	0.000968
Ptgs1	1227.5	-1.4	-1.51	1.12E-12	1.26E-10
AC125351.1	47.4	-1.4	-1.8	2.46E-06	5.24E-05
Fstl4	14.3	-1.4	-2.76	0.000124	0.001486
Plbd1	1881.3	-1.41	-1.54	2.06E-11	1.72E-09
Gm15931	220.2	-1.41	-1.6	2.87E-09	1.36E-07
Tcf7l2	320.1	-1.41	-1.64	2.26E-08	8.71E-07
Capn9	80.5	-1.41	-1.69	1.32E-07	4.12E-06
March1	226	-1.41	-1.68	2.64E-07	7.58E-06
Mcc	21.2	-1.41	-2.32	6.53E-05	0.000866
Etv1	13.7	-1.41	-2.38	6.82E-05	0.000895
AC160028.2	11.6	-1.41	-3.13	0.000179	0.002013
Nav1	825.1	-1.42	-1.59	2.42E-10	1.5E-08
Igf1	580.6	-1.42	-1.61	3.17E-09	1.47E-07
Cpne8	29	-1.42	-1.91	1.06E-05	0.000185
Csf1r	9890.8	-1.43	-1.54	1.16E-13	1.79E-11
Hpgd	485.4	-1.43	-1.57	1.74E-11	1.47E-09
Tmem26	319.6	-1.43	-1.58	5.08E-11	3.83E-09
Prss29	62.2	-1.43	-1.75	1.22E-07	3.86E-06
Ighv5-2	17.8	-1.43	-3.08	0.000154	0.001777
Cd209b	9.6	-1.43	-3.28	0.00016	0.001835
Axl	7411.5	-1.44	-1.56	4.08E-13	5.33E-11
Tbc1d9	512.6	-1.44	-1.57	1.2E-12	1.33E-10
Plpp3	349.2	-1.44	-1.62	4.78E-10	2.76E-08
Clec4a3	317.3	-1.44	-1.61	4.9E-10	2.82E-08
Scnn1a	79.5	-1.44	-1.72	1.08E-07	3.49E-06
Jhy	47.7	-1.44	-2.65	9.59E-05	0.001207
Nr1d1	927.5	-1.45	-1.54	0	1.35E-13
Itga9	882.5	-1.45	-1.6	8.13E-12	7.66E-10
Slc45a3	1350.4	-1.45	-1.59	2.01E-11	1.68E-09
Clec4n	902.8	-1.45	-1.62	1.39E-10	9.13E-09
Lrp4	160.7	-1.45	-1.7	9.85E-09	4.07E-07
Cacna1e	284.9	-1.45	-1.73	1.14E-07	3.62E-06
Mmp12	54	-1.45	-2.08	1.42E-05	0.000237
P2ry13	230.8	-1.46	-1.59	5.57E-13	6.92E-11
Nuak1	190.3	-1.46	-1.6	6.64E-13	7.9E-11
Adap2	260.1	-1.46	-1.72	4.52E-08	1.58E-06
Adgrg6	43.3	-1.46	-1.97	4.05E-06	8.05E-05

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Kcnj10	1374.3	-1.47	-1.58	4E-15	8.36E-13
Ppp1r9a	128.9	-1.47	-1.8	1.43E-07	4.44E-06
Rab30	116	-1.47	-2.11	1.86E-05	0.000297
Gfra2	1060.6	-1.48	-1.62	4.86E-12	4.82E-10
St6galnac2	280.3	-1.48	-1.68	7.6E-10	4.15E-08
2900052N01Rik	111.1	-1.48	-1.76	1.26E-08	5.15E-07
Slc8a1	117.6	-1.48	-1.88	7.25E-07	1.83E-05
Ighv1-39	28.6	-1.48	-2.7	9.84E-05	0.001231
Gpr137b	186	-1.49	-1.71	1.37E-09	7.01E-08
Trpm2	1260.6	-1.5	-1.66	3.19E-12	3.31E-10
Aatk	130.6	-1.5	-1.7	7.89E-11	5.6E-09
A530064D06Rik	30	-1.5	-1.98	7.1E-07	1.8E-05
Pyroxd2	242.8	-1.51	-1.68	9.76E-12	9.04E-10
Hcar2	808.2	-1.51	-1.74	8.34E-10	4.5E-08
Catsperg2	14.8	-1.51	-2.17	9.58E-06	0.00017
Slc40a1	4625.9	-1.53	-1.66	1E-14	1.89E-12
Mmp19	385.3	-1.53	-2.04	1.82E-06	4.01E-05
Mpzl1	469.8	-1.55	-1.7	2.45E-13	3.38E-11
Dnah2	262.4	-1.55	-1.85	3.81E-09	1.74E-07
Igkv6-15	223	-1.55	-2.65	3.22E-05	0.00047
Cadm1	1067.9	-1.56	-1.72	1.47E-13	2.19E-11
Ear2	200.6	-1.56	-1.76	4.64E-11	3.53E-09
Cd302	387	-1.57	-1.73	3.57E-13	4.7E-11
Lilra6	252.1	-1.58	-1.75	2.95E-13	3.94E-11
Cd163	1248.1	-1.58	-1.77	1.19E-11	1.05E-09
Lilra5	286.1	-1.58	-1.8	2.95E-11	2.34E-09
Trem14	1601.2	-1.59	-1.7	0	2E-15
Slc11a1	1813.6	-1.59	-1.72	4E-15	8.92E-13
Itgb5	955.2	-1.6	-1.73	1E-15	2.17E-13
Adgre1	1926.6	-1.6	-1.75	1.8E-14	3.26E-12
Rgl3	91.8	-1.6	-1.86	2.53E-10	1.57E-08
Ccr3	902.9	-1.61	-1.75	2E-15	5.62E-13
Siglec1	622.7	-1.61	-1.78	1.01E-12	1.15E-10
Slco2b1	818.9	-1.62	-1.75	1E-15	1.75E-13
Apod	9.3	-1.62	-3.32	2E-05	0.000315
C6	1221.3	-1.63	-1.78	1.5E-14	2.85E-12
Sirpb1b	32.1	-1.64	-2.12	2.61E-08	9.73E-07
Sirpb1a	105.6	-1.65	-1.88	3.33E-12	3.42E-10
Lhfp	173.9	-1.66	-1.92	3.96E-11	3.03E-09
Hmox1	16492.8	-1.66	-2.02	3.06E-09	1.43E-07
Gm2762	38.5	-1.66	-1.94	2.82E-08	1.04E-06
Pilra	854.5	-1.67	-1.82	1E-15	1.75E-13

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Angptl7	118.5	-1.67	-1.89	2.22E-12	2.38E-10
Matn2	73.1	-1.67	-2	5.51E-10	3.12E-08
Mertk	1388.9	-1.67	-2.06	6.31E-09	2.76E-07
Cxcl1	159.6	-1.67	-2.1	1.42E-08	5.76E-07
Adamdec1	659.5	-1.68	-1.83	0	1.2E-13
Tenm4	307.5	-1.68	-1.89	1.99E-13	2.82E-11
Bank1	715.4	-1.68	-1.96	5.8E-11	4.31E-09
Gm5150	271.8	-1.69	-1.83	0	9E-15
Igkv3-7	151.3	-1.69	-3.52	8.26E-06	0.000149
Aspa	38.6	-1.7	-2.48	1.27E-07	3.99E-06
Gdf15	168.7	-1.71	-2.33	5.3E-08	1.82E-06
Adam22	142.7	-1.72	-2.24	2.59E-08	9.71E-07
Vcam1	9147.6	-1.73	-1.91	6E-15	1.2E-12
Fjx1	45.8	-1.73	-3.32	1.23E-06	2.86E-05
Paqr9	427.9	-1.74	-1.96	4E-14	6.63E-12
Slc22a23	202.4	-1.75	-1.94	0	7.9E-14
Dlc1	173.9	-1.75	-2.01	6.48E-13	7.83E-11
Sema6a	113.4	-1.75	-2.11	8.35E-11	5.81E-09
Snx31	36.9	-1.75	-2.37	2.42E-08	9.17E-07
Mrc1	3441.3	-1.76	-1.96	3E-15	6.66E-13
Pira2	53.5	-1.76	-2.34	7.75E-09	3.32E-07
Siglece	886.5	-1.77	-1.91	0	0
Il1a	348.3	-1.77	-2.22	7.34E-10	4.02E-08
Cecr2	73.9	-1.77	-2.86	9.93E-07	2.39E-05
Pilrb1	346.4	-1.78	-2.02	4E-14	6.68E-12
Stra6l	77.8	-1.78	-2.07	1.68E-12	1.8E-10
Sash1	878.3	-1.8	-2.01	1E-15	1.46E-13
Hfe	584	-1.81	-2.02	2E-15	5.33E-13
Kirrel3	96.4	-1.82	-2.06	1E-14	1.9E-12
Gm14548	229.3	-1.82	-2.13	2.57E-12	2.7E-10
Gpd1	933.9	-1.84	-2.05	1E-15	1.85E-13
Ace	93.4	-1.84	-2.73	5.03E-08	1.75E-06
Pilrb2	349.2	-1.85	-2.1	4E-15	9.43E-13
Spic	2050.5	-1.86	-2.04	0	1E-15
Lrp1	4560.8	-1.86	-2.08	0	6.6E-14
Slc15a2	279.3	-1.89	-2.18	1.7E-14	3.03E-12
Vstm4	221	-1.89	-2.18	2E-14	3.63E-12
Abcc3	2372	-1.9	-2.04	0	0
Cd209a	127.2	-1.9	-2.44	1.68E-10	1.09E-08
Cd300e	272.8	-1.91	-2.34	7.55E-12	7.15E-10
Sned1	230.5	-1.92	-2.29	9.22E-13	1.07E-10
Myo18b	126.8	-1.96	-2.34	1.63E-13	2.4E-11

gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Ptprm	389.7	-1.98	-2.21	0	0
Kcnj16	221.3	-1.98	-2.22	0	4E-15
Gm6377	147.2	-1.99	-2.51	8.92E-12	8.32E-10
Mybpc2	29.1	-1.99	-3.53	2.54E-08	9.54E-07
Sema6d	939.7	-2.01	-2.3	0	2.2E-14
Cbr2	76.5	-2.01	-2.9	8.13E-10	4.42E-08
Itgad	5960.5	-2.04	-2.25	0	0
Adam23	301.5	-2.04	-2.27	0	0
Postn	510.9	-2.04	-2.28	0	1E-15
Cbln1	33.6	-2.04	-2.89	1.25E-10	8.28E-09
Rnf150	123.1	-2.07	-2.32	0	0
Hs3st2	297.9	-2.13	-2.53	0	1.3E-13
Fcna	5828.3	-2.2	-2.39	0	0
Col14a1	699.7	-2.25	-2.55	0	0
Akr1b7	148.5	-2.26	-2.71	0	9E-15
Wisp2	41.1	-2.27	-3.56	1.66E-11	1.41E-09
Adgre4	851.8	-2.37	-2.61	0	0
Nfasc	204.4	-2.38	-2.98	0	1.5E-14

[000462] Next, NIH DAVID analysis using the upregulated gene list was run. In both comparisons, cell cycle genes upregulation is the highest enrichment cluster [although SV + α 4-1BB mAb vs. SV has a lower enrichment score compared with SV plus α 4-1BB mAb vs. untreated samples (FIGs. 23B and 25). This indicates that SV + α 4-1BB mAb induced more potent T cell cycle progression compared with SV only. T cell proliferation is critical for an effective anti-tumor response against A20 lymphoma. The CD4/CD8 T cell ratio in untreated mice decreased markedly by day 28 after tumor inoculation (FIG. 26A-26B). In addition, Treg/CD8 T cell ratio increased by day 28, indicating impairment of T cell function (Figure 26C-26D). In other groups the T cell ratio remained constant due to proliferation.

[000463] CD69 is the earliest marker of immune system activation. SV plus α 4-1BB mAb treatment synergistically upregulated CD69 on day 2 (FIG. 23D). Additionally, KEGG GSEA indicates that T cell receptor signaling gene sets were enriched when comparing SV + α 4-1BB vs untreated samples (enrichment score = 0.35, Normalized Enrichment Score (NES) = 1.56, FDR q value = 0.17, nominal p value = 0) (FIG. 23E).

[000464] **SV plus α 4-1BB mAb stimulated cytotoxic T cell function**

[000465] To investigate the antitumor cytotoxicity of SV/ α 4-1BB treated splenocytes, f-Luc A20 lymphoma cells were co-cultured with splenocytes on day 7. The ratios explored between splenocytes and tumor cell were 40: 1, 20:1, 10:1. SV plus α 4-1BB treated splenocytes demonstrated the highest cytotoxicity among all groups, as calculated by the reduction of f-Luc activity (FIG. 27A). To understand if this response is induced by TAA or anti-viral immunity, the same experiment was performed using mice under treatment but without tumor inoculation. We found that SV plus α 4-1BB achieves the same effect as the combination treatment with tumor inoculation. This indicates that anti-tumor response on day 7 was not tumor specific. Accordingly, NKG2D, granzyme B and perforin were highly expressed in CD8 T cells from α 4-1BB treated mice. In addition, SV plus α 4-1BB in combination induced the highest expression of NKG2D and granzyme B in CD8 T cells. NKG2D, granzyme B and perforin upregulation was tumor independent because the same pattern was observed in all treatments without tumor inoculation (FIG. 27B-27C). Correspondingly, IPA indicates that gene sets of cytotoxic T cell development are significantly upregulated in SV plus α 4-1BB mAb. These genes include Gzmb (granzyme B), Prfl (perforin) and Klrk1 (NKG2D) (FIG. 27D). These data indicate that SV plus α 4-1BB mAb markedly enhanced cytotoxic T cell activity.

[000466] SV plus α 4-1BB mAb induced IFN γ production from T cells

[000467] Other upregulated genes in the SV plus α 4-1BB mAb combined treatment include STAT4 (FIG. 27D) and IL12rb1 (FIG. 28D), which are required for the development of Th1 cells from naive CD4⁺ T cells and IFN γ production (FIG. 27D) in response to IL-12 [Jacobson NG et al., J Exp Med. 1995]. Consistent with this observation, splenocytes from SV plus α 4-1BB mAb treatment produced significantly higher number of IFN γ spots compared with other groups, reaching peak production on day 7 (FIG. 28A, upper panel). After day 7, the response dampened but still remained at the highest level compared with other groups (FIG. 28A, lower panel). This is in line with increased IFN γ RNA levels. To identify if TAA or viral antigen induces IFN γ production on day 7, the same experiment was performed in mice not inoculated with tumor cells. For both SV or SV plus α 4-1BB treatment, the presence or absence of tumor did not significantly affect IFN γ levels (FIG. 29), confirming that IFN γ production on day 7 was mainly an anti-viral response. To identify whether T cells or antigen presentation cells (APCs) play the major role in IFN γ production, we harvested SV treated splenic T cells and naive T cells respectively. T cells from SV treated mice were co-cultured with naive APCs. Conversely, APCs

from SV treated mice were cultured with naive T cells. T cells from SV treated mice produced IFN γ when co-cultured with naive APC. Naive T cells produce much less IFN γ spots when cultured with SV infected APC. However, neither T cell nor APC alone could produce elevated numbers of IFN γ spots. These observations indicate that T cells play the dominant role in IFN γ production during SV infection (FIG. 30A). APCs are essential for helping T cells to produce IFN γ .

[000468] Next, to identify whether CD4 or CD8 T cells produce IFN γ , flow cytometric analysis was performed for cytokine analysis. Among splenocytes, 2–2.5% SV plus α 4-1BB mAb treated CD4 T cells produced IFN γ , which is significantly higher than other groups. Very low percentages of CD8 T cells produced IFN γ in all groups (FIG. 28B). There were much less IFN γ producing T cells after removing APC (FIG. 28B). Also, there was no difference among all groups for IFN γ production. This suggests that T cell-APC interaction is essential for IFN γ production. To test the antitumor IFN γ production activity of the purified T cells, they were co-cultured for 5 h with A20 cells, which express major histocompatibility complex (MHC) I and II molecules [Pizzoferrato E et al., *Int J Cancer*, 2004]. Only CD4 T cells from the SV plus α 4-1BB mAb group produced IFN γ after co-culture (FIG. 28C and 30B). This indicates that SV plus α 4-1BB mAb induces anti-tumor IFN γ production activity. Besides IFN γ , several Th1 associated genes were also upregulated in the T cells from SV plus α 4-1BB mAb treated groups. These include *Ccr5*, *Cxcr3*, *Havcr2* (*Tim3*), *IL12rb1* and *Klrc1* (Fig. 4d). T-bet is the key transcription factor which is essential for type 1 immune response (IFN γ production, T cell cytotoxicity) and memory T cell differentiation. In correspondence with the IFN γ expression findings, it was observed that SV plus α 4-1BB mAb coordinately upregulates T-bet in T cells on day 7 (FIG. 28E). This suggests that SV helps α 4-1BB boost the type 1 immune response, which is critical for controlling tumor growth. SV or α 4-1BB mAb alone could not induce high IFN γ production due to low T-bet upregulation. Eomesodermin (EOMES), another important transcription factor, is upregulated in activated T cells and is essential for memory CD8 T cell development. Both α 4-1BB mAb and SV plus α 4-1BB mAb induced high expression of EOMES on day 7 (FIG. 28F). The lack of both T-bet and EOMES results in a lower expression of CXCR3 in T cells and a drastic decrease in the number of tumor-infiltrating T cells [28]. The data disclosed herein are consistent with these observations. Elevated CXCR3 (FIG. 28D), T-bet and EOMES (FIGs. 28E and 28F) in T cells of the combined SV plus α 4-1BB mAb treated animals, were found.

[000469] SV and α 4-1BB mAb stimulated chemotaxis, adhesion and enhanced T cell infiltration and activation in tumor

[000470] Through RNA-Seq, a series of chemokines and chemokine receptors have been identified to be upregulated in SV plus α 4-1BB mAb (FIG. 31A). Among those molecules, CCR5 upregulation was confirmed by flow cytometry (FIG. 31B). CCR5 potentiates CD4 T helper cell functions boosting overall anti-tumor responses [Gonzalez-Martin A et al., Oncoimmunology, 2012]. SV plus α 4-1BB significantly was found to upregulate CD11a and ICAM-1(CD54). These two adhesion molecules are highly expressed on activated T cells. LFA-1 (CD11a/CD18)-ICAM-1 interaction is essential for the formation of immune synapses between T cell and APC [Walling BL et al., Front Immunol, 2018]. LFA-1 and ICAM-1 are also required for T cell-T cell homotypic aggregation and activation [Sabatos CA, et al., Immunity, 2008; Gerard A, et al., Nat Immunol. 2013]. α 4-1BB mAb stimulation induced significant upregulation of CD11a and ICAM-1 in both CD4 and CD8 T cells whereas SV does not (FIGs. 31C-31E). In addition, T cell costimulatory molecule, OX40, was also significantly upregulated in T cells of mice treated with α 4-1BB. (FIG. 31F, left). OX40 engagement promotes effector T cell function and survival [33 Croft M, et al., Immunol Rev. 2009]. ICOS, another CD4 T cell costimulatory molecule, was upregulated in SV or α 4-1BB alone but upregulated most in the SV plus α 4-1BB combination treatment, suggesting a synergistic effect exists (FIG. 31F, right).

[000471] TIL play a critical anti-tumor role and is an important marker for prognosis. Compared with untreated, the percentage of CD3 and CD8 T cells were increased about 2 fold after combination treatment (FIG. 31G). Ki67 were upregulated in those T cells which indicated active division (FIG. 32A). For untreated TIL, the frequency of Foxp3⁺ Treg cells was the highest (FIG. 32B) and CD8/Treg ratio was the lowest (FIG. 31H). Treatment enhanced the T-bet and EOMES expression in T cells (FIG. 32C-32D). NKG2D and granzyme B were highly upregulated in tumor infiltrating CD8 T cells (FIG. 31I, and 32E). Overall, these data indicate that combination treatment enhanced T cell infiltration, division, activation, cytotoxicity and downregulated the inhibitory Treg population.

[000472] SV and α 4-1BB mAb synergistically enhanced oxidative phosphorylation

[000473] T cell activation requires a quick consumption of energy through both enhanced glycolysis and oxidative phosphorylation [Wahl DR et al., Immunol Rev., 2012]. Metabolic switch is a major feature of T cell activation and memory T cell development [van der Windt GJ

et al., Immunol Rev., 2012]. GSEA KEGG analysis identified that the glycolysis gene set is upregulated in SV plus α 4-1BB vs. untreated samples (FIG. 33A). This process quickly produces ATP and supports T cell migration and cytotoxicity in hypoxic or acidic microenvironments. IP A confirms that SV plus α 4-1BB mAb synergistically enhanced oxidative phosphorylation (FIG. 33B).

[000474] Both oxygen consumption rate (OCR, represents oxidative phosphorylation) and extracellular acidification rate (ECAR, represents glycolysis) of all groups (FIG. 33C) was assessed. Compared with other groups, SV plus α 4-1BB significantly increased both OCR and ECAR. This indicates that both glycolysis and oxidative phosphorylation are activated in T cells of animals treated with SV plus α 4-1BB.

[000475] SV plus low dose α 4-1BB mAb cured A20 tumor bearing mice

[000476] To reduce the potential risk of cytotoxicity and expense of treatment with SV vectors plus α 4-1BB, the study disclosed herein explored whether low doses of α 4-1BB mAb and fewer injections would be as effective in curing tumor bearing mice as the higher doses and frequencies used in our initial studies. As demonstrated (FIG. 34A and 34B), A20 tumor bearing mice can be completely cured by SV (3 times per week for 3 weeks) plus a low dose of α 4-1BB mAb (50 μ g per week for 3 weeks). This reduces both the SV and α 4-1BB mAb dosing requirements. The reduced dose of α 4-1BB mAb would be helpful, as well, in preventing the α 4-1BB mAb induced liver toxicity reported by some investigators [Bartkowiak T, et al., Clin Cancer Res., 2018].

[000477] All tumor cured mice acquired long lasting antitumor immunity

[000478] To investigate the memory response to A20 lymphoma, naive and tumor cured mice were inoculated with 3×10^6 A20 tumor cells. Only mice that had survived more than 4 months after 1st time of tumor challenge were chosen. In all tumor cured mice, we found that A20 lymphoma was completely rejected whereas naive mice were susceptible to A20 inoculation (FIG. 35A).

[000479] To confirm anti-tumor specificity has been elicited, IFN γ production of purified T cells in the presence or absence of tumor cells was measured by Elispot assay. T cells were isolated from naive and cured mice under SV plus α 4-1BB treatment (4 months after treatment finished). Isolated T cells were co-cultured with A20 and CT26 tumor cells respectively. Co-

culturing with A20 cells dramatically enhanced IFN γ production, whereas co-culturing with CT26 cells only slightly enhanced IFN γ production (FIG. 35B).

[000480] Next, cytotoxicity to both naive and cured mice under SV plus α 4-1BB treatment (the same method as FIG. 27A) was measured. Compared with naive, cured mice had enhanced cytotoxicity to A20 lymphoma cells, but not to CT26 tumor cells. To confirm that this is mediated by T cells, the same experiment was done using purified T cells. Cured mice had enhanced cytotoxicity compared with naive mice (FIG. 35C).

[000481] To better understand differences between this memory T cell response and the initial treatment responses as observed on day 7, RNA-Seq was performed by using purified splenic T cells from all re-challenged groups. In T cells of these re-challenged mice we found only a few differentially expressed genes among the three treated groups (Table 5), indicating that tumor cured mice develop a very similar T cell gene expression profile regardless of treatment method. Compared with untreated, KEGG analysis indicates that TCR signaling is the highest upregulated pathway in SV plus α 4-1BB group (FIG. 35D), indicating that continuously enhanced TCR signaling is critical for maintaining antitumor immunity.

[000482] **Table 5:** The SD expressed gene lists among all tumor cured mice groups.

SV+ α 4-1BB recha vs SV rechal					
gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Arl5c	1644.9	-0.7	-0.71	4.57E-07	0.014007
SV+ α 4-1BB rechal vs α 4-1BB rechal					
gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Prdm16	86.7	-1.85	-2.48	4.63E-07	0.001563
Scamp5	106.1	-1.59	-1.99	3.98E-06	0.010743
Klri1	117.3	-1.57	-1.98	5.79E-06	0.013016
Ighv2-2	457.3	-1.52	-1.94	1.05E-05	0.020265
Kcnj10	439.7	-1.45	-1.76	1.24E-05	0.020949
Slc40a1	1111.8	-1.41	-1.69	1.53E-05	0.022994
Trbv29	826.4	-1.3	-1.36	6.35E-12	8.57E-08
Tmcc3	416.3	-1.23	-1.35	1.28E-07	0.000575
Abcc3	1020.9	-1.2	-1.36	2.12E-05	0.02863
Arl5c	1644.9	-0.76	-0.77	2.43E-08	0.000164
SV rechal vs. α 4-1BB rechal					
gene	baseMean	log2FC	log2FCunshrunk	pvalue	padj
Gzmk	1000.8	-1.78	-2.44	1.81E-06	0.033784
Penk	2127.9	-1.35	-1.6	6.37E-06	0.038894
Smoc2	154.2	-1.85	-2.98	8.65E-06	0.038894

Spag6	6.7	-1.59	-7.14	8.74E-06	0.038894
Ighv3-5	85.6	-1.86	-3.04	1.08E-05	0.038894
Wipf3	150.6	-1.84	-5.01	1.25E-05	0.038894

[000483] The conventional view of oncolytic virus therapy against tumors is that it requires selective infection of cancer cells resulting in the induction of cancer cell lysis and apoptosis. TAAs, released from dead tumor cells, attract and further stimulate an antitumor immune response. The study described herein found that encoding a TAA is not necessary for SV vectors plus α 4-1BB mAb therapy to be fully successful. SV vectors lacking an A20 lymphoma TAA were able to treat A20 lymphoma and, in combination with α 4-1BB mAb, eradicated the growing tumors. This is particularly important when effective immune reactive TAAs are unknown. It is possible that the immunotherapeutic response of SV vectors plus α 4-1BB mAb is independent of whether a tumor is “cold” (i.e., having few TAAs or mutation-specific neoantigens capable of promoting robust T cell activation) or “hot.”

[000484] The study describe herein showed that both NKG2D (KLRK1) and granzyme B are highly expressed under combination treatment. This massive nonspecific activation is critical for controlling tumor growth at an early time point (day 7). This step is also important for inducing anti-tumor specificity that is mediated by TAAs released from dead tumor cells due to nonspecific killing. After tumor regression, T cells from treated animals were able maintain the ability to produce IFN γ and acquired immunological memory to rapidly reject A20 lymphoma rechallenges. IFN γ production from purified T cells of cured mice was significantly enhanced after encountering A20 tumor cells. This demonstrates that anti-tumor specificity is fully established in cured mice. Upregulated molecular pathways of responsive T cells induced by SV vectors and α 4-1BB mAbs alone and in combination were identified and compared in the study described herein. The combination of SV and α 4-1BB mAb has a synergistic effect and represents a potent and robust therapeutic treatment able to cure B lymphomas and provide long term protection in a preclinical model.

[000485] In conclusion, SV vectors in combination with α 4-1BB mAb completely eradicated a B-cell lymphoma in a preclinical mouse model, a result that could not be achieved with either treatment alone. Tumor elimination involves a synergistic effect of the combination that significantly boosts T cell cytotoxicity, IFN- γ production, migration, tumor infiltration and oxidative phosphorylation. In addition, all mice that survived after treatment developed long

lasting antitumor immunity. The studies disclosed herein provides a novel, alternative method for B cell lymphoma treatment and describes a rationale to help translate SV vectors plus agonistic mAbs into clinical applications.

[000486] Example 4: Sindbis viral vector expressed NY-ESO-1 and IL-12 enhances survival of subjects with established tumors

[000487] The study described herein investigates the effect of administering a tumor associated antigen and an immunostimulatory molecule, as expressed by a Sindbis viral vector on anti-tumor response and survival in a subject with an established tumor. Previous studies, demonstrated vectors encoding TAAs, such as NY-ESO-I, could cure CT26-NY-ESO-1 tumors [Galon J, et al., Nature reviews Drug discovery 2019; Gupta S, et al., Frontiers in oncology 2017]. However, while this approach has been effective in enhancing the immune response to and clearance of established tumors of colon and prostate cancers, the efficacy in curing other cancers, e.g. ovarian cancer has been limited. Therefore, an approach of administering a combination of a SV expressed immunostimulatory molecule, IL-12 along with the SV-NY-ESO-1, to a subject with an established tumor was tested.

[000488] Combination of NY-ESO-1 and IL-12 expressed by separate Sindbis viral vectors enhances survival of subjects with established tumors

[000489] The study described herein investigates the effect of administering IL-12 and NY-ESO-1, both expressed by separate Sindbis viral vectors, on established tumors. C57/Bl6 albino (female) mice re-injected with Alm5-2Fluc-17 ovarian cancer cells to establish a tumor (FIG. 19), and treated with either a SV vector expressing IL-12 (SV-IL-12), a SV vector expressing NY-ESO-1 (SVNYESO) or a 50% mix of a SV-IL-12 and a SVNYESO (SV-NYESO_SV-IL12).

[000490] A Sindbis replicon expressing NYESO-1 cDNA (SV-NYESO1) was made by PCR amplification of the NYESO-1 gene from the pReceiver-M02 plasmid. Expression of the NYESO-1 gene was confirmed by western blot. NYESO-1 was detected by western blot following standard protocol, using as a primary antibody the anti-NYESO-1 clone E978 (Upstate) at a dilution 1/5,000 in Tris-buffered saline-Tween (TBS-T) with 5% non-fat milk. SV-IL12 plasmid used in this study has been published in 2002 [Tseng JC et al., J Natl Cancer Inst. 2002]. To construct a Sindbis viral vector containing genes for interleukin 12 (IL-12), the Sindbis viral vector SinRep/2PSG was first constructed, which contains a secondary subgenomic

promoter that is responsive to the Sindbis replicase. Two DNA oligonucleotide primers (sequence 5' CGCGTAAAGCATCTCTACGGTGGTCCTAATAGTGCATG-3'; SEQ ID NO: 29) and its complementary strand 5'CACTATTAGGACCACCGTCGAGATGCTTTA-3'; SEQ ID NO: 30) containing the subgenomic promoter sequence were annealed and ligated into the MluI and SphI sites of the SinRep plasmid. The murine IL-12 α subunit gene (mp35; ATCC 87596) and the IL-12 β subunit gene (mp40; ATCC 87595) were subcloned into the MluI and the StuI sites of SinRep/2PSG, respectively, to produce the Sin-Rep/IL12 plasmid.

[000491] As expected the SV-IL-12 treatment group showed a better percentage survival of mice with tumor over the SVNYESO treatment group and the untreated (control) group. However, a synergistically higher showed enhanced percentage survival rate was observed in the SV-NYESO_SV-IL12 in comparison to the SV-IL-12 treatment group (FIG. 36). The results described herein clearly show the possibility of using a combination of SV vectors expressing IL-12 and NY-ESO-1, for treatment of cancers that may be resistant to treatment with a SV expressing a tumor associated antigen.

[000492] Combination of NY-ESO-1 and IL-12 expressed by the same Sindbis viral vectors enhances survival of subjects with established tumors

[000493] The study described herein investigates the effect of administering IL-12 and NY-ESO-1, both expressed by the same Sindbis viral vector, on established tumors. C57/Bl6 albino (female) mice re-injected with Alm5-2Fluc-17 ovarian cancer cells to establish a tumor (FIG. 19), and treated with either a SV vector expressing IL-12 (SV-IL-12), a SV vector expressing NY-ESO-1 (SVNYESO) or a Sindbis viral vector that expresses both IL-12 and NYESO (SV-NYESO_SGP2_IL12). As shown in FIG. 36, the SV-IL-12 treatment group showed a better percentage survival of mice with tumor over the SVNYESO treatment group and the untreated (control) group. As expected, the SVNYESO treatment group and the untreated (control) group showed similar survival rate, thereby showing that certain tumors are resistant to treatment with a SV expressing a tumor associated antigen (TAA) like NY-ESO-1. A synergistically higher enhanced percentage survival rate was observed in the SV-NYESO_SGP2_IL12 treatment group, in comparison to the SV-IL-12 treatment group (FIG. 37).

[000494] The study described herein, provides plasmid constructs for expressing NY-ESO-1, IL-12 and anti-OX40 in a SV vector. The study described herein, provides plasmid constructs encoding IL-12 α and β subunits (FIG.38), anti-OX40 IgG2a heavy and light chains (FIG. 39), a

single chain antibody to OX40 (OX40 ScFv) (FIG. 40), a human NY-ESO-1 (FIG. 41) and an OX40 ligand fused to a Fc peptide (OX40L-Fc T2A) and a NY-ESO-1 with a termination peptide sequence T2A in between (FIG. 42).

[000495] In summary, the results of the study described herein clearly show the possibility of using a SV vectors expressing both IL-12 and NY-ESO-1, for treatment of cancers that may be resistant to treatment with a SV expressing a tumor associated antigen.

What we claim is:

1. A method for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of (a) a Sindbis viral vector and (b) an antibody directed against a co-stimulatory molecule, or a nucleic acid encoding same; or an antibody to an immune system agonist molecule, or a nucleic acid encoding same.
2. The method of claim 1, wherein the antibody is selected from the group consisting of anti-OX40 antibody, anti-4-1BB antibody, anti-CD28 antibody, anti-GITR antibody, anti-CD137 antibody, anti-cd37 antibody, and anti-HVEM antibody.
3. The method of claim 1, wherein the Sindbis viral vector comprises at least one nucleic acid encoding an immunostimulatory or an immunomodulatory protein.
4. The method of claim 2, wherein the immunostimulatory or immunomodulatory protein is IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-34, IL-35 or IL-36.
5. The method of claim 3, wherein the immunostimulatory or immunomodulatory protein is IL-12.
6. The method of claim 1, wherein the Sindbis viral vector and the antibody induce an immune response in a tumor associated antigen (TAA) nonspecific manner.
7. A method for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and (b) an anti-OX40 monoclonal antibody or a nucleic acid encoding same, thereby treating cancer in the subject.

8. The method of claim 7, wherein the Sindbis viral vector comprising the nucleic acid encoding interleukin-12 further comprises the nucleic acid encoding the anti-OX40 monoclonal antibody.
9. The method of claim 7, comprising administering a Sindbis viral vector comprising the nucleic acid encoding interleukin-12 and administering a Sindbis viral vector comprising the nucleic acid encoding the anti-OX40 monoclonal antibody.
10. The method of claim 7, wherein the nucleic acid encoding interleukin-12 comprises the nucleic acid sequence encoding interleukin 12 alpha subunit of SEQ ID NO: 1, the nucleic acid encoding interleukin 12 beta subunit of SEQ ID NO: 2, or a combination thereof.
11. The method of claim 7, wherein the nucleic acid encoding the anti-OX40 monoclonal antibody comprises
- a nucleic acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence selected from of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:32, SEQ ID NO:36, SEQ ID NO:40, SEQ ID NO:44 and SEQ ID NO:48 encoding an anti-OX40 antibody heavy chain,
 - a nucleic acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequence of SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:38, SEQ ID NO:42, SEQ ID NO:46 and SEQ ID NO:50 encoding an anti-OX40 antibody light chain,
 - or any combination of heavy chain nucleic acid sequence or light chain nucleic acid sequence thereof.
12. The method of claim 7, wherein the nucleic acid encoding the anti-OX40 monoclonal antibody comprises a nucleic acid encoding an anti-OX40 antibody heavy chain amino acid sequence, an anti-OX40 antibody light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13, or a combination thereof.

13. The method of claim 7, wherein the Sindbis viral vector and the anti-OX40 monoclonal antibody are administered systemically.
14. The method of claim 7, wherein the Sindbis viral vector and the anti-OX40 monoclonal antibody are administered parenterally.
15. The method of claim 7, wherein the Sindbis viral vector and the anti-OX40 monoclonal antibody are administered intraperitoneally.
16. The method of claim 7, wherein the Sindbis viral vector is replication defective.
17. The method of claim 7, wherein the cancer comprises a solid tumor.
18. The method of claim 7, wherein the cancer is colon cancer, prostate cancer or ovarian cancer.
19. A Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a nucleic acid encoding an anti-OX40 monoclonal antibody.
20. The Sindbis viral vector of claim 19, wherein the nucleic acid encoding interleukin-12 comprises the nucleic acid sequence encoding interleukin 12 alpha subunit of SEQ ID NO: 1, the nucleic acid encoding interleukin 12 beta subunit of SEQ ID NO: 2, or a combination thereof.
21. The Sindbis viral vector of claim 19, wherein the nucleic acid encoding the anti-OX40 monoclonal antibody comprises
 - a nucleic acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence selected from of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:32, SEQ ID NO:36, SEQ ID NO:40, SEQ ID NO:44 and SEQ ID NO:48 encoding an anti-OX40 antibody heavy chain,
 - a nucleic acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequence of SEQ ID NO:8, SEQ ID NO:12,

SEQ ID NO:34, SEQ ID NO:38, SEQ ID NO:42, SEQ ID NO:46 and SEQ ID NO:50 encoding an anti-OX40 antibody light chain,

or any combination of heavy chain nucleic acid sequence or light chain nucleic acid sequence thereof.

22. A composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and (b) an anti-OX40 monoclonal antibody or a nucleic acid encoding same.

23. A composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and (b) a Sindbis viral vector comprising a nucleic acid encoding an anti-OX40 monoclonal antibody.

24. A method for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 or (b) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a nucleic acid encoding NY-ESO-1, thereby treating cancer in the subject.

25. The method of claim 24, wherein the nucleic acid encoding interleukin-12 comprises the nucleic acid sequence encoding interleukin 12 alpha subunit of SEQ ID NO: 1, the nucleic acid encoding interleukin 12 beta subunit of SEQ ID NO: 2, or a combination thereof.

26. The method of claim 24, wherein the nucleic acid encoding NY-ESO-1 comprises a nucleic acid sequence of SEQ ID NO: 14.

27. The method of claim 24, wherein the (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 or (b) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a nucleic acid encoding NY-ESO-1 are administered systemically.

28. The method of claim 24, wherein the (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 or (b) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a nucleic acid encoding NY-ESO-1 are administered parenterally.
29. The method of claim 24, wherein the (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 or (b) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a nucleic acid encoding NY-ESO-1 are administered intraperitoneally.
30. The method of claim 24, wherein the Sindbis viral vector is replication defective.
31. The method of claim 24, wherein the cancer comprises a solid tumor.
32. The method of claim 24, wherein the cancer is colon cancer, prostate cancer or ovarian cancer.
33. A Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and a nucleic acid encoding an NY-ESO-1.
34. The Sindbis viral vector of claim 33, wherein the nucleic acid encoding interleukin-12 comprises the nucleic acid sequence encoding interleukin 12 alpha subunit of SEQ ID NO: 1, the nucleic acid encoding interleukin 12 beta subunit of SEQ ID NO: 2, or a combination thereof.
35. The Sindbis viral vector of claim 33, wherein the nucleic acid encoding the NY-ESO-1 comprises a nucleic acid sequence of SEQ ID NO: 14.
36. A composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding interleukin-12 and (b) a Sindbis viral vector comprising a nucleic acid encoding an NY-ESO-1.

37. A method for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of (a) a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 and (b) an anti-OX40 monoclonal antibody or a nucleic acid encoding same, thereby treating cancer in the subject.

38. The method of claim 37, wherein the Sindbis viral vector comprising the nucleic acid encoding NY-ESO-1 further comprises the nucleic acid encoding the anti-OX40 monoclonal antibody.

39. The method of claim 37, comprising administering a Sindbis viral vector comprising the nucleic acid encoding NY-ESO-1 and administering a Sindbis viral vector comprising the nucleic acid encoding the anti-OX40 monoclonal antibody.

40. The method of claim 37, wherein the nucleic acid encoding NY-ESO-1 comprises a nucleic acid sequence of SEQ ID NO: 14.

41. The method of claim 37, wherein the nucleic acid encoding the anti-OX40 monoclonal antibody comprises

a nucleic acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence selected from of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:32, SEQ ID NO:36, SEQ ID NO:40, SEQ ID NO:44 and SEQ ID NO:48 encoding an anti-OX40 antibody heavy chain,

a nucleic acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequence of SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:38, SEQ ID NO:42, SEQ ID NO:46 and SEQ ID NO:50 encoding an anti-OX40 antibody light chain,

or any combination of heavy chain nucleic acid sequence or light chain nucleic acid sequence thereof.

42. The method of claim 37, wherein the nucleic acid encoding the anti-OX40 monoclonal antibody comprises a nucleic acid encoding an anti-OX40 antibody heavy chain amino acid sequence, an anti-OX40 antibody light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13, or a combination thereof.
43. The method of claim 37, wherein the Sindbis viral vector and the anti-OX40 monoclonal antibody are administered systemically.
44. The method of claim 37, wherein the Sindbis viral vector and the anti-OX40 monoclonal antibody are administered parenterally.
45. The method of claim 37, wherein the Sindbis viral vector and the anti-OX40 monoclonal antibody are administered intraperitoneally.
46. The method of claim 37, wherein the Sindbis viral vector is replication defective.
47. The method of claim 37, wherein the cancer comprises a solid tumor.
48. The method of claim 37, wherein the cancer is colon cancer, prostate cancer or ovarian cancer.
49. A Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 and a nucleic acid encoding an anti-OX40 monoclonal antibody.
50. The Sindbis viral vector of claim 49, wherein the nucleic acid encoding the NY-ESO-1 comprises the nucleic acid sequence of SEQ ID NO: 14.
51. The Sindbis viral vector of claim 49, wherein the nucleic acid encoding the anti-OX40 monoclonal antibody comprises
a nucleic acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence selected from of SEQ ID NO:6, SEQ ID

NO:10, SEQ ID NO:32, SEQ ID NO:36, SEQ ID NO:40, SEQ ID NO:44 and SEQ ID NO:48 encoding an anti-OX40 antibody heavy chain,

a nucleic acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequence of SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:34, SEQ ID NO:38, SEQ ID NO:42, SEQ ID NO:46 and SEQ ID NO:50 encoding an anti-OX40 antibody light chain,

or any combination of heavy chain nucleic acid sequence or light chain nucleic acid sequence thereof.

52. The Sindbis viral vector of claim 49, wherein the nucleic acid encoding the anti-OX40 monoclonal antibody comprises a nucleic acid encoding an anti-OX40 antibody heavy chain amino acid sequence, an anti-OX40 antibody light chain amino acid sequence, that binds to a target antigen of the amino acid sequence of SEQ ID NO: 13, or a combination thereof.

53. A composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 and (b) an anti-OX40 monoclonal antibody or a nucleic acid encoding same.

54. A composition comprising (a) a Sindbis viral vector comprising a nucleic acid encoding NY-ESO-1 and (b) a Sindbis viral vector comprising a nucleic acid encoding an anti-OX40 monoclonal antibody.

55. A method for treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount (a) a Sindbis viral vector and (b) an anti-4-1BB monoclonal antibody or a nucleic acid encoding same, thereby treating cancer in the subject.

56. The method of claim 55, wherein the Sindbis viral vector and the anti-4-1BB monoclonal antibody are administered systemically.

57. The method of claim 55, wherein the Sindbis viral vector and the anti-4-1BB monoclonal antibody are administered parenterally.
58. The method of claim 55, wherein the Sindbis viral vector and the anti-4-1BB monoclonal antibody are administered intraperitoneally.
59. The method of claim 55, wherein the Sindbis viral vector is replication defective.
60. The method of claim 55, wherein the cancer is a hematologic cancer.
61. The method of claim 55, wherein the cancer is a B cell lymphoma.

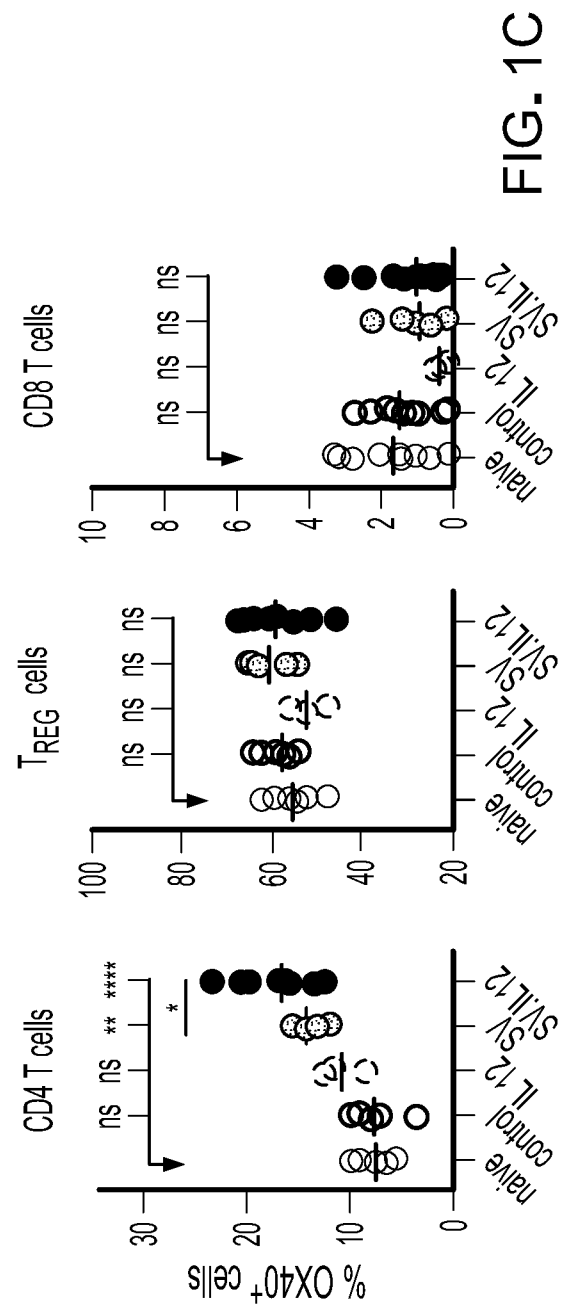
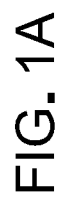


FIG. 1C

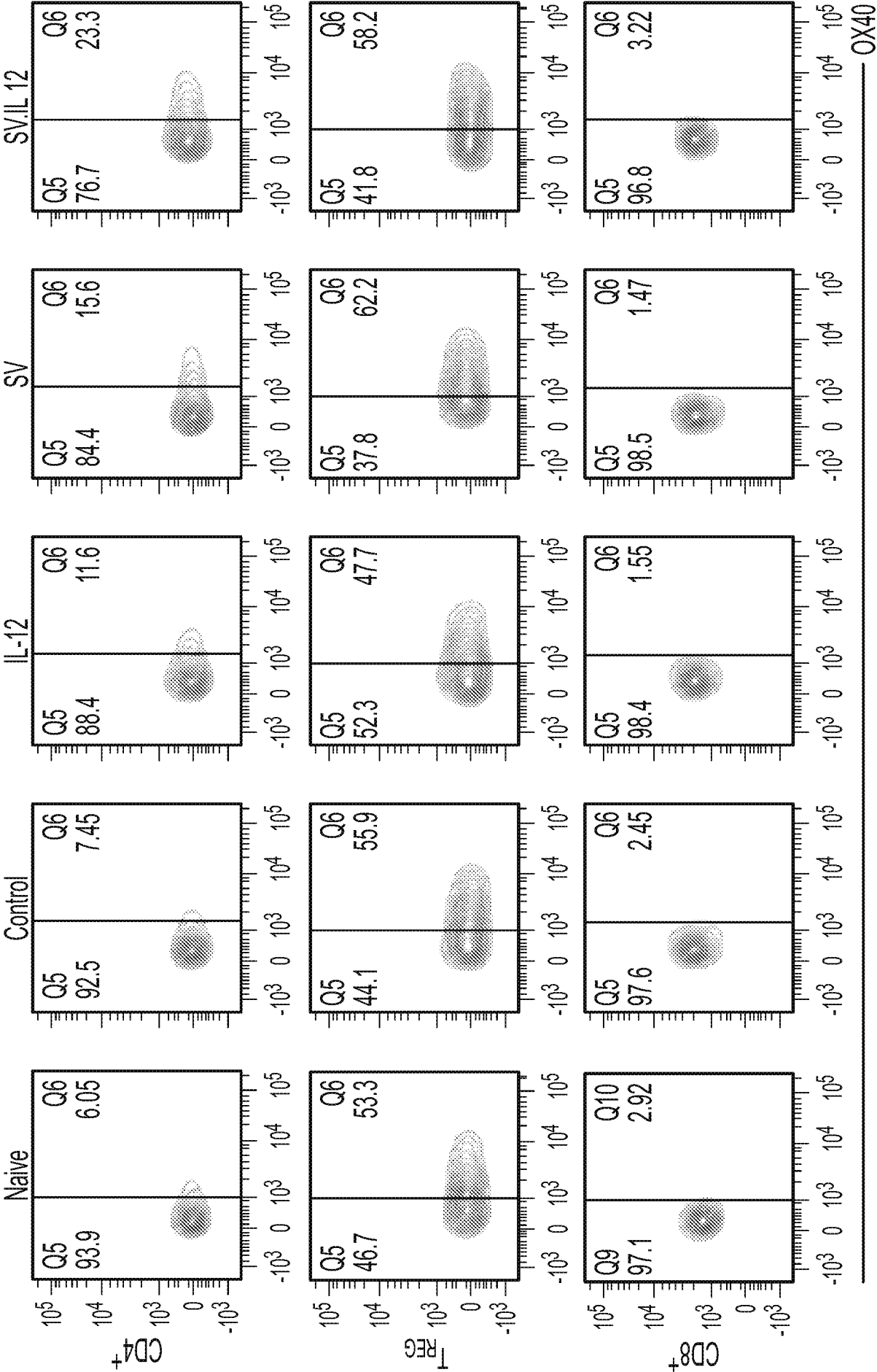
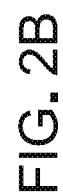


FIG. 1D



4/73

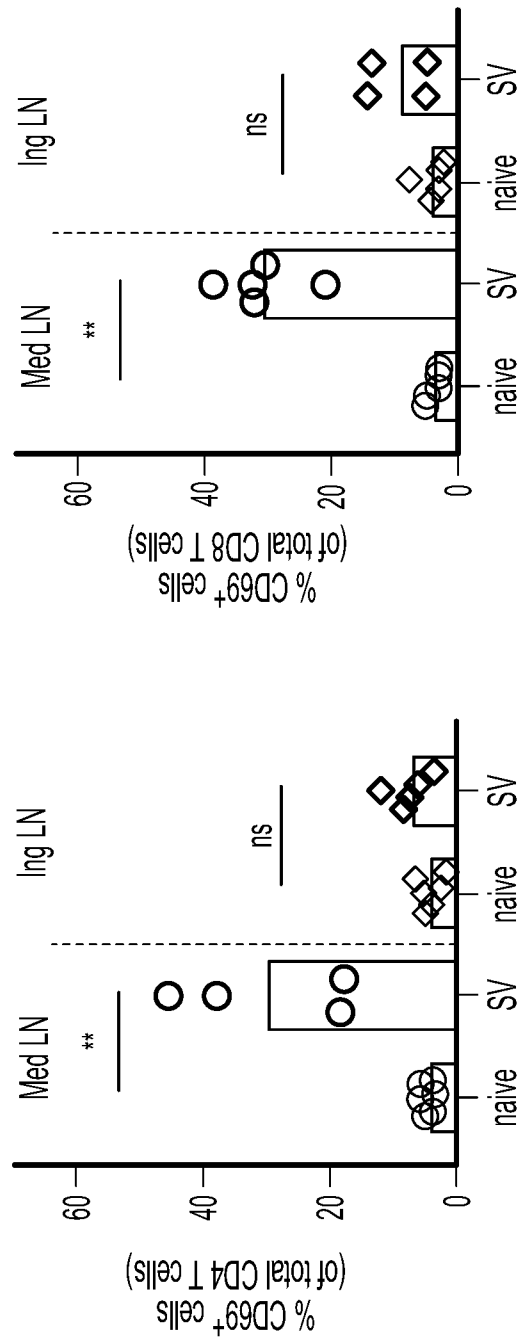
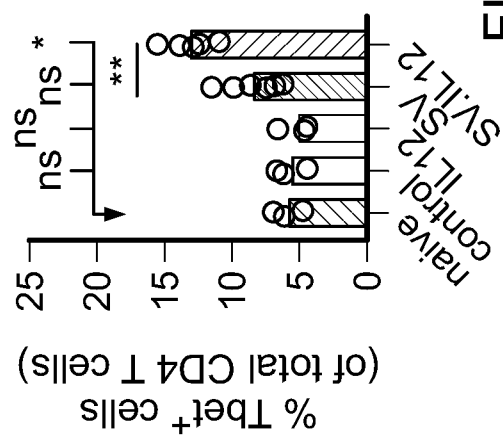
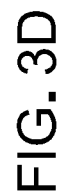
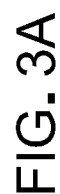


FIG. 2C



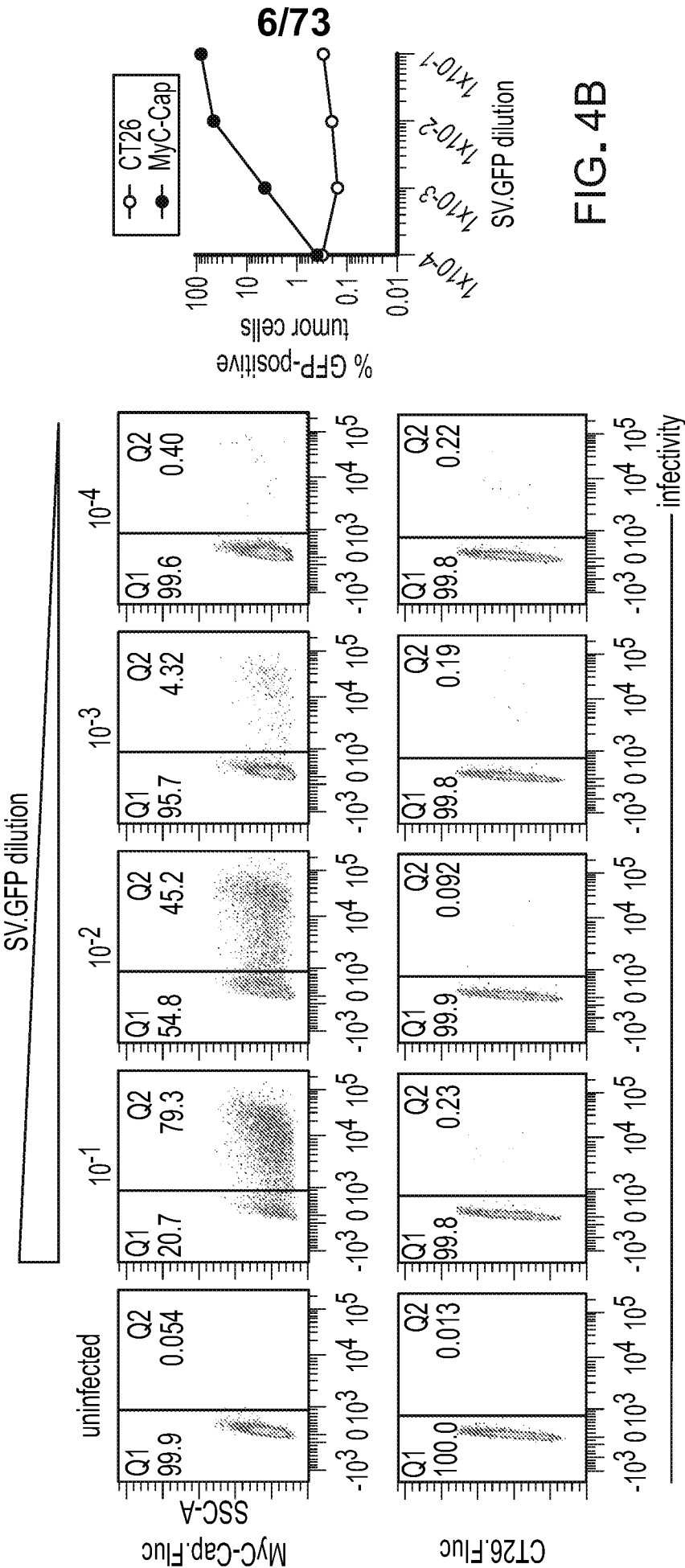


FIG. 4A

FIG. 4B

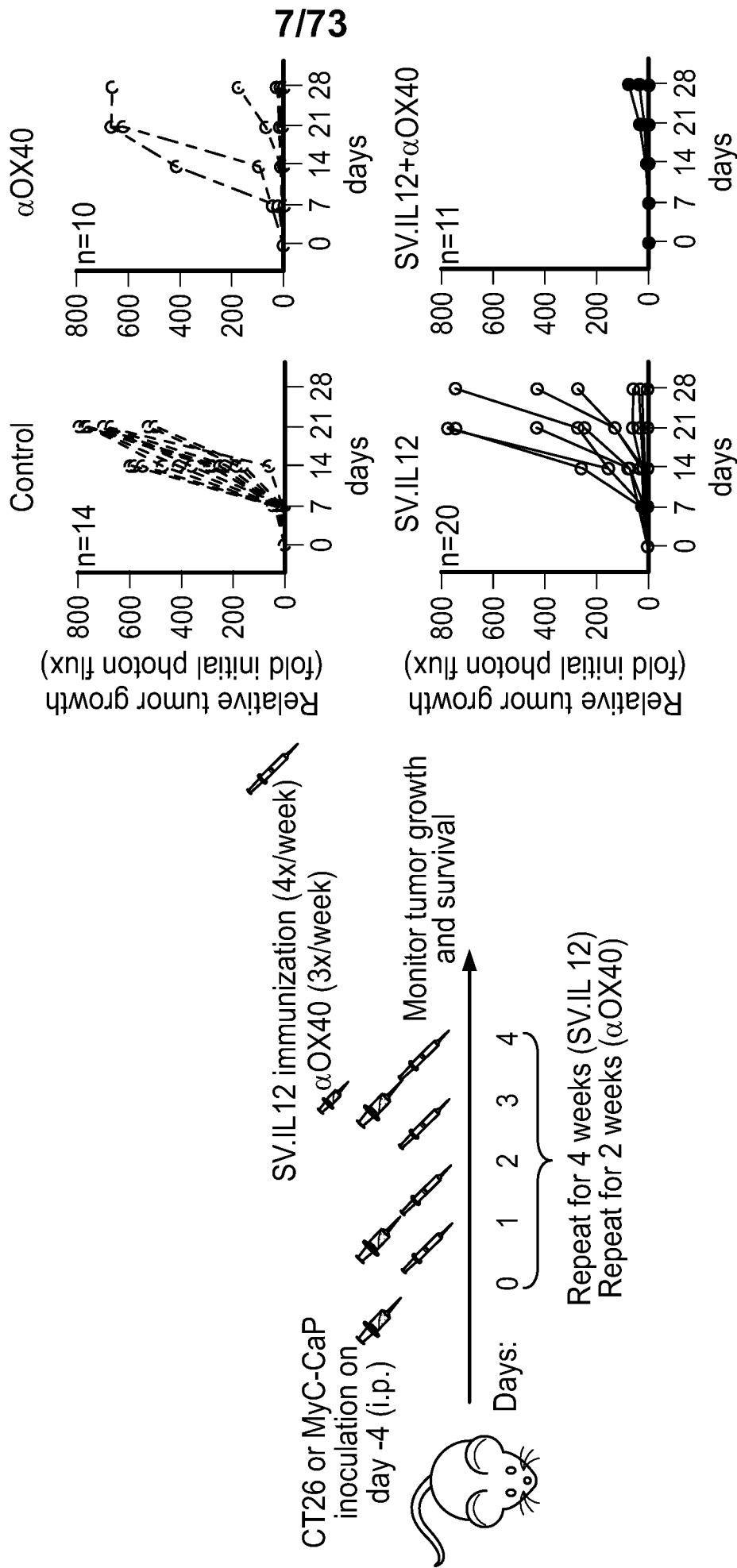


FIG. 5B

FIG. 5A

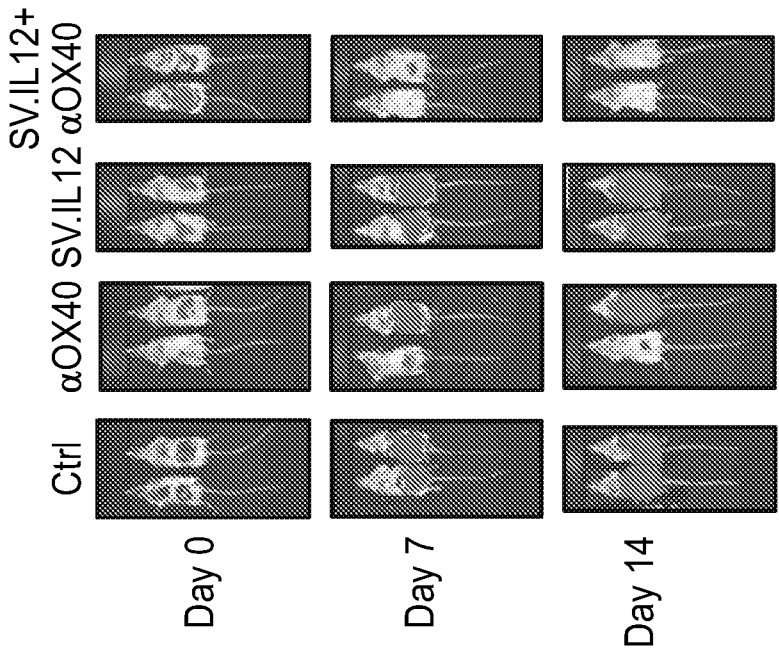
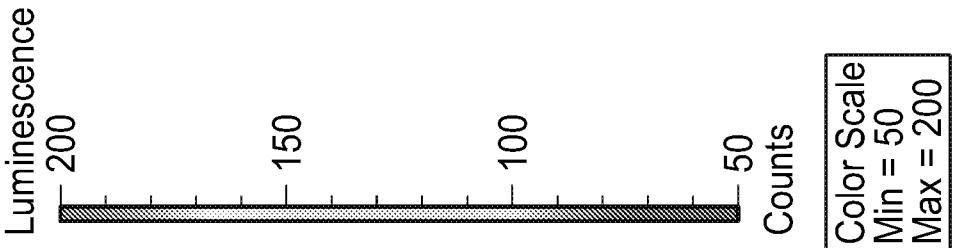
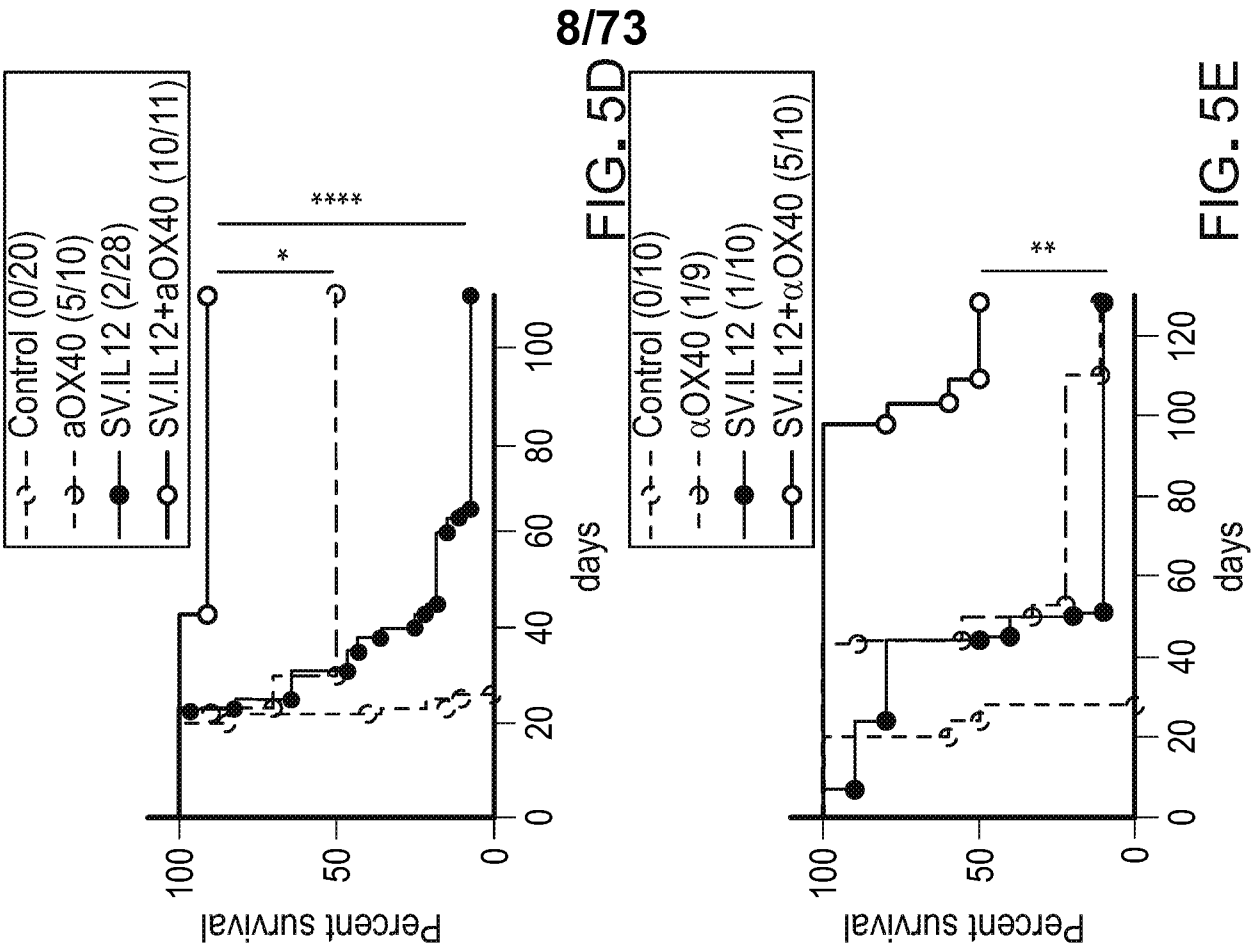


FIG. 5E

FIG. 5C

9/73

MyC-CaP.Fluc

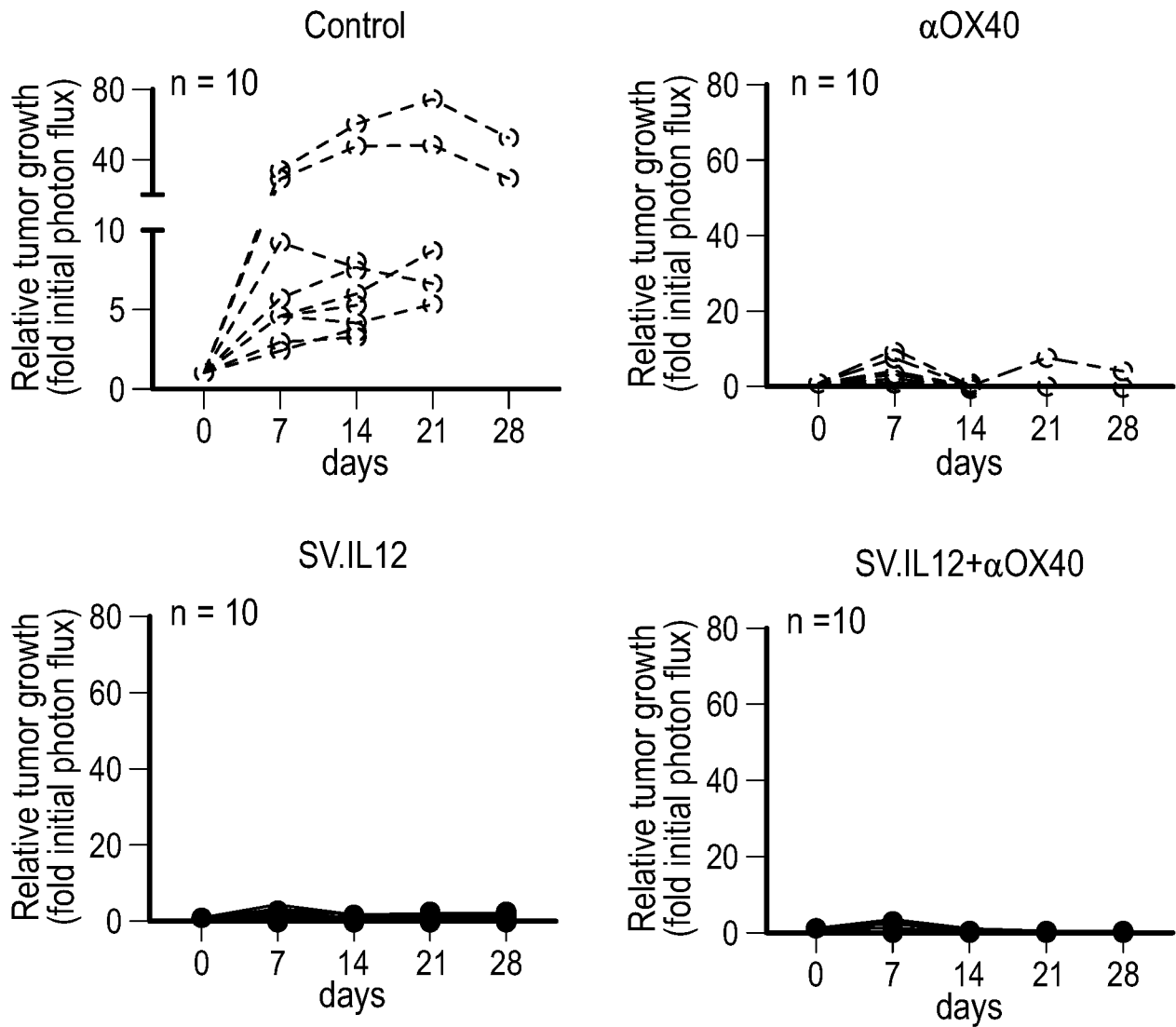


FIG. 6A

10/73

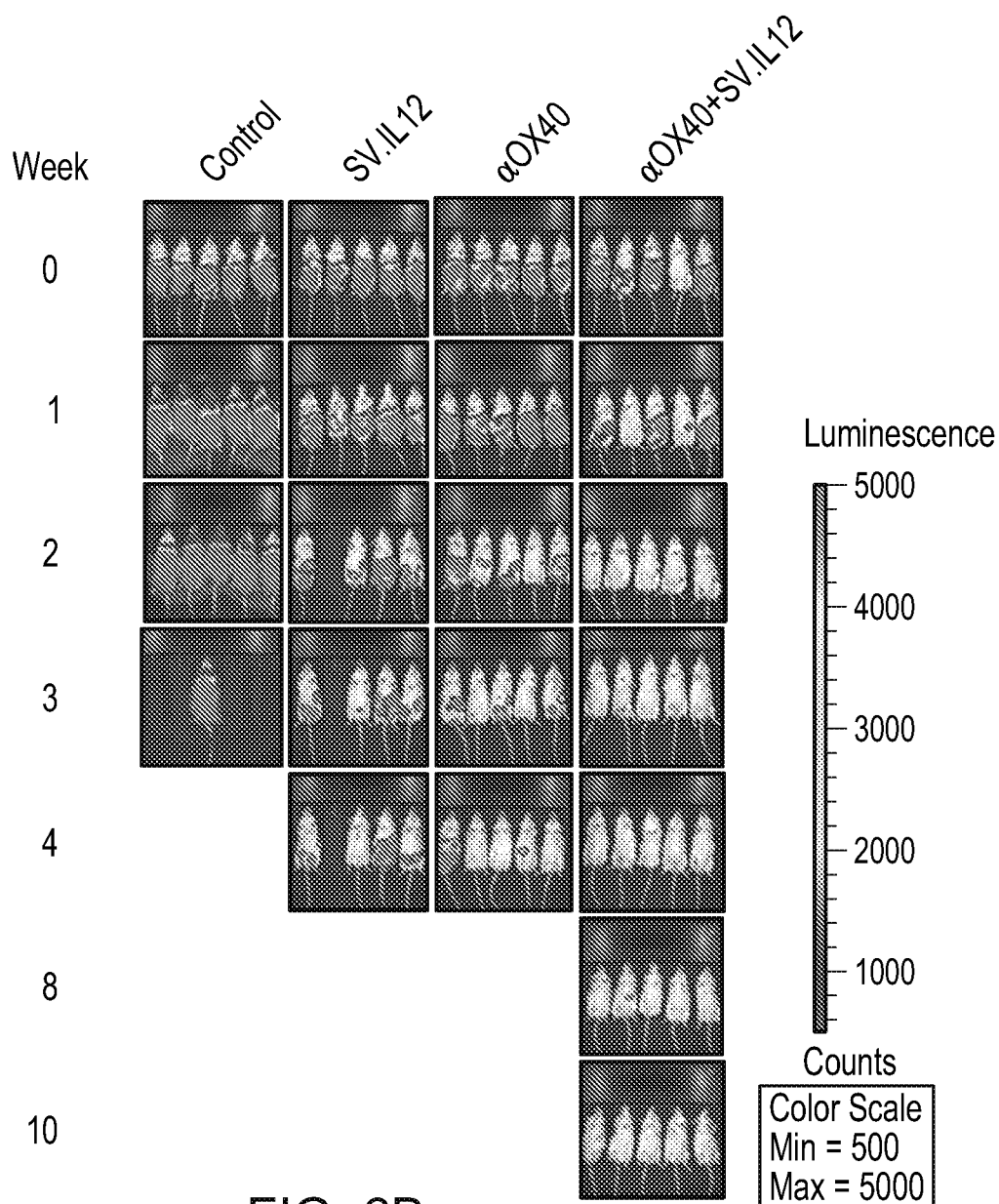
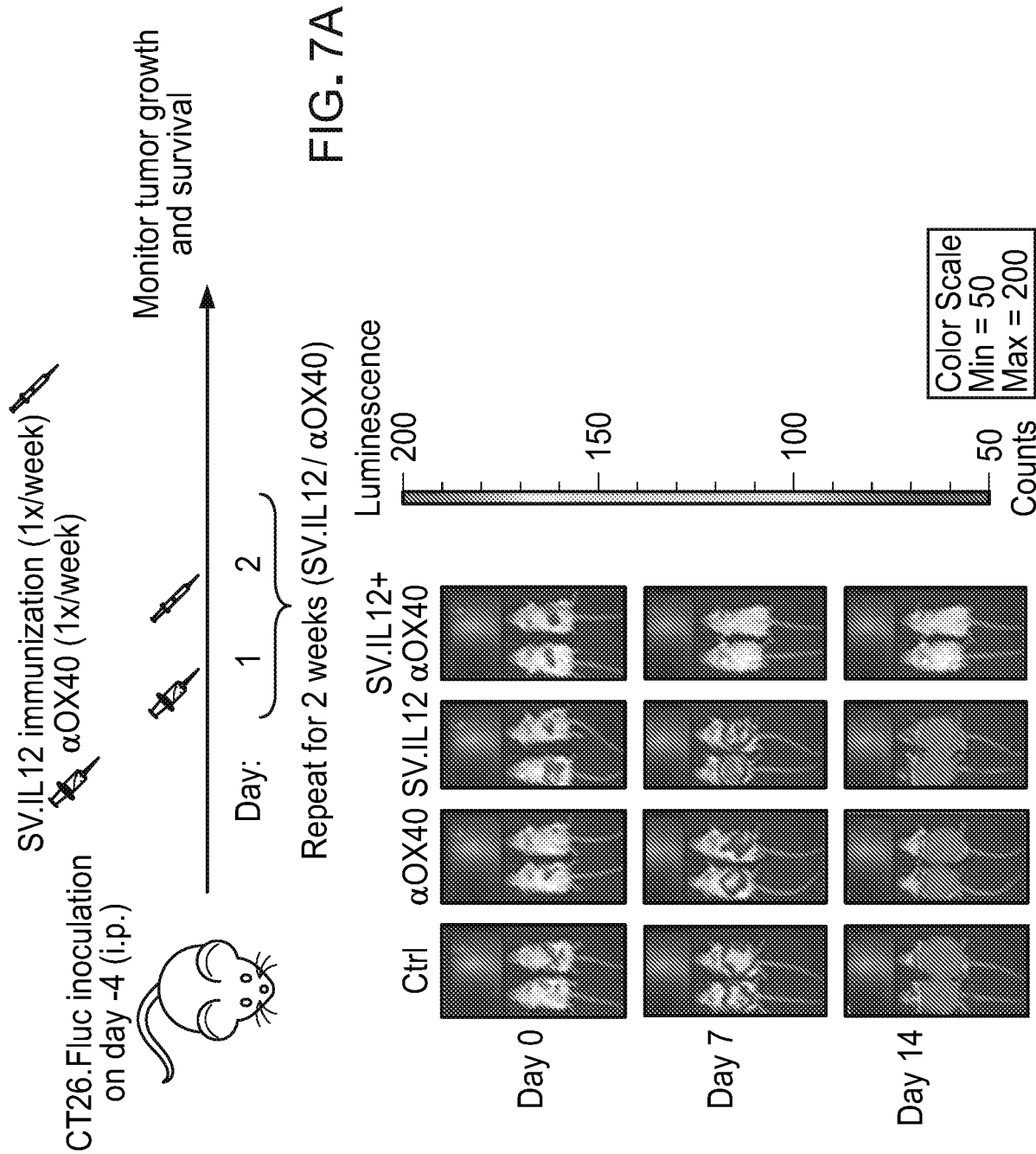


FIG. 6B



CT26.Fluc

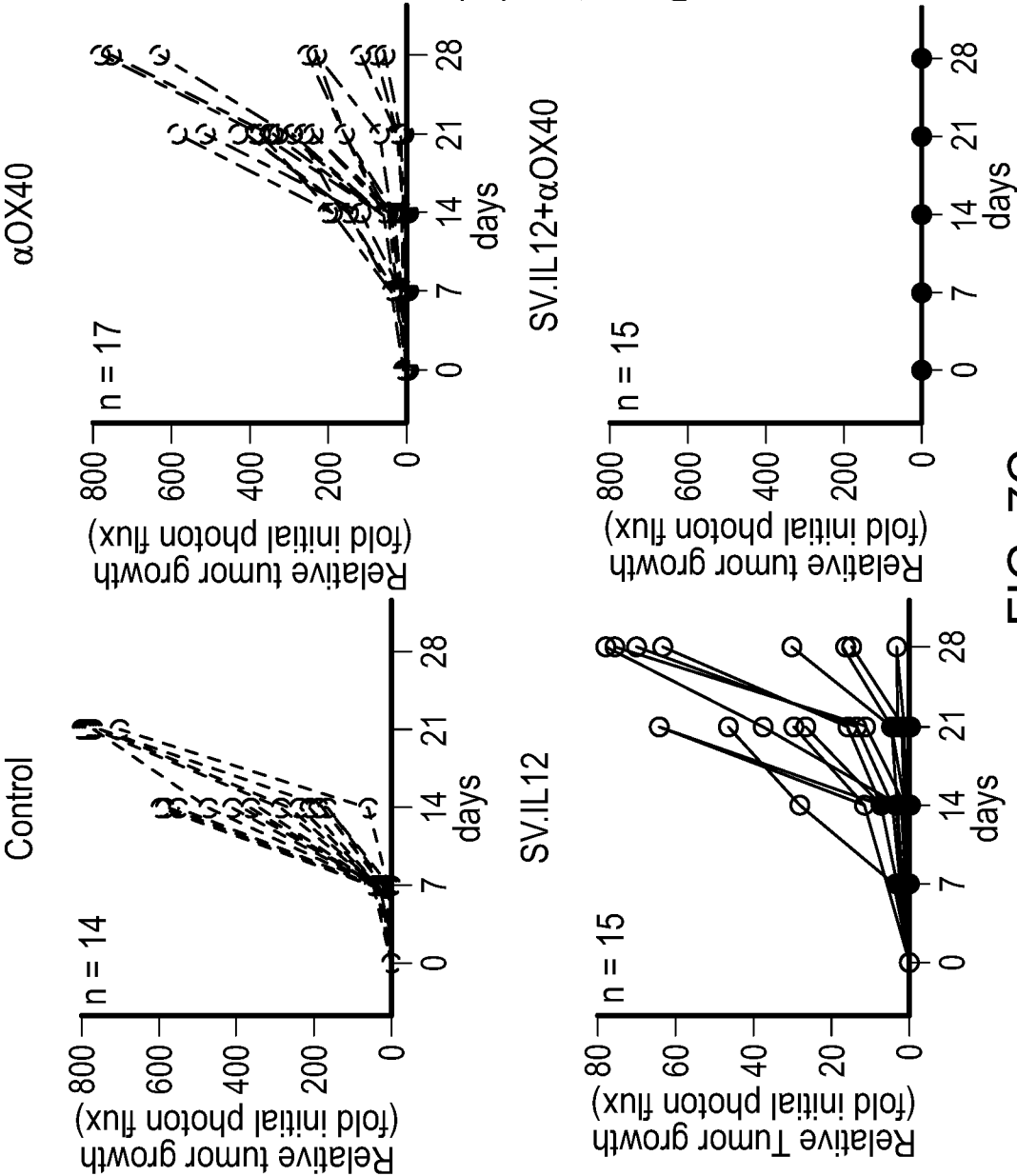


FIG. 7C

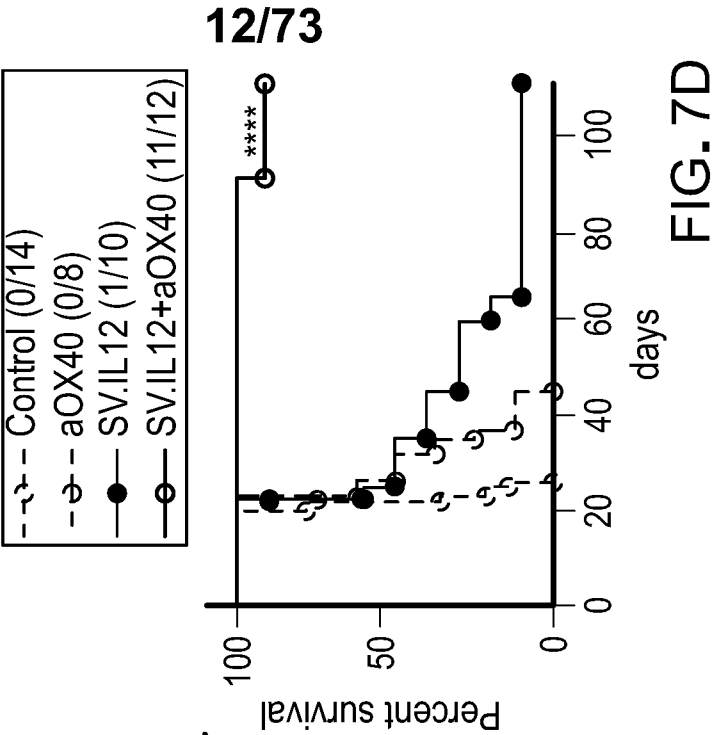


FIG. 7D

12/73

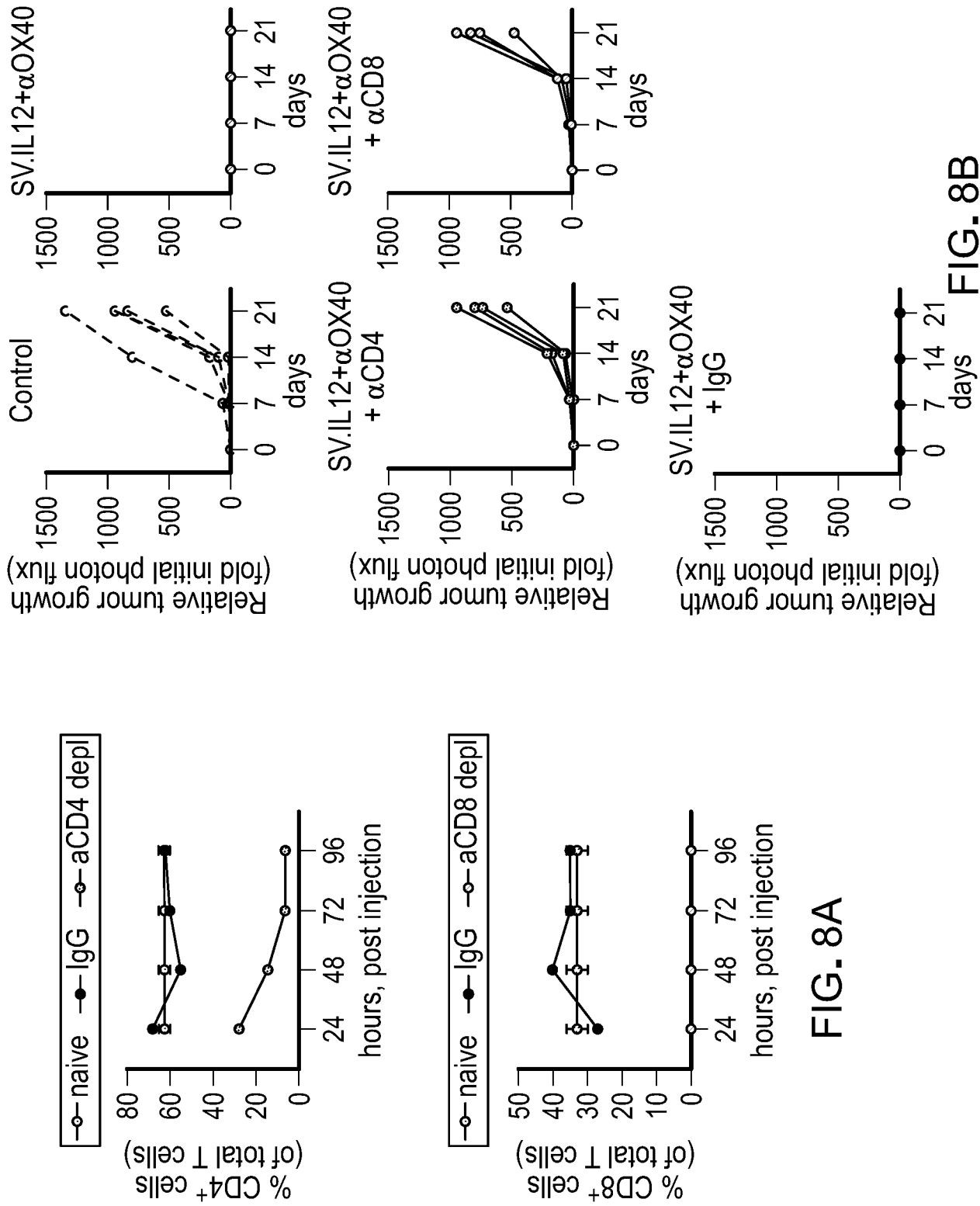


FIG. 8A

FIG. 8B

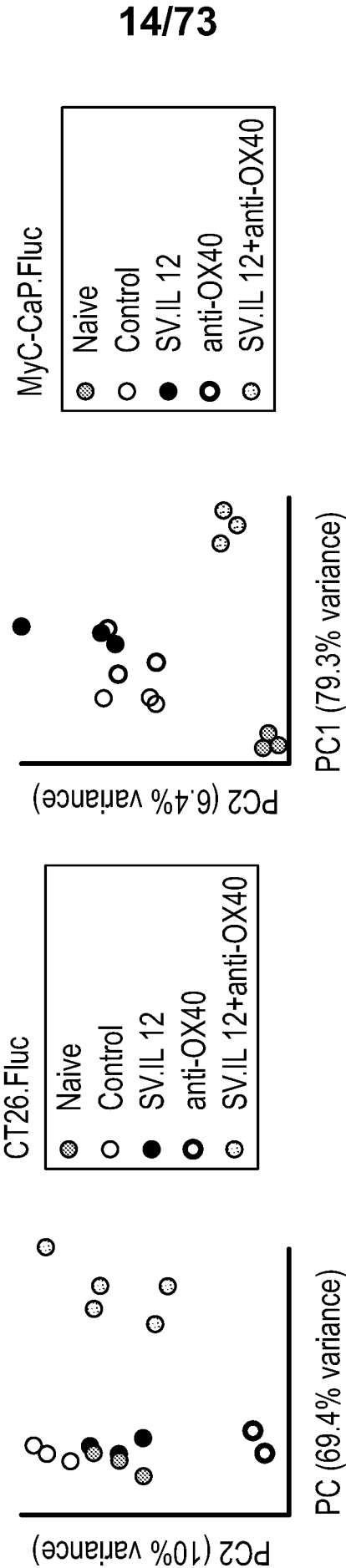


FIG. 9A

FIG. 9B

15/73

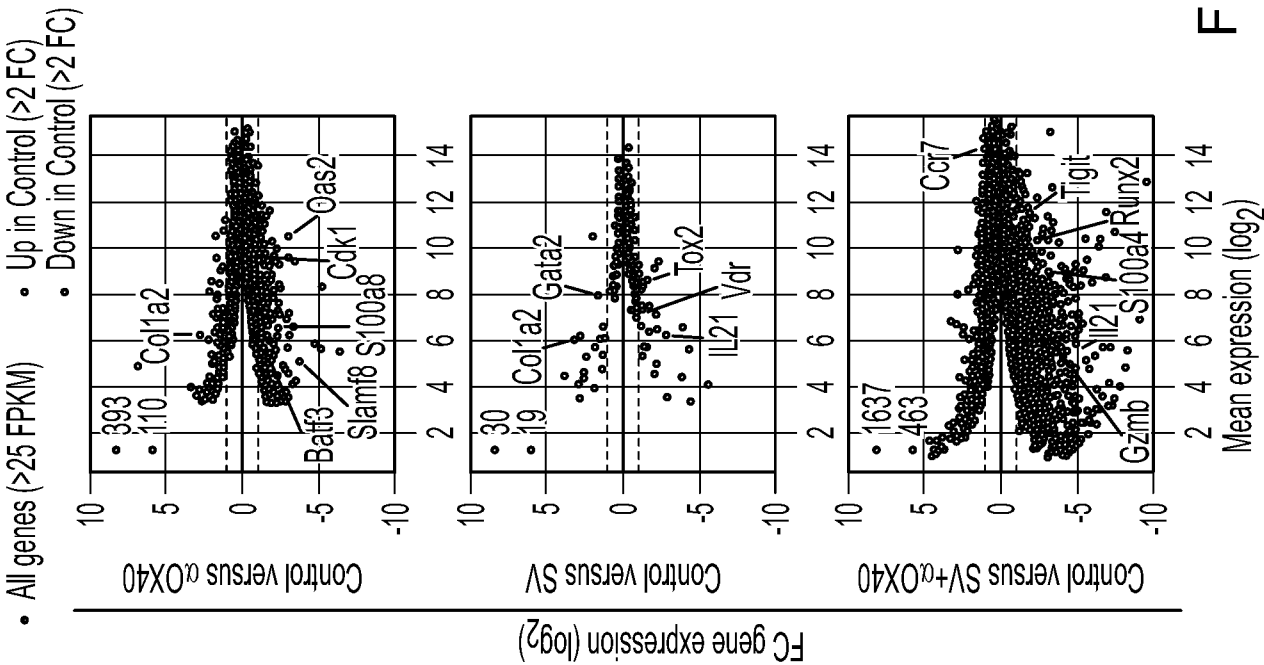


FIG. 9C

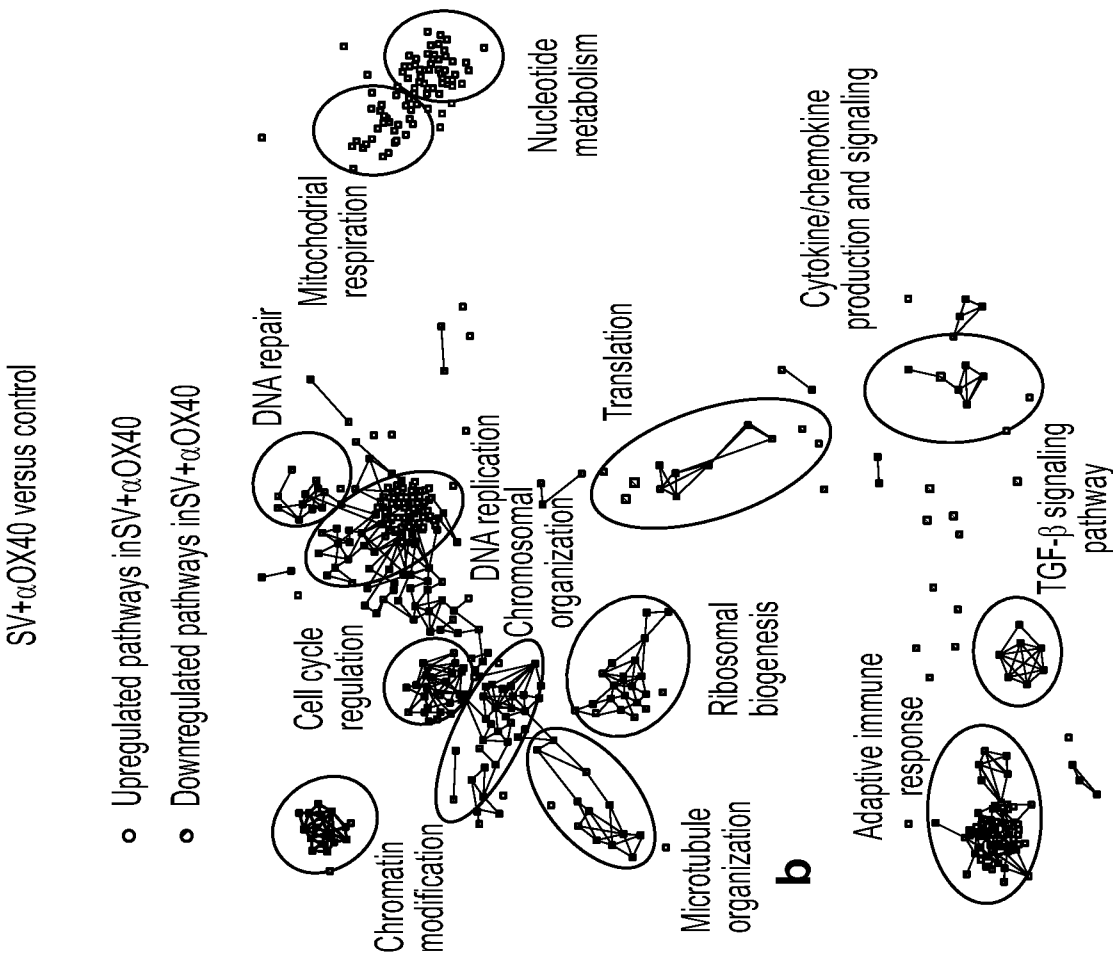


FIG. 9D

16/73

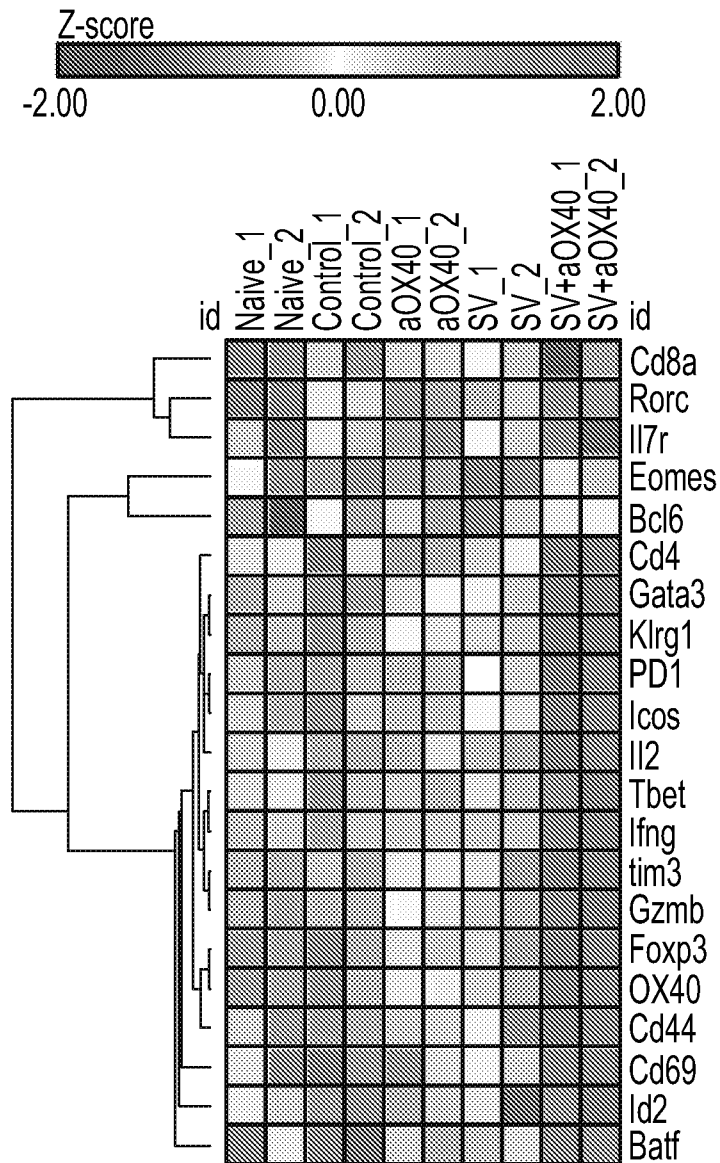


FIG. 9E

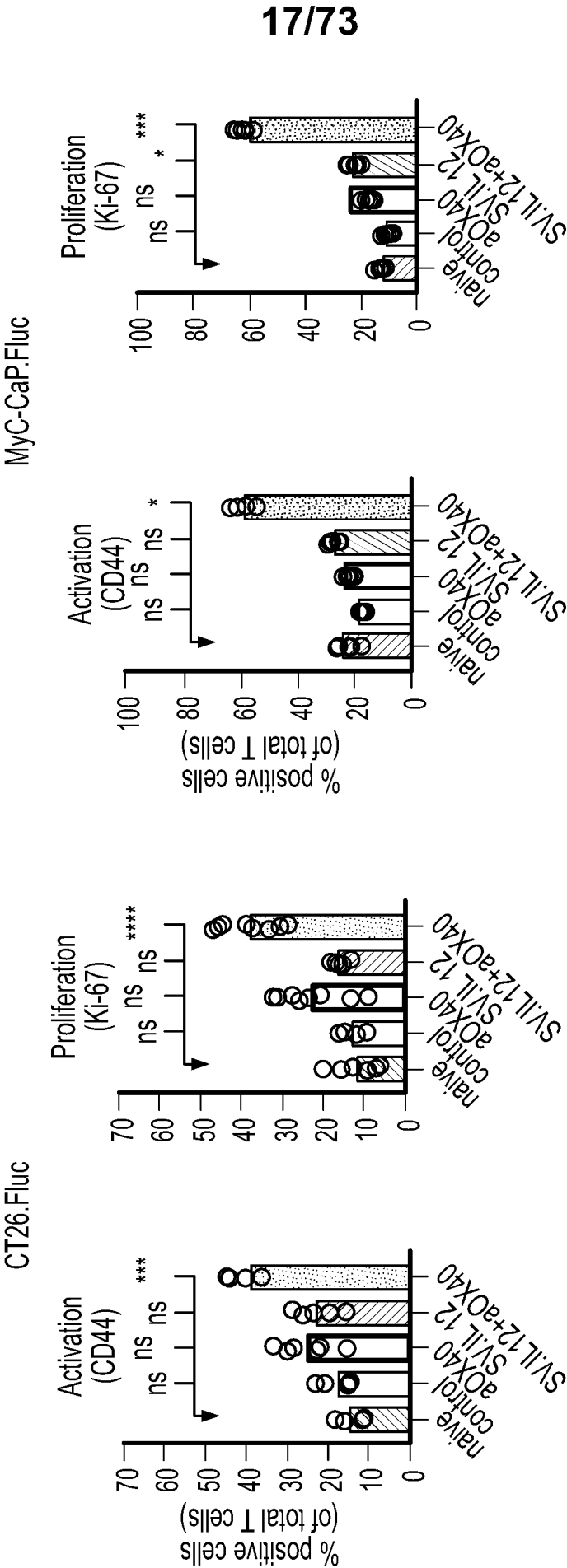
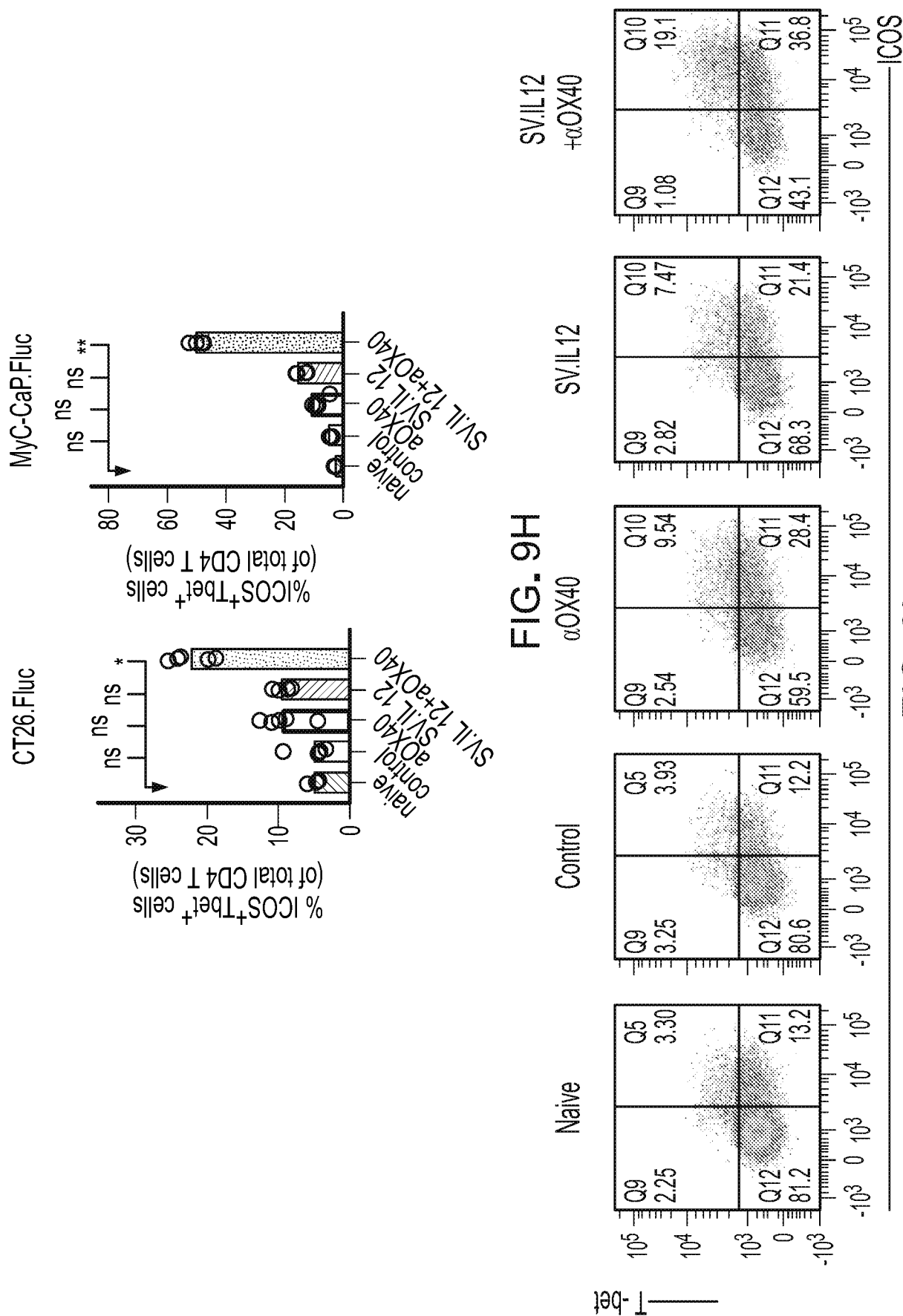
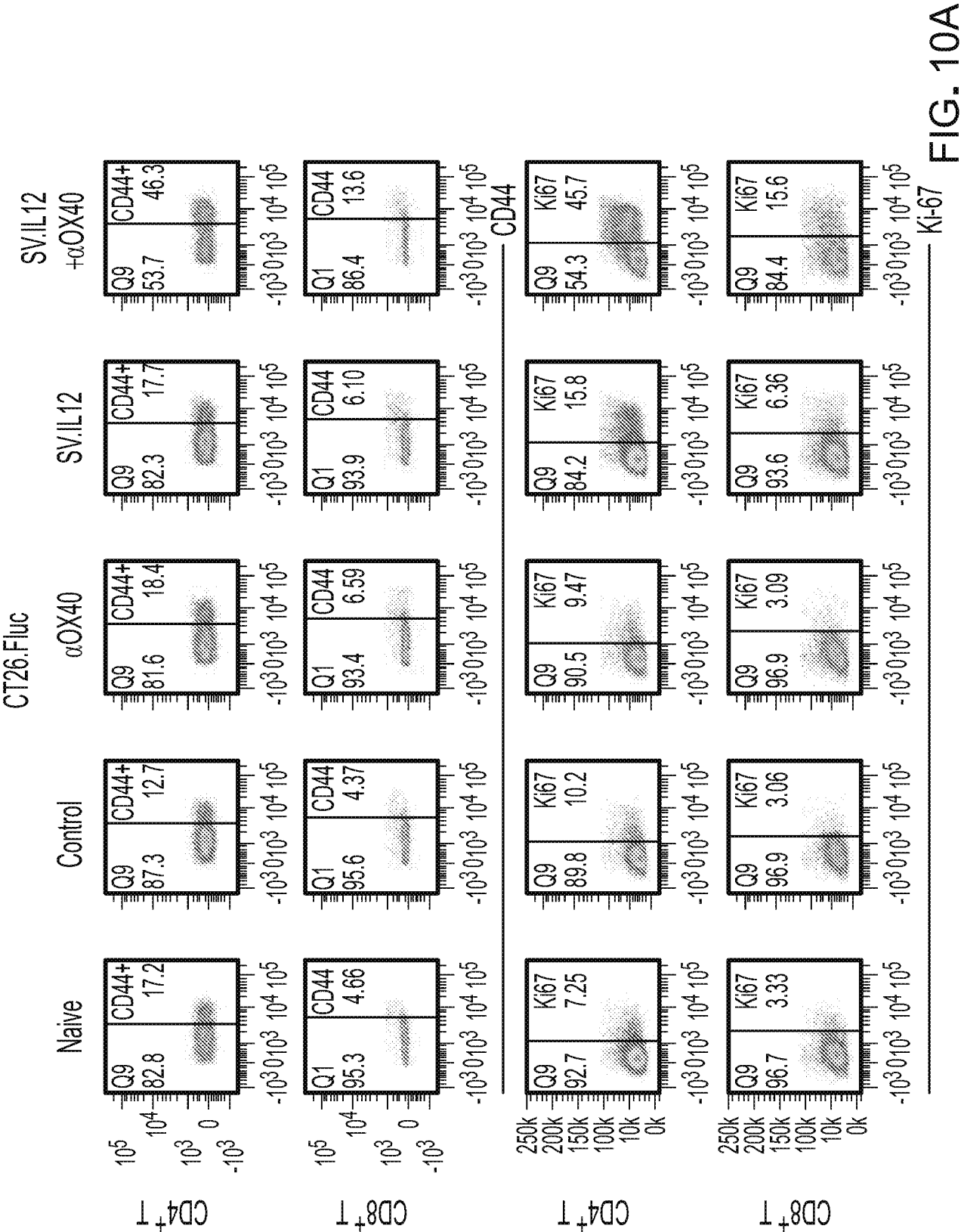


FIG. 9G

FIG. 9F





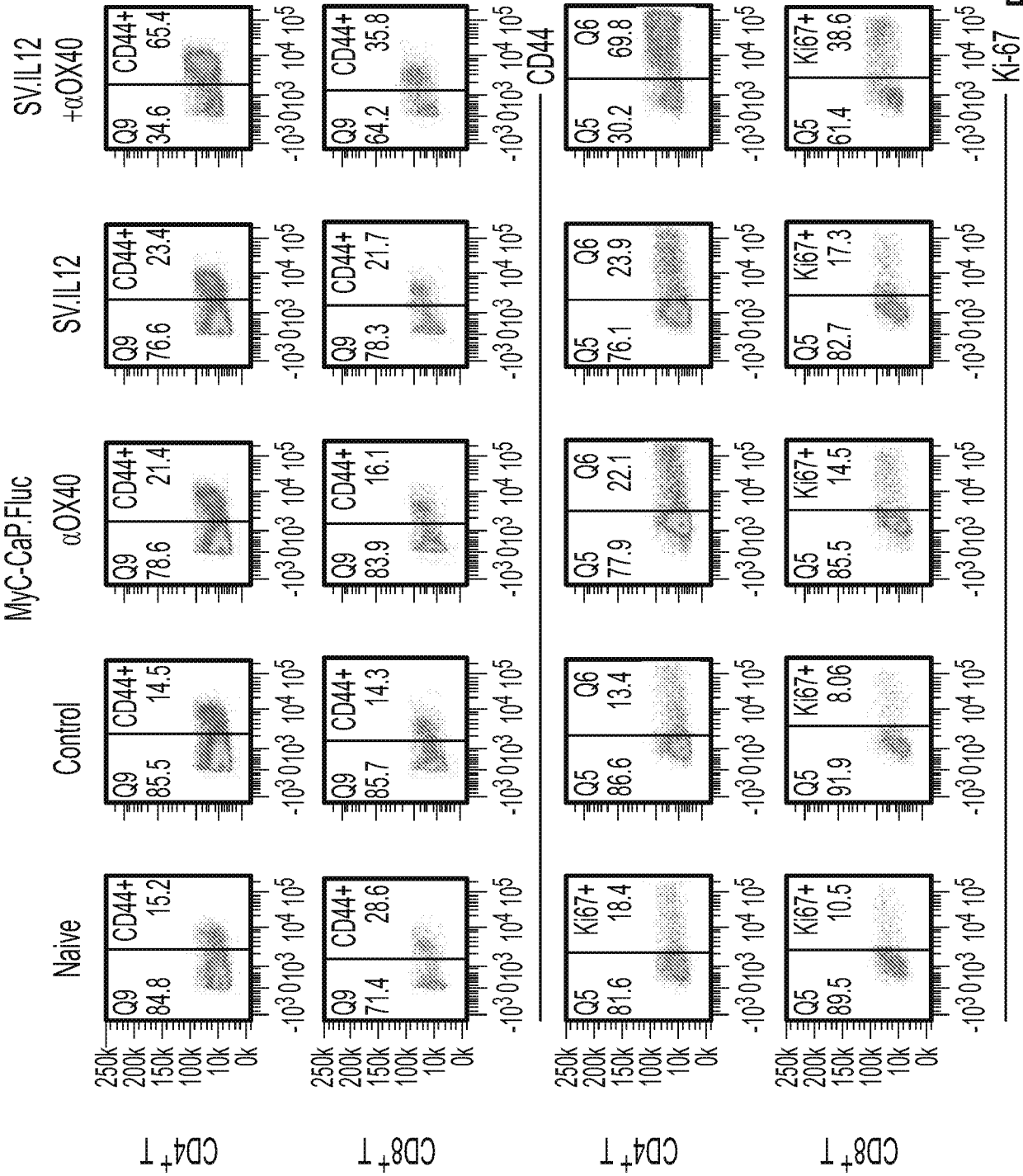


FIG. 10B

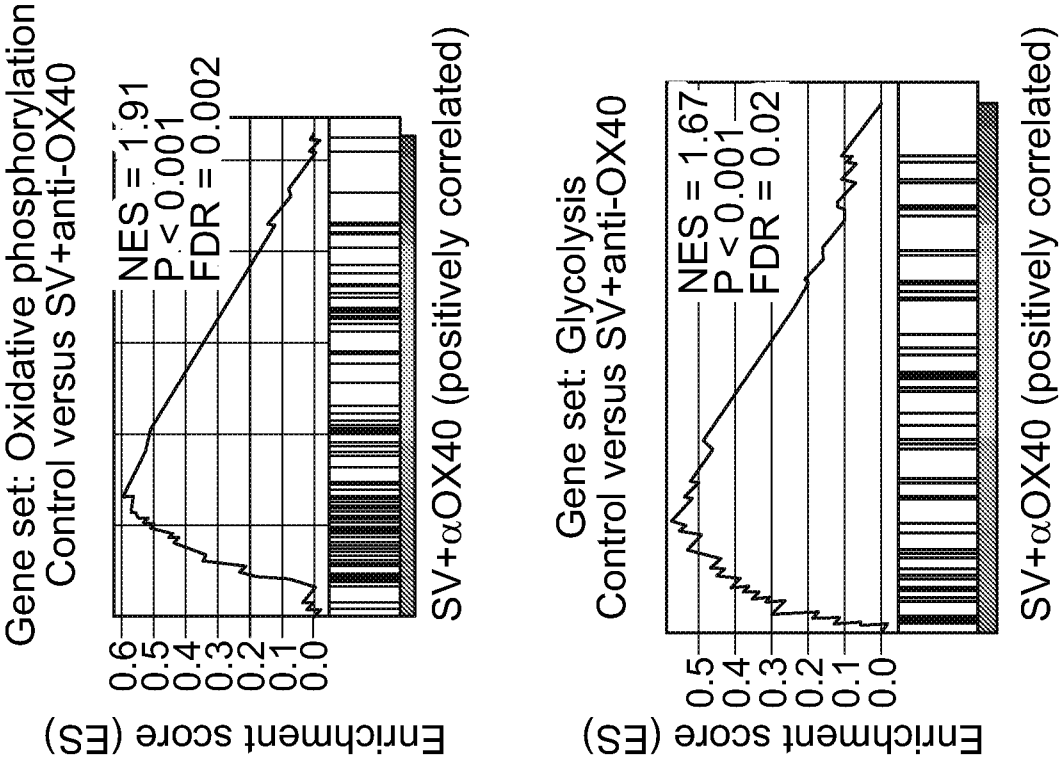


FIG. 11A

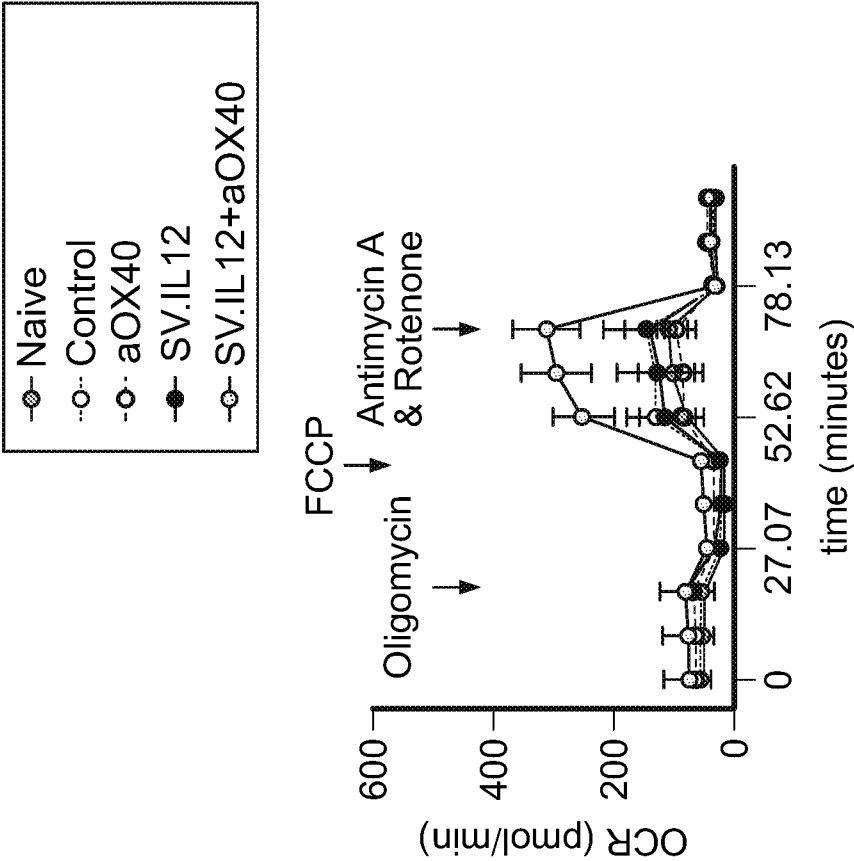


FIG. 11B

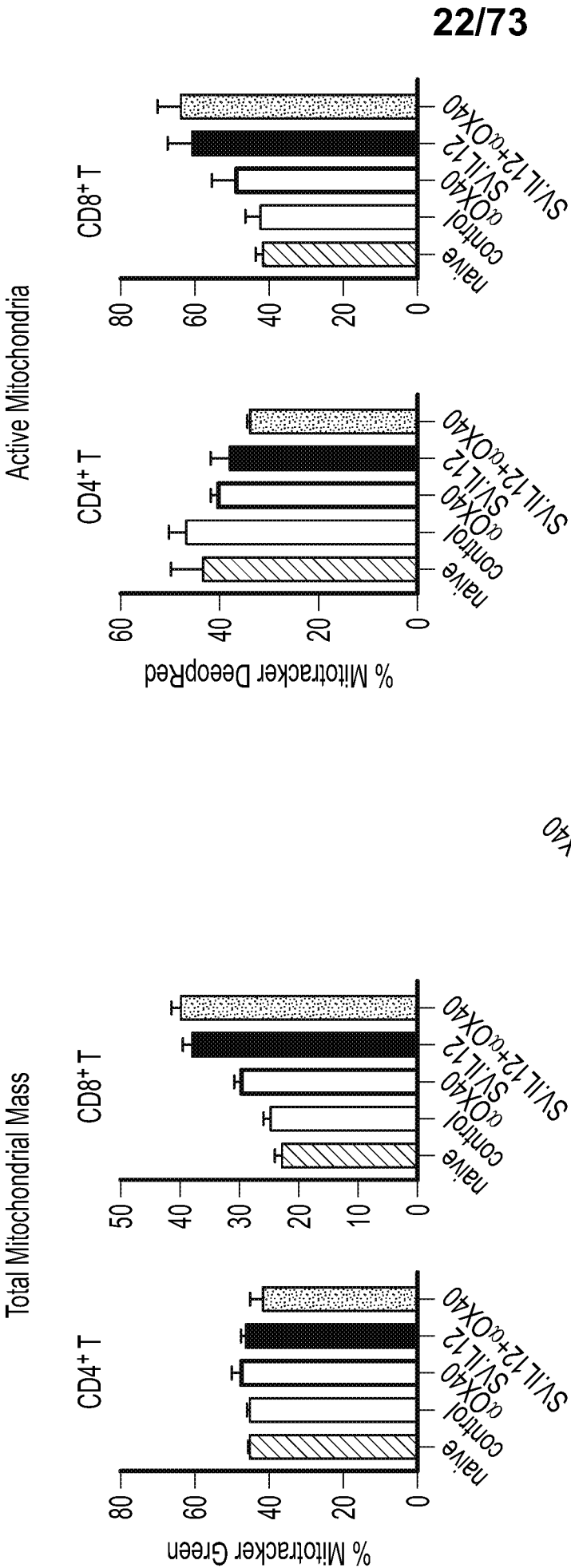


FIG. 11D

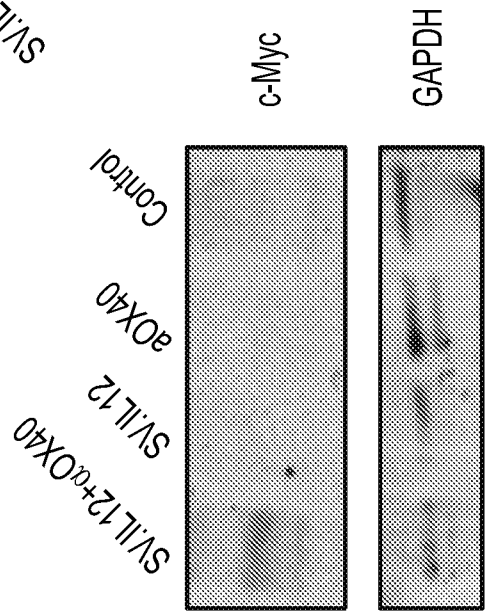


FIG. 11E

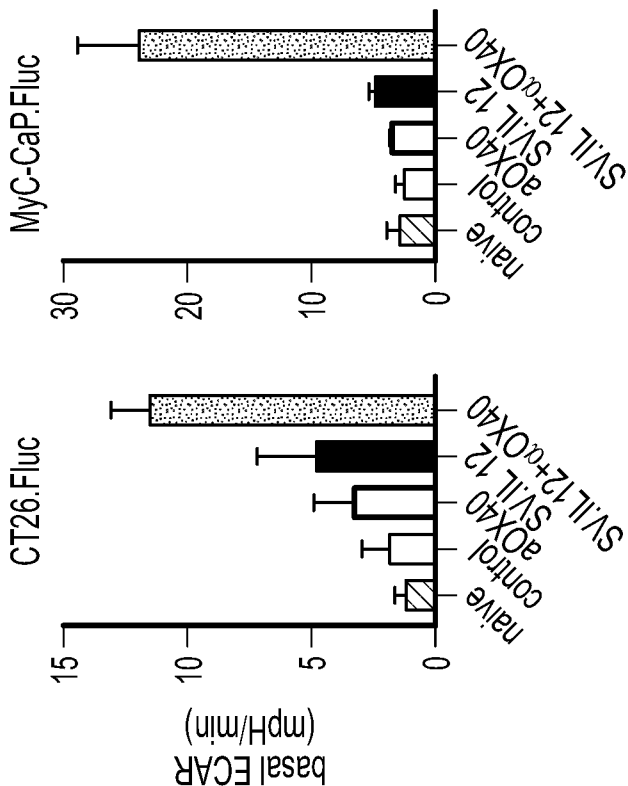


FIG. 11F

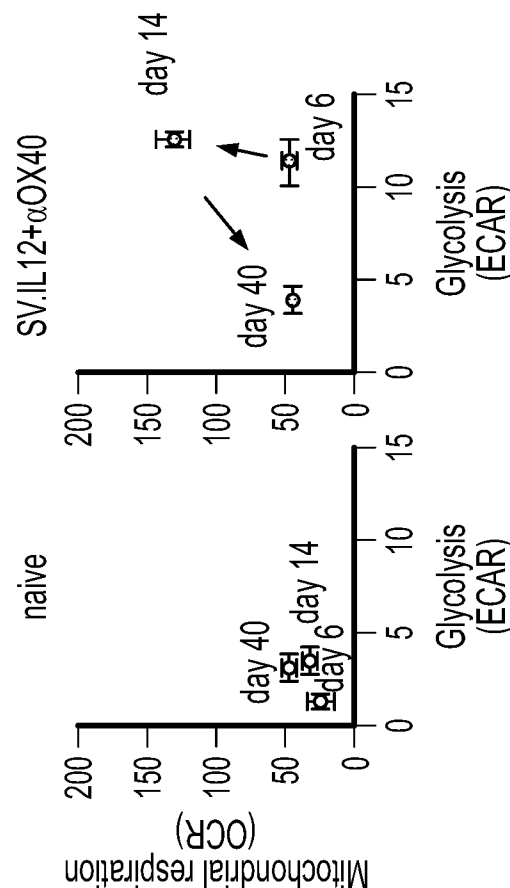


FIG. 11G

24/73

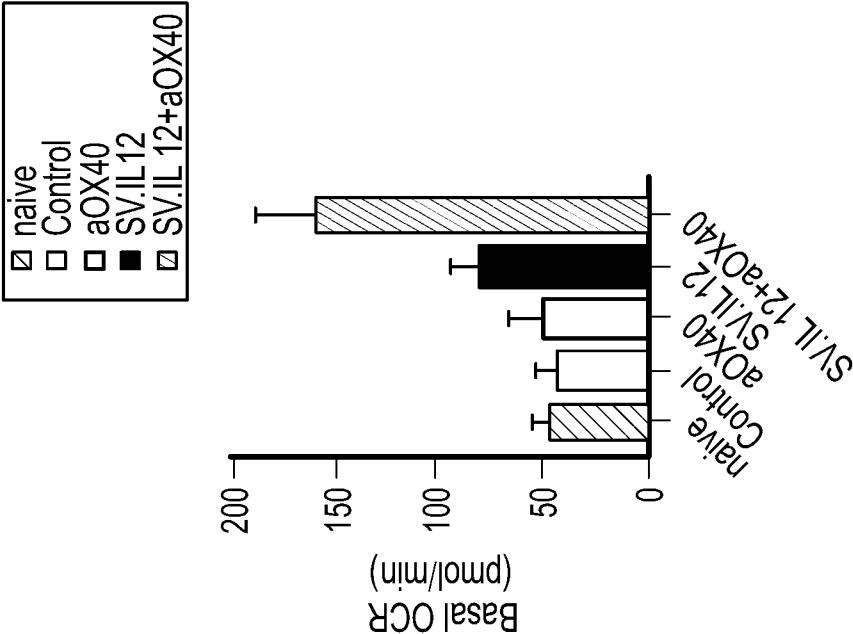


FIG. 12B

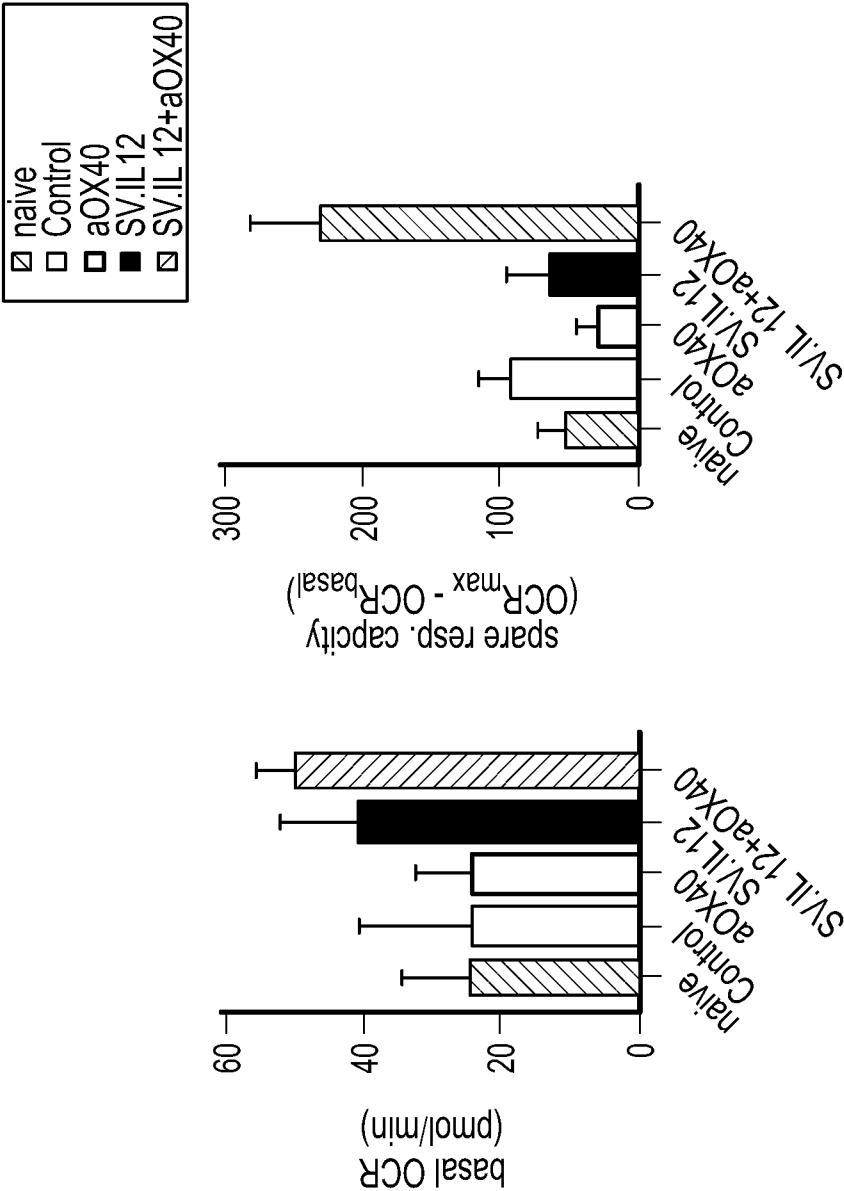


FIG. 12A

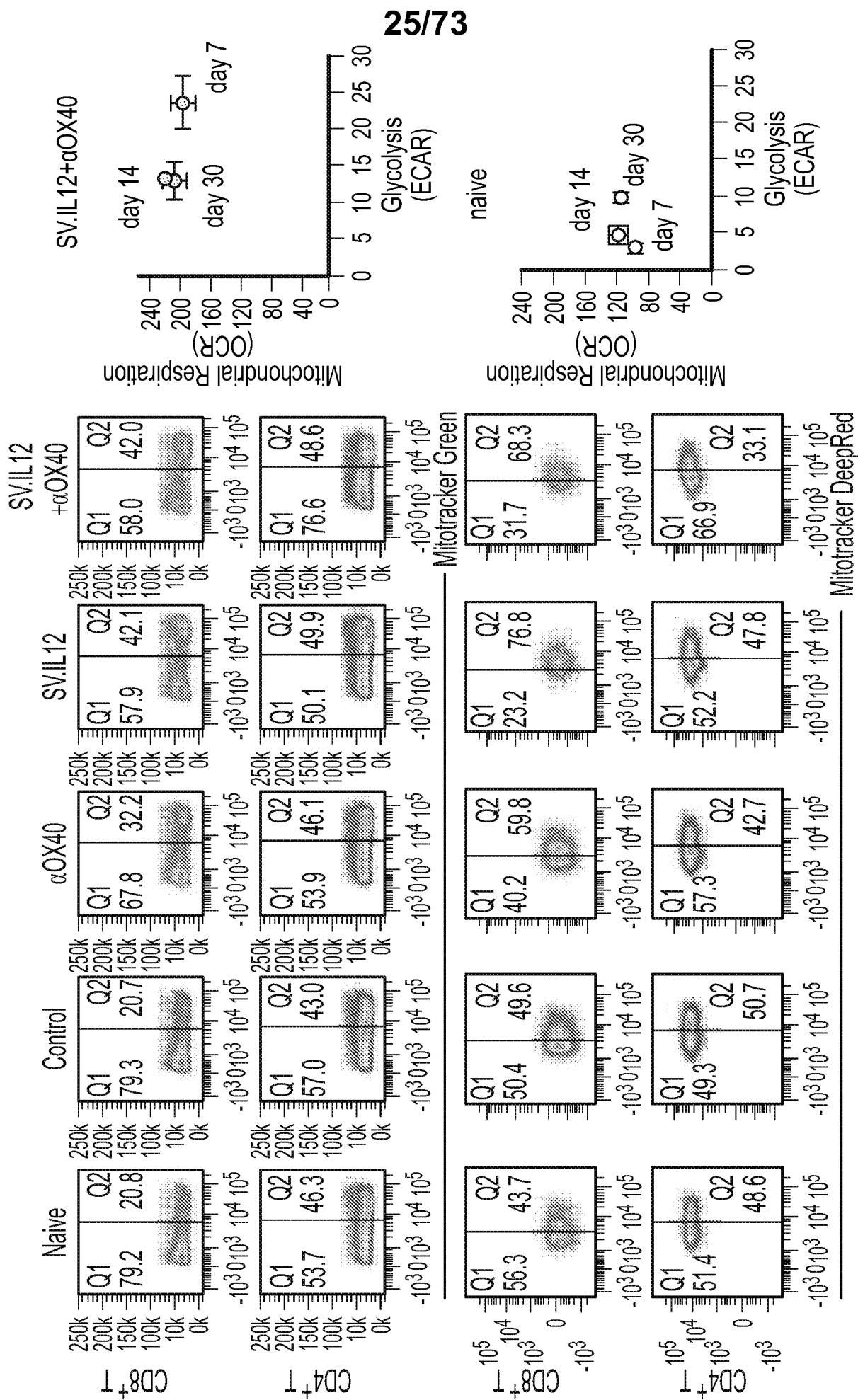
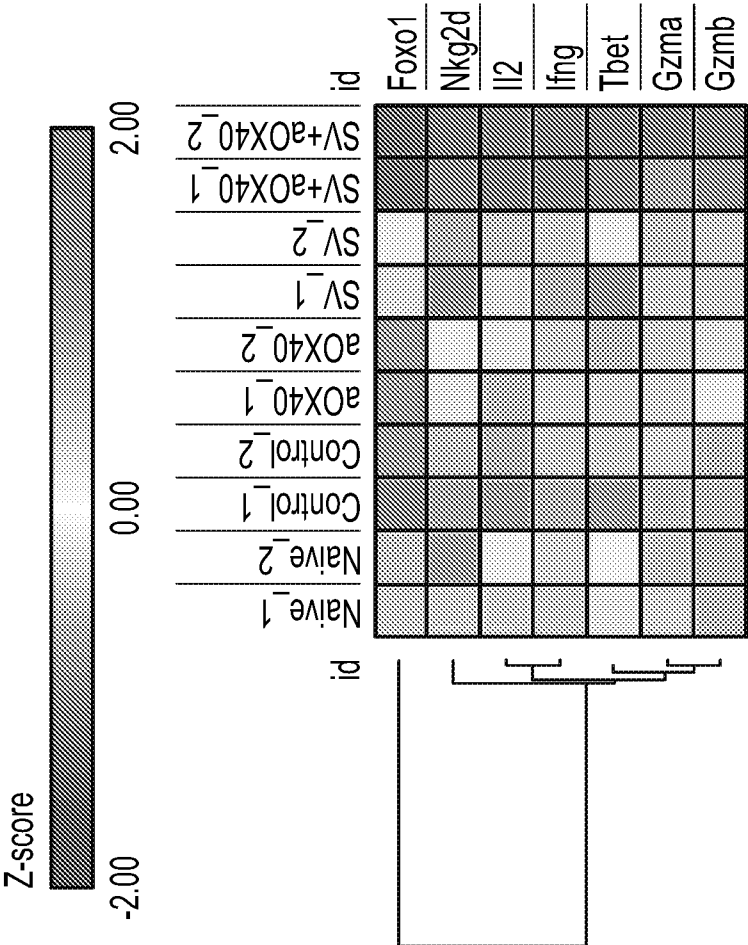
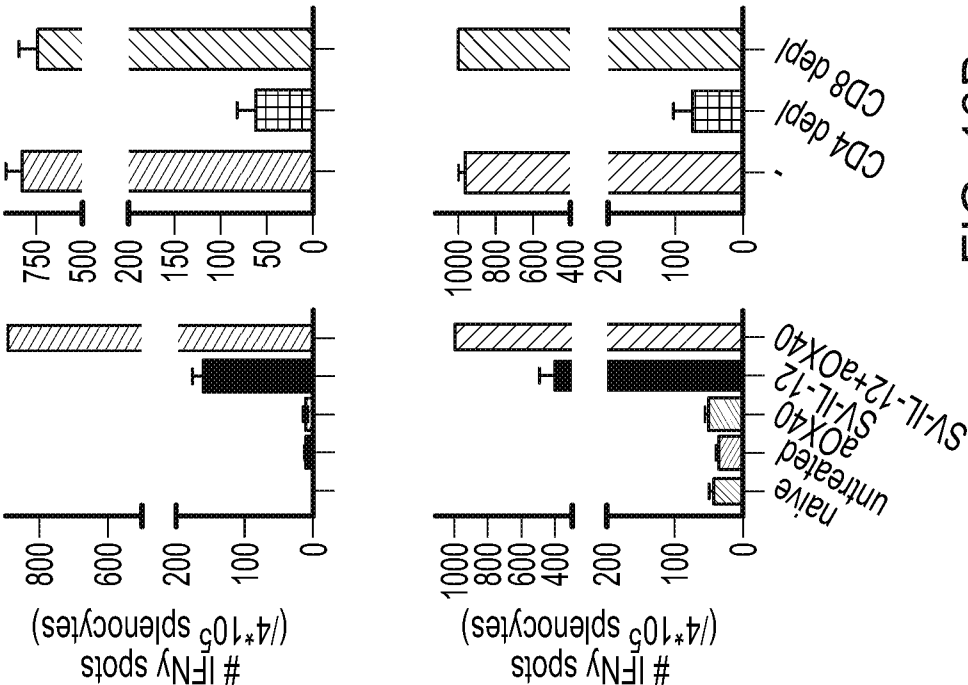


FIG. 12D

FIG. 12C

26/73



27/73

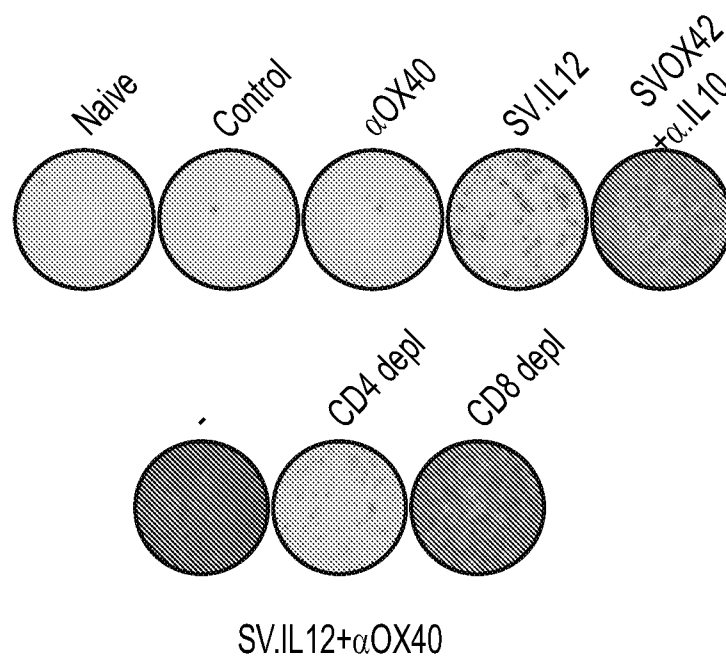
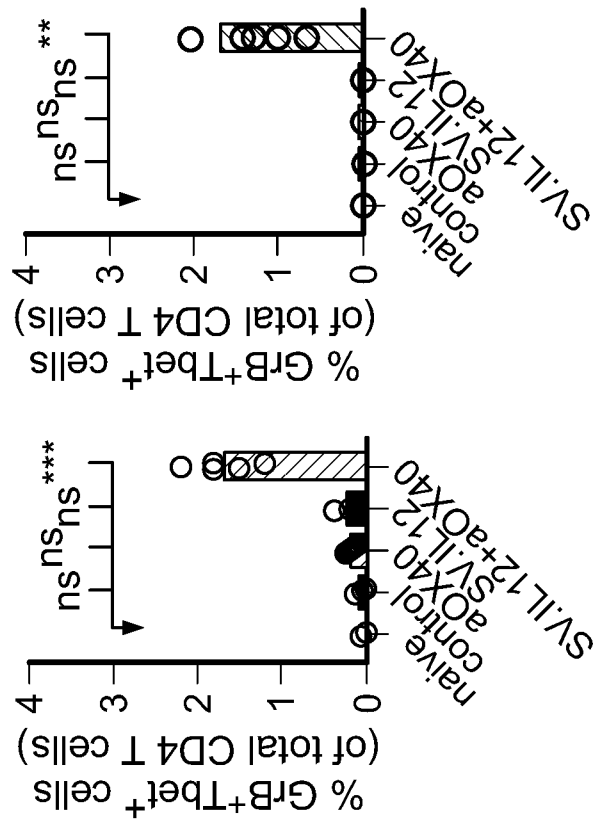
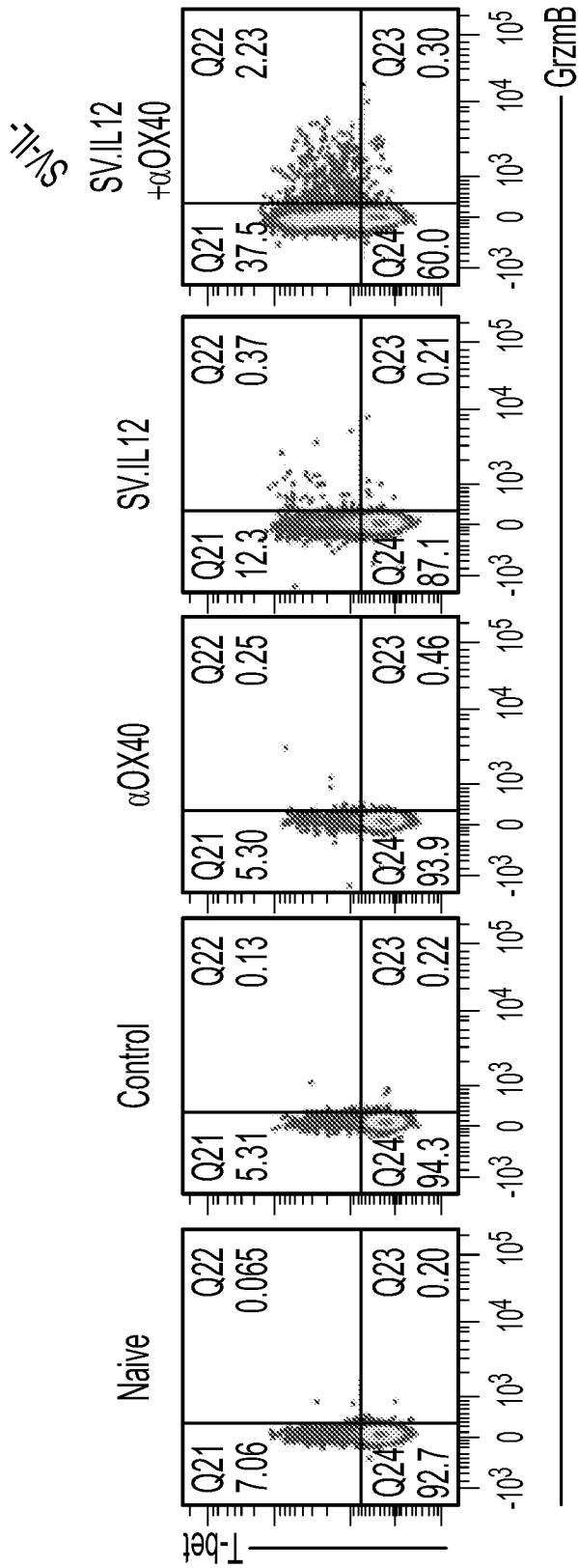


FIG. 13C



29/73

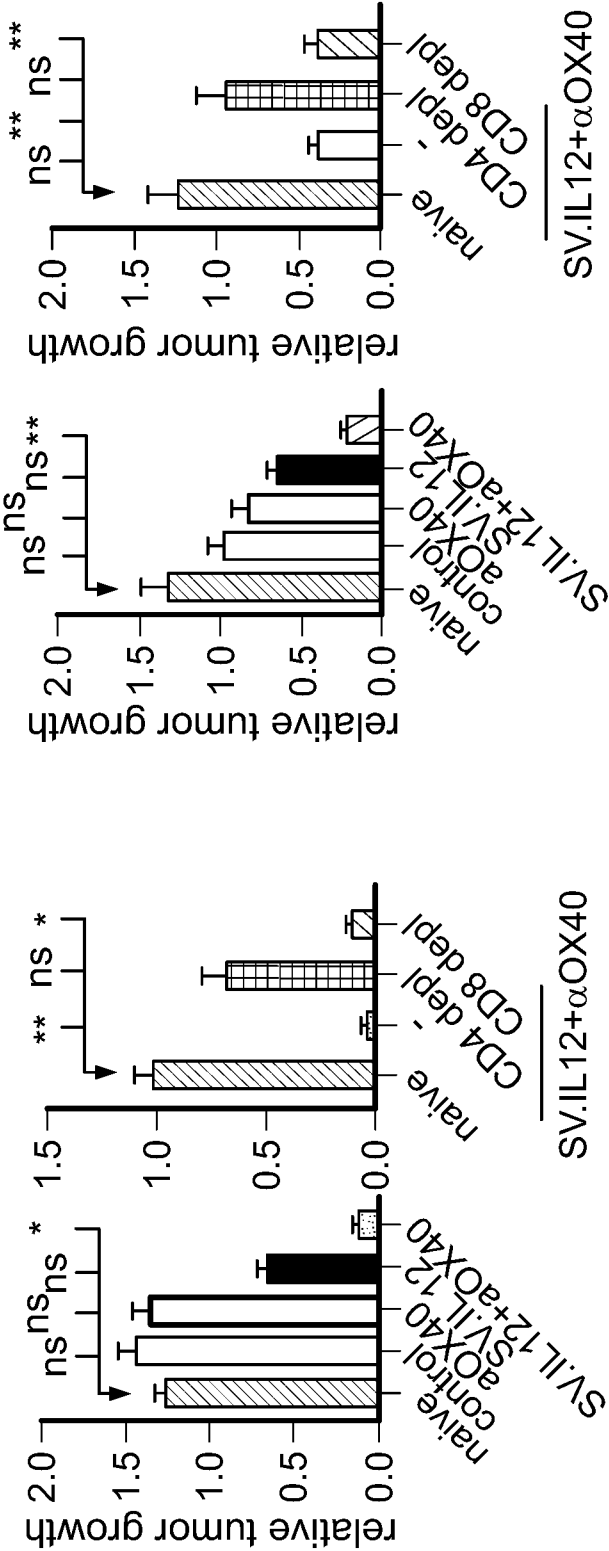
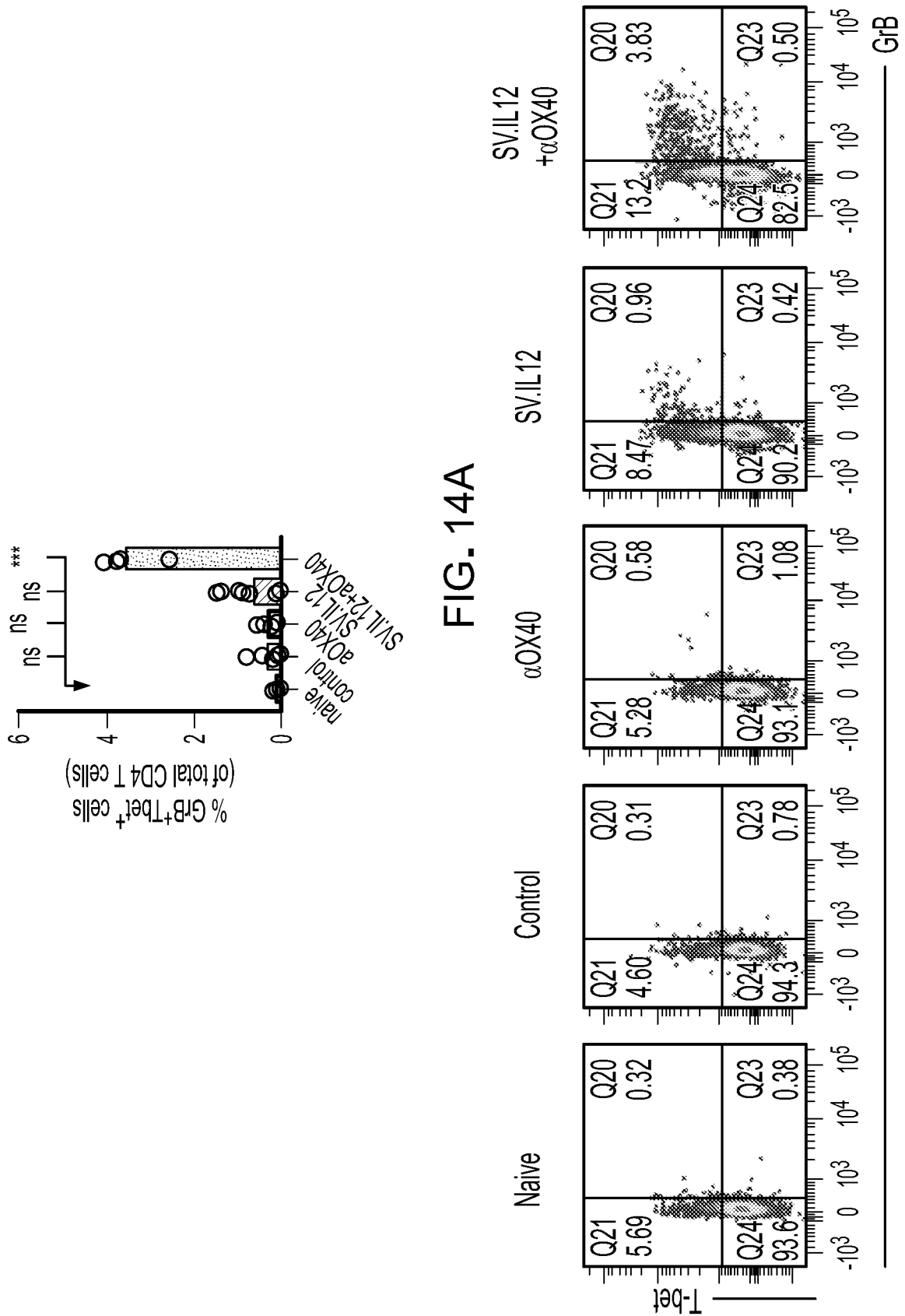


FIG. 13G

FIG. 13F

SUBSTITUTE SHEET (RULE 26)



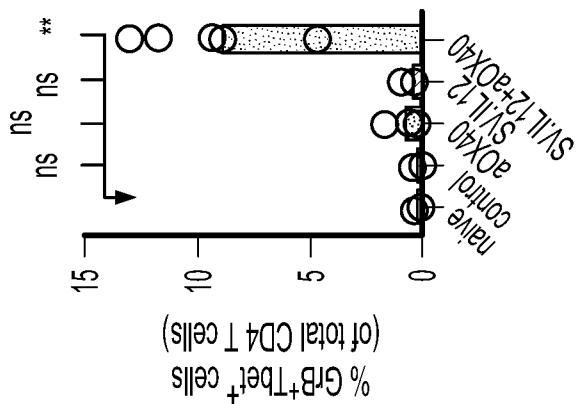


FIG. 14C

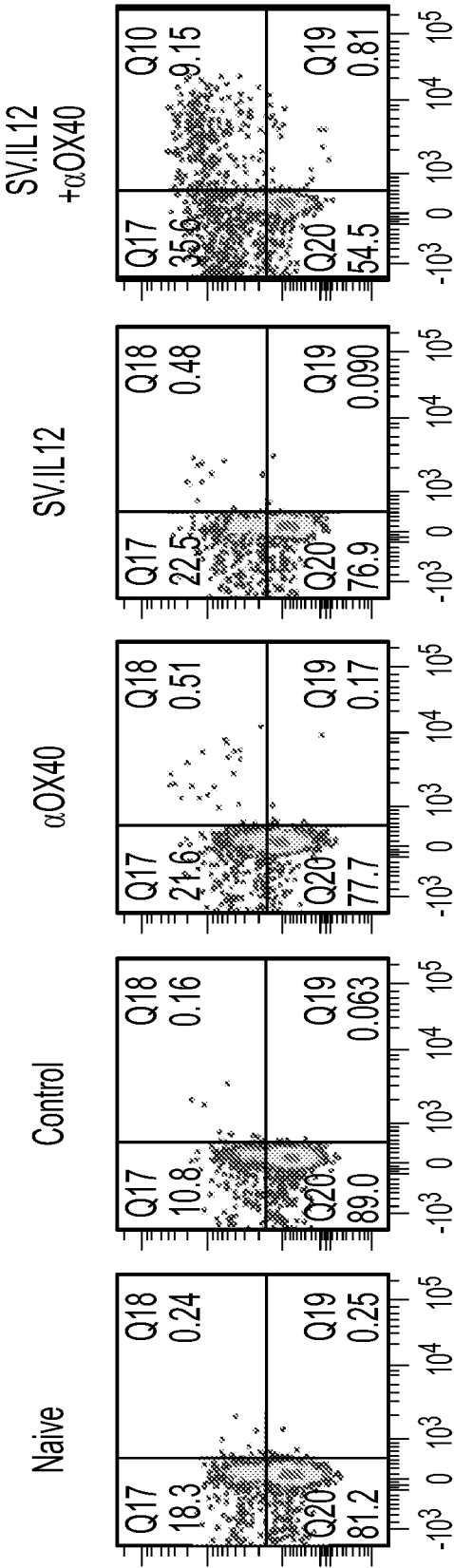


FIG. 14D

32/73

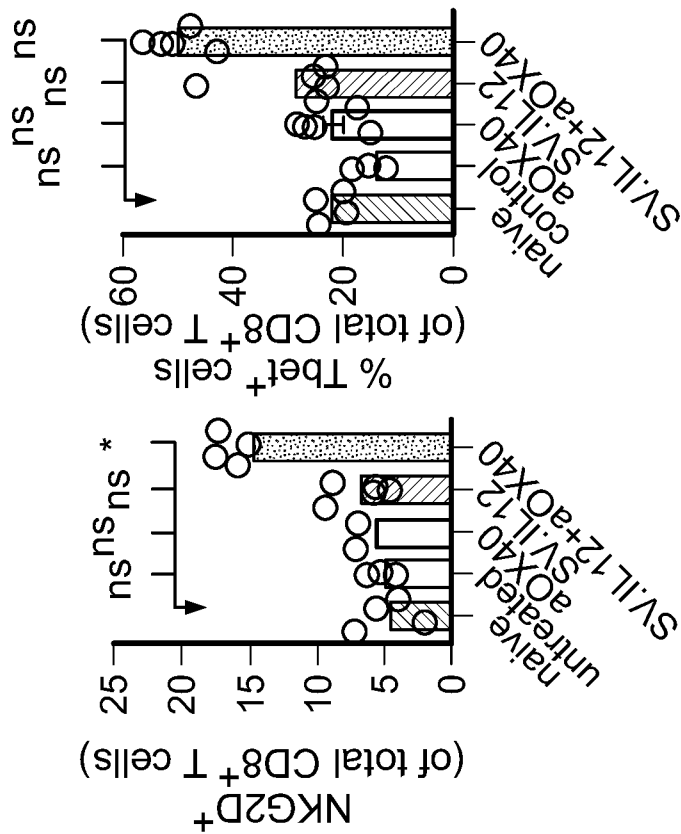


FIG. 14F

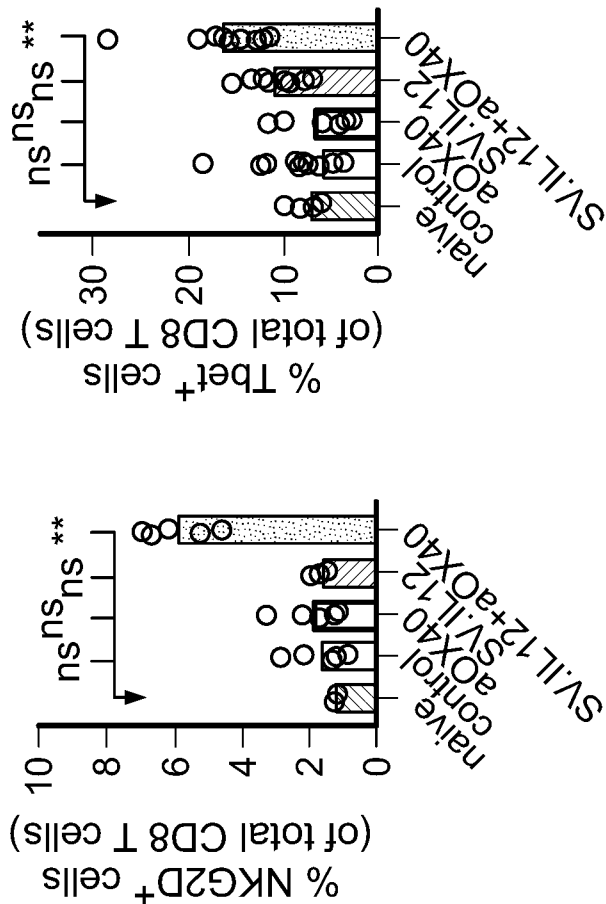
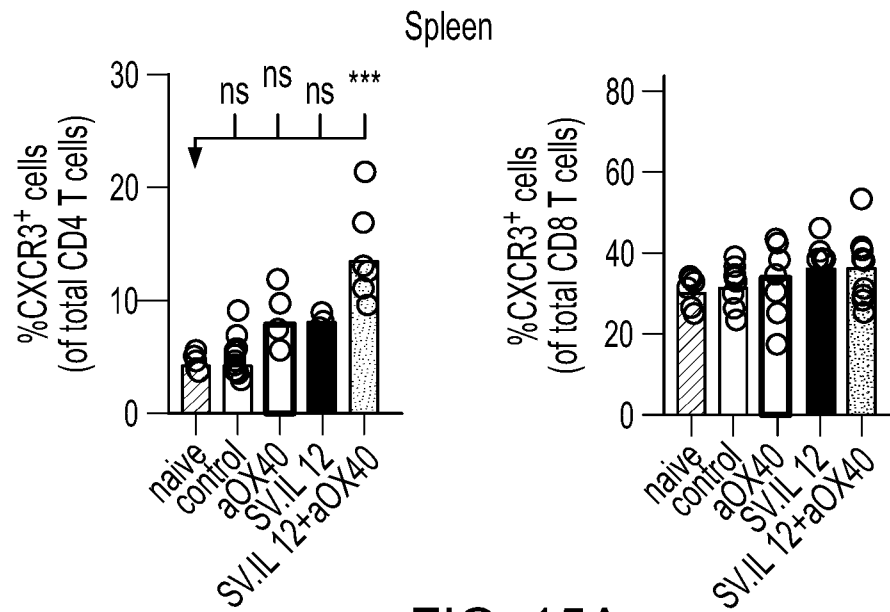
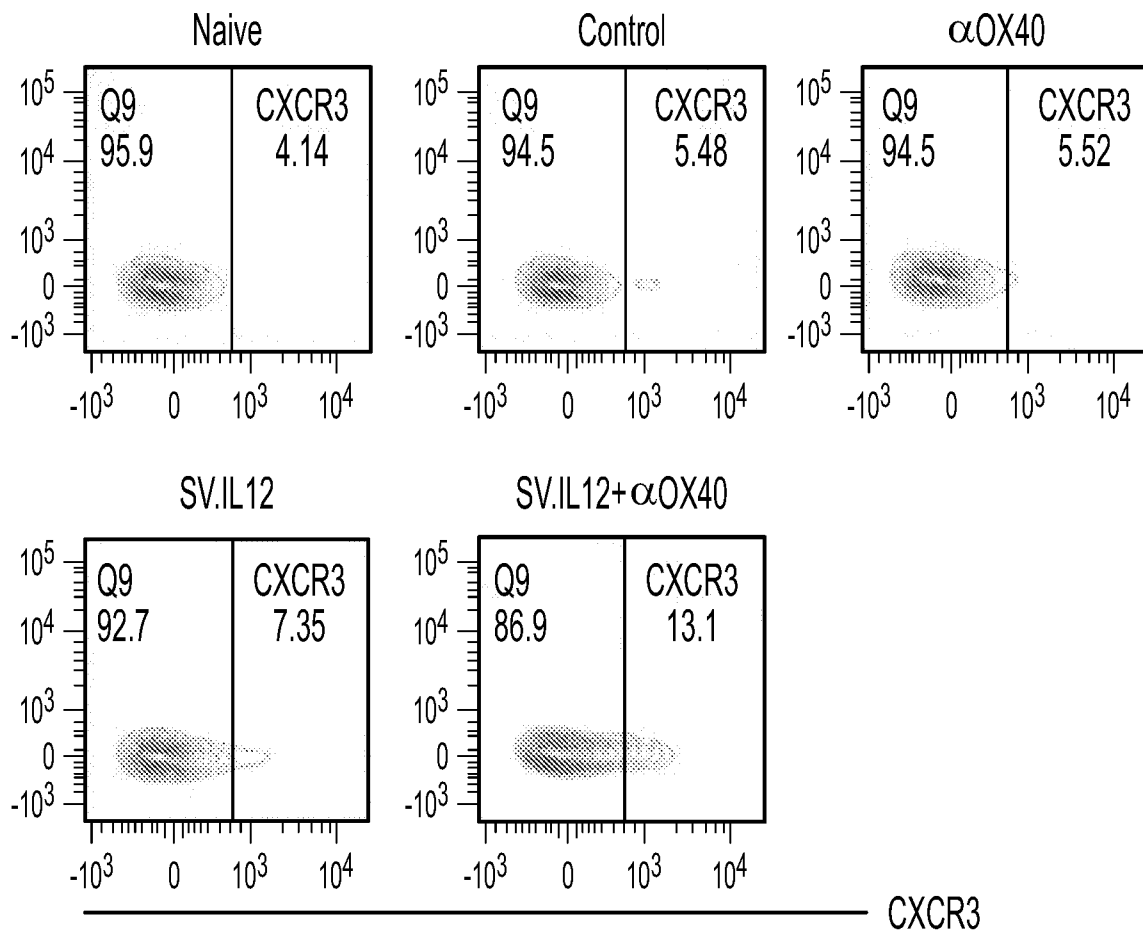
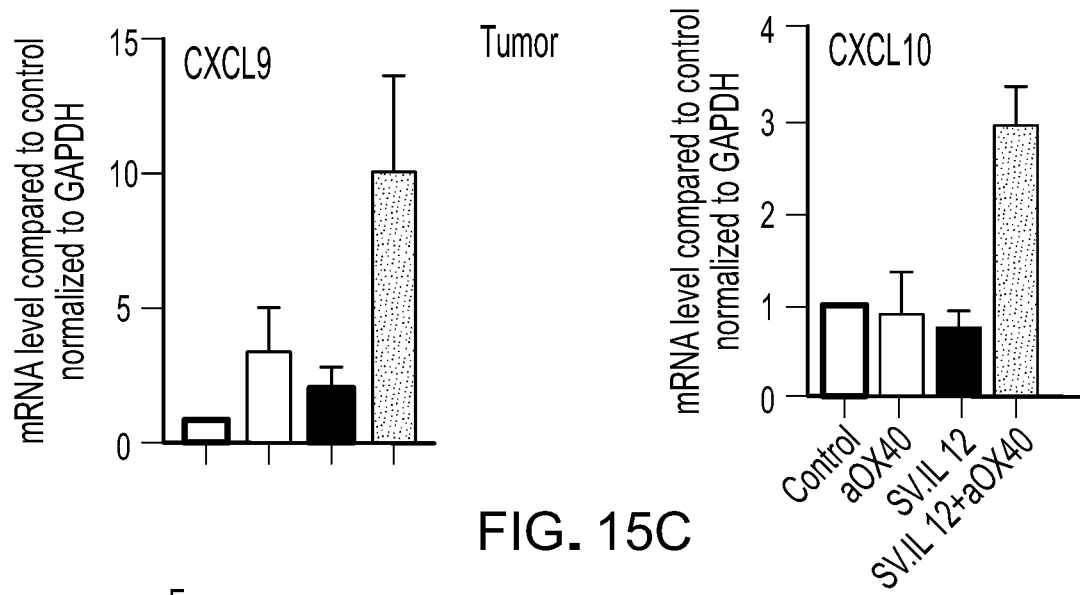
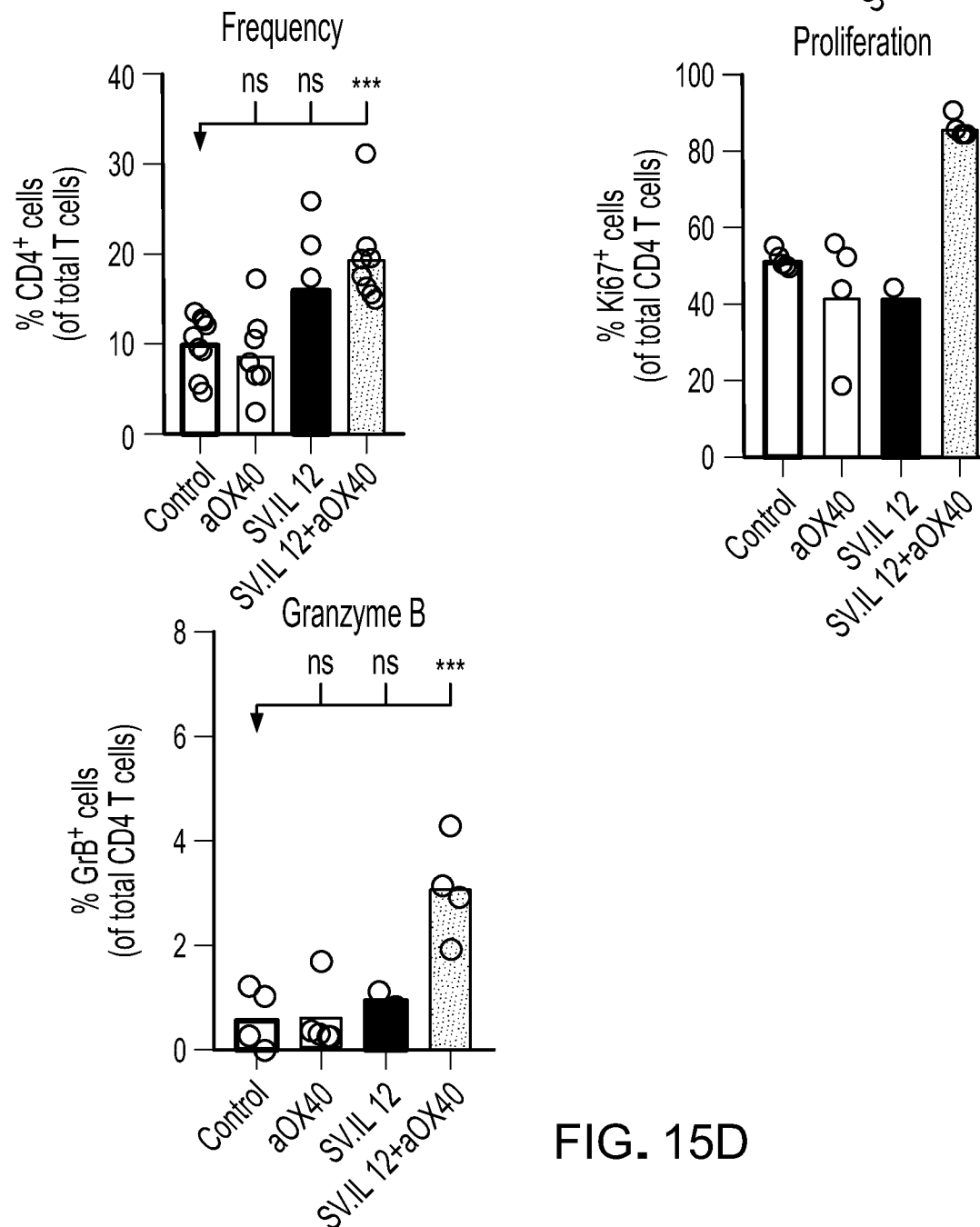


FIG. 14E

33/73**FIG. 15A****FIG. 15B**

34/73**FIG. 15C****FIG. 15D**

35/73

CT26.Fluc

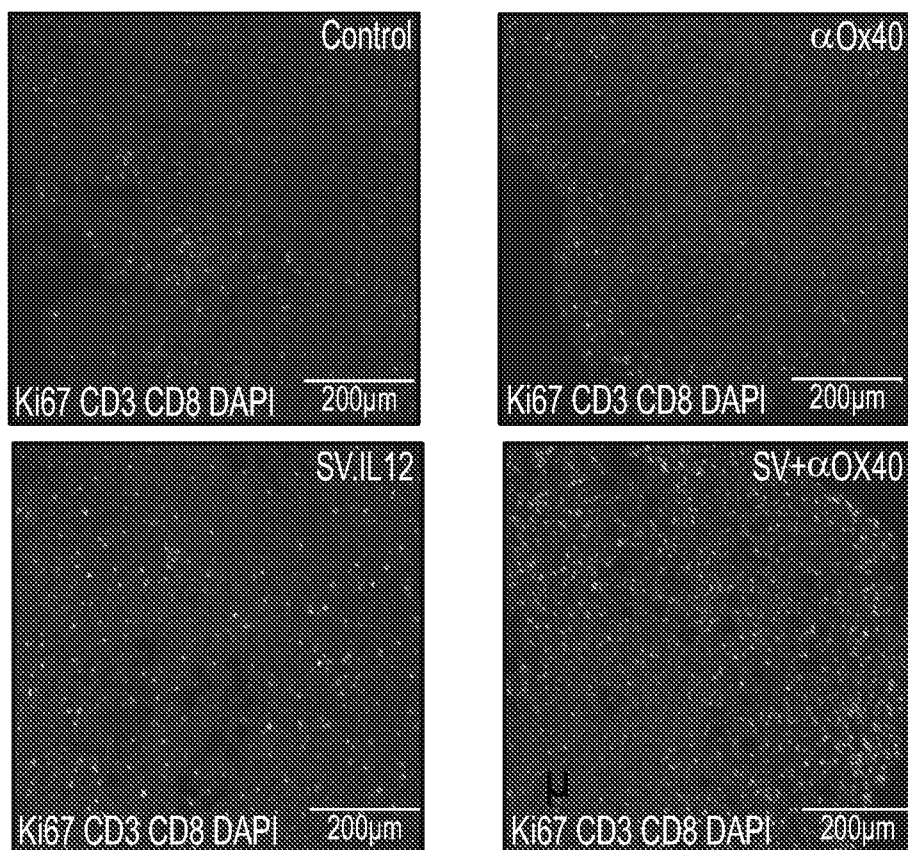


FIG. 15E

MyC-CP.Fluc

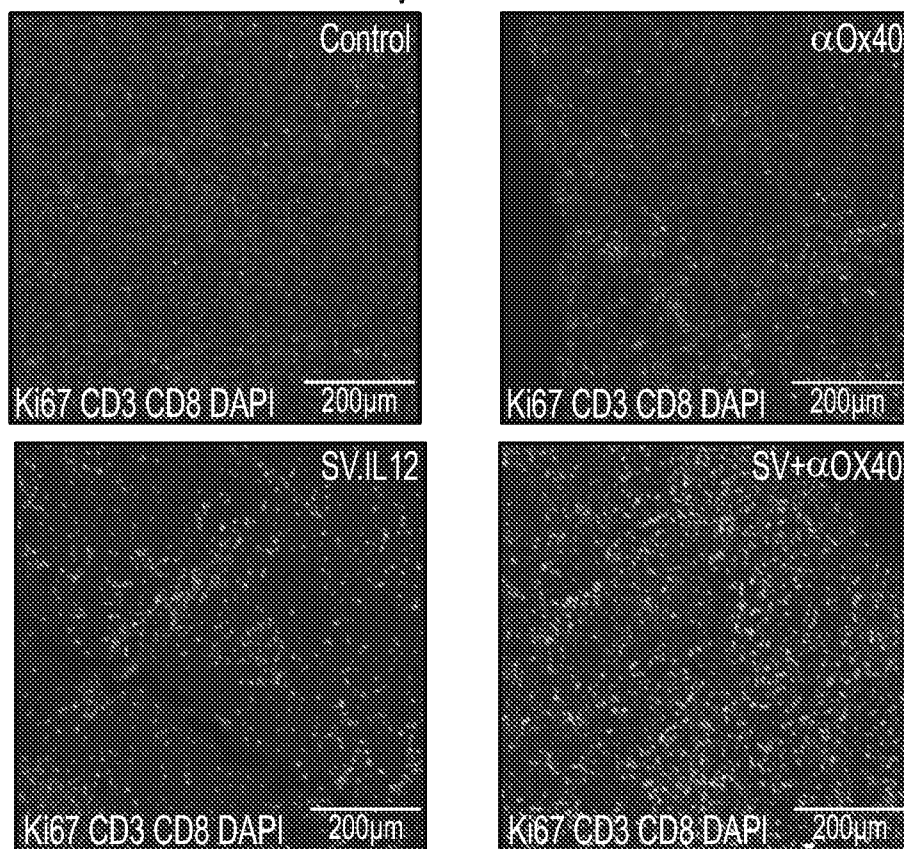
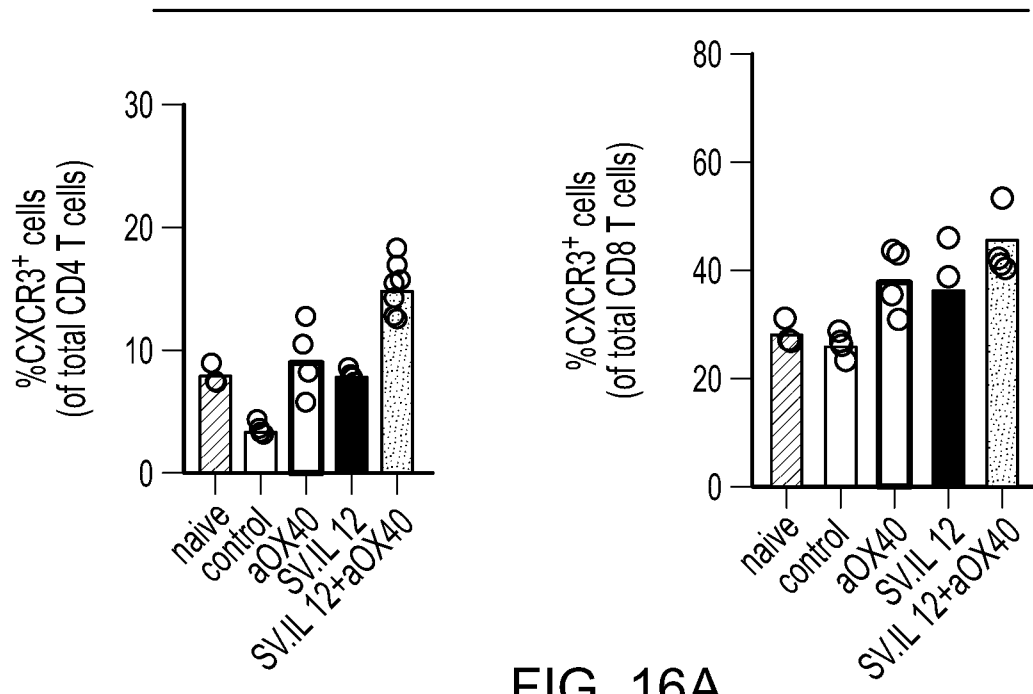


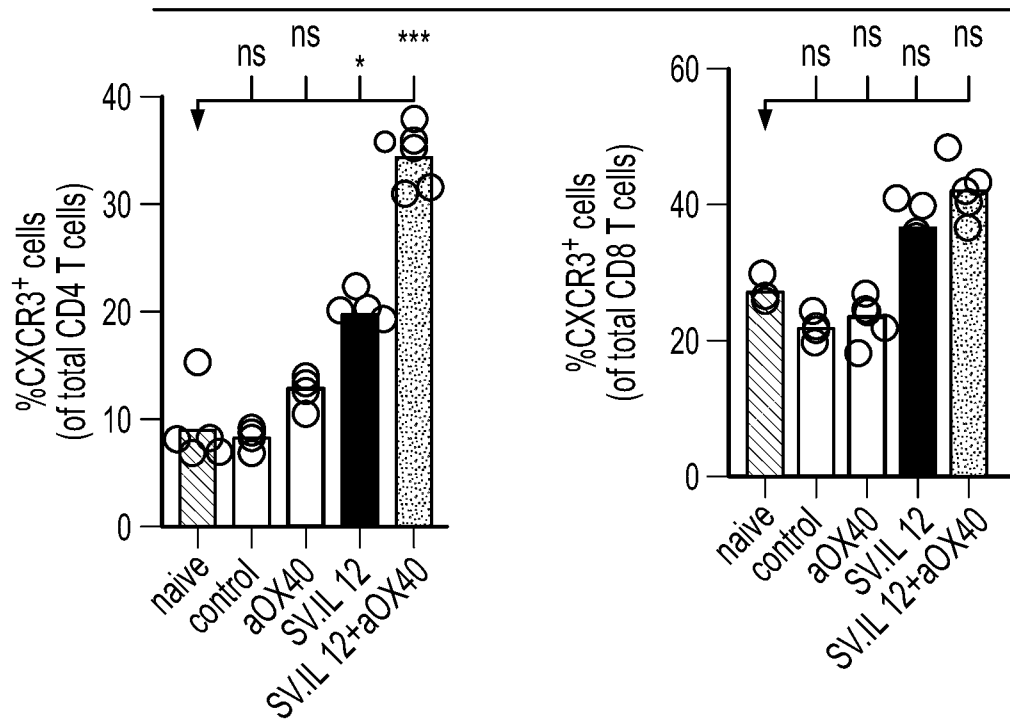
FIG. 15F

36/73

Spleen (Day 14)

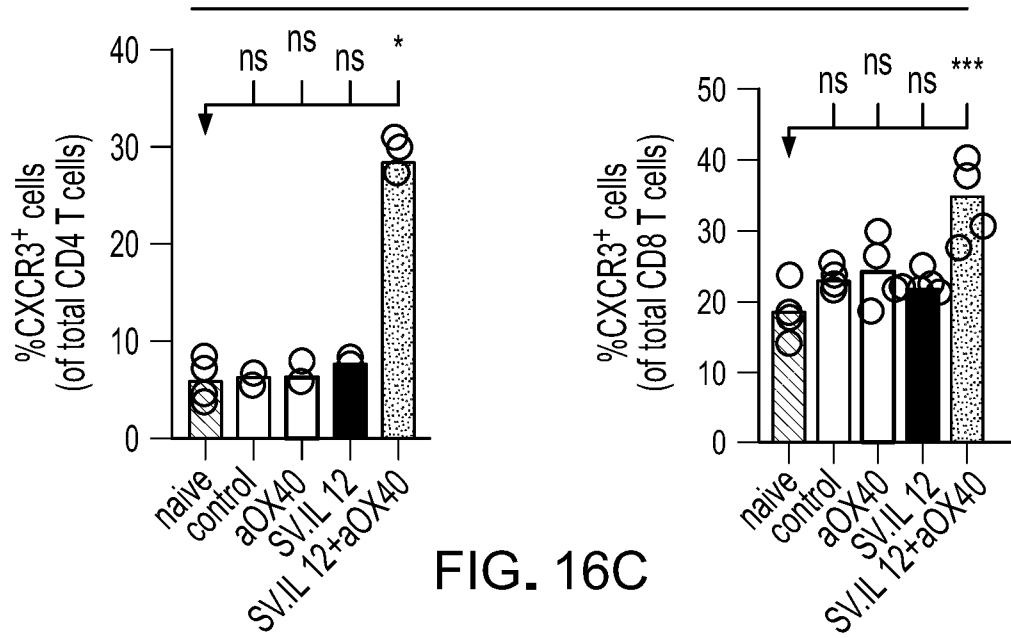
**FIG. 16A**

Spleen (Day 7)

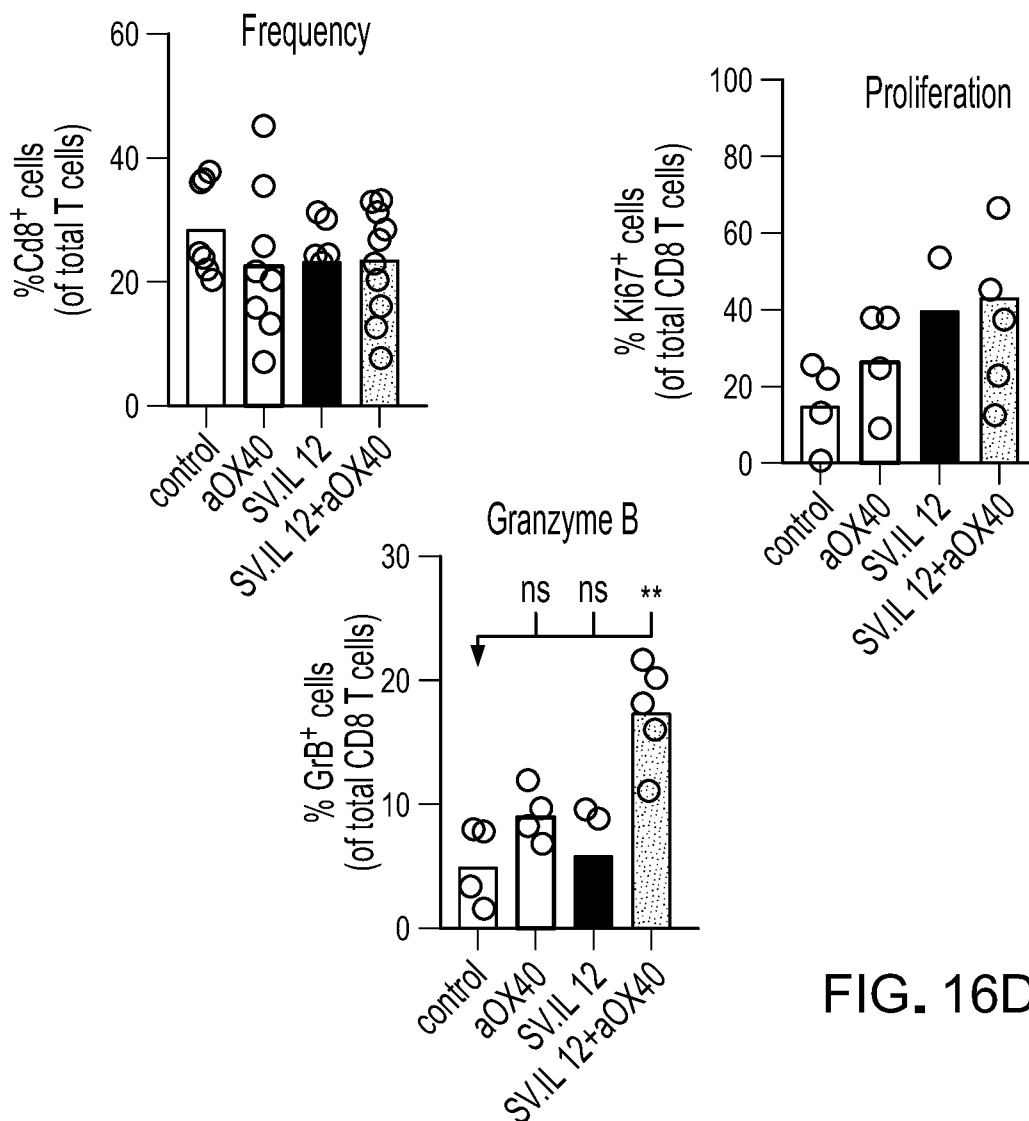
**FIG. 16B**

37/73

Spleen (Day 14)



Tumor (Day 14)



38/73

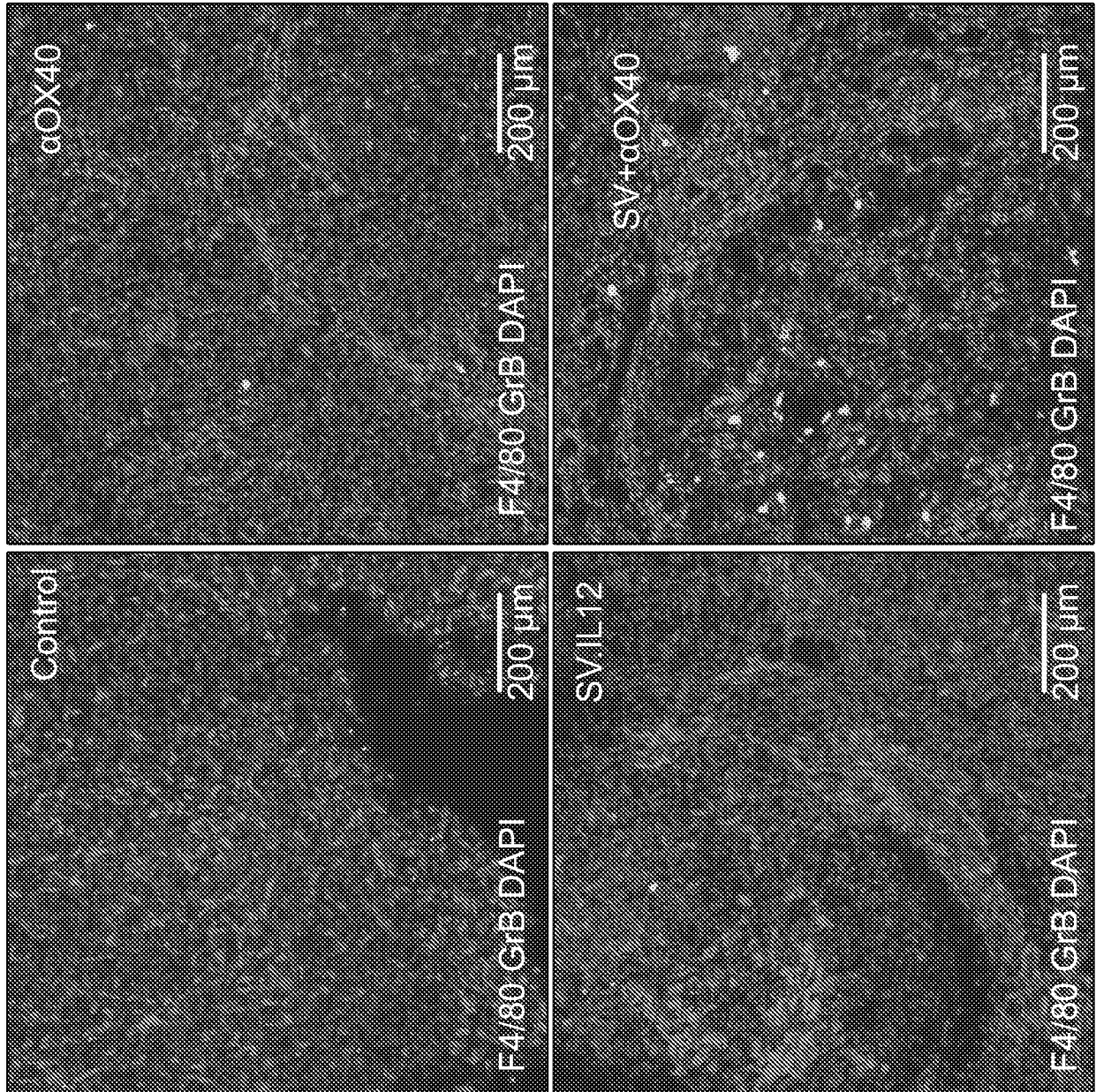


FIG. 17

39/73

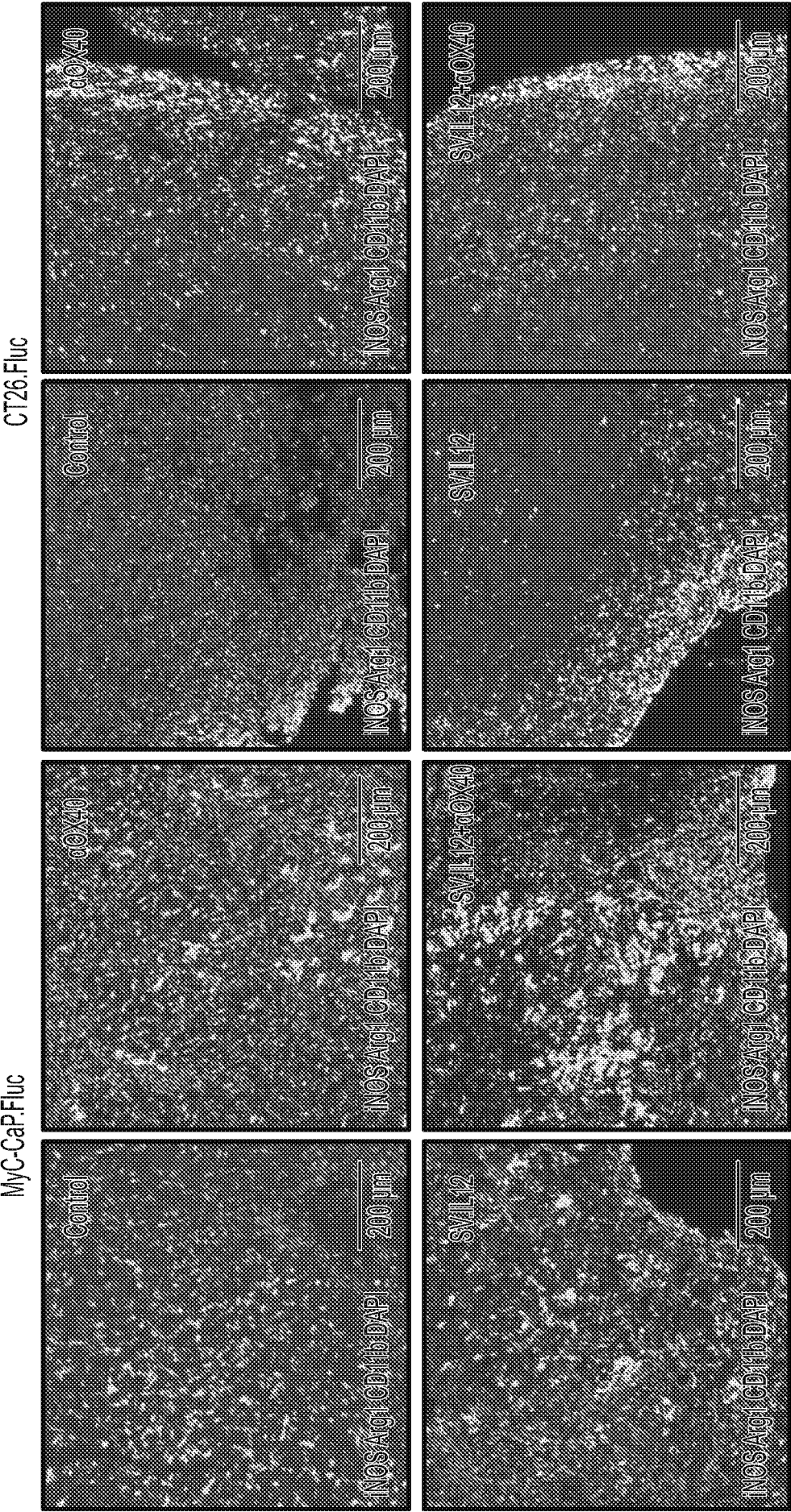


FIG. 18B

FIG. 18A

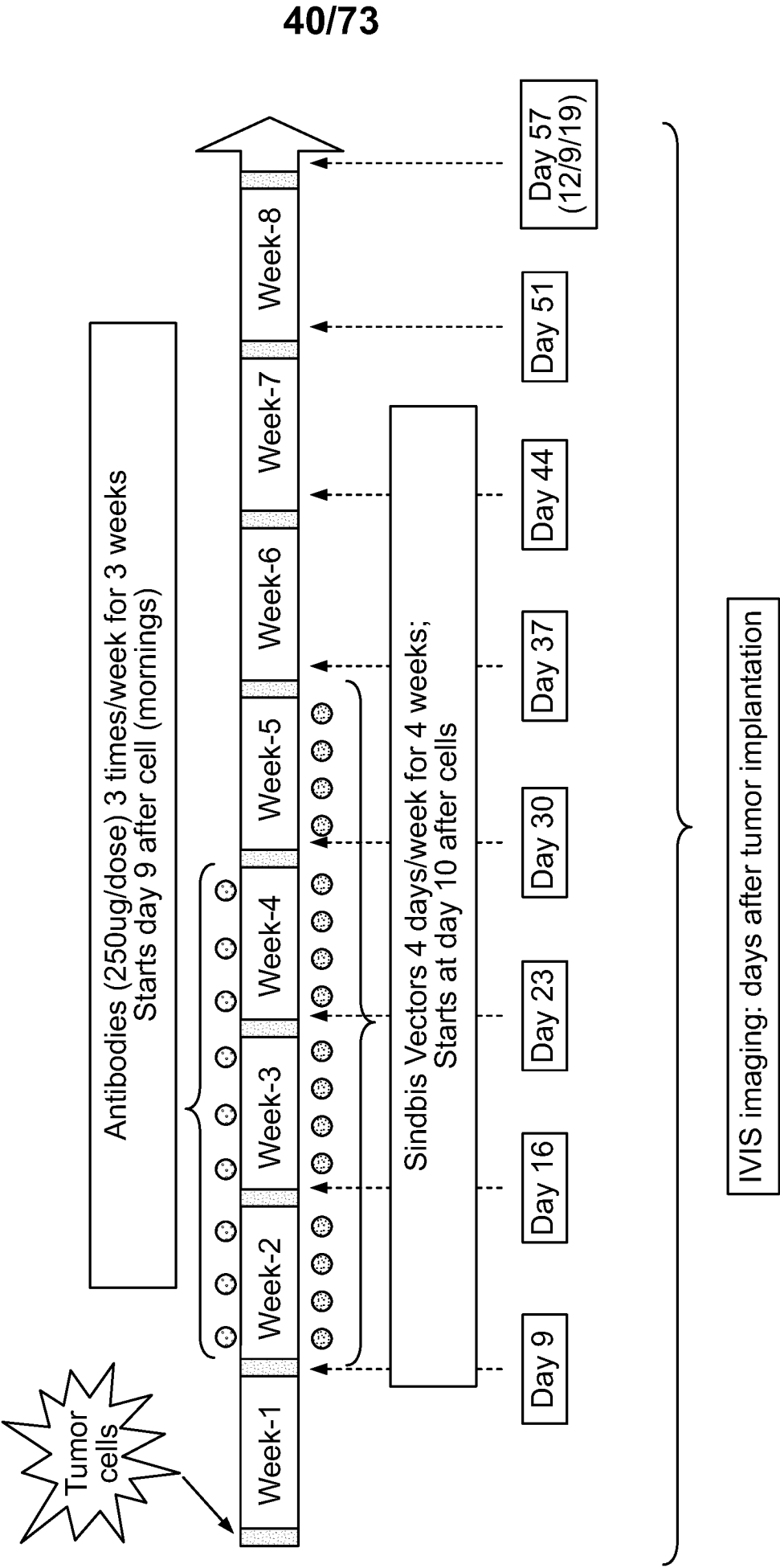


FIG. 19

41/73

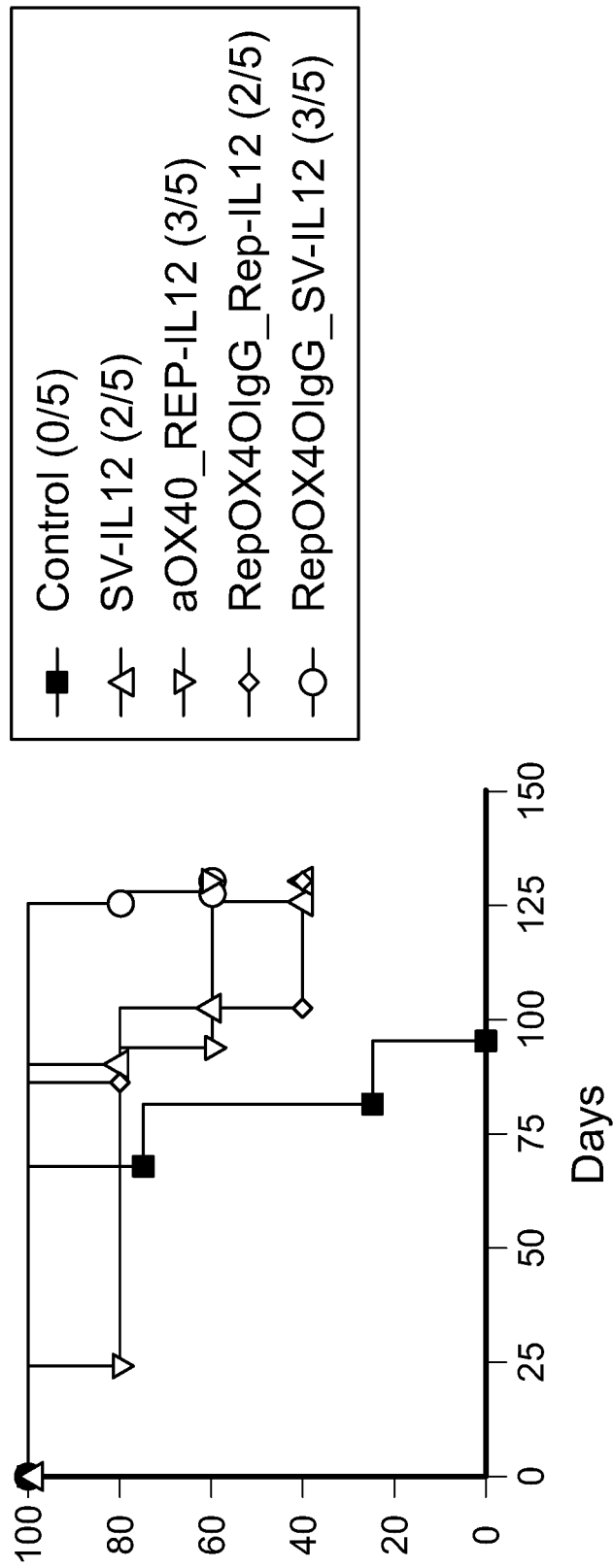
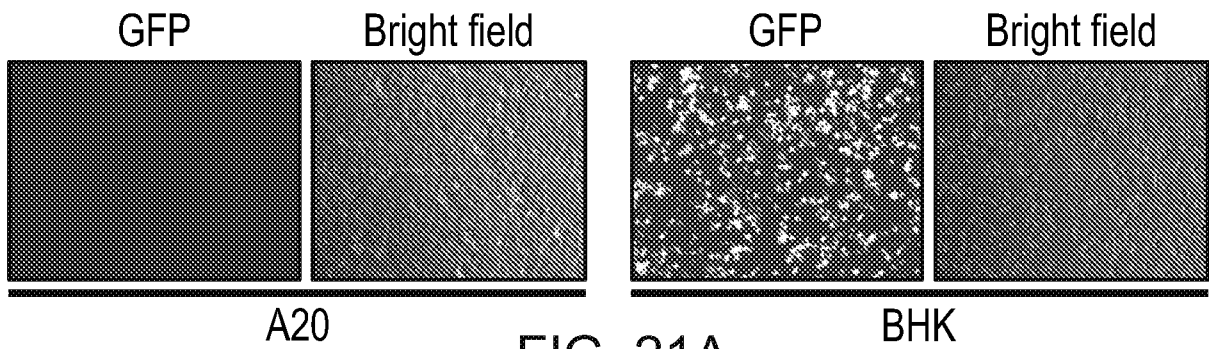
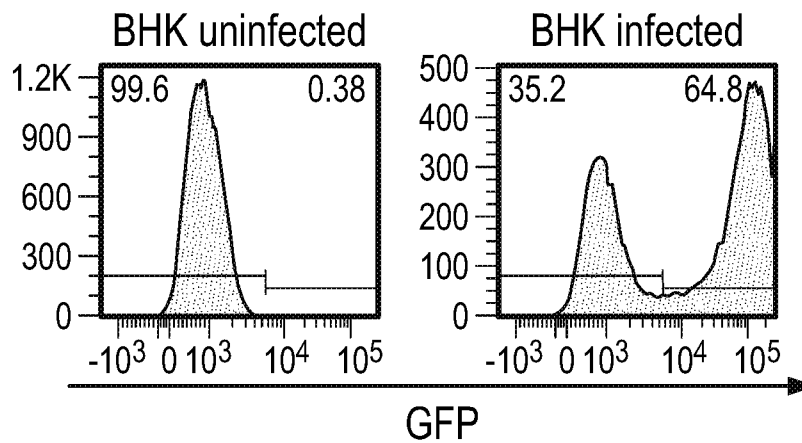
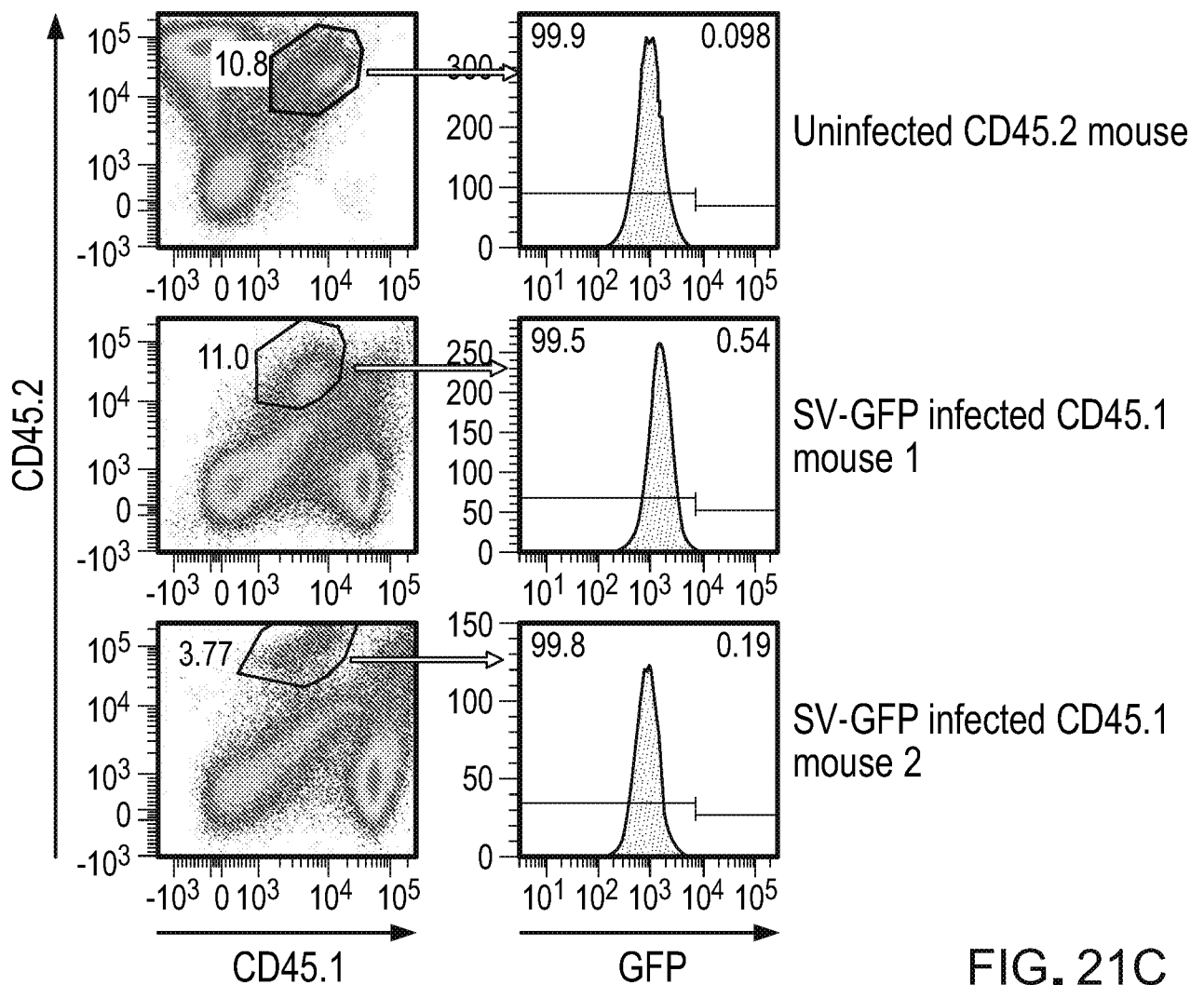


FIG. 20

42/73**FIG. 21A****FIG. 21B****FIG. 21C**

43/73

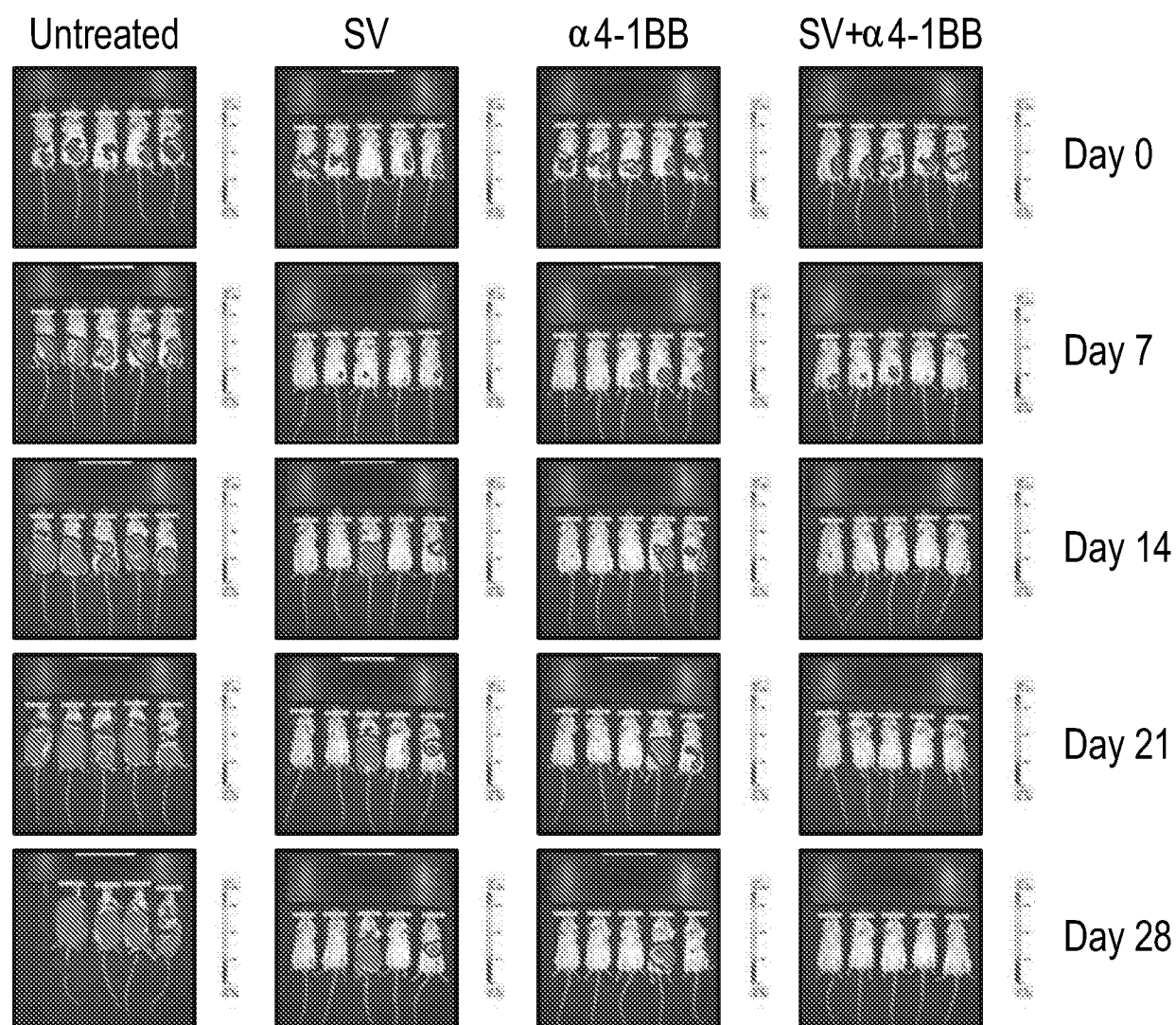


FIG. 22A

44/73

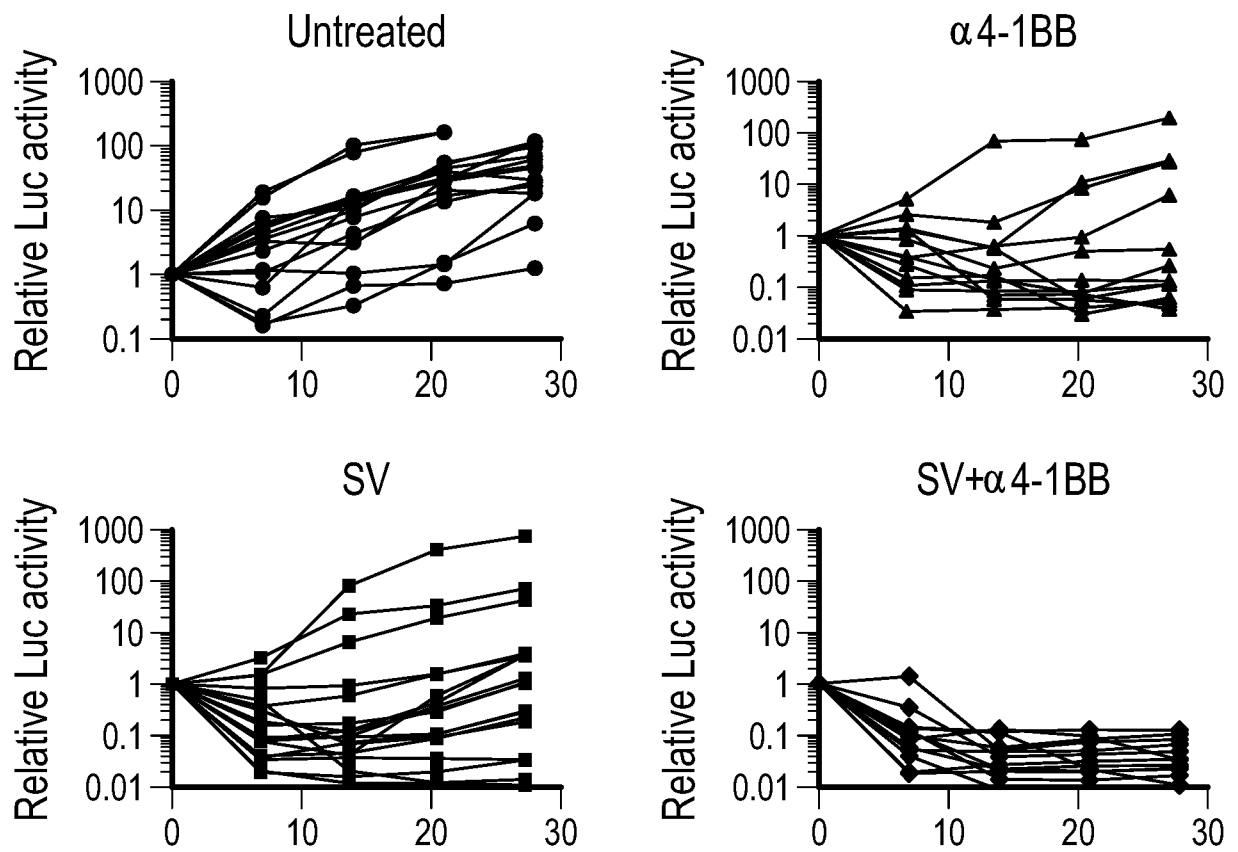


FIG. 22B

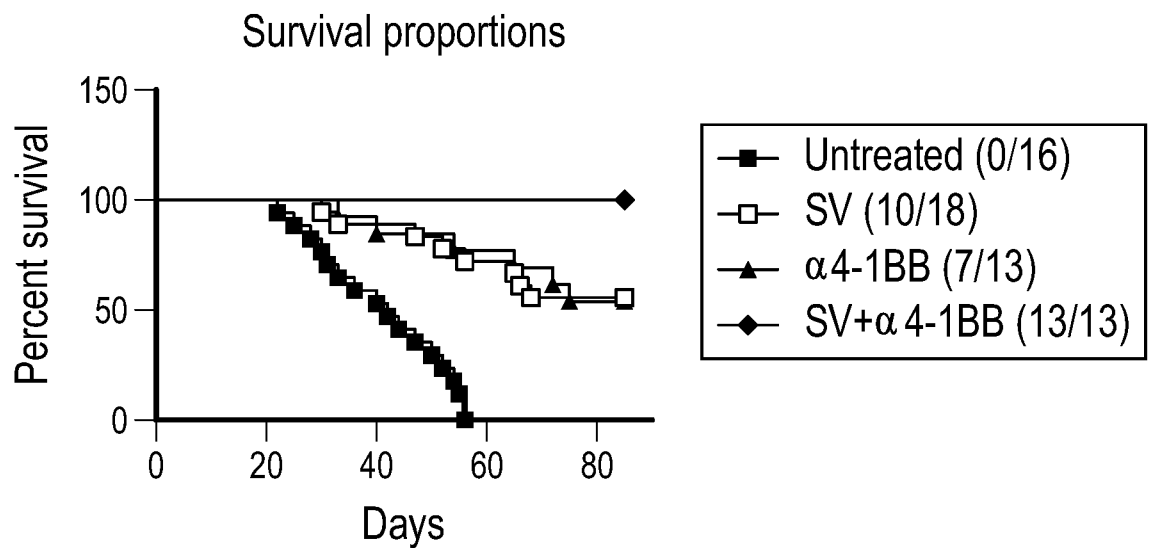
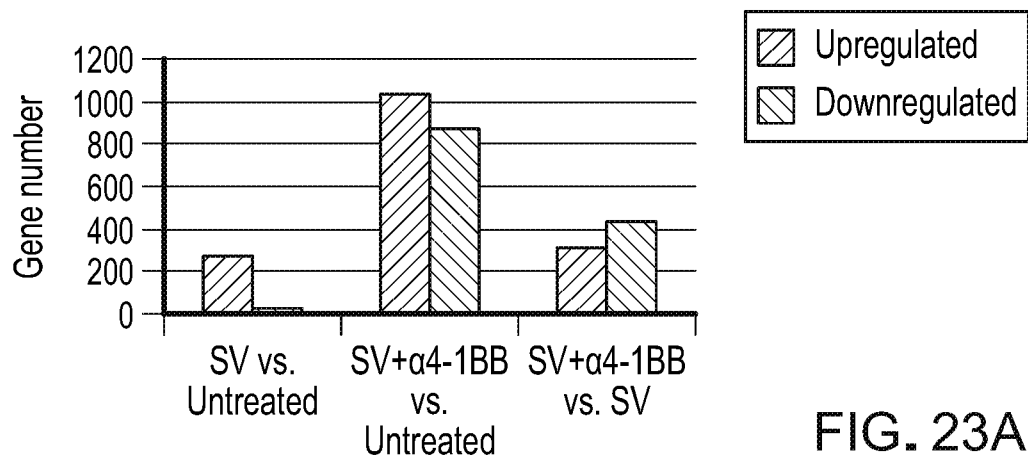
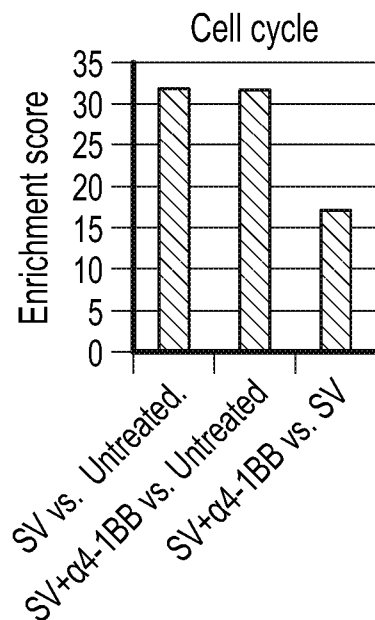
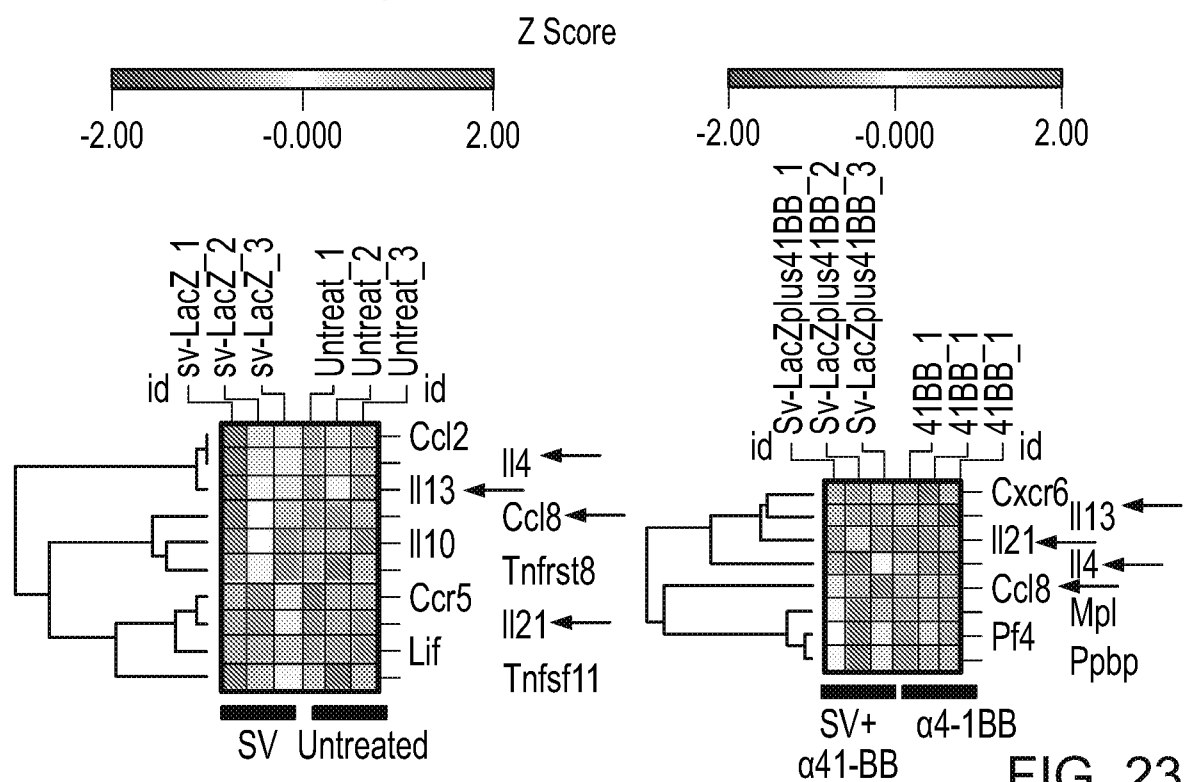


FIG. 22C

45/73**FIG. 23A****FIG. 23B****FIG. 23C**

46/73

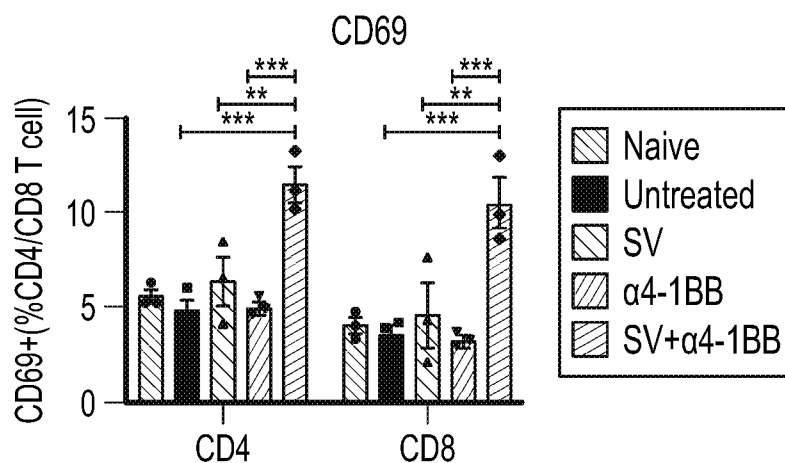
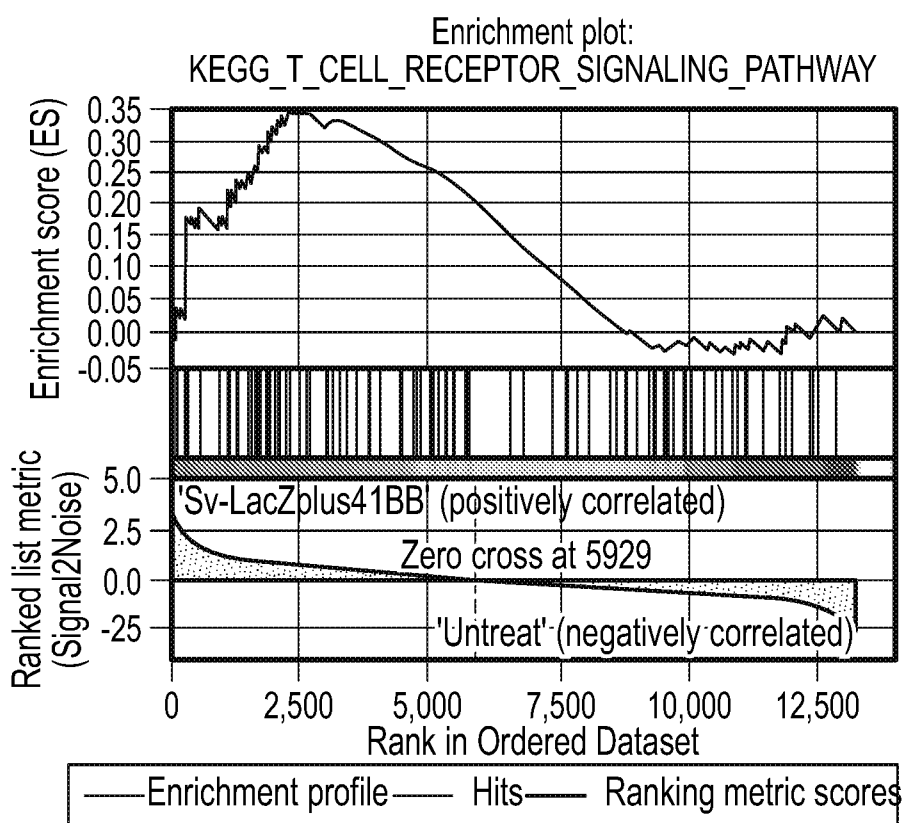


FIG. 23D



Enrichment score (ES): 0.35
 Normalized Enrichment Score (NES)= 1.56
 Nominal p value: 0
 False discovery rate (FDR) q -value: 0.17

FIG. 23E

47/73

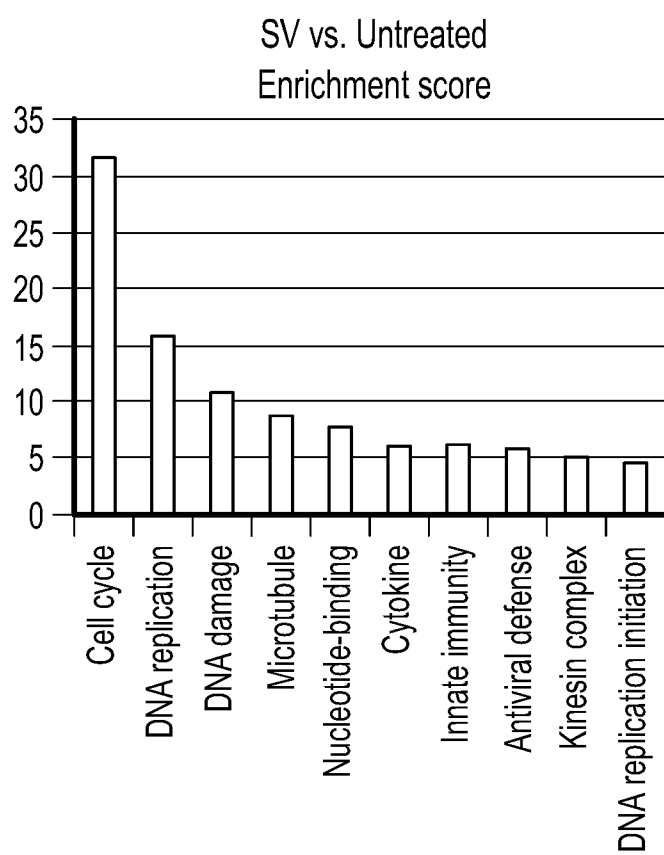


FIG. 24A

48/73

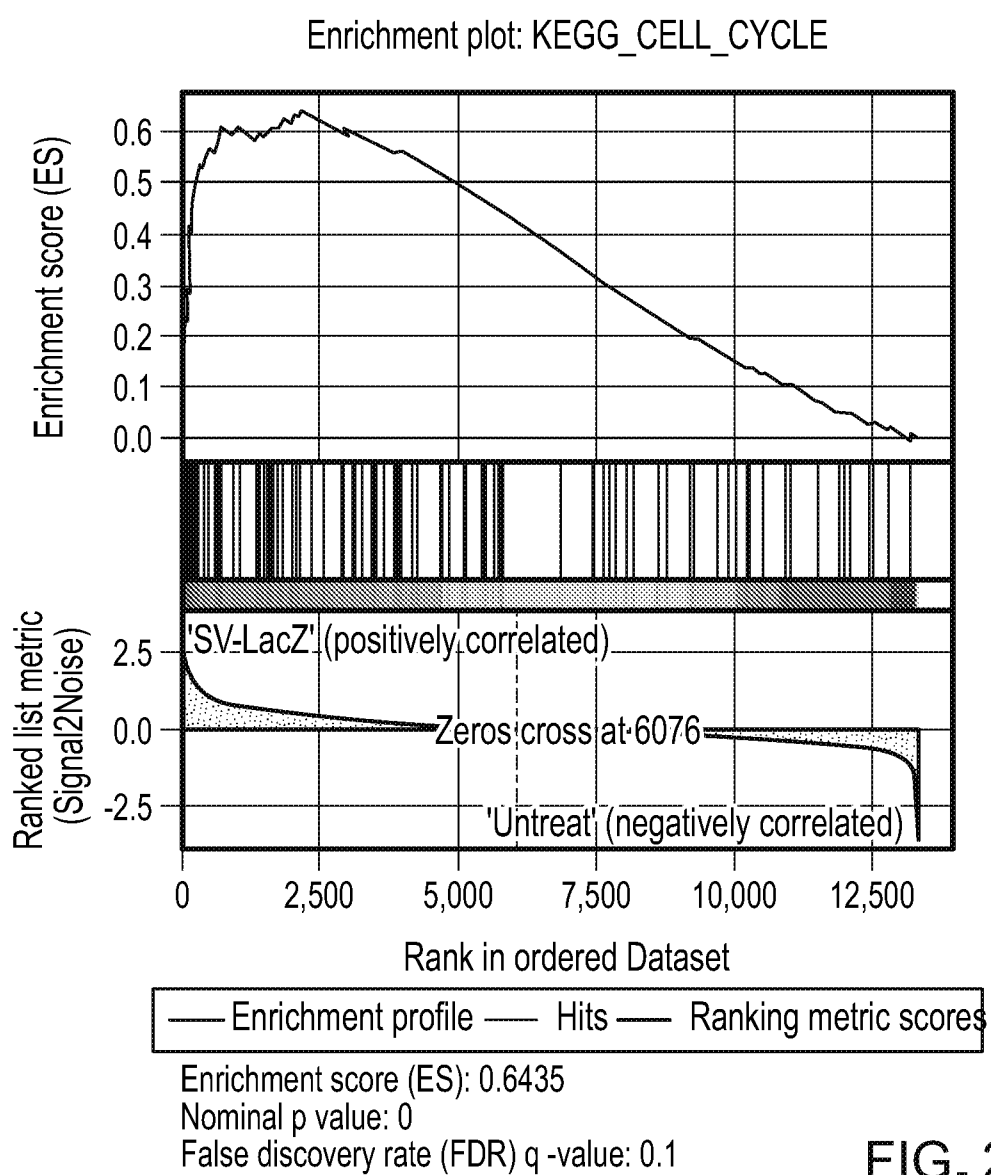
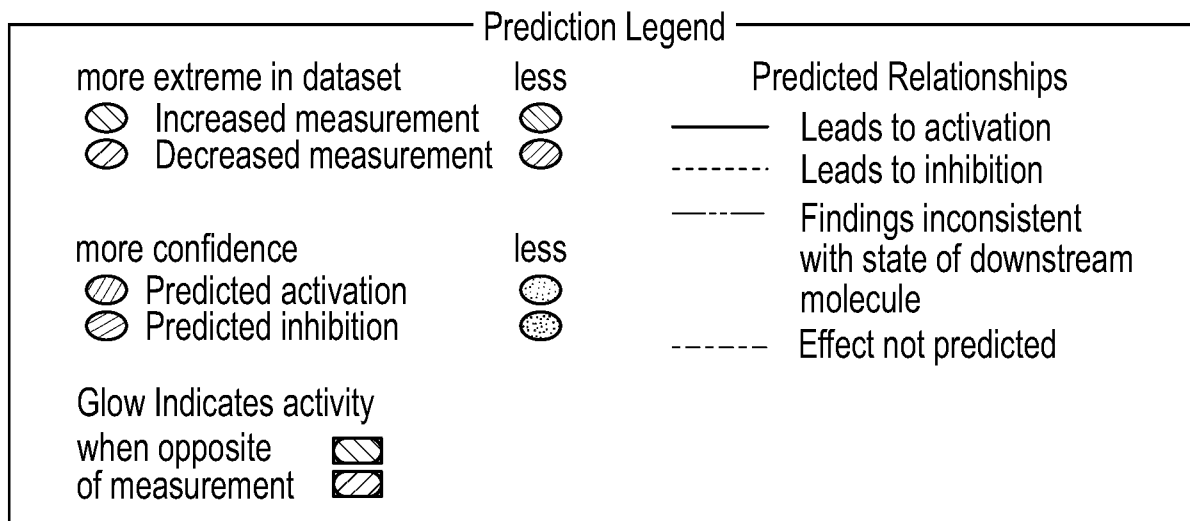
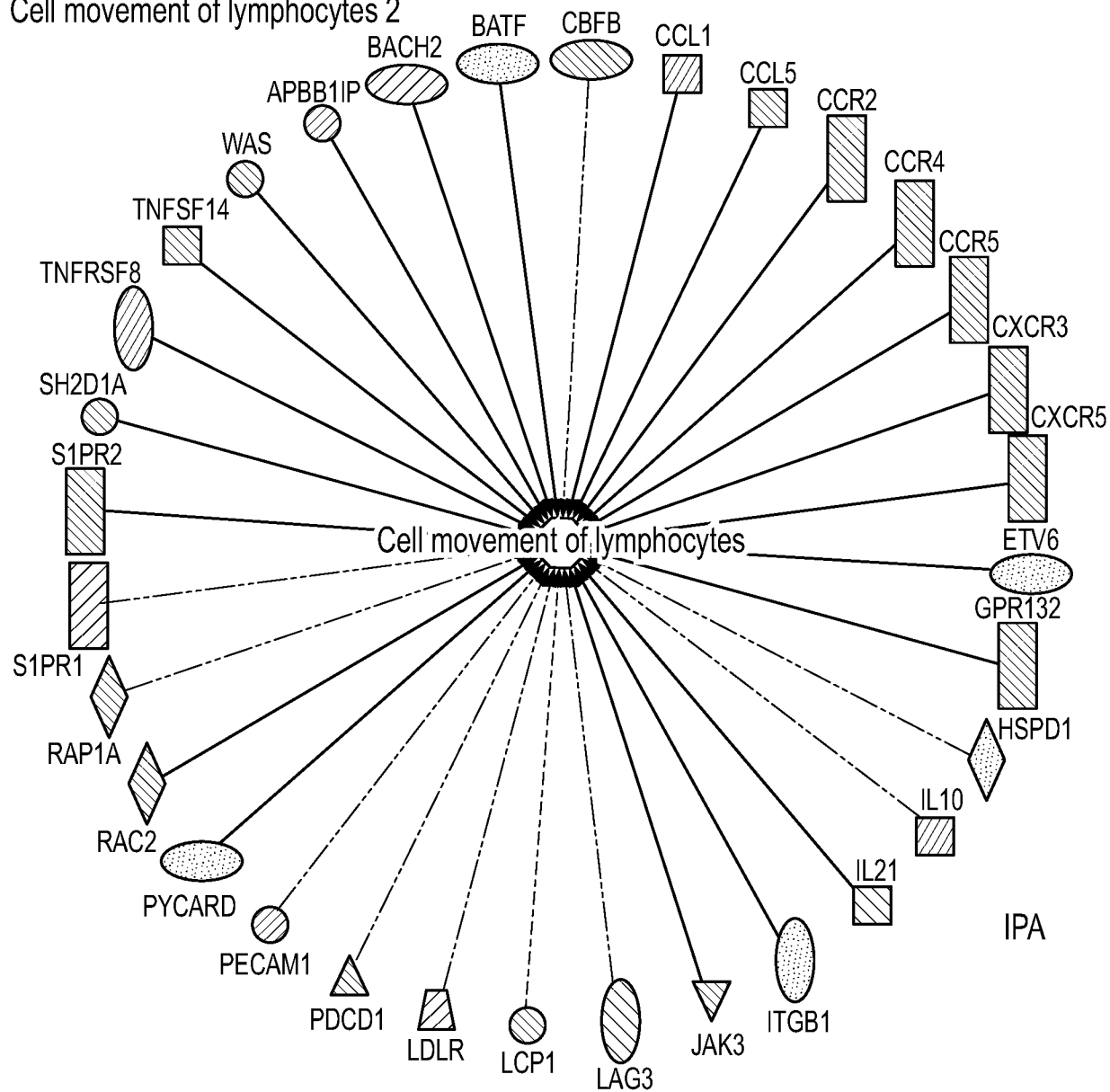


FIG. 24B

49/73

Cell movement of lymphocytes 2

**FIG. 24C**

50/73

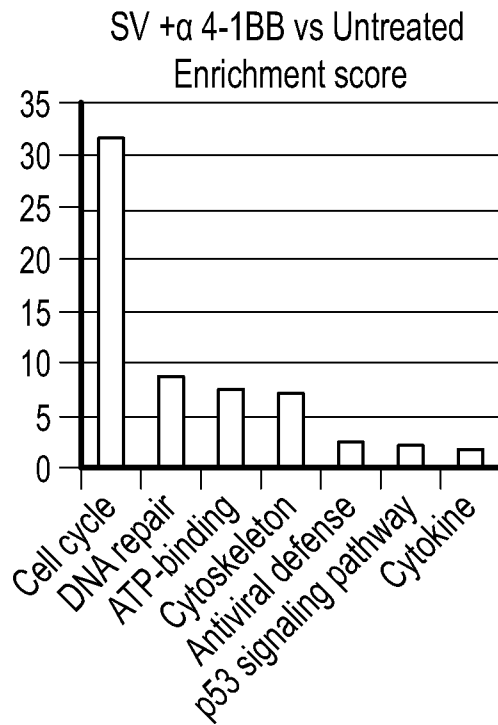


FIG. 25A

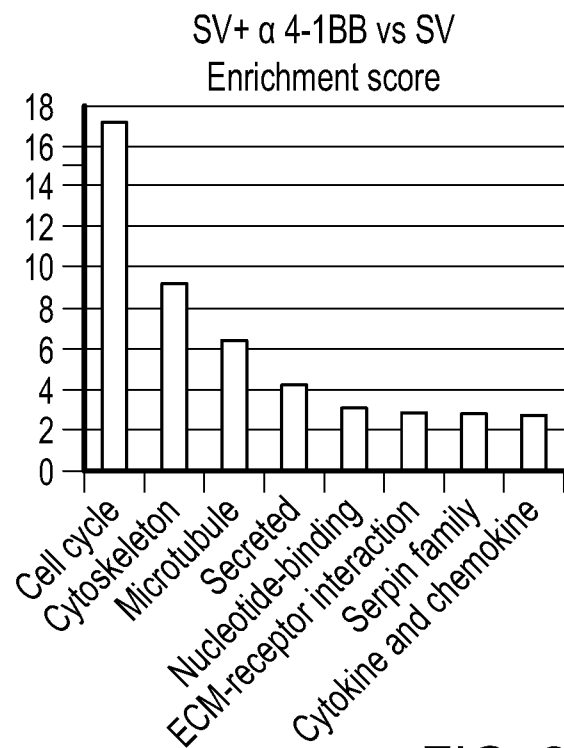


FIG. 25B

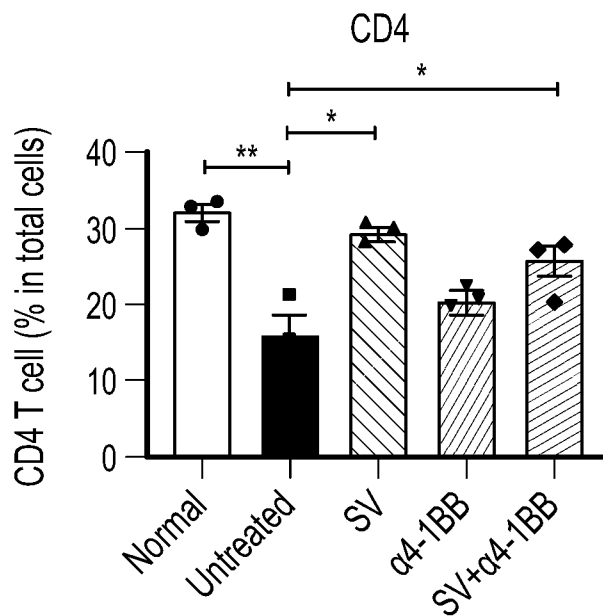


FIG. 26A

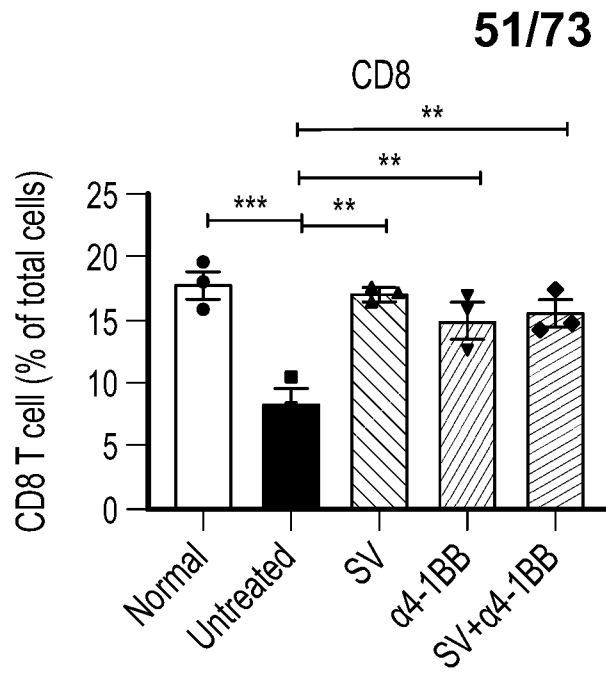


FIG. 26B

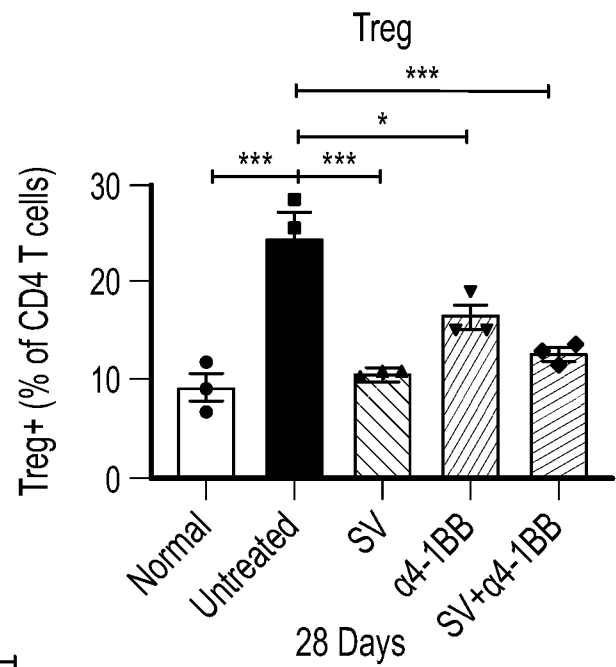


FIG. 26C

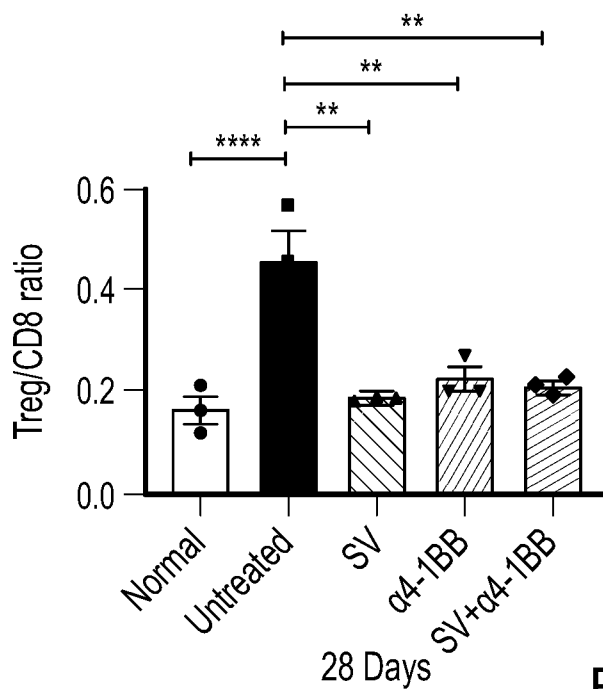
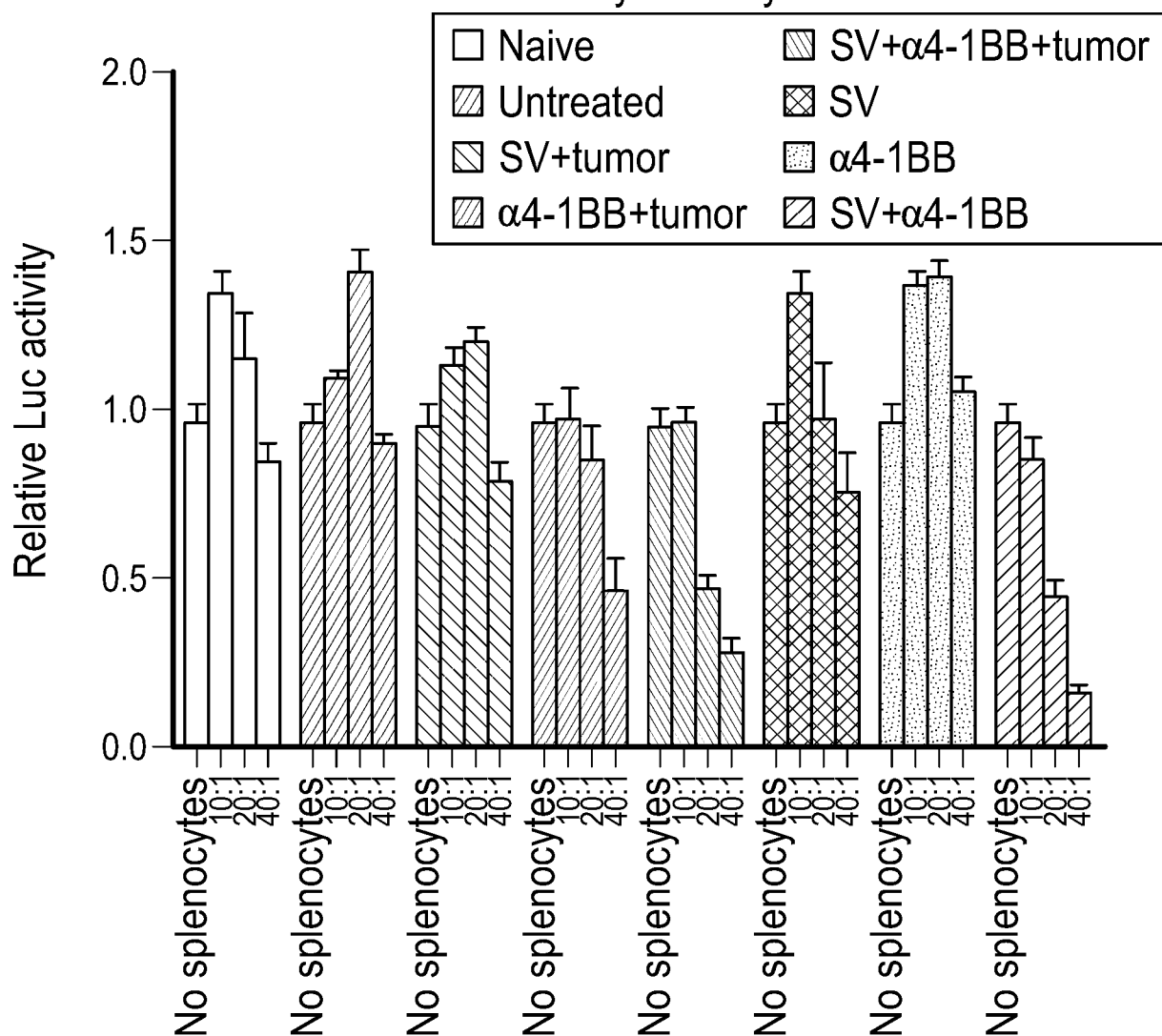
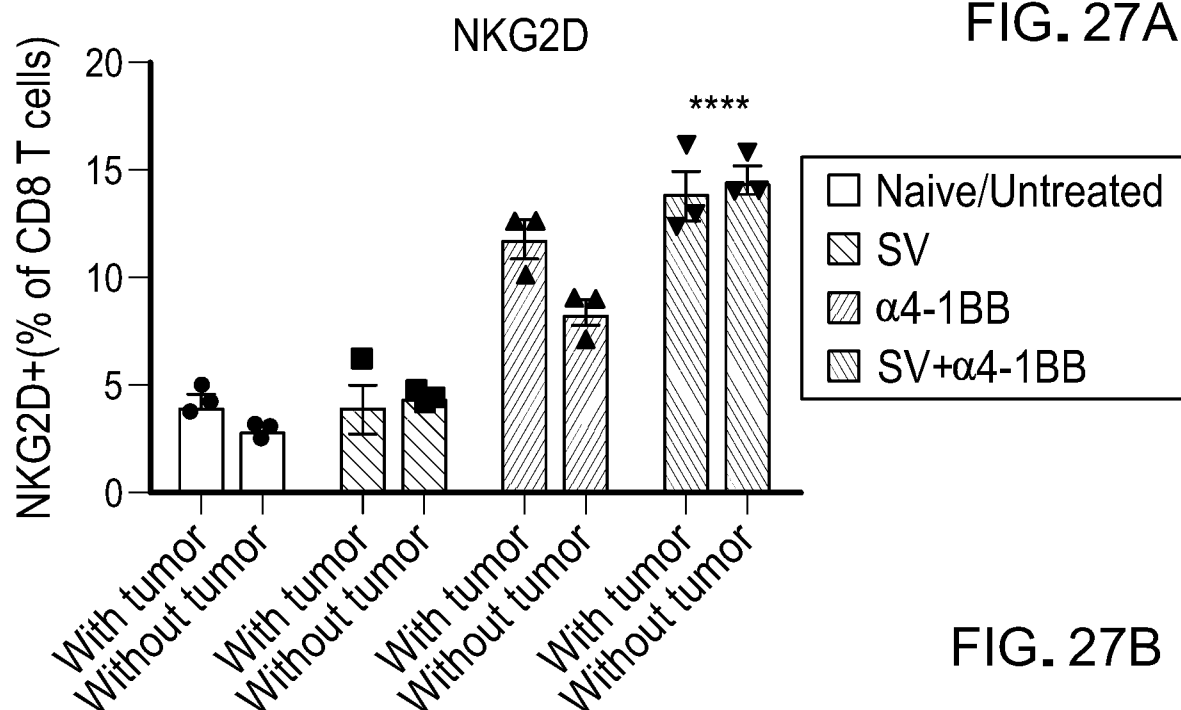


FIG. 26D

52/73**Cytotoxicity****FIG. 27A****FIG. 27B**

53/73

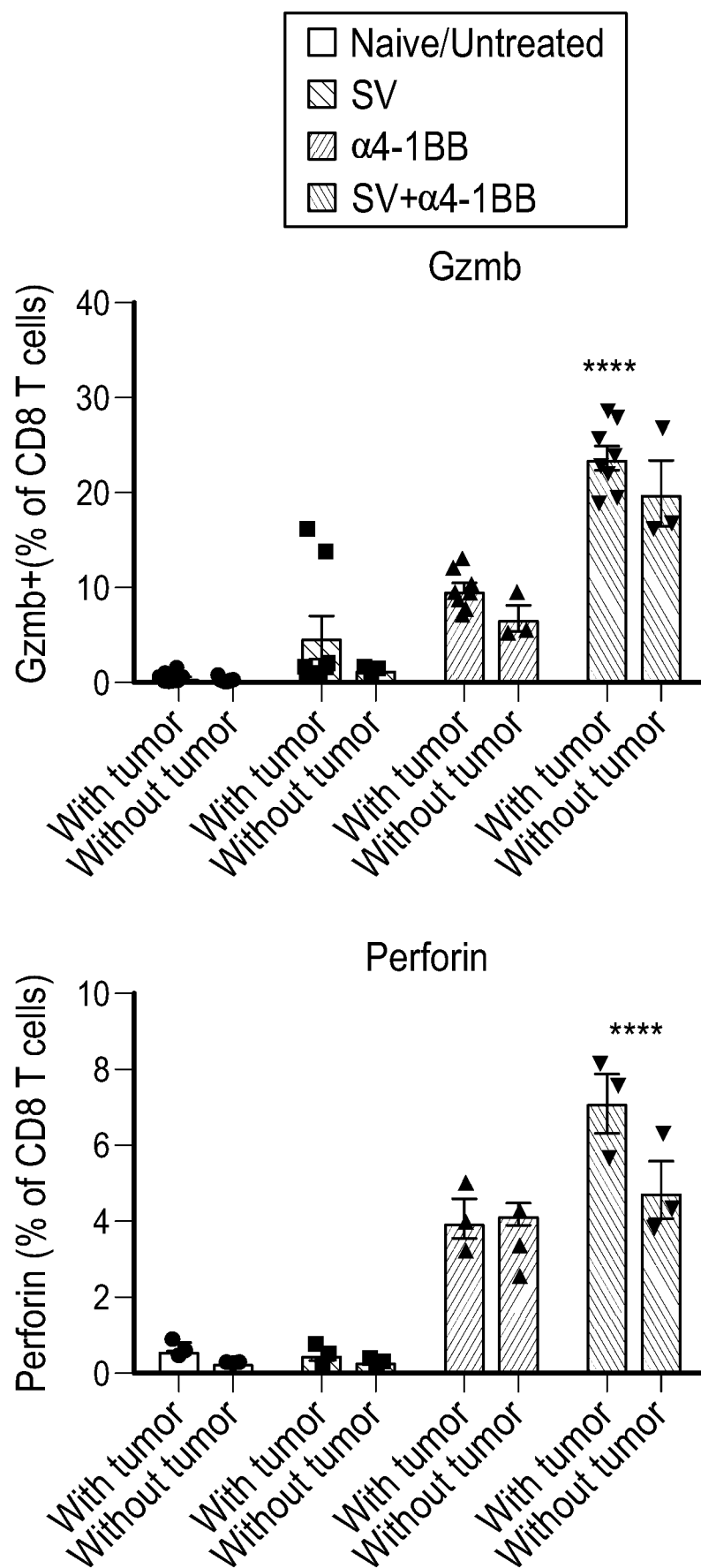


FIG. 27C

54/73

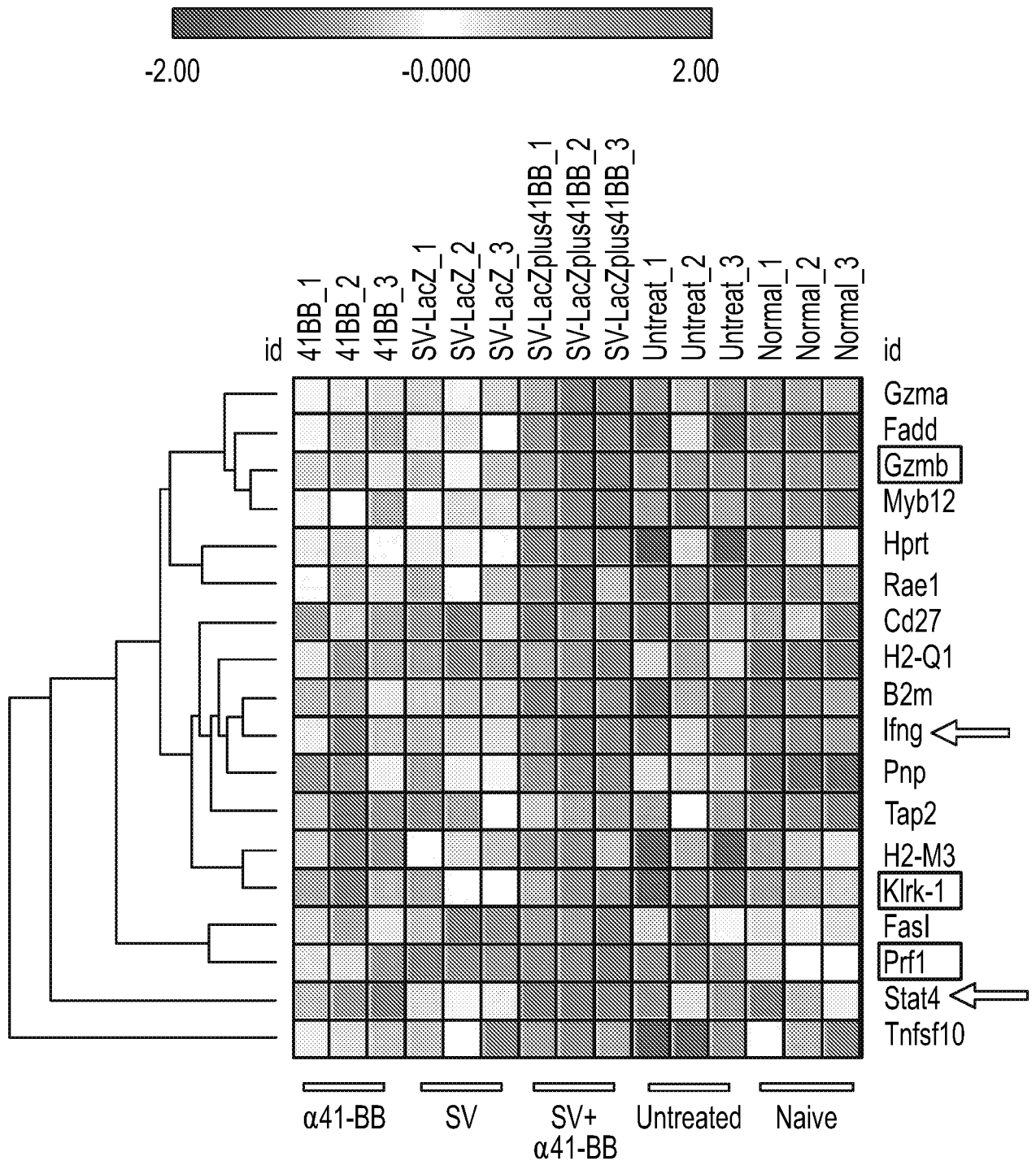


FIG. 27D

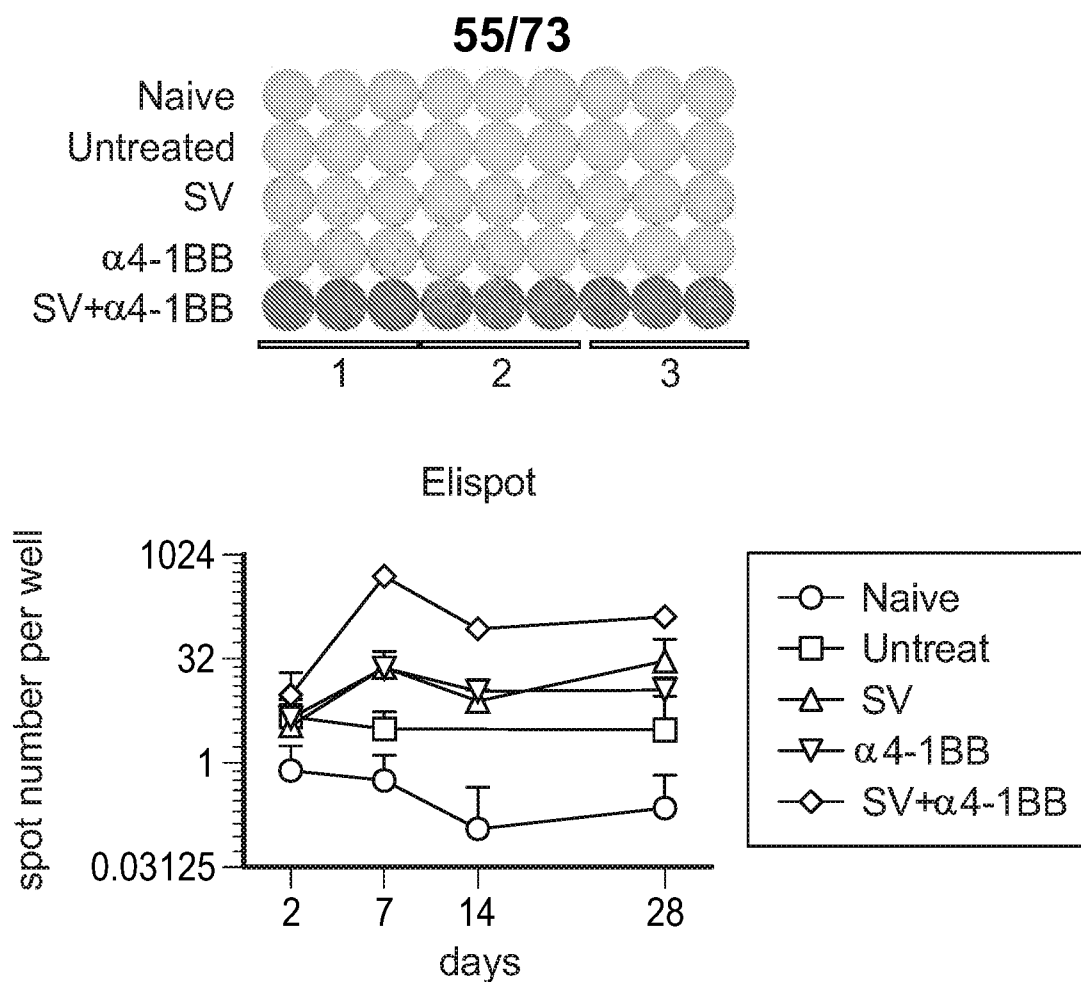


FIG. 28A

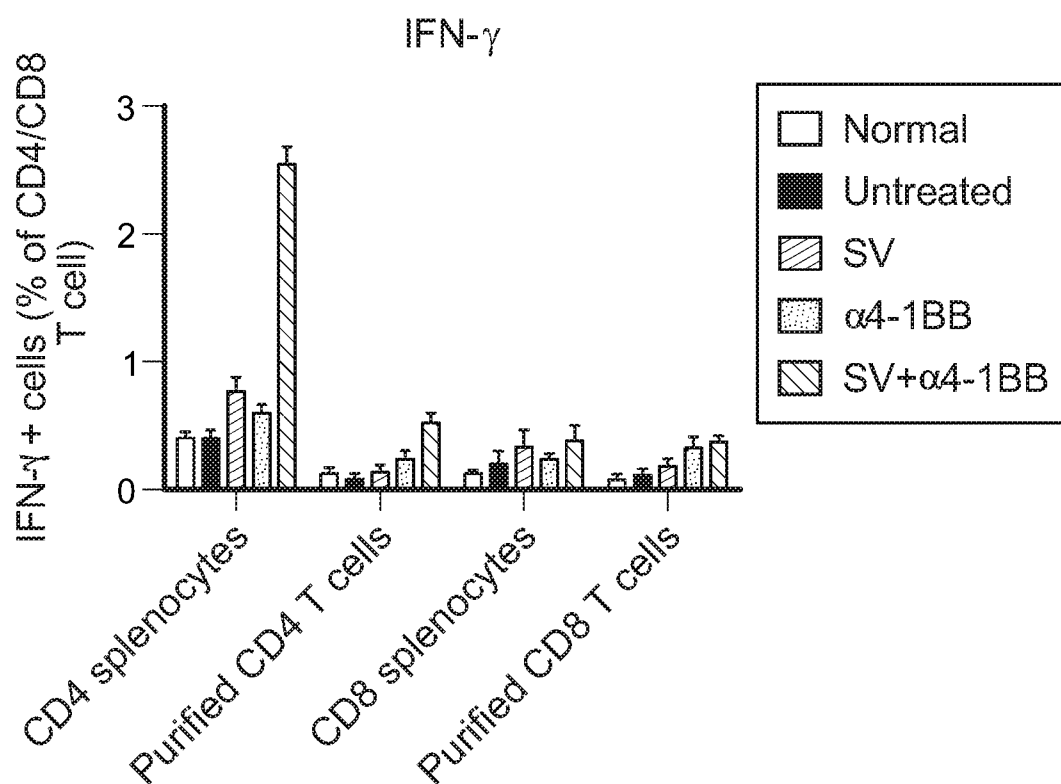


FIG. 28B

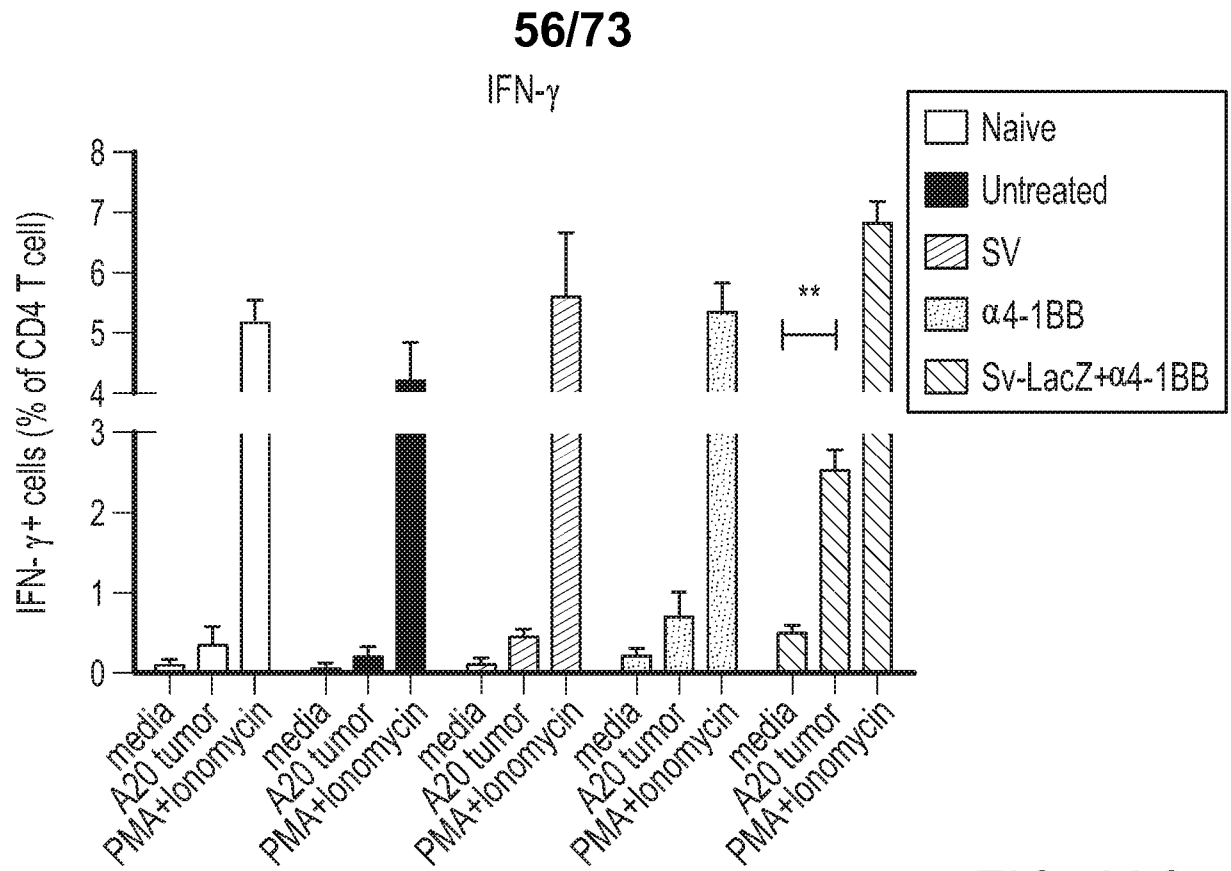


FIG. 28C

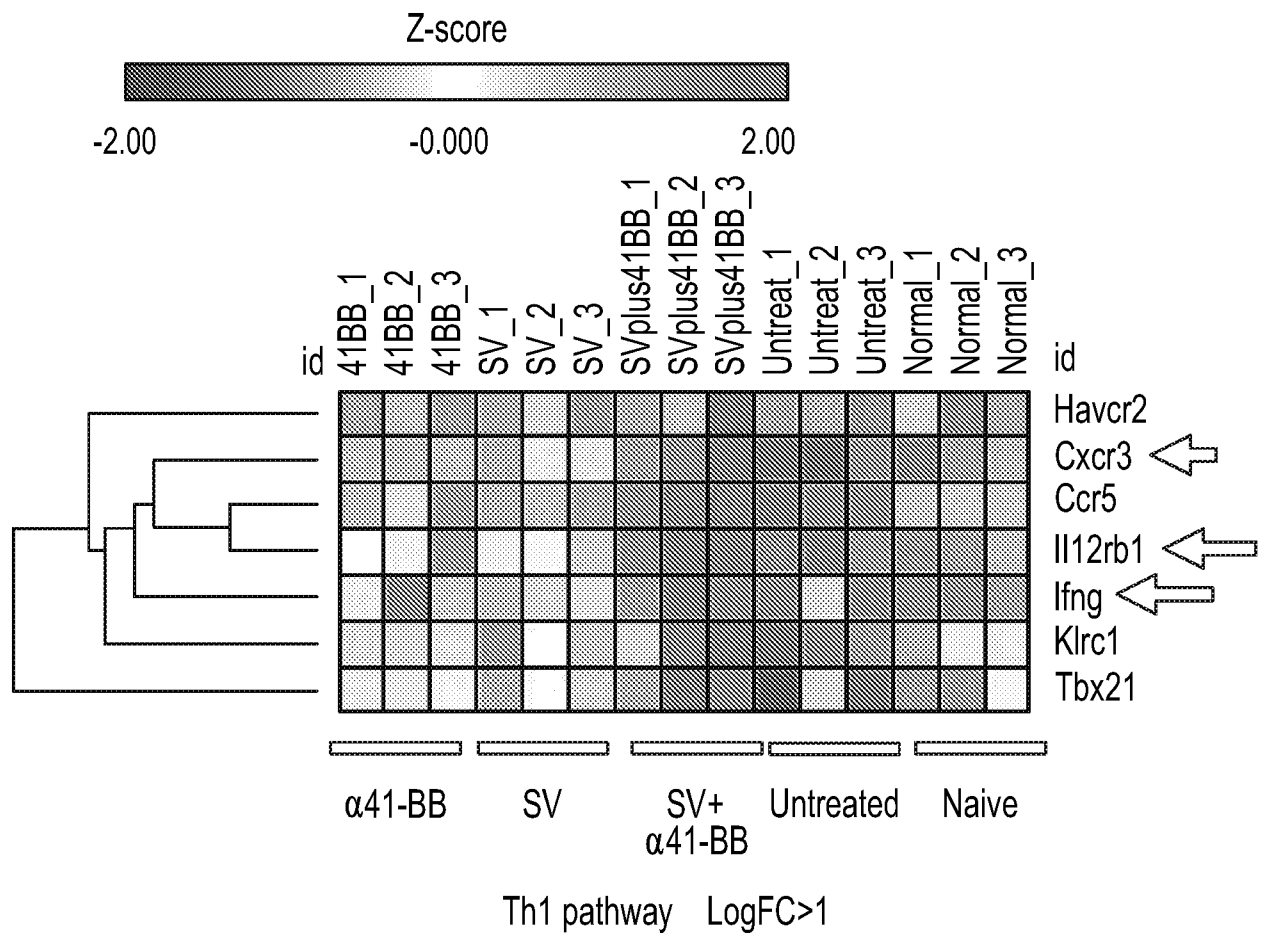


FIG. 28D

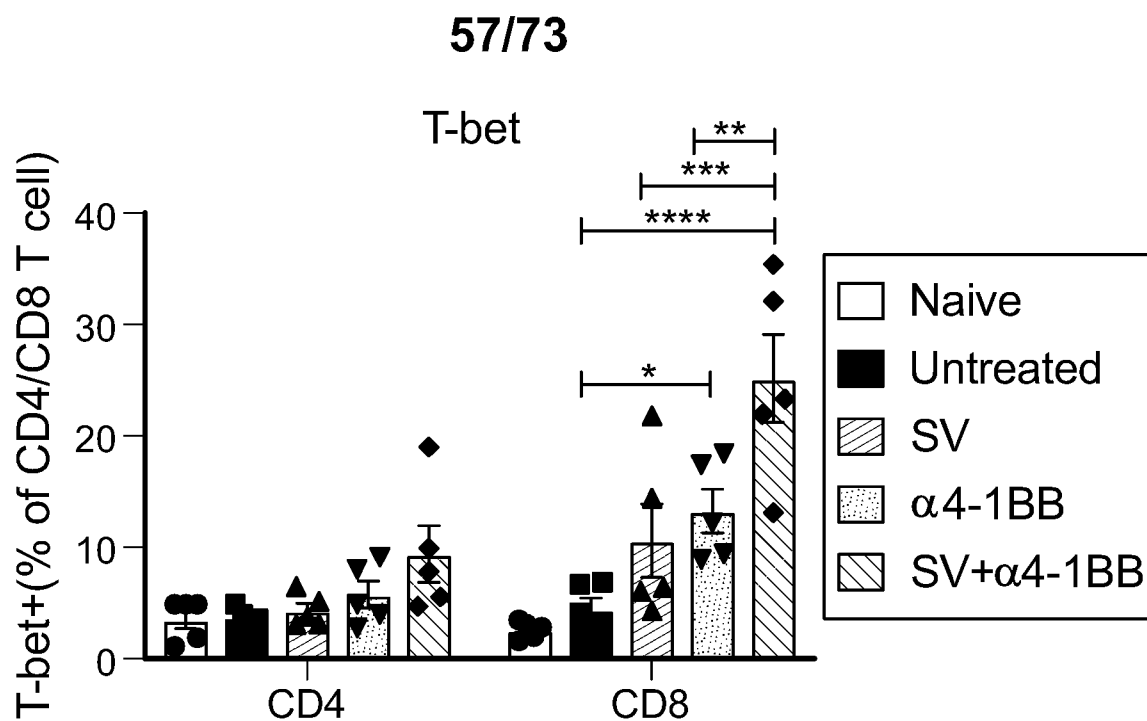


FIG. 28E

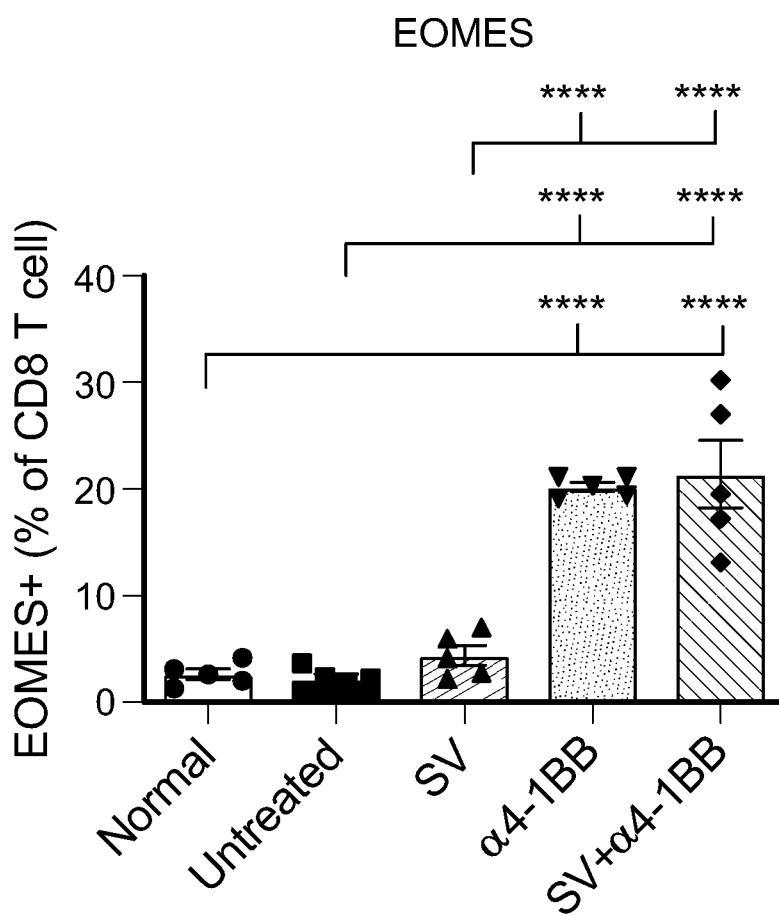


FIG. 28F

58/73

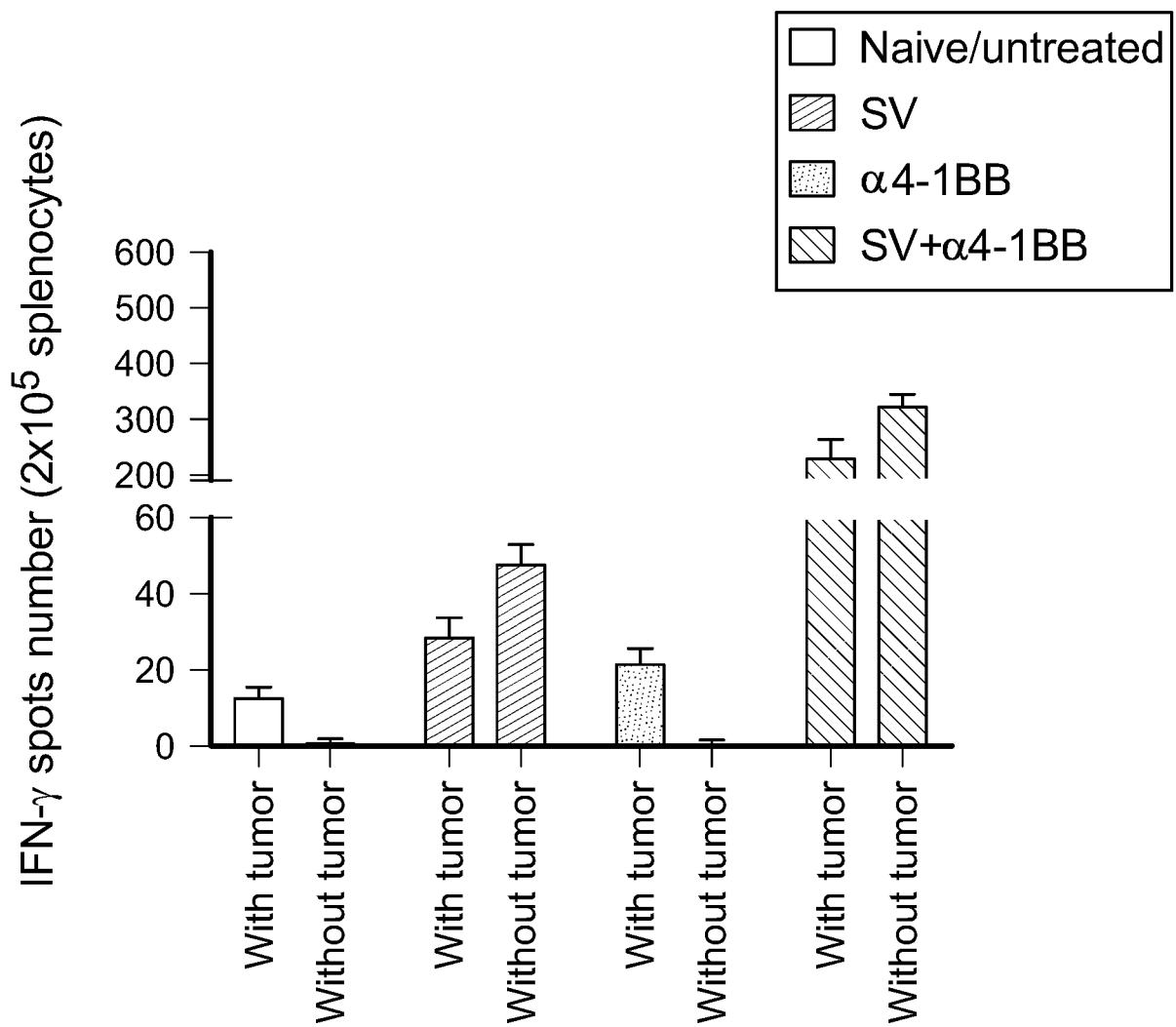


FIG. 29

59/73

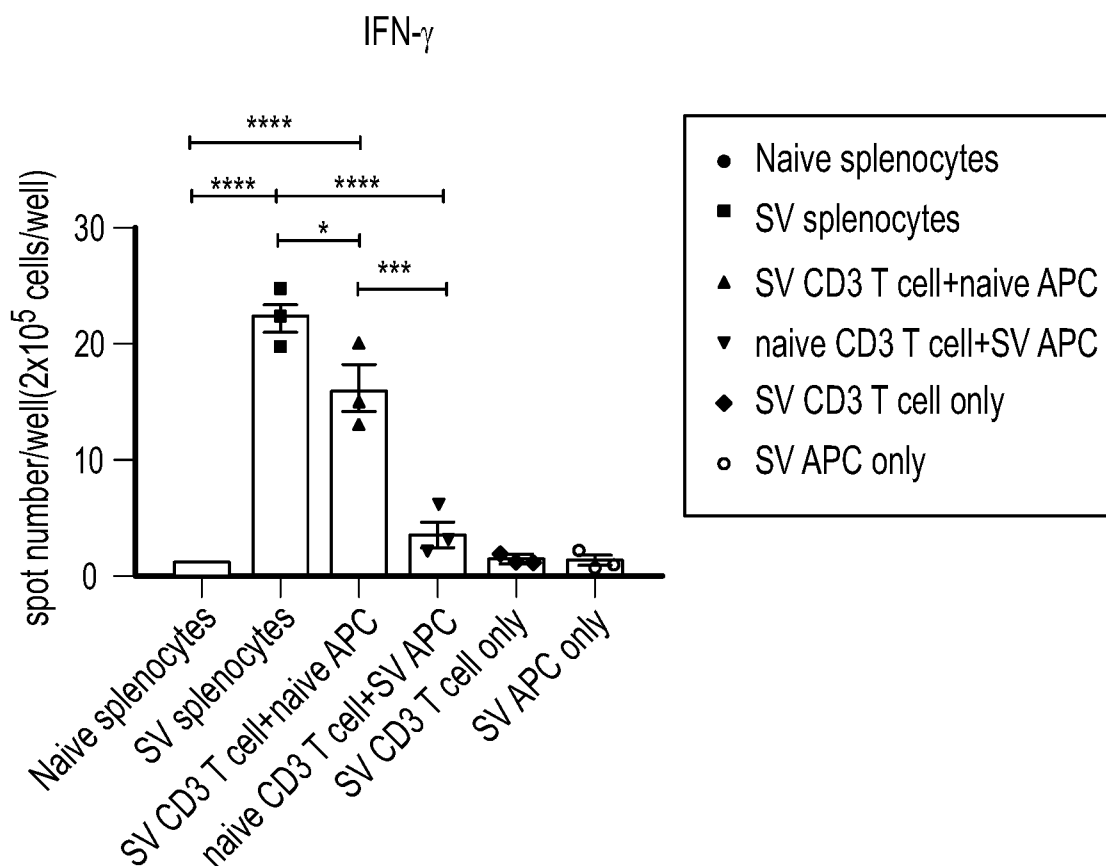


FIG. 30A

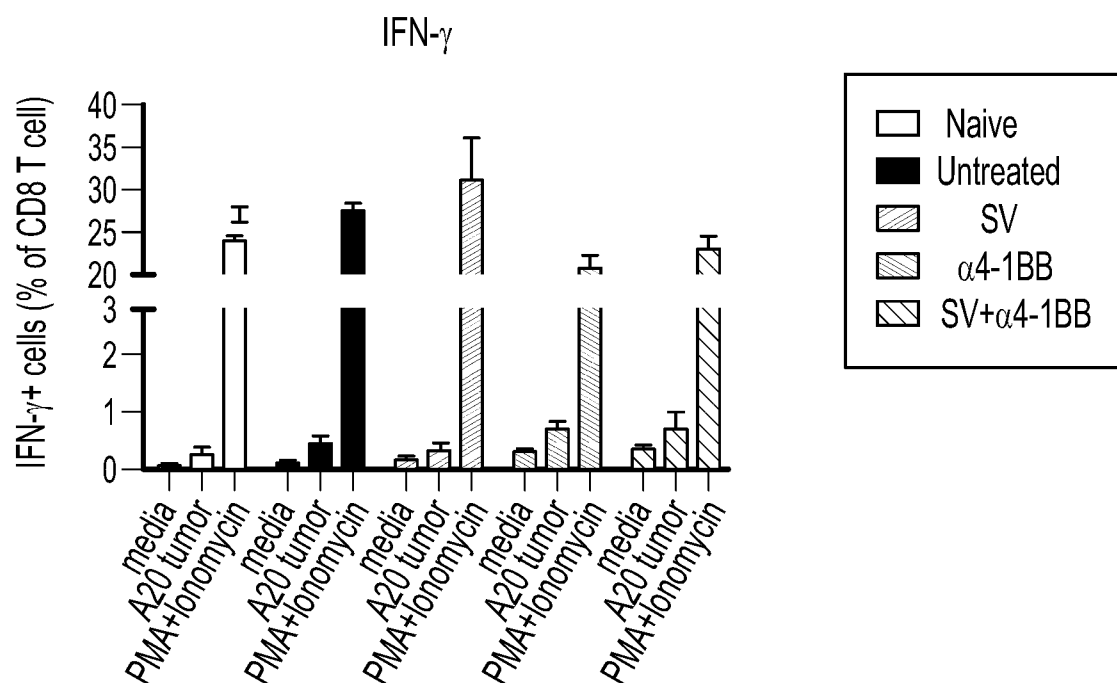


FIG. 30B

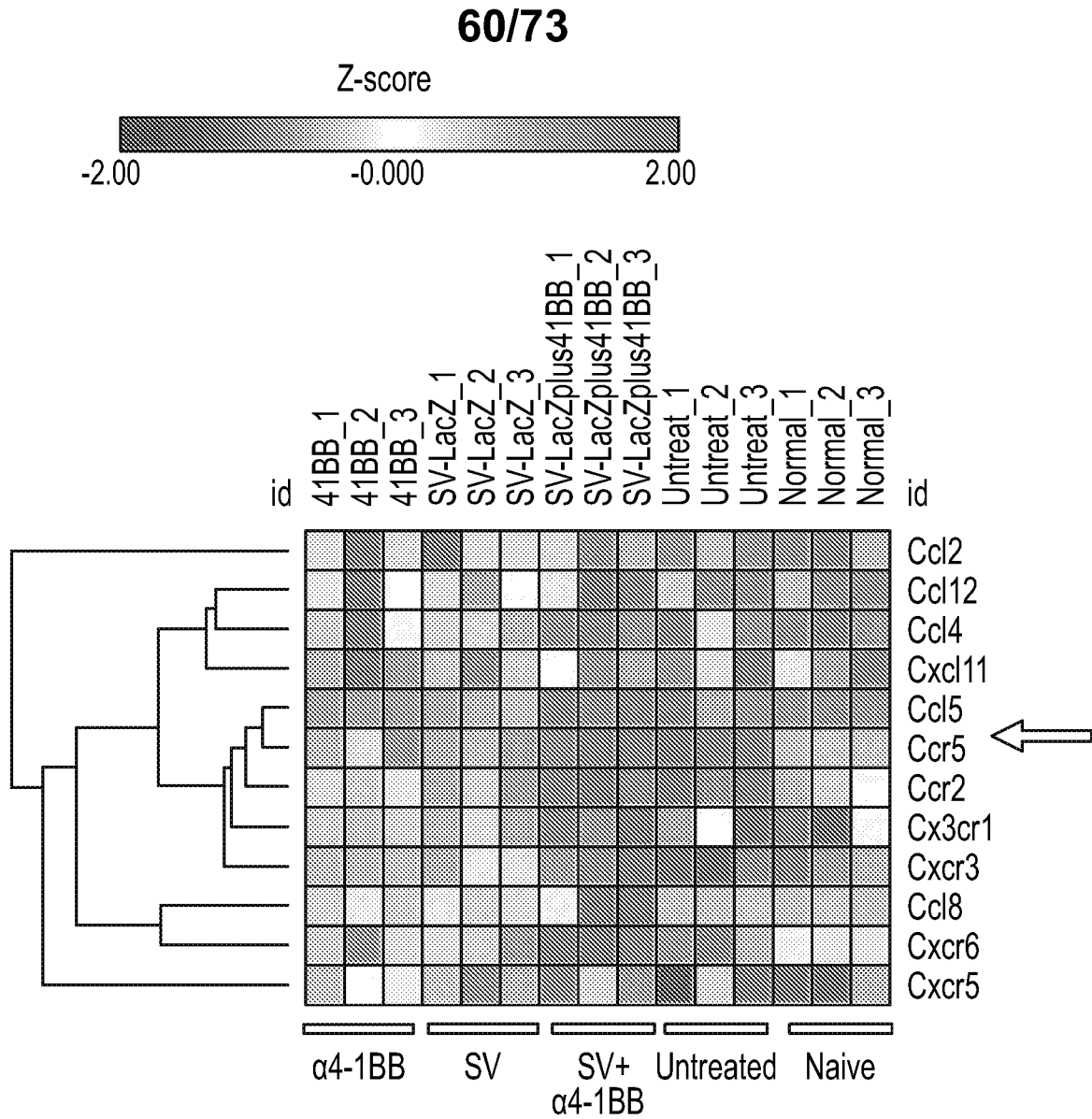


FIG. 31A

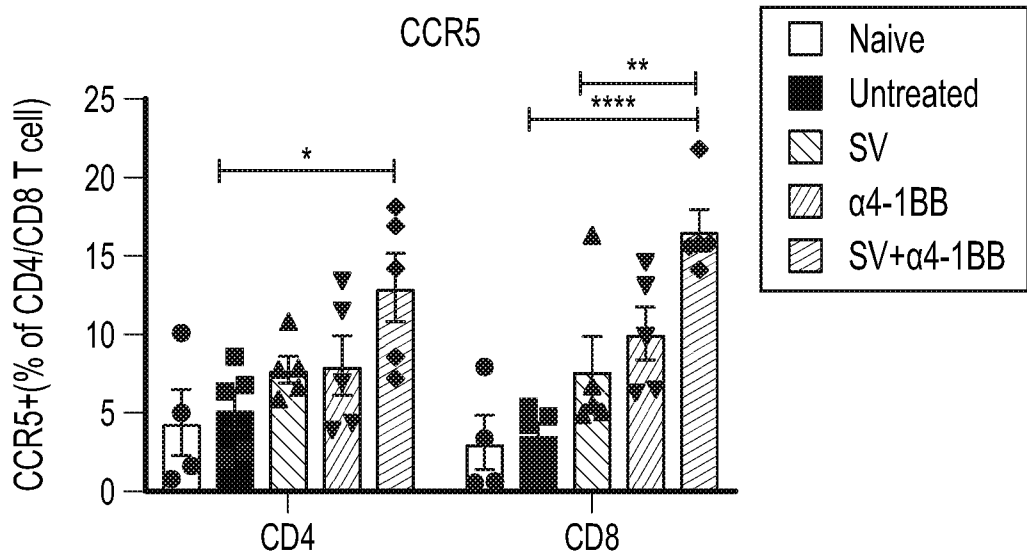
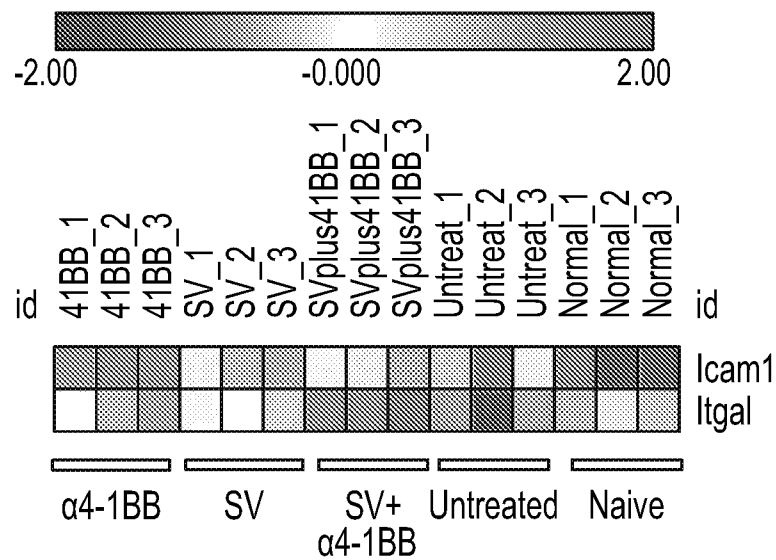
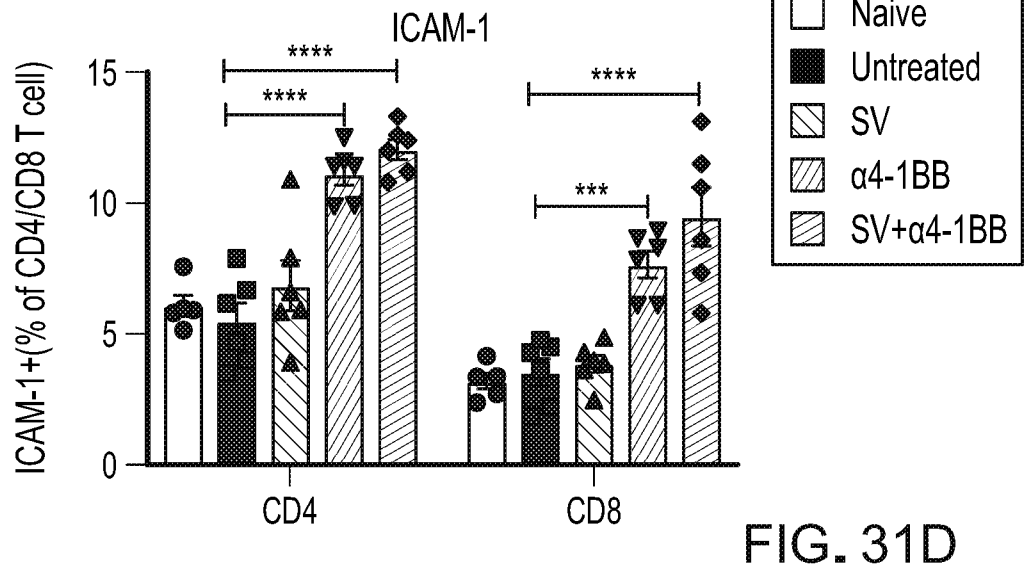
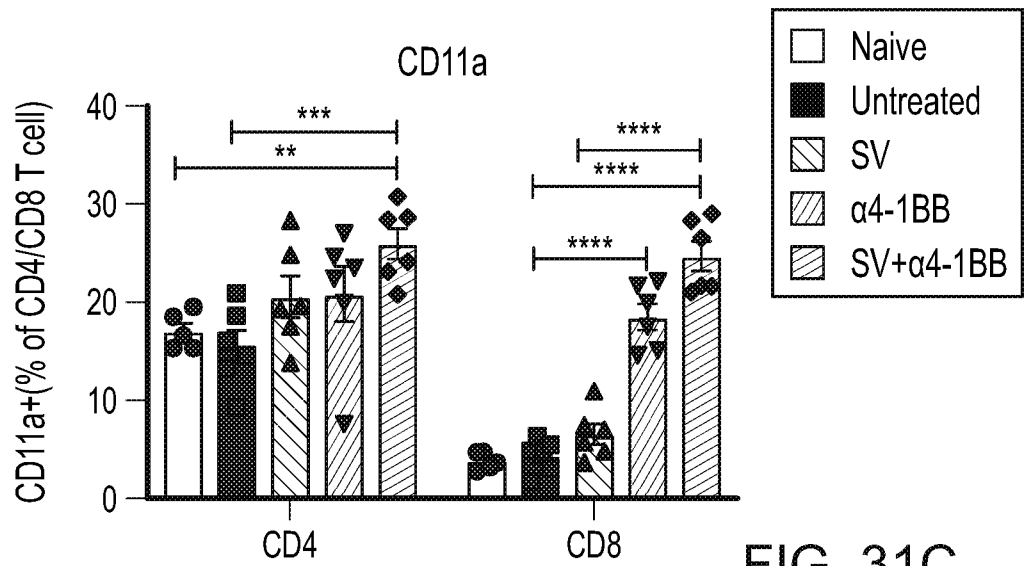


FIG. 31B

61/73

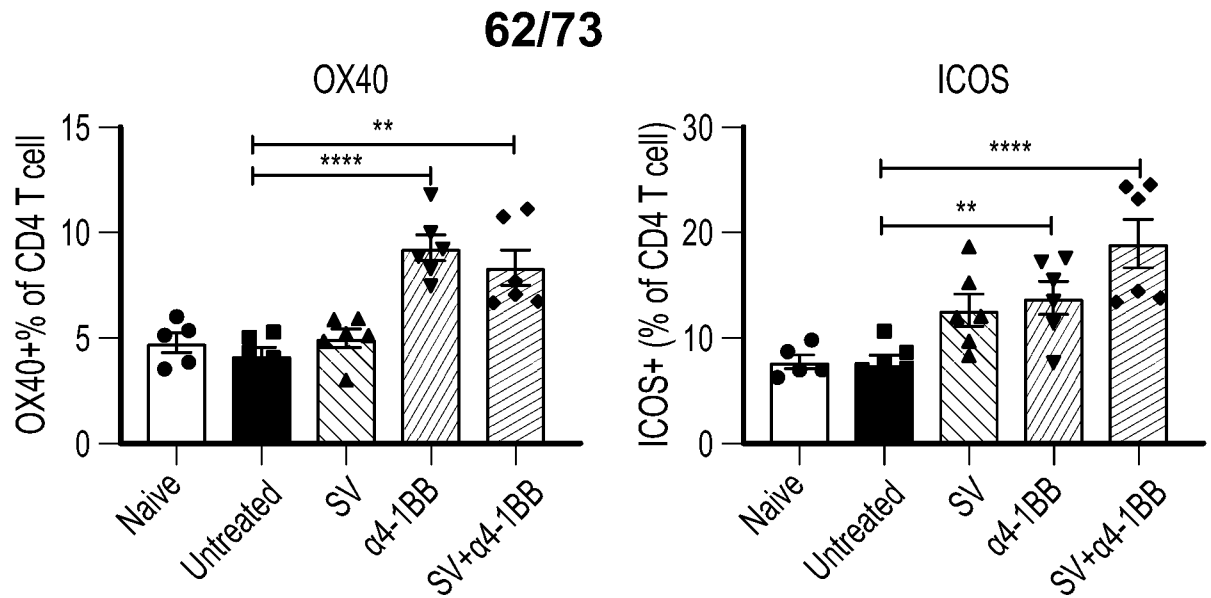


FIG. 31F

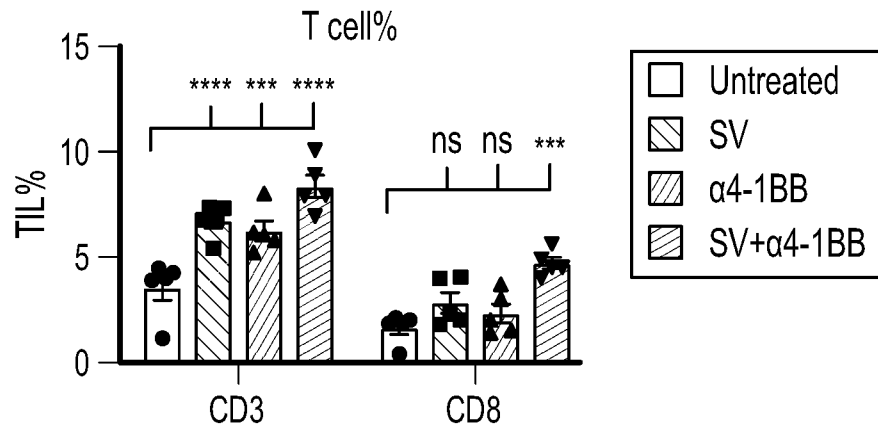


FIG. 31G

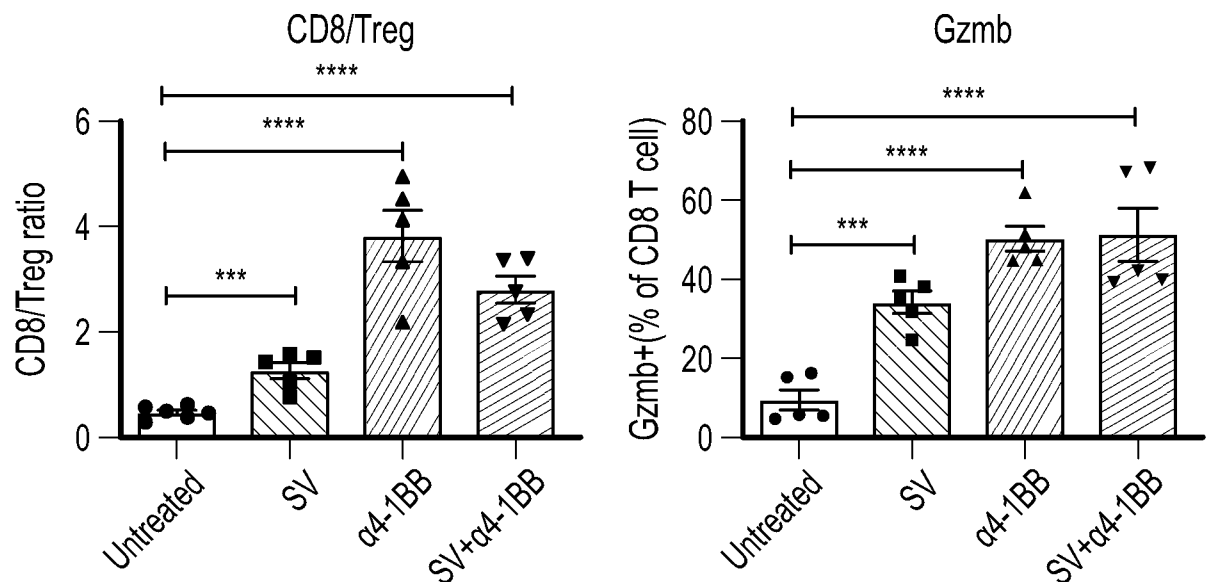


FIG. 31H

FIG. 31I

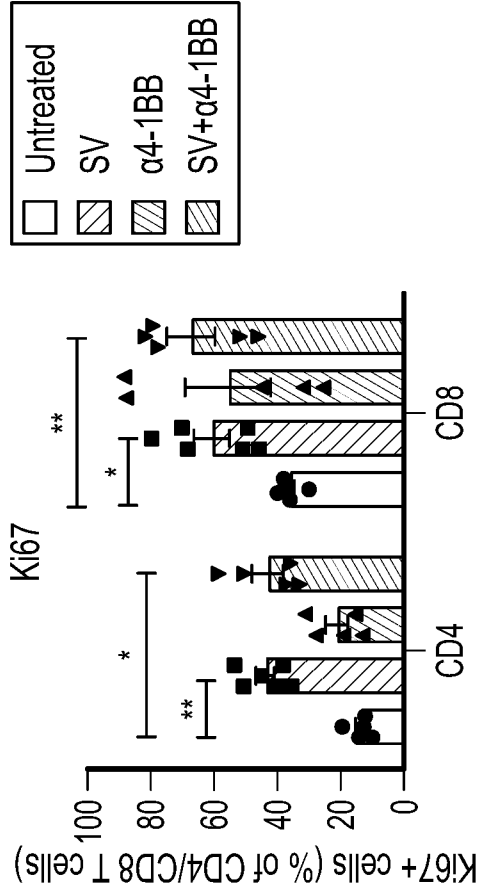


FIG. 32A

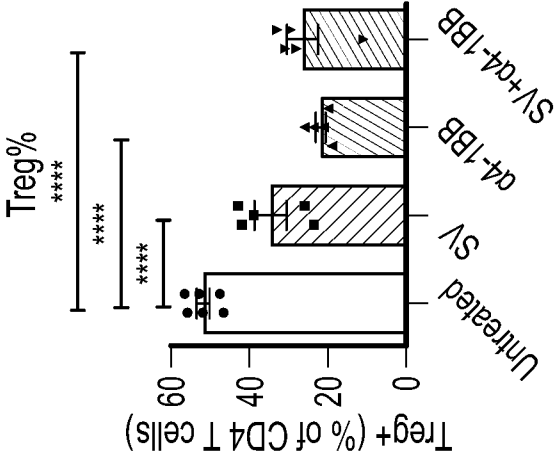


FIG. 32B

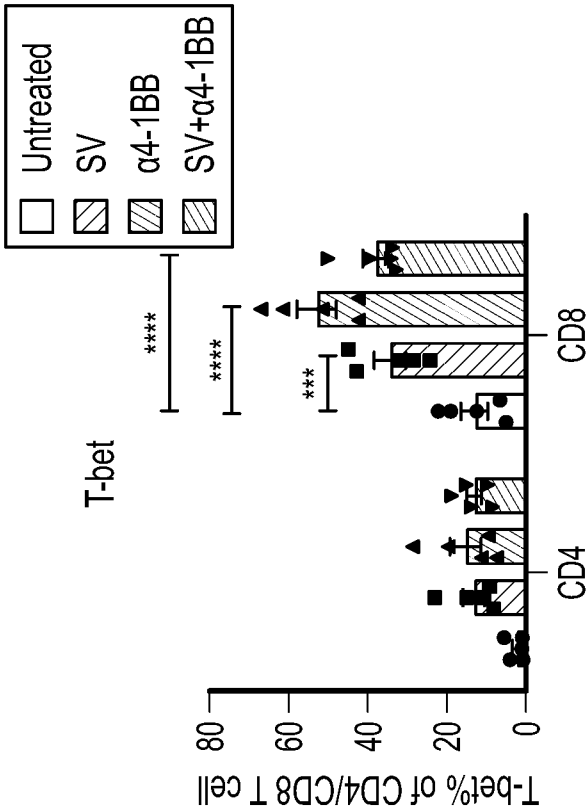


FIG. 32C

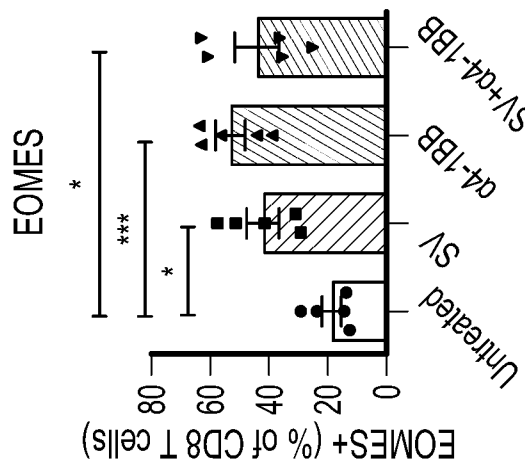


FIG. 32D

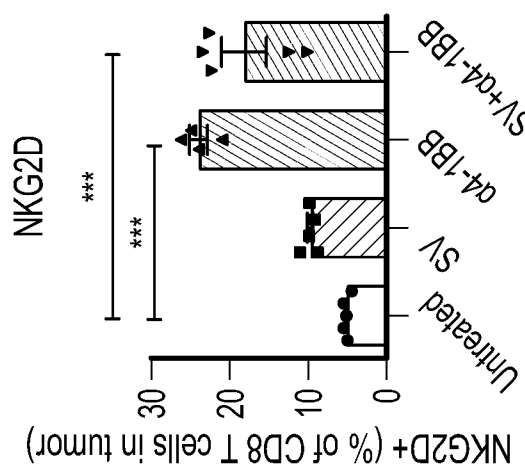
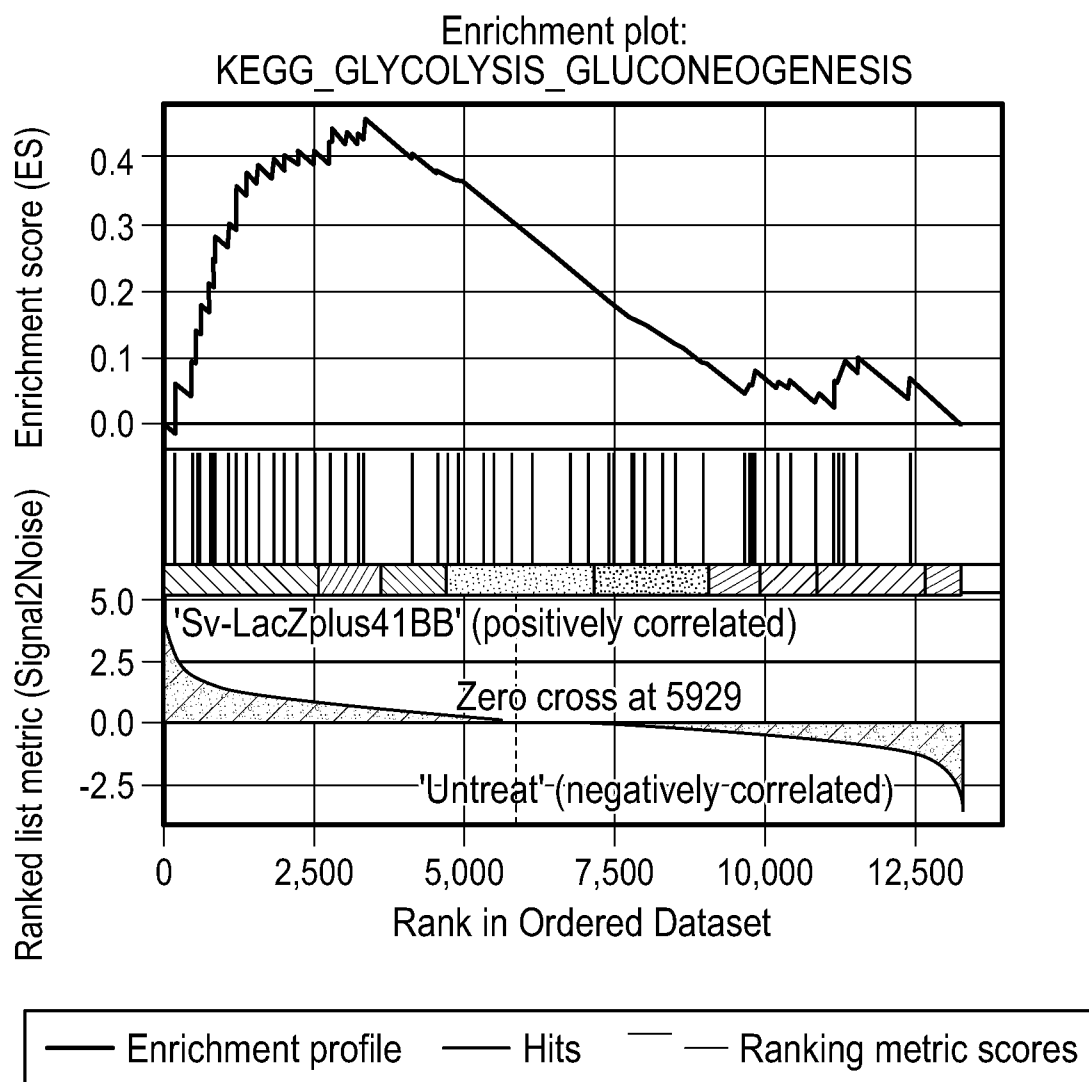


FIG. 32E

64/73



Enrichment score (ES): 0.46
Normalized Enrichment Score (NES): 1.51
Nominal p value:0
False discovery rate (FDR) q-value:0.18

FIG. 33A

65/73

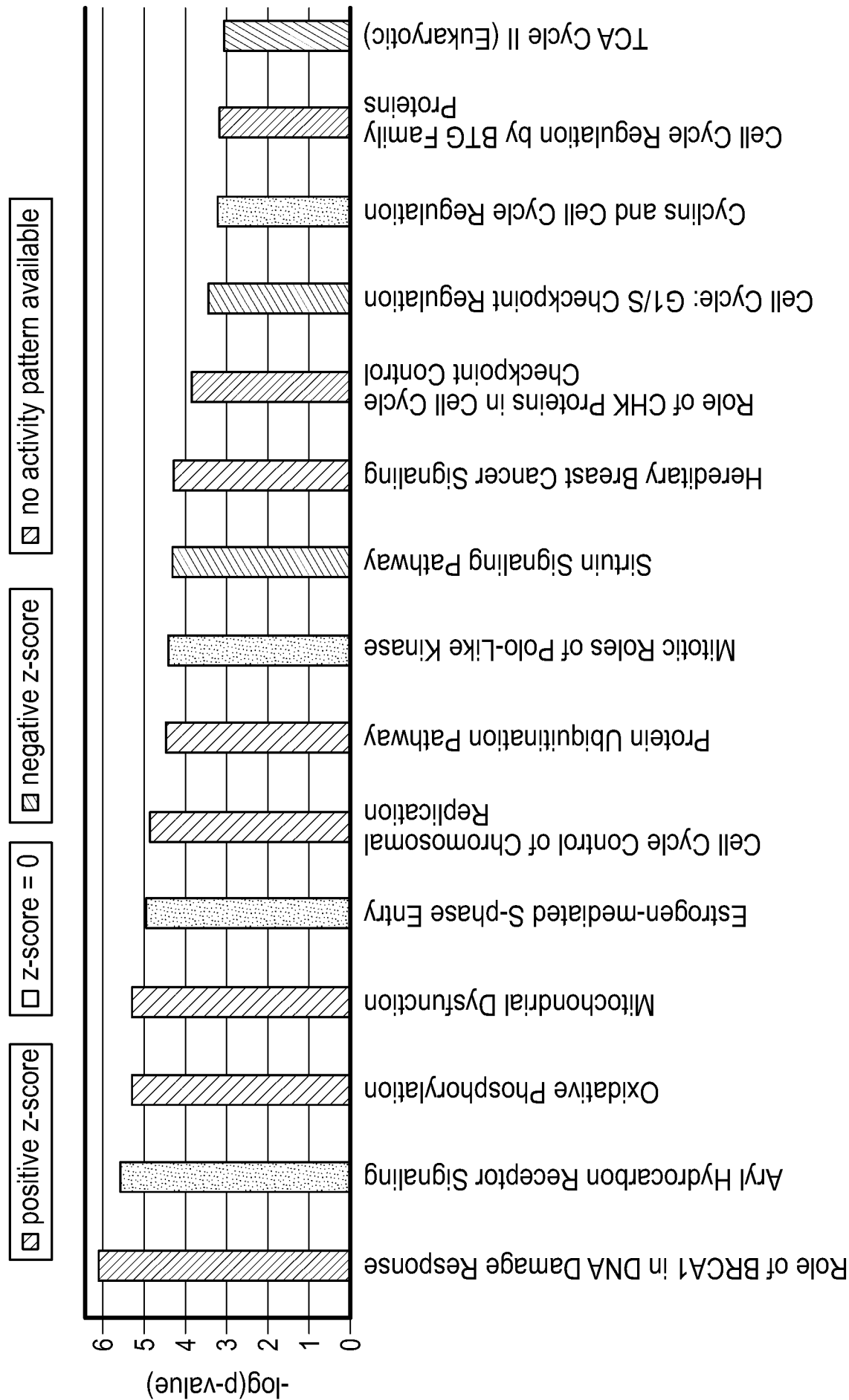
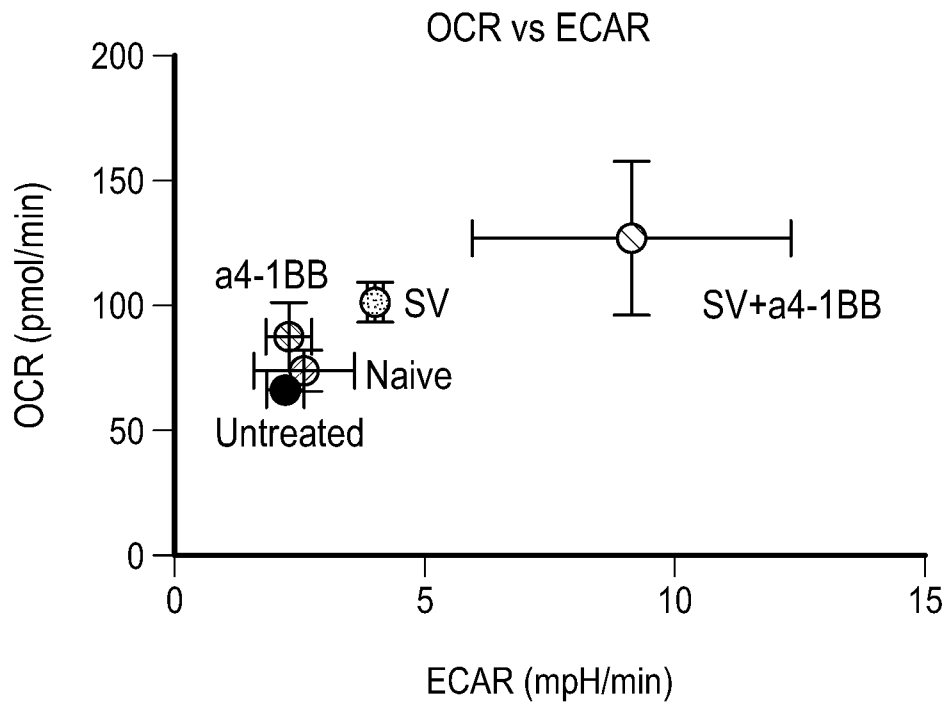
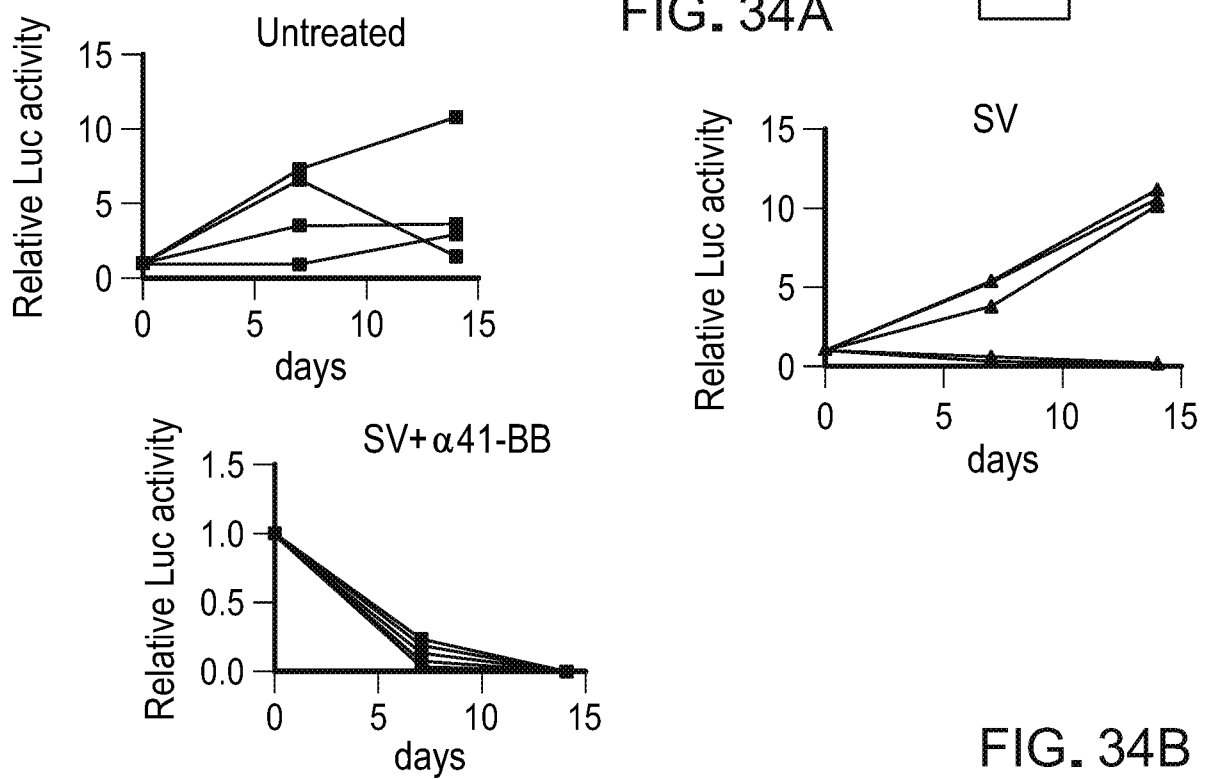
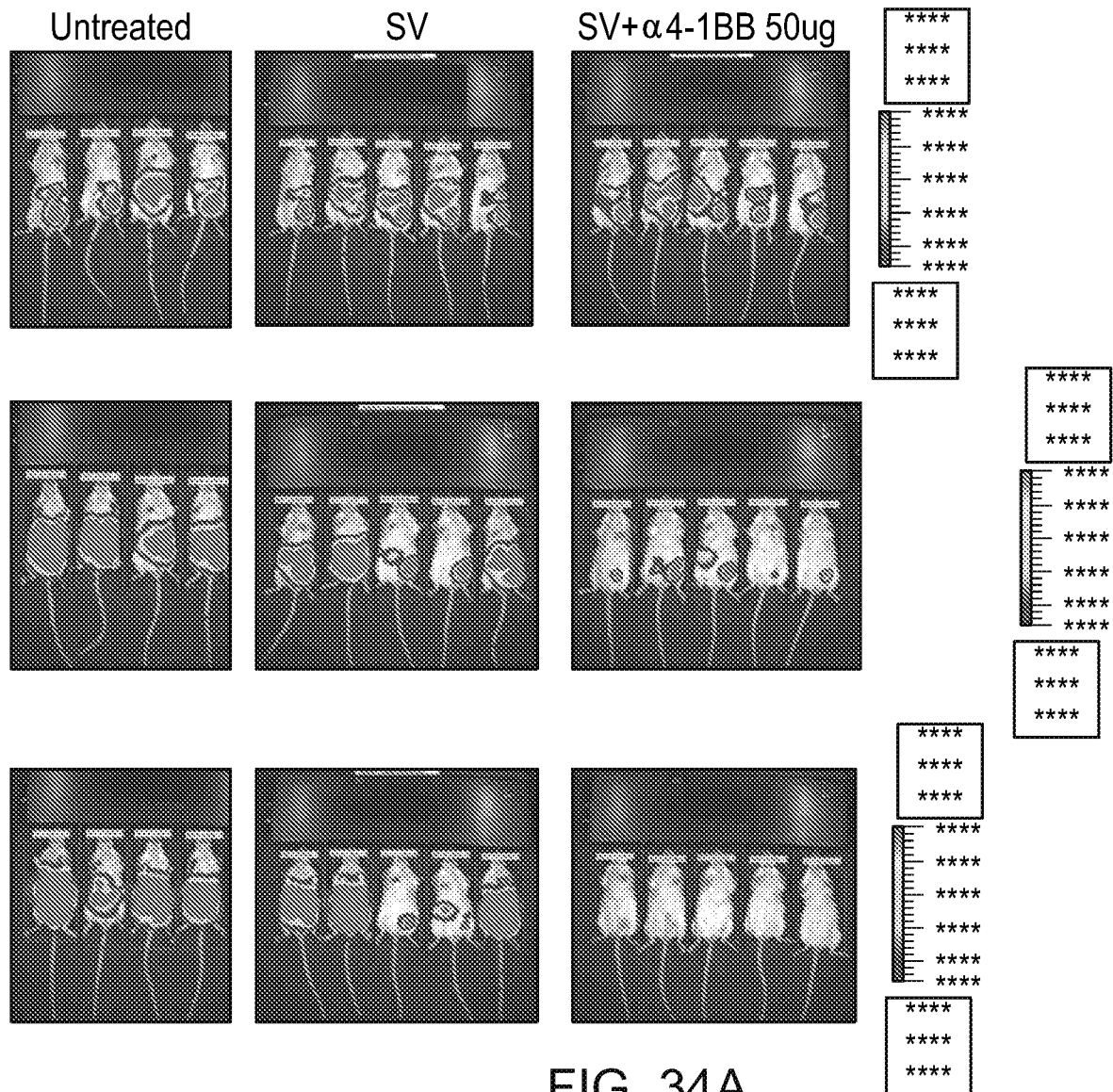


FIG. 33B

66/73**FIG. 33C**

67/73



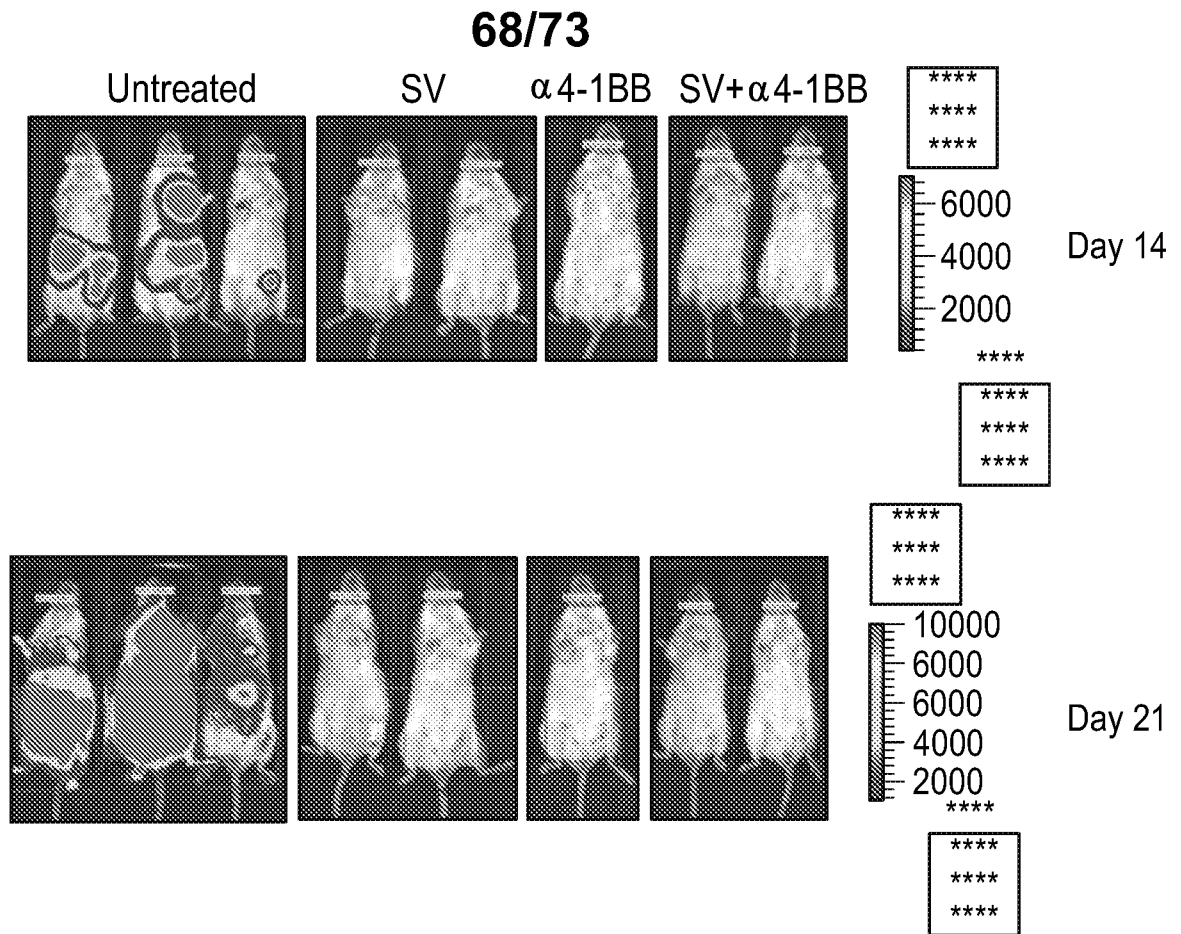


FIG. 35A

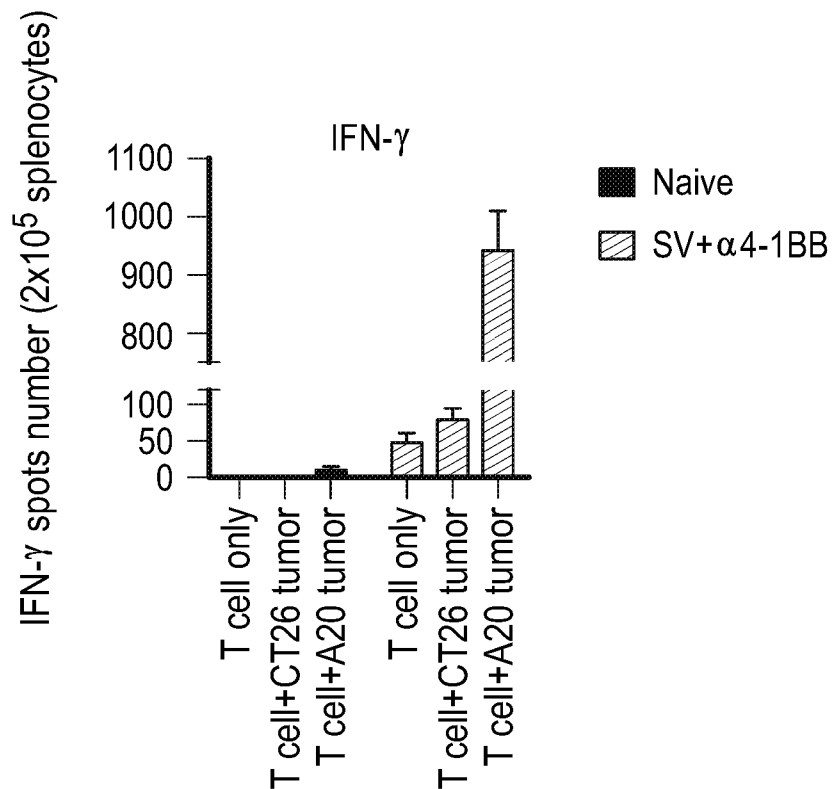


FIG. 35B

69/73

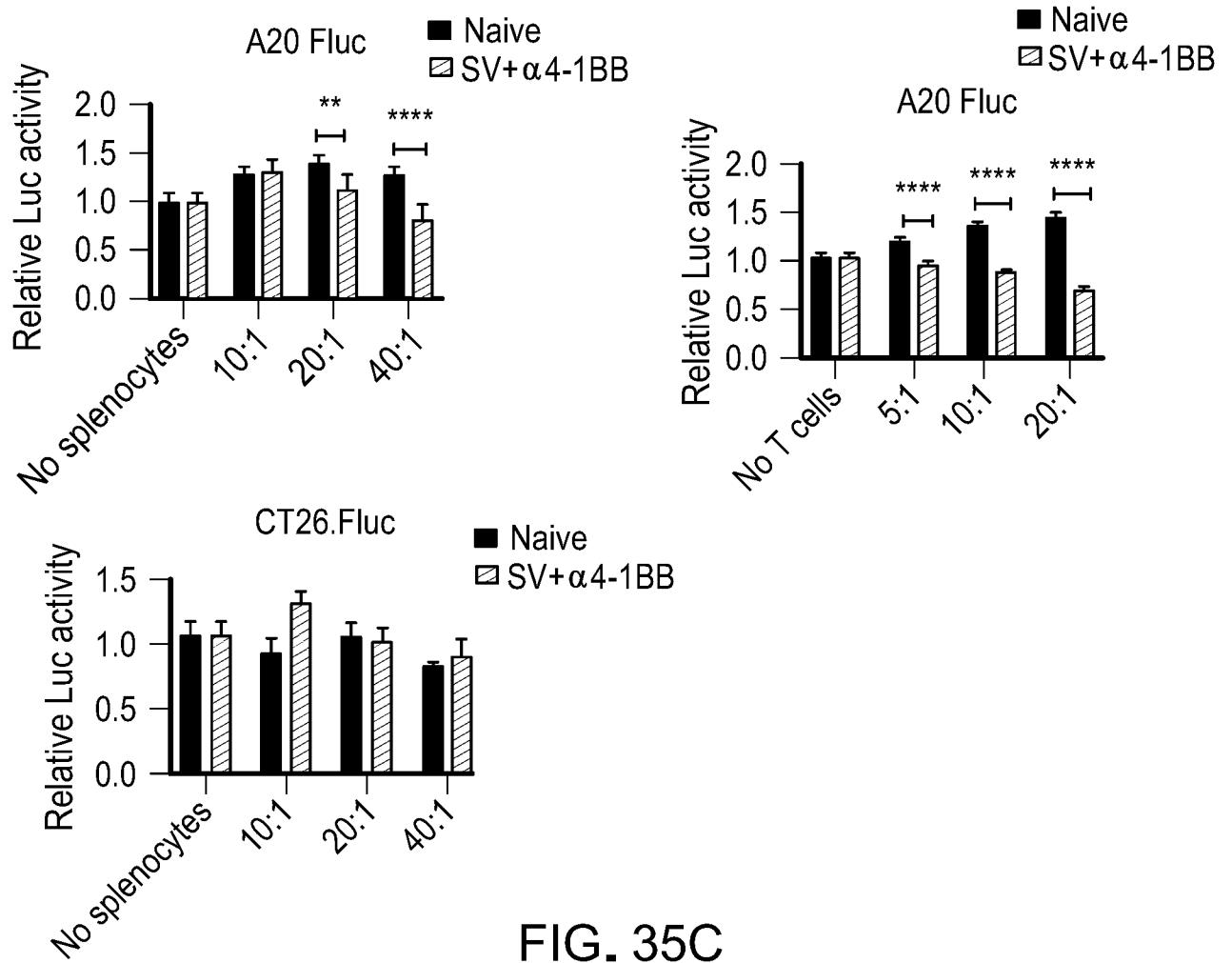


FIG. 35C

KEGG upregulated pathway

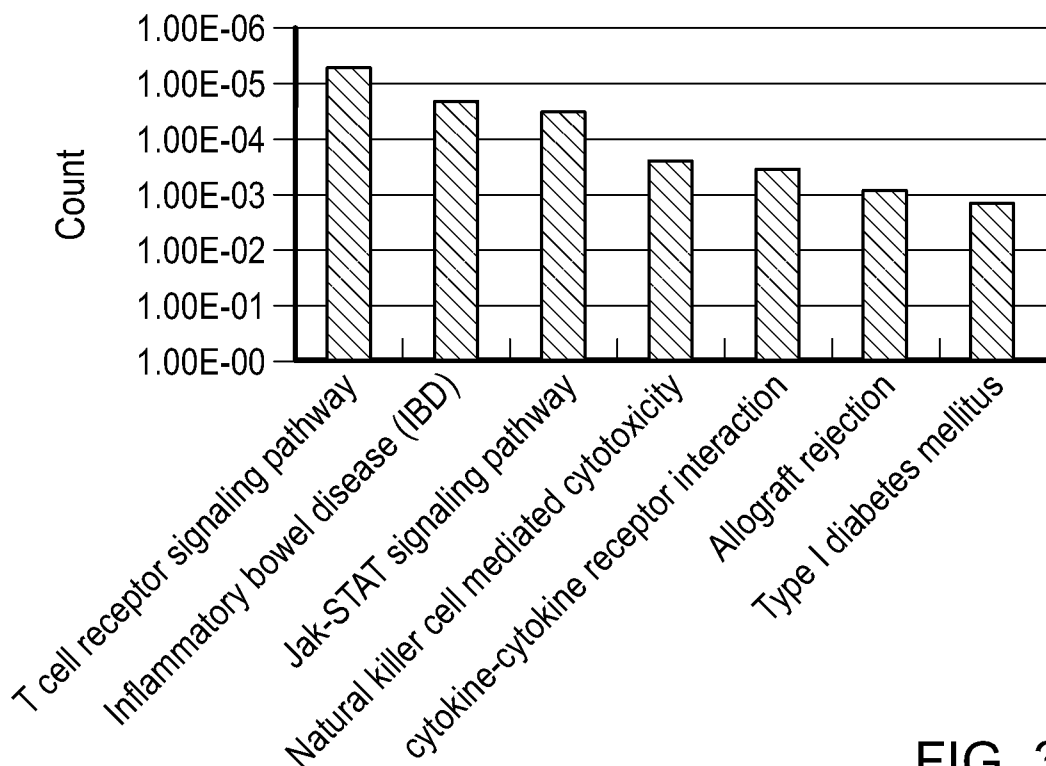
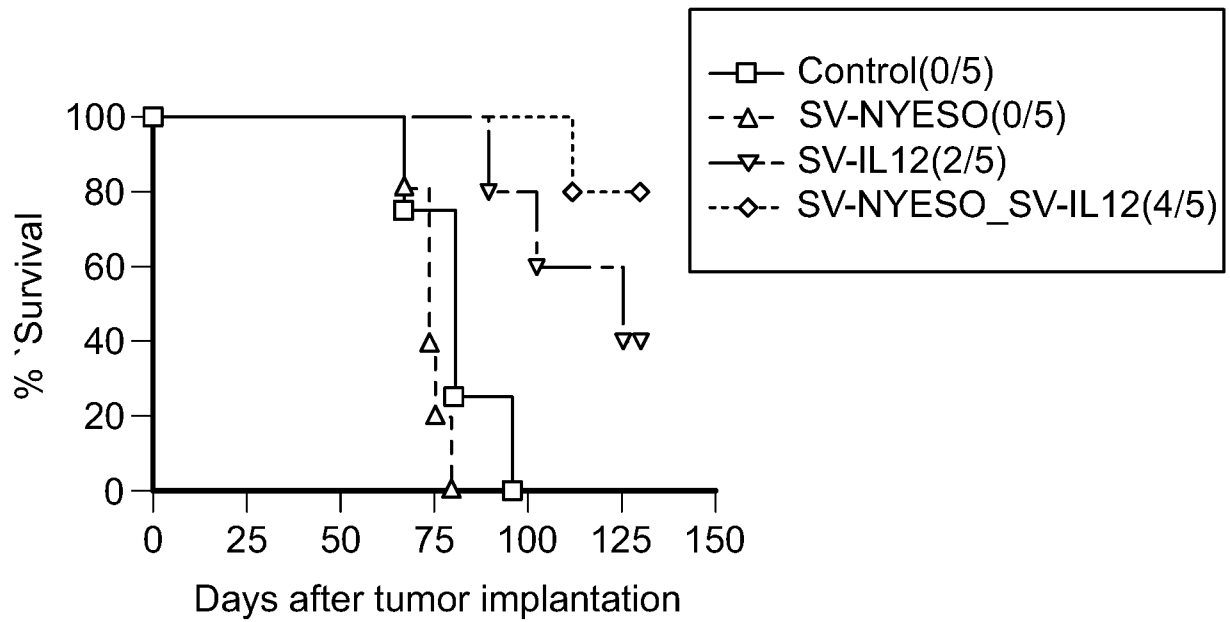
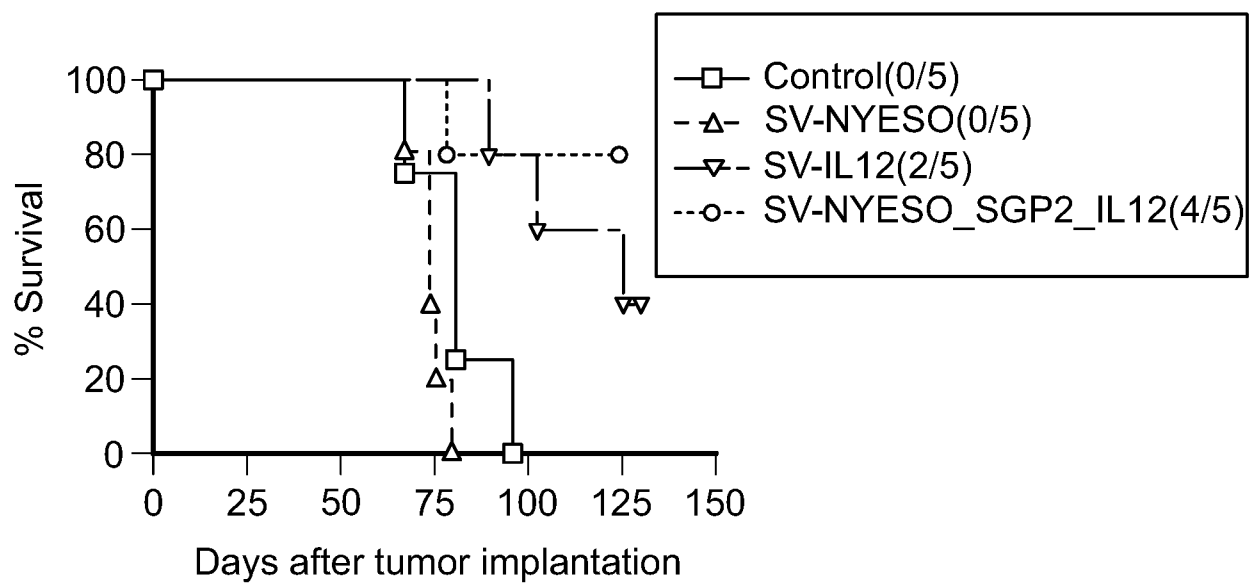
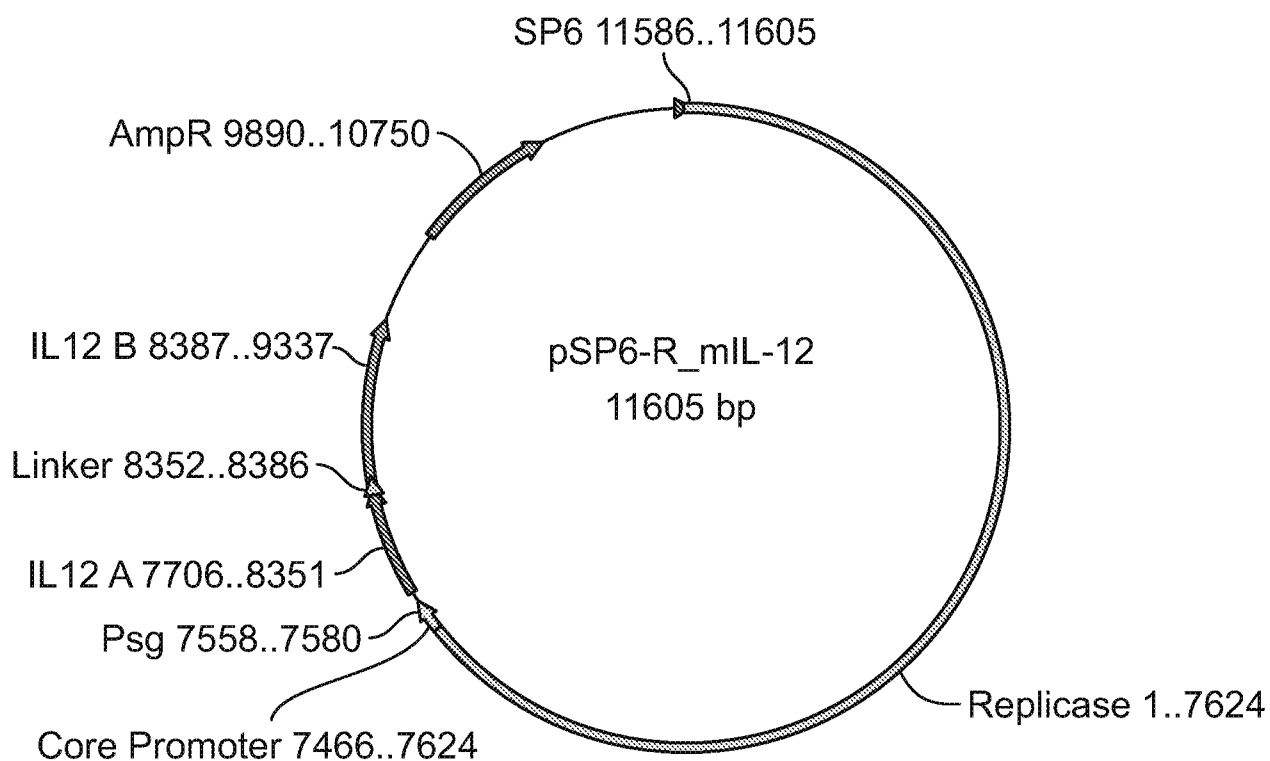
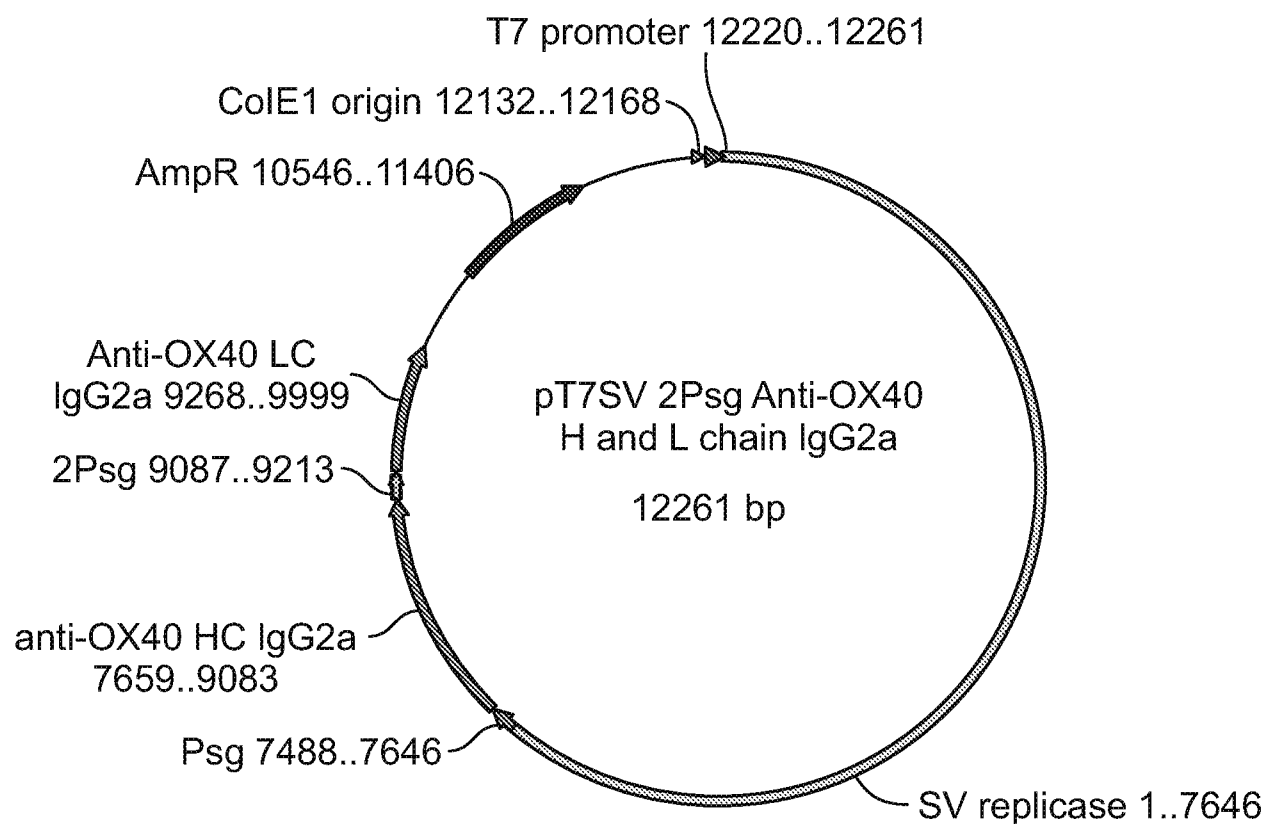


FIG. 35D

70/73**FIG. 36****FIG. 37**

71/73**FIG. 38****FIG. 39**

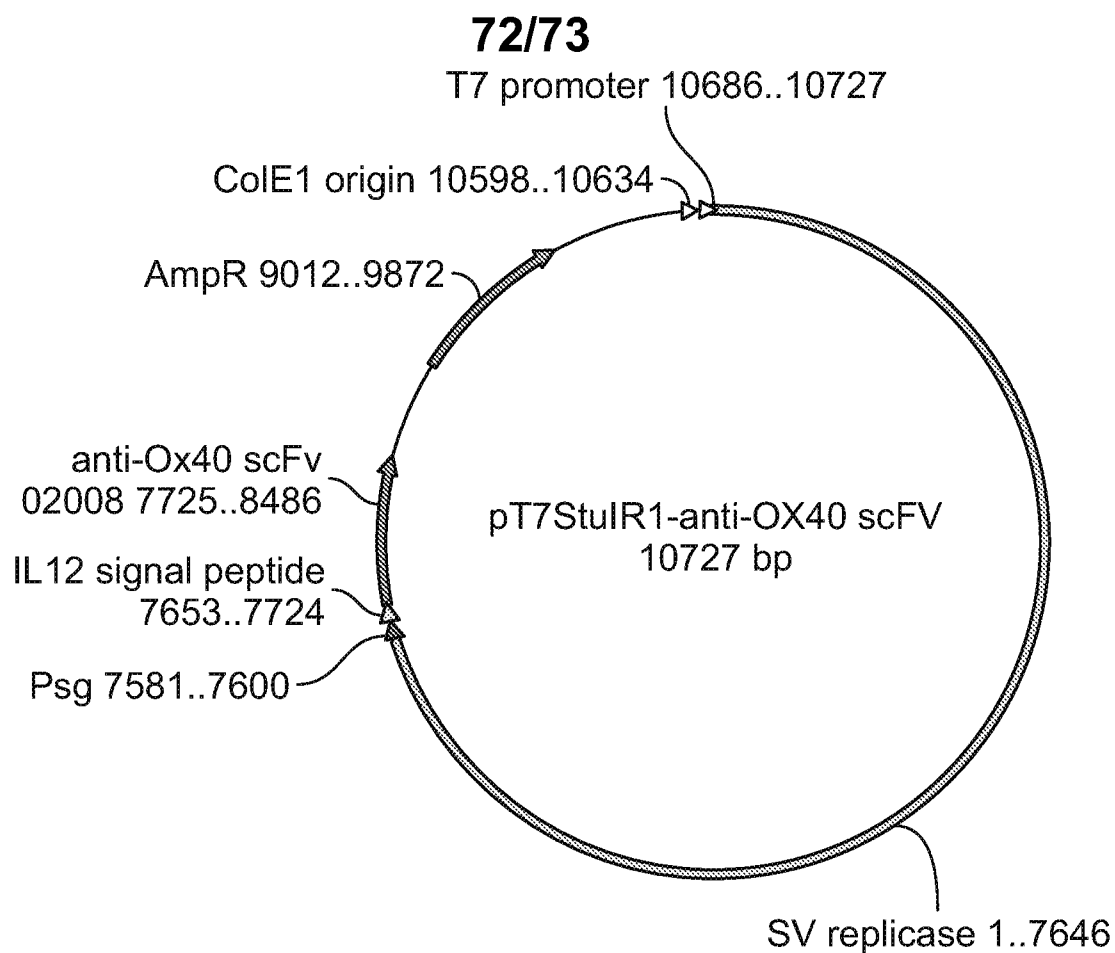


FIG. 40

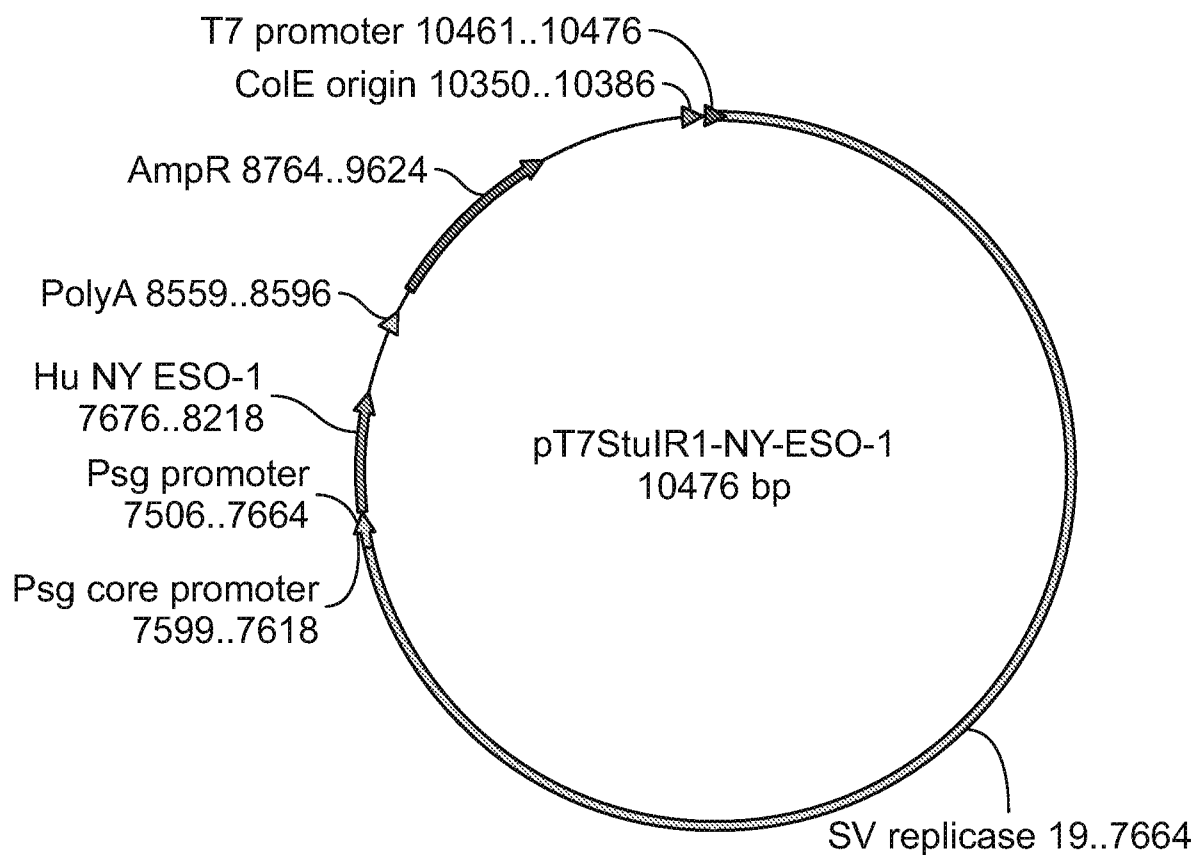
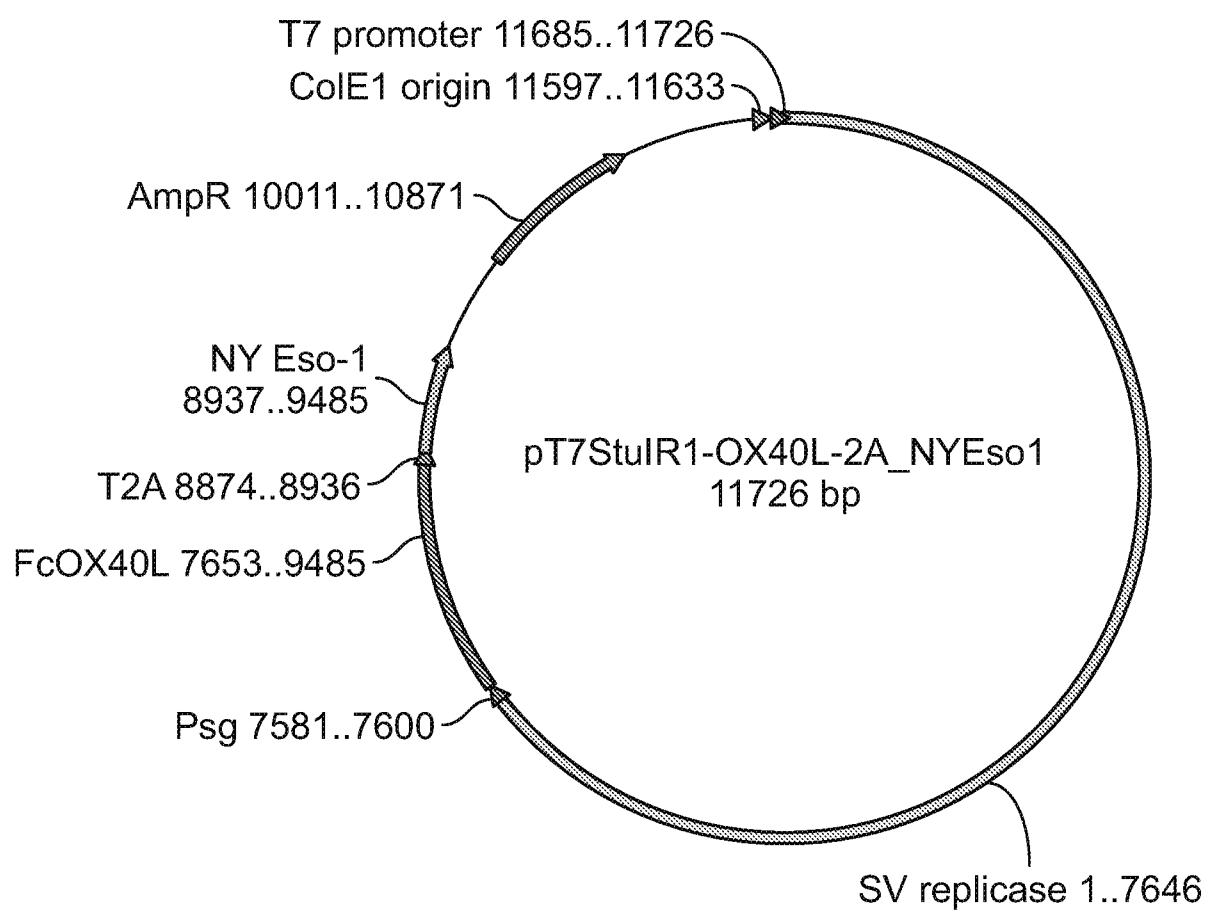


FIG. 41

73/73**FIG. 42**

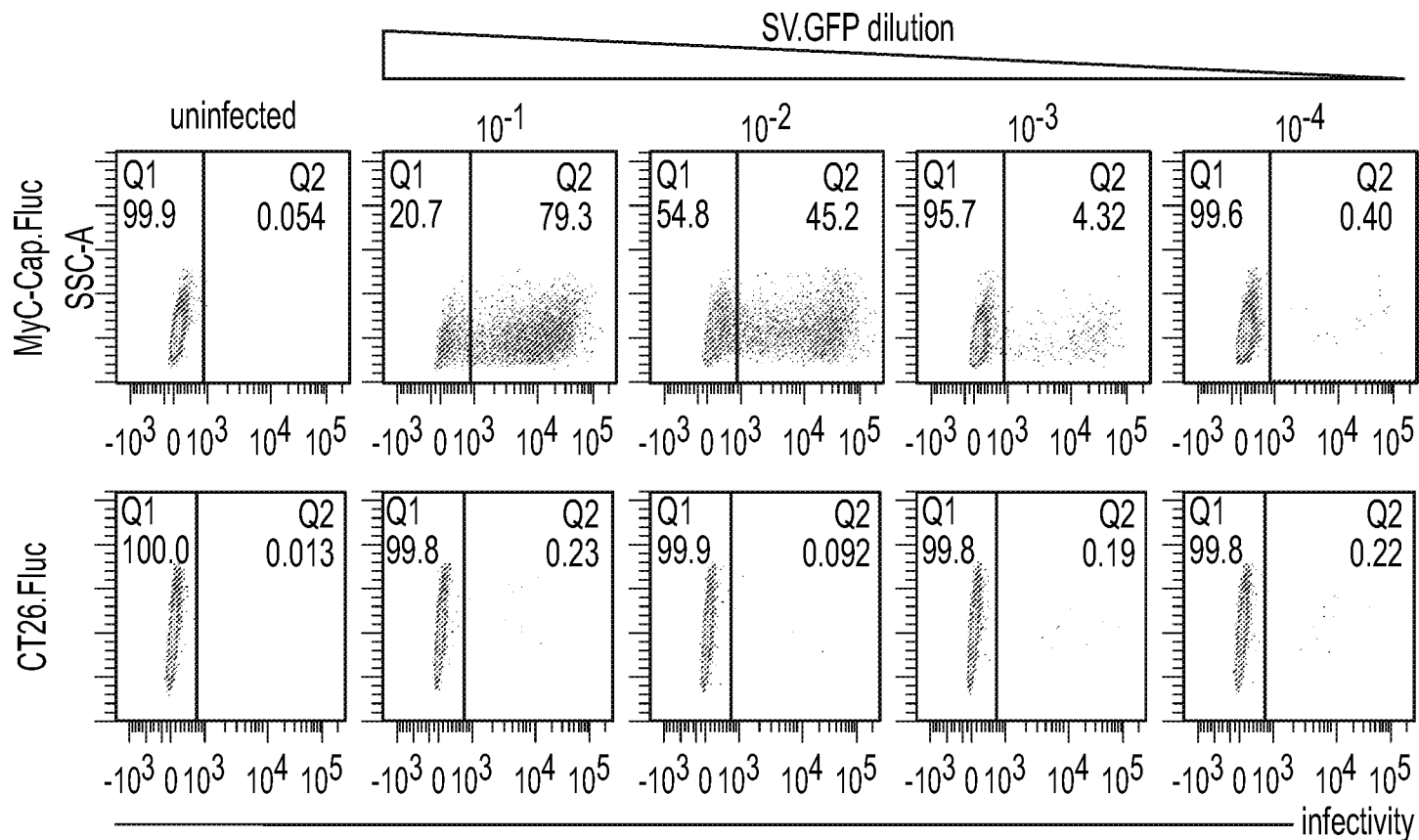


FIG. 4A