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(54) Title: VIRUS ENCODING AN ANTI-TCR-COMPLEX ANTIBODY OR FRAGMENT

(57) Abstract: The present disclosure relates to a replication deficient oncolytic viral vector or replication capable oncolytic virus encoding an antibody or a binding fragment thereof to the antigen-specific T-cell receptor complex (TCR) for expression on the surface of a cancer cell, pharmaceutical compositions comprising the same, and use of any one of the same in treatment, particularly in the treatment of cancer.

VIRUS ENCODING AN ANTI-TCR-COMPLEX ANTIBODY OR FRAGMENT

The present disclosure relates to an oncolytic virus comprising a transgene encoding at least an agonistic antibody or binding fragment thereof to the antigen-specific T-cell receptor complex (TCR), such as an anti-CD3 antibody (including a binding fragment thereof), for expression on the surface of a cancer cell, compositions comprising the same and use of said virus and composition for the treatment of cancer.

The present application claims priority from GB1522334.0, GB1607463.5, GB1617207.4 and GB1617206.6, each of which are incorporated into this application in their entirety.

BACKGROUND

Cancer is still a huge social burden to society in terms of the hardship and suffering of patients and their loved ones, and also in terms of the high financial cost of treating, caring for and supporting patients. It is now thought that the immune systems of healthy individuals clear cancerous cells routinely. However, in those patients with cancer one or more of the defense mechanisms involved in this clearance is/are down regulated or turned off completely.

It is now known that tumors change their microenvironment to make it more permissive to their growth. This occurs by the tumor releasing extracellular signals that, for example, promote tumor angiogenesis and/or induce local immune suppression or immune tolerance.

The T-cell receptor complex (TCR) on T-cells recognizes a particular antigen specifically when the antigen is in the form of peptide presented on a major histocompatibility (MHC) molecule on a cell surface. The T-cell receptor complex (TCR) comprises an antigen-binding heterodimer of alpha and beta or gamma and delta chains, together with the immunoglobulin family proteins CD3 epsilon, delta, gamma and zeta chains. The surface CD3 epsilon, delta and gamma chains play important roles in assembly of the TCR complex and stable surface expression. The zeta chain homodimer is almost completely intracellular and functions as the key signaling molecule of the complex when the TCR complex is cross-linked physiologically by antigen-MHC complexes on cell surfaces or by pharmacological modalities, for example antibodies to the TCR complex such as an anti-CD3 antibody (including a binding fragment thereof).

It is clear from many different preclinical and clinical studies that the microenvironment within tumours can suppress the development and activity of anti-tumour immune responses, with a wide variety of mechanisms being shown to potentially play a role. In particular immuno-suppressive mechanisms ultimately prevent T-cell responses from mediating the killing of tumour cells. Suppressive mechanisms may include the exclusion of T-cells from entering tumour tissues, inhibiting activation or activity of T-cells that do enter the tumour and the modulation of tumour cell proteins which reduces the ability of T-cells to recognize or respond to them. The importance of such immunosuppressive pathways in supporting tumour progression has been particularly highlighted by the clinical efficacy shown by antibodies to receptors in two such suppressive pathways, CTLA4 and PD-1/PDL1, which has led to their marketing approval for the treatment of melanoma and other cancers.

Whilst adoptive transfer of T cells stimulated *in vitro* has been proposed to provide activated cytolytic T cells, for example for the treatment of cancers such as a nasopharyngeal cancer, this can be a difficult, expensive and inconvenient therapy. Furthermore, sometimes these therapies are only effective in specific patient sub-groups.

Some clinical trials have been performed with cancer antigens, for example MAGE, in the form a vaccine containing a cancer antigen and adjuvant, such as monophosphoryl A and CpG. However, these approaches have failed to deliver the high clinical successes that were anticipated. Thus true *in vivo* stimulation or activation of T cells focused on cancer cells for the treatment of cancer is not a practical reality and the present time.

The present inventors have generated data showing that cancer cells that are made to express at least an agonistic anti-TCR antibody or binding fragment on their surface are dealt with more effectively by the immune system than unmodified cancer cells which don't express the protein on their surface.

Whilst not wishing to be bound by theory the present inventors believe that making the cancer cell express at least an agonistic anti-TCR antibody is a way of focusing or kickstarting a patient's immune system to fight the cancer, for example the anti-TCR antibody or binding fragment thereof may engage and/or activate T cells. Such activation of T-cells that physiologically would recognize cancer-specific antigens, including patient-specific neoantigens, can also lead to generation of effector and memory T-cell progeny that can migrate to regions of the same tumour or other tumour sites (e.g. metastases) not expressing an agonistic anti-TCR antibody or fragment thereof. Thus this therapy has the potential to generate an extended immune response in the form of activated T cells to cancer cells expressing their physiological cancer-specific antigen to fight the cancer systemically in the patient.

Clearly a cancer treatment that engages the body's own immune responses to fight the cancer would be extremely advantageous. Furthermore, the therapy is very focused on cancer cells and thus the off-targets effects and toxicities are likely to be much less than traditional therapies, such as chemotherapy.

The cancer cell can be made to express an anti-TCR antibody or binding fragment thereof by infecting the cancer cell with a replication competent oncolytic virus or a replication deficient oncolytic viral vector encoding an anti-TCR antibody or a binding fragment thereof.

SUMMARY OF THE DISCLOSURE

The present disclosure provides:

1. A replication deficient oncolytic viral vector or replication capable oncolytic virus encoding an antibody or a binding fragment thereof to the antigen-specific T-cell receptor complex (TCR) (such as an anti-CD3 antibody or binding fragment thereof) for expression on the surface of a cancer cell.
2. A replication deficient oncolytic viral vector or replication capable oncolytic virus according to paragraph 1, wherein the virus is replication competent.
3. A replication deficient oncolytic viral vector or replication capable oncolytic virus according to paragraph 1, wherein the virus is replication deficient.

4. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraph 1 to 3 wherein the encoded antibody further comprises a transmembrane domain or a GPI anchor.
5. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 4, wherein the transmembrane domain is selected from a sequence shown in SEQ ID NO: 10 to 14.
6. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of claims 1 to 5, wherein the oncolytic virus is selected from an adenovirus (such as Ad5, Ad3, Ad11, Ad35, Ad11/Ad3 and Ad5/3 or a derivative thereof), herpes simplex virus (such as HSV-1), vesicular stomatitis virus, reovirus, vaccina virus, Seneca valley virus, coxsackievirus, measles, Maraba virus and Newcastle disease virus, or for example selected from Oncorine (H101), Talimogene laparepvec, Reolysin, Pexastimogene devacirepvec (JX-594), Cavatak, Seprehvir,CGTG-102, MV-NIS, PV701, CL-ONC1, CG0070, and enadenotucirev (EnAd).
7. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 6, wherein the oncolytic virus is an adenovirus.
8. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 7, wherein the oncolytic adenovirus is a group B virus, such as Ad11 or a derivative thereof.
9. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 8, wherein the oncolytic adenovirus is EnAd.
10. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 7, wherein the oncolytic adenovirus is a group C virus, such as Ad5 or a derivative thereof.
11. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 6, wherein the oncolytic virus a herpes virus, such as HSV-1.
12. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 6, wherein the oncolytic virus is a vaccina virus.
13. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 6, wherein the oncolytic virus is a measles virus.
14. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraph 1 to 13, wherein the antibody or binding fragment is selected from the group comprising a full length antibody, a Fab, modified Fab, Fab', modified Fab', F(ab')2, Fv, single domain antibodies, scFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies, humabodies, disulfide stabilised forms of any one of the same and epitope-binding fragments thereof.
15. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 1 to 14 wherein the antibody binding fragment is a single chain Fv.

16. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of claims 1 to 15, wherein the oncolytic virus encodes at least one further transgene.
17. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 16, wherein the oncolytic virus encodes at least two further transgenes.
18. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 16 or 17, wherein the further transgene(s) encodes a protein independently selected from the group comprising a cytokine, a chemokine, an antagonistic antibody molecule or binding fragment thereof, an agonistic antibody molecule or binding fragment thereof, an immunomodulator, an enzyme and combinations thereof.
19. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 18, wherein at least one further transgene encodes an antibody molecule or a binding fragment thereof (for example which may be in a surface expressed form and/or secreted form).
20. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 19, wherein the antibody molecule or binding fragment is an inhibitor.
21. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to paragraph 20, wherein the inhibitor is an inhibitor of an angiogenesis factor or an inhibitor of a T cell deactivating factor.
22. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 19 or 21 wherein the antibody molecule or binding fragment comprises a binding domains specific to an antigen independently selected from CTLA-4, PD-1, PD-L1, PD-L2, VISTA, B7-H3, B7-H4, HVEM, ILT-2, ILT-3, ILT-4, TIM-3, LAG-3, BTLA, LIGHT or CD160, for example CTLA-4, PD-1, PD-L1 and PD-L2,
23. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 19 or 23 wherein the antibody molecule or binding fragment comprises a binding domains specific to an antigen independently selected from FGF, VEGF, VEGFR and NRP-1, PDGF, PDGFR, TGF-β, endoglin, TGF-β receptors, CCL2, $\alpha_V\beta_3$, $\alpha_V\beta_5$, $\alpha_5\beta_1$, VE-cadherin, CD31, ephrin, a plasminogen activator, plasminogen activator inhibitor-1, AC133, 1D1/1D3, class 3 semaphorins and combinations thereof.
24. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 19 to 21, wherein the antibody molecule or binding fragment thereof (in particular a secreted antibody as opposed to a cell surface expressed antibody) comprises a binding domain specific to a cancer antigen, for example MAGE, PRAME, NYESO, WT-1, CA-125, tyrosinase, MUC-1, CEA, alphafetoprotein (AFP), HER1, HER2, HER3, HER4, CD52, CD30, CD19 and combinations thereof.

25. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraph 19 to 24, wherein antibody molecule or binding fragment is an agonist.
26. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to paragraph 25, wherein the agonist is independently selected from the group comprising an antibody molecule or binding fragment comprising a binding domain specific to CD40, CD40 ligand (also known as CD154), GITR, OX40, CD27, 4-1BB and a combination thereof, such as CD40, GITR, OX40, CD27 and 4-1BB.
27. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to paragraph 26 wherein the antibody molecule or binding fragment thereof comprises a binding domain specific to CD40L.
28. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to paragraph 26 or 27 wherein the antibody molecule or binding fragment thereof comprises a binding domain specific to CD40.
29. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 26 to 28 wherein the antibody molecule or binding fragment thereof comprises a binding domain specific to GITR.
30. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 26 to 29 wherein the antibody molecule or binding fragment thereof comprises a binding domain specific to OX40.
31. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 26 to 30 wherein the antibody molecule or binding fragment thereof comprises a binding domain specific to CD27.
32. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 26 to 31 wherein the antibody molecule or binding fragment thereof comprises a binding domain specific to 4-1BB.
33. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraph 18 to 32, where the immunomodulator is a membrane-associated protein ligand for an immune cell surface receptors independently selected from the group comprising CTLA-4, PD-1, PD-L1, PD-L2, VISTA, B7-H3, B7-H4, HVEM, ILT-2, ILT-3, ILT-4, TIM-3, LAG-3, BTLA, LIGHT or CD160, for example CTLA-4, PD-1, PD-L1, PD-L2, CD16, CD25, CD33, CD332, CD127, CD31, CD43, CD44, CD162, CD301a, CD301b and Galectin-3, FLT-3, FLT-3 ligand, TLRs, TLR ligands, CCR7, CD1a, CD1c, CD11b, CD11c, CD80, CD83, CD86, CD123, CD172a, CD205, CD207, CD209, CD273, CD281, CD283, CD286, CD289, CD287, CXCR4, GITR Ligand, IFN-α2, IL-12, IL-23, ILT1, ILT2, ILT3, ILT4, ILT5, ILT7, TSLP Receptor, CD141, CD303, CADM1, CLEC9a, XCR1 and CD304, OX40, OX40 ligand, CD27, CD28, CD30, CD40, CD40 ligand, CD70, CD137, GITR, 4-1BB, ICOS,ICOS ligand and combinations thereof.

34. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to paragraph 33, wherein membrane-associated protein ligand for an immune cell surface receptor is or binds to CTLA-4.
35. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to paragraph 33 or 34, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to PD-1.
36. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 35, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to PD-L1.
37. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 36, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to PD-L2.
38. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 37, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to VISTA.
39. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 38, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to B7-H3.
40. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 39, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to B7-H4.
41. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 40, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to HVEM.
42. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 41, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to ILT-2.
43. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 42, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to ILT-3.
44. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 43, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to ILT-4.
45. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 44, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to TIM-3.
46. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 45, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to LAG-3.

47. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 46, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to BTLA.
48. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 47, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to LIGHT.
49. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 48, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD160.
50. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 49, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD16.
51. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 50, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD25.
52. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 51, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD33.
53. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraph 33 to 52, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD332.
54. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 53, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD127.
55. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 54, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD31.
56. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 55, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD43.
57. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 56, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD44.
58. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 57, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD162.
59. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 58, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD301a.

60. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 59, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds CD301b.
61. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 60, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds Galectin-3.
62. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 61, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds FLT-3.
63. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 62, wherein the membrane-associated protein ligand for an immune cell surface receptor is FLT-3 ligand.
64. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 63, wherein the membrane-associated protein ligand for an immune cell surface receptor is a TLR.
65. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 64, wherein the membrane-associated protein ligand for an immune cell surface receptor is a TLR-ligand.
66. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 65, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CCR7.
67. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 66, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD1a.
68. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 67, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD1c.
69. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 68, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD11b.
70. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 69, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD11c.
71. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 70, wherein the membrane-associated protein ligand for an immune cell surface receptor is CD80.
72. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 71, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD83.

73. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 72, wherein the membrane-associated protein ligand for an immune cell surface receptor is CD86.
74. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 73, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD123.
75. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 74, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD172a.
76. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 75, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD205.
77. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 76, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD207.
78. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 77, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD209.
79. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 78, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD273.
80. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 79, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD281.
81. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 80, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD283.
82. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 81, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD286.
83. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 82, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD289.
84. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 83, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD287.
85. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 84, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CXCR4.

86. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 85, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to GITR ligand.
87. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 86, wherein the membrane-associated protein ligand for an immune cell surface receptor binds to IFN-α2.
88. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 87, wherein the membrane-associated protein ligand for an immune cell surface receptor is IL-12.
89. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 88, wherein the membrane-associated protein ligand for an immune cell surface receptor is IL-23.
90. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 89, wherein the membrane-associated protein ligand for an immune cell surface receptor binds to ILT1.
91. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 90, wherein the membrane-associated protein ligand for an immune cell surface receptor binds to ILT5.
92. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 91, wherein the membrane-associated protein ligand for an immune cell surface receptor binds to ILT7.
93. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 92, wherein the membrane-associated protein ligand for an immune cell surface receptor binds to TSLP Receptor.
94. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 93, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD141.
95. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 94, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD303.
96. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 95, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CADM1.
97. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 96, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CLEC9a.
98. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 96, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to XCR1.

99. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 98, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD304.

100. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 99, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to OX40.

101. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 100, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to OX40 ligand.

102. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 101, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD27.

103. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 102, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD28.

104. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 103, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD30.

105. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 104, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD40.

106. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 105, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD40 ligand.

107. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 106, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD70.

108. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 107, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to CD137.

109. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 108, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to GITR.

110. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 109, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to 4-1BB.

111. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 110, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to ICOS.

112. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of paragraphs 33 to 111, wherein the membrane-associated protein ligand for an immune cell surface receptor is or binds to ICOS ligand.

113. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraph 18 to 112, wherein at least one further transgene encodes a cytokine (including secreted and/or cell membrane associated molecule).

114. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraph 17 to 113, wherein a second further transgene encodes a cytokine (including secreted or cell membrane associated molecules).

115. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraph 18 to 114, wherein the encoded cytokine is selected from TNF alpha super family (TNFRSF includes TNF-alpha, TNF-C, OX40L, CD154, FasL, LIGHT, TL1A, CD70, Siva, CD153, 4-1BB ligand, TRAIL, RANKL, TWEAK, APRIL, BAFF, CAMLG, NGF, BDNF, NT-3, NT-4, GITR ligand, EDA-A, EDA-A2), TGF-beta superfamily, IL-1 family (i.e. IL-1 and IL-8), IL-2 family, IL-10 family, IL-17 family, interferon family.

116. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 115, wherein the cytokine is TNF-alpha.

117. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 115 or 116, wherein the cytokine is TNF-C.

118. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 117, wherein the cytokine is OX40L.

119. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 118, wherein the cytokine is CD154.

120. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 119, wherein the cytokine is FasL.

121. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 120, wherein the cytokine is LIGHT.

122. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 121, wherein the cytokine is TL1A.

123. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 122, wherein the cytokine is CD70.

124. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 123, wherein the cytokine is Siva.

125. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 124, wherein the cytokine is CD153.

126. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 125, wherein the cytokine is 4-1BB ligand.

127. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 126, wherein the cytokine is TRAIL.

128. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 127, wherein the cytokine is RANKL.
129. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 128, wherein the cytokine is TWEAK.
130. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 129, wherein the cytokine is APRIL.
131. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 130, wherein the cytokine is BAFF.
132. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 131, wherein the cytokine is CAMLG.
133. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 132, wherein the cytokine is NGF.
134. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 133, wherein the cytokine is BDNF.
135. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 134, wherein the cytokine is NT-3.
136. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 135, wherein the cytokine is GITR ligand.
137. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 136, wherein the cytokine is EDA-A.
138. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 137, wherein the cytokine is EDA-A2.
139. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 138, wherein the cytokine is from the TGF-beta superfamily.
140. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 139, wherein the cytokine is IL-1.
141. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 140, wherein the cytokine is IL-8.
142. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 141, wherein the cytokine is IL-2.
143. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 142, wherein the cytokine is IL-10.
144. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 143, wherein the cytokine is IL-17.
145. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 115 to 144, wherein the cytokine is from the interferon family.

146. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 114 or 115 wherein the encoded cytokine is independently selected from TNF-alpha, IL-1, IL-8, IL-10, IL-17, interferon, LIGHT, TL1A, Siva, TRAIL, RANKL, TWEAK, APRIL, NGF, BDNF, NT-3, and EDA-A2.

147. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 114, 115 or 146wherein the encoded cytokine is independently selected from TNF-C, OX40L, CD154, FasL, CD70, CD153, 4-1BB ligand, and EDA-A.

148. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraph 18 to 147, wherein the cytokine is selected from the group comprising IL-2, IFN-alpha, IFN-beta, IFN-gamma, Flt3 ligand, GM-CSF, IL-15, and IL-12.

149. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 148, wherein the cytokine is IL-2.

150. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 148 or 149, wherein the cytokine is IFN-alpha.

151. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 148 to 150, wherein the cytokine is IFN-beta.

152. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 148 to 151, wherein the cytokine is IFN-gamma.

153. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 148 to 152, wherein the cytokine is Flt3 ligand.

154. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 148 to 153, wherein the cytokine is GM-CSF.

155. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 148 to 154, wherein the cytokine is IL-15.

156. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 148 to 154, wherein the cytokine is IL-12.

157. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraph 21 to 156, wherein at least one further transgene encodes a chemokine.

158. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraph 21 to 34, wherein the chemokine is selected from the group comprising MIP-1 alpha, RANTES, IL-8, CCL5, CCL17, CCL20, CCL22, CXCL9, CXCL10, CXCL11, CXCL13, CXCL12, CCL2, CCL19 and CCL21.

159. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 158, wherein the chemokine encoded is MIP-1 alpha.

160. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 158 or 159, wherein the chemokine encoded is RANTES.

161. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 158 to 160, wherein the chemokine encoded is IL-8.

162. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 158 to 161, wherein the chemokine encoded is CCL5.

163. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 158 to 162, wherein the chemokine encoded is CCL17.

164. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 158 to 163, wherein the chemokine encoded is CCL20.

165. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 158 to 164, wherein the chemokine encoded is CCL22.

166. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 158 to 165, wherein the chemokine encoded is CXCL9.

167. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 158 to 166, wherein the chemokine encoded is CXCL10.

168. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 158 to 167, wherein the chemokine encoded is CXCL11.

169. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 158 to 168, wherein the chemokine encoded is CXCL12.

170. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 158 to 169, wherein the chemokine encoded is CXCL13.

171. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 158 to 170, wherein the chemokine encoded is CCL2.

172. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 158 to 171, wherein the chemokine encoded is CCL19.

173. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of paragraphs 158 to 172, wherein the chemokine encoded is CCL21.

174. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to paragraph 159, wherein the virus comprises transgenes encoding a cytokine and chemokine combination selected from i) MIP-1α and Flt3, and ii) MIP-1α and IFNα.

175. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of claims 1 to 174, wherein the anti-CD3 antibody or binding fragment has at least the binding domain comprising a VH and a VL regions from muromonab-CD3 (OKT3), otelixizumab, teplizumab or visilizumab.

176. A method of treating a cancer patient by *in vivo* stimulation of T cells, for example T cells in the cancer cell environment, to focus on cancerous cells by:

administering a therapeutically effective amount of a replication deficient oncolytic viral vector or oncolytic replication capable virus encoding an antibody or a binding fragment thereof to the antigen-specific T-cell receptor complex (TCR),

wherein the virus or viral vector selectively infects said cancerous cells and expresses on the surface of the cell the said encoded anti-TCR antibody or binding fragment.

177. A method of treating a cancer patient according to paragraph 176 by:
administering a therapeutically effective amount of a replication deficient oncolytic viral vector or oncolytic replication capable virus encoding an anti-CD3 antibody or a binding fragment thereof,
wherein the virus or viral vector selectively infects said cancerous cells and expresses on the surface of the cell the said encoded anti-CD3 antibody or binding fragment.

178. A method of treating a cancer patient comprising administering a therapeutically effective amount of a replication deficient oncolytic viral vector or oncolytic replication capable virus encoding an anti-TCR antibody or binding fragment thereof (such as anti-CD3 antibody or a binding fragment thereof), for expression on the surface of a cancer cell.

179. A method according to any one of paragraph 176 to 178, wherein the virus is replication competent.

180. A method according to any one of paragraph 176 to 178, wherein the virus is replication deficient.

181. A method according to any one of paragraphs 176 to 180, wherein the oncolytic virus or viral vector is selected from an adenovirus (such as Ad5, Ad3, Ad11, Ad35, Ad11/Ad3 and Ad5/3 based viruses), herpes simplex virus (such as HSV-1), reovirus, vaccina virus, Seneca valley virus, coxsackie virus, measles virus, Maraba virus and Newcastle disease virus.

182. A method according to paragraph 181, wherein the virus or viral vector is selected from the group comprising Oncorine (H101), Talimogene laparepvec, Reolysin, Pexastimogene devacirepvec (JX-594), Cavatak, Seprehvir,CGTG-102, MV-NIS, PV701, CL-ONC1, CG0070, and enadenotucirev (EnAd).

183. A method according to paragraph 181, wherein the oncolytic virus or viral vector is an adenovirus.

184. A method according to paragraph 183, wherein the oncolytic virus or viral vector is a group B virus, for example Ad11 or a derivative thereof.

185. A method according to paragraph 184, wherein the oncolytic virus or viral vector is enadenotucirev.

186. A method according to any one of paragraph 176 to 185, wherein the antibody or binding fragment is selected from the group comprising a full length antibody, a Fab, modified Fab, Fab', modified Fab', F(ab')2, Fv, single domain antibodies, scFv, bi, tri or tetra-valent antibodies, bis-scFv, diabodies, triabodies, tetrabodies, humabodies, disulfide stabilised forms of any one of the same and epitope-binding fragments thereof.

187. A method according to paragraph 186, wherein the antibody binding fragment is a single chain Fv.

188. A method according to any one of paragraph 176 to 187, wherein the oncolytic virus or viral vector encodes a further transgene.

189. A method according to paragraph 188, wherein the further transgene encodes a protein independently selected from the group comprising a cytokine, a chemokine, an antagonistic antibody molecule or fragment thereof, an agonistic antibody molecule or fragment thereof and combinations thereof.

190. A method according to paragraph 189, wherein the cytokine is selected from the group comprising IL-2, IFN-alpha, IFN-beta, IFN-gamma, Flt3 ligand, GM-CSF, IL-15, IL-12 and combinations thereof.

191. A method according to paragraph 189 or 190, wherein the chemokine is selected from the group comprising MIP-1 alpha, RANTES, IL-8, CCL5, CCL17, CCL20, CCL22, CXCL9, CXCL10, CXCL11, CXCL13, CXCL12, CCL2, CCL19, CCL21 and combinations thereof.

192. A method according to any one of paragraph 189 to191, wherein a cytokine and chemokine combination of MIP-1α and Flt3 or MIP-1α and IFNα is encoded in the virus.

193. A method according to any one of claims 176 to 192, wherein the anti-CD3 antibody or binding fragment has at least the binding domain comprising a VH and a VL regions from muromonab-CD3 (OKT3), otelixizumab, teplizumab or visilizumab.

194. A method according any one of paragraph 176 to 193, wherein the anti-CD3 antibody or binding fragment thereof comprises a transmembrane domain or a GPI anchor.

195. A method according to paragraph 194, wherein the transmembrane is selected from a sequence shown in SEQ ID NO: 10 to 14.

196. A method according to any one of paragraph 176 to 195, where a linker sequence is employed between the transmembrane domain and the antibody or binding antibody fragment, such as scFv (e.g. myc tag sequence, CD80, CD86 membrane proximal sequences, immunoglobulin sequences).

In one embodiment one or more transgene encoded by the virus of the present disclosure are under the control of an endogenous promoter, in particular where the virus is an adenovirus.

In one embodiment one or more transgene encoded by the virus of the present disclosure are under the control of an exogenous promoter such as a CMV promoter, in particular where the virus is a non-adenovirus.

In one embodiment there is provided a virus (replication deficient or replication competent virus comprising a transgene cassette disclosed here, including disclosed as part of complete virus sequence).

In one embodiment an adenovirus of the present disclosure does not encode a B7 protein nor does it express an active fragment thereof.

In one embodiment a protein or peptide encoded by a virus of the present disclosure for surface expression on a cancer cell is an immunogenic protein, for example a non-human protein.

In a one aspect the present disclosure provides a method of treating a cancer patient by stimulating *in vivo* T cells to focus on cancerous cells by administering a therapeutically effective amount of a replication deficient oncolytic viral vector or replication competent oncolytic virus

encoding an anti-TCR antibody or a binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof), for expression on the surface of said cancerous cells.

Thus there is provided a method of treating a cancer patient by *in vivo* stimulation of T cells, for example T cells in the cancer cell environment, to focus on cancerous cells by:

administering a therapeutically effective amount of a replication deficient oncolytic viral vector or oncolytic replication competent virus encoding an anti-TCR antibody or a binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof), wherein the virus or viral vector selectively infects said cancerous cells and expresses on the surface of the cell the said encoded anti-TCR antibody or binding fragment.

Also provided is a replication deficient oncolytic viral vector or a replication competent oncolytic virus encoding an anti-TCR antibody or a binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof), for expression on the surface of cancerous cells, for use in treatment of a cancer patient by *in vivo* stimulation of T cells, in particular T cells in the cancer cell environment, to focus on said cancerous cells. As described herein the virus or viral vector selectively infects said cancerous cells and expresses on the surface of the cell the said encoded anti-TCR antibody or binding fragment (such as an anti-CD3 antibody or binding fragment thereof).

In one embodiment there is provided a replication deficient oncolytic viral vector or a replication competent oncolytic virus encoding a an anti-TCR antibody or a binding fragment thereof, encoding a an anti-TCR antibody or a binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof), for expression on the surface of cancerous cells for use in the manufacture of a medicament for the treatment cancer by *in vivo* stimulation of T cells, in particular T cells in the cancer cell environment, to focus on said cancerous cells.

In a second independent embodiment there is provided a method of treating a cancer patient comprising administering a therapeutically effective amount of a replication deficient oncolytic viral vector or oncolytic replication competent virus encoding an anti-TCR antibody or a binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof), for expression on the surface of a cancer cell, for example with the proviso that when the virus is a replication competent oncolytic adenovirus then: a transgene or transgenes therein are under the control of an exogenous promoter, and/or the virus does not encode a B7 protein or an active fragment thereof.

Thus there is provide a replication deficient oncolytic viral vector or oncolytic replication competent virus encoding an anti-TCR antibody or a binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof), for expression on the surface of a cancer cell, for use in the treatment of cancer, for example with the proviso that when the virus is a replication competent oncolytic adenovirus then: a transgene or transgenes therein is/are under the control of an exogenous promoter, and/or the virus does not encode a B7 protein or an active fragment thereof.

In one embodiment a replication deficient oncolytic viral vector or oncolytic replication competent virus encoding an anti-TCR antibody or a binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof), for expression on the surface of a cancer cell, for the manufacture of a medicament for the treatment of cancer, for example with the proviso that when the virus is a replication competent oncolytic adenovirus then: a transgene or transgenes therein

are under the control of an exogenous promoter, and/or the virus does not encode a B7 protein or an active fragment thereof.

In an independent aspect there is provided a replication deficient oncolytic viral vector or replication competent oncolytic virus encoding an anti TCR antibody or binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof) for expression on the surface of a cancer cell.

Thus in a third aspect there is provided a replication deficient oncolytic viral vector or replication competent oncolytic virus encoding an anti-TCR antibody or binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof) for expression on the surface of a cancer cell, for example with the proviso that when the virus is a replication competent oncolytic adenovirus then: a transgene or transgenes therein are under the control of an exogenous promoter, and/or the virus does not encode a B7 protein or an active fragment thereof.

In one embodiment of the present disclosure the virus is replication competent. In one embodiment the present disclosure is not a replication competent oncolytic adenovirus.

In one embodiment of the present disclosure the viral vector, which is replication deficient, for example deleted in part or all of a gene essential for viral replication, for example the E1 region in adenoviruses.

In one embodiment of the disclosure the virus or viral vector is attenuated.

In one embodiment of the disclosure the oncolytic virus or viral vector is selected from an adenovirus (such as Ad5, Ad3, Ad11, Ad11/Ad3 and Ad5/3), herpes simplex virus (such as HSV-1), reovirus, vaccina virus, Seneca valley virus, coxsackie virus, Maraba virus, measles virus, vesicular stomatitis virus and New Castle disease virus, including mutated versions, variants or derivatives thereof comprising a transgene.

In one embodiment the virus or viral vector is selected from the group comprising Oncorine (H101), Talimogene, Reolysin, Pexastimogene devacirepvec (JX-594), Cavatak, Seprehvir,CGTG-102, MV-NIS, PV701, CL-ONC1, CG0070, and Enadenotucirev (EnAd), including mutated versions, variants or derivatives thereof comprising a transgene.

In one embodiment of the disclosure the oncolytic virus is an adenovirus, for example a group B virus, such as Ad11, more specifically EnAd.

In one embodiment of the disclosure the oncolytic virus is not an adenovirus, for example is not a group B adenovirus, such as is not Ad11, more specifically is not EnAd.

In one embodiment the anti-TCR antibody or binding fragment thereof is specific to the TCR alpha, beta, gamma or delta proteins that are directly involved in antigen binding or a combination thereof, for example an anti-TCR beta chain antibody.

In one embodiment the anti-TCR antibody or binding fragment is specific to CD3 delta, epsilon, gamma or a combination thereof, in particular is specific to CD3 epsilon.

In one embodiment the binding domain comprises a VH and a VL from an antibody muromonab-CD3 (also known as OKT3), otelixizumab (also known as TRX4), teplizumab (also known as hOKT3γ1(Ala-Ala)), or visilizumab.

In one embodiment of the disclosure when the replication competent virus is an adenovirus the binding domain of the agonistic anti-TCR antibody or binding fragment thereof does not comprise a VH and a VL from an antibody muromonab-CD3 (also known as OKT3), otelixizumab (also known as TRX4), teplizumab (also known as hOKT3γ1(Ala-Ala)), or visilizumab.

In one embodiment of the disclosure the oncolytic virus or viral vector encodes a further transgene, for example a further transgene encoding a protein selected from the group comprising a cytokine, a chemokine, an antagonistic antibody molecule or fragment thereof, an agonistic antibody molecule, or fragment thereof, an immunomodulator and combinations thereof.

In one embodiment of the disclosure the oncolytic virus or viral vector encodes at least two further transgenes, for example a further transgene encoding a protein independently selected from the group comprising a cytokine, a chemokine, an antagonistic antibody molecule or fragment thereof, an agonistic antibody molecule, or fragment thereof, an immunomodulator and combinations thereof.

In one embodiment the further transgene encodes an antibody or binding fragment thereof.

An antibody binding fragment which may be encoded by an oncolytic virus or viral vector of the disclosure in one embodiment is independently selected from a Fab, modified Fab, Fab', modified Fab', F(ab')2, Fv, single domain antibodies, scFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies, disulfide stabilised forms of any one of the same and epitope-binding fragments thereof, in particular a scFv.

In one embodiment the antibody encoded is a full length antibody.

In one embodiment of the disclosure the further transgene encodes an antibody or binding fragment which activates T cells, for example is agonist which stimulates T cell signalling, such an antibody or binding fragment specific to CD28, in particular an agonist specific to CD28.

In one embodiment of the disclosure, the further transgene encodes a cytokine, for example a secreted or membrane associated cytokine.

Cytokine as employed herein means low-molecular-weight proteins that can regulate the nature, intensity and duration of immune response by binding to specific receptors on target cells and exerting a variety of effects on lymphocytes and/or other cells. As used herein, the term cytokine includes secreted versions of cell surface ligands or receptors, including fusion proteins (for example molecules fused to an immunoglobulin Fc domain).

In one embodiment according to the present disclosure the cytokine encoded is selected from the group comprising IL-2, IFN-alpha, IFN-beta, IFN-gamma, Flt3 ligand, GM-CSF, IL-15, IL-12 and combinations thereof.

In one embodiment according to the present disclosure the encoded cytokine is independently selected from the TNF alpha super family (TNFRSF includes TNF-alpha, TNF-C,

OX40L, CD154, FasL, LIGHT, TL1A, CD70, Siva, CD153, 4-1BB ligand, TRAIL, RANKL, TWEAK, APRIL, BAFF, CAMLG, NGF, BDNF, NT-3, NT-4, GITR ligand, EDA-A, EDA-A2), TGF-beta superfamily, IL-1 family (i.e. IL-1 and IL-8), IL-2 family, IL-10 family, IL-17 family, interferon family.

In one embodiment according to the present disclosure the chemokine is selected from the group comprising MIP-1 alpha, IL-8, CCL5, CCL17, CCL20, CCL22, CXCL9, CXCL10, CXCL11, CXCL13, CXCL12, CCL2, CCL19, CCL21 and combinations thereof.

In one embodiment according to the present disclosure the chemokine is selected from the group comprising CXCR2, CCR2, CCR4, CCR5, CCR6, CCR7, CCR8, CXCR3, CXCR4, CXCR5 and CRTH2.

In one embodiment the further transgenes encode a first antibody or binding fragment thereof and a second antibody or binding fragment thereof, referred to herein an antibody/antibody combination.

In one embodiment the further transgene or transgenes encode an antibody or binding fragment and a cytokine, referred to herein as an antibody/cytokine combination.

In one embodiment the further transgene or transgenes encodes a first cytokine and a second cytokine, referred to herein as a cytokine/cytokine combination.

In one embodiment the further transgene or transgenes encode a first chemokine and a second chemokine, referred to herein as a chemokine/chemokine combination.

In one embodiment the further transgene or transgenes encode a chemokine and cytokine, referred to herein as cytokine/chemokine combination.

Where there are multiple transgenes one will generally encode one entity such as the cytokine or antibody etc. and a different gene will encode the entity, for example the chemokine or antibody etc.

Thus in one embodiment according to the present disclosure the oncolytic virus or viral vector encodes a cytokine, chemokine combination, for example MIP-1α and Flt3 or MIP-1α and IFNα.

In one embodiment the anti-TCR antibody or binding fragment has at least the binding domain comprising a VH and a VL regions from muromonab-CD3 (OKT3) the variable regions for which are shown in SEQ ID NO: 1 and 2 and a single chain Fv version of which is shown in SEQ ID NO: 3, otelixizumab, teplizumab (the variable regions for which are shown in SEQ ID NO: 6 and 7) or visilizumab.

In one embodiment a transgene encoded by the virus, for example the anti-TCR antibody or binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof) comprises a transmembrane domain or a GPI anchor.

In one embodiment the anti-CD3 antibody or binding fragment is only surface expressed, that it is not expressed as a secreted protein.

In one embodiment a combination of a transmembrane domain and a secretory signal sequence is employed to express a protein encoded by the virus (for example as described herein)

on the surface of an infected cancer cell. The present inventors have shown that the proteins encoded are expressed only on cells which are permissive to infection by the virus, i.e. cancer cells.

In one embodiment the fragment employed to express the protein on the surface of the infected cancer cell (such as the transmembrane fragment) is selected from the group comprising TM Domain Sequences (Minimal portions) given in SEQ ID NO: 10, 11, 12, 13 or 14:

SEQ ID NO:	Name	SEQUENCE
10	PDGFR Receptor A	AVLVLLVIVIISLIVLVVIW
11	PDGFR Receptor B	VVISAILALVVLTIISLIILI
12	INSULIN-LIKE GROWTH FACTOR 1	IIIGPPLIFVFLFSVVIGSIYLFL
13	IL6-R	SSSVPLPTFLVAGGSLAFGTLLCIAIVL
14	CD28	FWVLVVVGGVLACYSLLVTVAFIIFWV

In one embodiment the transmembrane domain is from a B7 protein.

In one embodiment the oncolytic virus or viral vector according to the present disclosure, in particular a replication competent adenovirus, such as Ad11 or EnAd does not encode a B7 protein nor an active fragment thereof.

In one embodiment the treatment is by *in vivo* stimulation of T cells, for example T cells in the cancer cell environment, to focus on cancerous cell.

SUMMARY OF THE FIGURES

Figure 1 shows a representation of a T cell receptor (example with alpha-beta antigen recognition chains)

Figure 2 shows schematics of transgene cassettes for viruses expressing human CD80 (Figure 2A), co-expressing human IFNα and human CD80 (Figure 2B), co-expressing OKT3 scFv and human CD80 (Figure 2C), co-expressing human Flt3L, human MIP1α and human IFNα (Figure 2D), co-expressing human Flt3L, human MIP1α and human CD80 (Figure 2E), co-expressing human IFNα, human MIP1α and human CD80 (Figure 2F), and a schematic of the open reading frame (ORF) or the OKT3 scFv (Figure 2G)

Figure 3 shows schematics of the NG-348A (Figure 3A), NG-420 (Figure 3B) and NG-420A (Figure 3C) transgene cassettes

Figure 4 shows high CD80 expression by 48 hours on the cell surface of A549 tumour cells infected with either NG-347 or NG-348 viruses but little or no CD80 expression following EnAd infection

Figure 5 shows high CD80 expression by 48 hours on the cell surface of DLD-1 tumour cells infected with NG-348 viruses but little or no CD80 expression following EnAd infection

Figure 6 shows CD80 expression on $EpCam^+$ A549 cells infected with NG-348 and co-cultured with human $CD3^+$ T-cells, but not when infection was with EnAd

Figure 7 shows CD25 is upregulated on human $CD3^+$ T-cells following co-culture with NG-348 infected A549 cells, but not when infection was with EnAd (Figure 7A), with both the percentage of $CD25^+$ cells (Figure 7B) and the level of CD25 expression per cell (Figure 7C) was increased.

Figure 8 shows CD25 is upregulated on both $CD4^+$ and $CD4^-$ (primarily CD8) human $CD3^+$ T cell subsets following co-culture with NG-348 infected A549 cells, but not when infection was with EnAd.

Figure 9 shows low level of HLA-DR expression on human $CD3^+$ T cells following co-culture with NG-348 or EnAd infected A549 cells

Figure 10 shows induction of CD107a expression on the surface of live, $CD3^+$ T cells following co-culture with NG-348 infected A549 cells, but not when infection was with EnAd.

Figure 11 shows induction of CD107a expression on the surface of both $CD4^+$ and $CD4^-$ $CD3^+$ T cell subsets following co-culture with NG-348 infected A549 cells, but not when infection was with EnAd.

Figure 12 shows induction of IL-2 (Figure 12A) and IFNγ (Figure 12B) production by $CD3^+$ T cells following co-culture with NG-348 infected A549 cells, but no IL-2 and only low levels of IFNγ when infection was with EnAd.

Figure 13 shows induction of IFNγ production by both $CD4^+$ (Figure 13A) and $CD8^+$ (Figure 13B) $CD3^+$ T cells following co-culture with NG-348 infected A549 cells, but no ($CD4^+$ cells) or low ($CD8^+$ cells) IFNγ when infection was with EnAd.

Figure 14 shows genome replication and hexon gene expression (mRNA levels) for EnAd, NG-347, and NG-348 in MRC-5 fibroblast cells compared to A549 tumour cells

Figure 15 shows CD80 and anti-CD3-scFv transgene mRNA and CD80 trasngene protein (flow cytometry) expression for virus NG-348 in MRC-5 fibroblast cells compared to A549 tumour cells

Figure 16 shows CD80 transgene mRNA and CD80 transgene protein for virus NG-347 in MRC-5 fibroblast cells compared to A549 tumour cells.

Figure 17 shows mRNA and secreted protein levels of MIP1α and IFNα generated by virus NG-347 in MRC-5 fibroblast cells compared to A549 tumour cells.

Figure 18 shows genome replication and hexon gene expression (mRNA levels) for EnAd, NG-347, and NG-348 in purified human T-cell cultures.

Figure 19 shows CD80 and anti-CD3 scFv transgene mRNA and protein expression (flow cytometry) for virus NG-348 in human T-cells compared to A549 tumour cells

Figure 20 shows CD80 transgene mRNA and CD80 transgene protein for virus NG-347 in purified human T-cells compared to A549 tumour cells

Figure 21 shows IFNα and MIP1α transgene mRNA generated by virus NG-347 in T cells compared to A549 tumour cells

Figure 22 shows NG-347 and NG-348 genome replication and hexon gene expression by human PBMCs compared to A549 tumour cells

Figure 23 shows CD80 and anti-CD3 scFv mRNA generated by virus NG-348 by PBMCs compared to A549 tumour cells

Figure 24 shows CD80, IFNα and MIP1α mRNA generated by virus NG-347 by PBMCs compared to A549 tumour cells

Figure 25 shows the similar activation of human dendritic cells by EnAd, NG-347 and NG-348 virus particles, as measured by down-regulation of CD14 expression and upregulation of CD80 on the cell surface

Figure 26 shows similar particle-mediated MIP1α and IFNα protein secretion from PBMCs cultured with NG-348 (A) or NG-347 (B) compared to EnAd.

Figure 27 shows NG-347 or NG-348 genome replication in co-cultures or T-cells or PBMCs with MRC-5 fibroblast cells compared to co-cultures with A549 tumour cells

Figure 28 shows INFγ secreted by PBMCs or T-cells co-cultured with MRC-5 fibroblast cells compared to A549 tumour cells, and treated with EnAd or virus NG-348.

Figure 29 shows MIP1α and IFNα secreted by human dendritic cells treated with EnAd, NG-347 or NG-348 virus particles

Figure 30 shows NFκB and IFN reporter gene activation in JurkatDual reporter T-cells co-cultured with EnAd, NG-347 or NG-348 infected A549 tumour cells

Figure 31 shows NF-κB-luciferase reporter activity generated by JurkatDual reporter T-cells co-cultured with EnAd, NG-347, NG-348 or NG-420 treated A549 HCT-116, DLD and HT29 tumour cells

Figure 32 shows NF-κB-luciferase reporter activity generated by JurkatDual cells co-cultured with either A549 or HT29 tumour cells infected with virus NG-348 and virus NG-420 as a function of virus particles added

Figure 33 shows the pharmacokinetics of EnAd and virus NG-348 in blood; blood cytokine levels after exposure to EnAd or virus NG-348; tissue biodistribution of EnAd or NG-348 viruses 6 or 24 hours after IV administration to CD1 mice

Figure 34 shows the pharmacokinetics in blood of EnAd, NG-347 and NG-348 viruses following IV administration to CB17-SCID mice bearing a subcutaenous HCT-116 tumour xenograft.

Figure 35 shows the tissue distribution of EnAd, NG-347 and NG-348 viruses 6 hours post intravenous dosing in tumour-bearing CB17-SCID mice, and virus genomes in HCT-116 tumour xenografts at day 7 and day 14-21 following intravenous or intra-tumoral dosing of EnAd, NG-347 and NG-348

Figure 36 shows virus hexon mRNA generated in HCT-116 tumour xenografts by EnAd, NG-347 or NG-348 viruses on day 7 or 14-21 following intravenous or intra-tumoral dosing

Figure 37 shows mRNA levels for hexon and CD80 transgene in HCT-116 tumour xenografts 7 or 21 days following intravenous dosing with virus NG-348

Figure 38 shows mRNA levels for a transgenes encoding anti-CD3 scFv and CD80 in HCT-116 tumour xenografts 7 or 14-21 days following IV dosing with virus NG-348

Figure 39 shows mRNA levels of MIP1α and IFNα transgenes in HCT-116 tumour xenografts 7 or 14-21 days following intravenous dosing with virus NG-347

Figure 40 shows CD80 protein expression in HCT-116 tumour xenografts 7 and 21 days following an intravenous dose of virus NG-348; and shows MIP1α and CD80 protein expression in HCT-116 tumours following an intravenous dose of virus NG-347.

Figure 41 shows CD69 is upregulated on more human $CD3^+$ T-cells following co-culture with NG-347 infected A549 cells than when infection was with EnAd

Figure 42 shows induction of IFNγ production by human $CD3^+$ T cells following co-culture with NG-347 infected A549 cells, but not when infection was with EnAd

Figure 43 shows comparable oncolytic potency (Figures 43) and infectivity (Figure 43 table) of EnAd, and NG-348 viruses in an HT-29 cytotoxicity assay

Figure 44 shows the signalling pathway of the TNF superfamily, from a Nature Reviews Immunology **3**, 745-756 (September 2003).

Figure 45 shows a schematic of the recombinant HSV-1virus, deleted for ICP-34.5 and with a transgene (labelled as X) incorporated

Figures 46 A-E shows transgene expression and T cell activation by HSV-X. Expression of the GFP reporter and of CD80 was evaluated by Flow cytometry, measuring the geometric mean fluorescence intensity and the percentage of positive cells, for each parameter. Expression of CD80 was only observed only in cells infected with HSV-X-8.1.

Figures 46 F-H show transgene expression and T cell activation upon infection of Vero cells by HSV-X. T cell activation was only observed upon stimulation with cells infected with HSV-X-8.1, which confirms functional expression of the transgene.

Figures 47 A-I show the evaluation of transgene expression in tumour target cells by flow cytometry analysis of CD69 expression. All 8 subclones were able to induce activation of T-cells as assessed by CD69 upregulation in tumour cells.

Figure 48A-B show the evaluation of expression of CD80 and GFP in tumour target cells by flow cytometry analysis.

Figure 49A-B shows expression of CD69 measured by flow cytometry.

Figure 50 A-C shows T-cell mediated killing was evaluated with XCelligence for a period of 5-6 days

Figure 51A-B shows the construction of the shuttle plasmid and details of the cloning.

Figure 52A-B shows CD80 expression by flow cytometry, measuring the geometric mean fluorescence intensity and the percentage of positive cells, for each parameter.

Figure 52C-D shows expression of the GFP reporter gene evaluated by flow cytometry at two different concentrations (1:10 and 1:100). GFP was expressed only by the recombinant viruses WR-434 and Wy-434.

Figure 52E-F shows both recombinant viruses were also able to express CD80, unlike the wild type control viruses.

Figure 53A-B shows T cell activation was evaluated by flow cytometry of CD69 expression for HT29 cells and SKOV cells.

Figure 54A-D shows expression of CD80 and GFP measured in infected tumour cells, and CD69 measured on lymphocytes.

Figure 55A-C show T-cell mediated killing evaluated with XCelligence for a period of 5-6 days. Untreated cells (solid black lines), or those incubated with T-cells but no virus (black dotted line), show healthy growth that reaches a high level after about 72h (for both types of cancer cell) and then stabilises.

Figure 56A shows cell viability assessed by observation of CPE in the cell monolayers. NG-348, Ad5-348 and Ad5 all showed significant CPE by 48 hrs post-infection with 100 ppc (NG-348 or Ad5) or 1 in 10 and 1 in 50 dilution of virus stock (Ad5-348).

Figure 56B-C shows quantification of the number of detected virus genomes per cell for two cells lines HT-29 and A549. The data indicates that NG-348 and Ad5-348 replicated in both cell lines

Figure 57A-B shows analysis of CD80 expression at 24, 48 and 72 hrs post infection. CD80 could be detected on the surface of Ad5-348 and NG-348 treated A549 cells and HT-29.

Figure 58A-B shows flow cytometry analysis of T cell activation assessed by expression of the T cell activation marker CD25 and the marker for T cell degranulation, CD107a on live, $CD3^+$, single cells.

Figure 58C shows detection of IFNγ expression, co-culture supernatants were diluted into 5% BSA/PBS assay buffer (in a range of 1:20 to 1:50) and ELISA was carried out using the human IFNγ Quantikine kit (R and D systems). The concentration of secreted IFNγ was determined by interpolating from the standard curve.

Figure 58D shows IFNγ expression assayed by ELISA from co-cultures of HSV and vaccinia virus infected HT-29 cells with T cells, described in examples 2 and 4. ELISA was carried out using the human IFNγ Quantikine kit (R and D systems).

Figure 59-72 shows various amino acid and polynucleotide sequence.

SUMMARY OF THE SEQUENCE LISTING

The present application contains 127 sequences in the associated sequence listing.

SEQ ID NO: 1 Amino acid sequence of the VH region of the antibody OKT3.

SEQ ID NO: 2 Amino acid sequence of the VH region of the antibody OKT3.

SEQ ID NO: 3 Amino acid sequence of the scFv construct contain OKT3 VH and VL.

SEQ ID NO: 4 Amino acid sequence of membrane anchored version of the scFv of SEQ ID NO: 3

SEQ ID NO: 5 Amino acid sequence of SEQ ID NO: 4, with V5 tag.

SEQ ID NO: 6 Amino acid sequence of teplizumab VH.

SEQ ID NO: 7 Amino acid sequence of teplizumab VL.

SEQ ID NO: 8 Amino acid sequence of teplizumab heavy chain.

SEQ ID NO: 9 Amino acid sequence of teplizumab light chain.
SEQ ID NO: 10 Amino acid sequence of PDGFR Receptor A.
SEQ ID NO: 11 Amino acid sequence of PDGFR Receptor B.
SEQ ID NO: 12 Amino acid sequence of Insulin-Like growth factor 1.
SEQ ID NO: 13 Amino acid sequence of IL-6R.
SEQ ID NO: 14 Amino acid sequence of CD28.
SEQ ID NO: 15 Amino acid sequence of PDGFR TM Domain.
SEQ ID NO: 16 Amino acid sequence of c-myc tag.
SEQ ID NO: 17 Amino acid sequence of c-myc tag with spacers.
SEQ ID NO: 18 Amino acid sequence of PDGFR TM Domain with N-terminal c-myc tag.
SEQ ID NO: 19 Amino acid sequence of HuVH human VH leader sequence
SEQ ID NO: 20 Amino acid sequence of a linker.
SEQ ID NO: 21 Polynucleotide sequence of EnAd Genome
SEQ ID NO: 22 to 30 Amino acid sequence of hinge linker sequence.
SEQ ID NO: 31 to 70 Amino acid sequence of a flexible linker sequence.
SEQ ID NO: 71 to 86 Amino acid sequence of a linker sequence.
SEQ ID NO: 87 Polynucleotide sequence of E2B region of the EnAd GENOME (BP 10355-5068).
SEQ ID NO: 88 Polynucleotide sequence of a non-coding sequence suitable for inclusion into BX.
SEQ ID NO: 89 Polynucleotide sequence of a non-coding sequence suitable for inclusion into BY.
SEQ ID NO: 90 & 91 Polynucleotide sequence of a splice acceptor sequence.
SEQ ID NO: 92 Polynucleotide sequence comprising a start codon.
SEQ ID NO: 93 Polynucleotide sequence of IRES.
SEQ ID NO: 94 Amino acid sequence of high efficiency self-cleavable P2A peptide sequence.
SEQ ID NO: 95 Amino acid sequence of high efficiency self-cleavable F2A peptide sequence.
SEQ ID NO: 96 Amino acid sequence of high efficiency self-cleavable E2A peptide sequence.
SEQ ID NO: 97 Amino acid sequence of high efficiency self-cleavable T2A peptide sequence.
SEQ ID NO: 98 Amino acid sequence of human CD80 amino acid sequence.
SEQ ID NO: 99 Polynucleotide sequence of a polyadenylation sequence (SV40 late polyA sequence).
SEQ ID NO: 100 Polynucleotide of NG-348 virus genome sequence.
SEQ ID NO: 101 Amino acid of V5 TAG
SEQ ID NO: 102 Polynucleotide of NG-348A virus genome sequence.
SEQ ID NO: 103 Polynucleotide of NG-420 virus genome sequence.
SEQ ID NO: 104 Polynucleotide of NG-420A virus genome sequence.
SEQ ID NO: 105 Polynucleotide of NG-444-R in pUC57-Kan plasmid.
SEQ ID NO: 106 Polynucleotide of 434-MVA-GFP+transgene.
SEQ ID NO: 107 Polynucleotide of 1863-MVA-GFP+transgene.

SEQ ID NO: 108	Polynucleotide 1864-MVA-GFP+transgene.
SEQ ID NO: 109	Polynucleotide CMV promoter.
SEQ ID NO: 110	Polynucleotide Ad5-348 genome.
SEQ ID NO: 111	Amino acid sequence human interferon-a amino acid sequence.
SEQ ID NO: 112	Amino acid sequence human soluble Flt3 ligand amino acid sequence.
SEQ ID NO: 113	Amino acid sequence human macrophage inflammatory protein 1a amino acid sequence (LD78b isoform).
SEQ ID NO: 114	Amino acid sequence membrane anchored form of the anti-human CD3 single chain Fv.
SEQ ID NO: 115	Polynucleotide sequence of NG-330 virus genome.
SEQ ID NO: 116	Polynucleotide sequence of NG-343 virus genome.
SEQ ID NO: 117	Polynucleotide sequence of NG-345 virus genome.
SEQ ID NO: 118	Polynucleotide sequence of NG-346 virus genome.
SEQ ID NO: 119	Polynucleotide sequence of NG-347 virus genome.
SEQ ID NO: 120	Polynucleotide of E3 region from EnAd.
SEQ ID NO: 121	Polynucleotide of a non-coding sequence suitable for inclusion into BY.
SEQ ID NO: 122	Polynucleotide sequence of NG-348 virus genome.
SEQ ID NO: 123	Polynucleotide sequence of membrane tethered OKT3-scFv.
SEQ ID NO: 124	Polynucleotide sequence of transgene cassette from NG-348.
SEQ ID NO: 125	Polynucleotide sequence of fully synthetic EnAd genome with incorporated cloning site for transgene cassette insertion as in plasmid pEnAd2.4
SEQ ID NO: 126	Polynucleotide sequence encoding human CD80.
SEQ ID NO: 127	Polynucleotide sequence encoding transmembrane from of anti-CD3 scFv.

DETAILED DISCLOSURE

T cells in the cancer environment include T cells inside a tumor and T cells in the vicinity of the tumor, for example T cells outside the tumor but able to engage the tumor or cancerous cell, in particular physically engage the tumor or cancerous cell.

There is evidence to suggest that the T cell infiltrate into tumors and once inside they are deactivated. Advantageously the method of the present disclosure is capable of activating T cells inside the tumor. In one embodiment the T cells inside the tumor are activated by oncolytic virus or viral vector according to the present disclosure.

In one embodiment a transmembrane tether or anchor sequence employed in the present disclosure comprises a PDGFR TM domain (e.g.ala513-arg561), such as shown in the sequence of SEQ ID NO: 15.

The transmembrane domains employed herein may be advantages in relation how the protein is expressed and/or in that the properties of the cell membrane after the expression of the protein thereon are not adversely affected.

In one embodiment a tether or anchor sequence employed in the present disclosure comprises a tag attached to a PDGF receptor or fragment thereof, such as PDGFR TM domain. Suitable tags include His-tags, Flag-tags, c-myc tag and the like. More specifically the tether or anchor may comprise a c-myc tag for example of SEQ ID NO: 16 EQKLISEEDL followed by a PDGFR TM domain is employed, for ala513-arg561), such as shown in the sequence of SEQ ID NO: 15

In one embodiment the c-myc tag comprises a spacer or spacer amino acids at the 3' and/or 5' end, for example as shown in the sequence of SEQ ID NO: 17 gsEQKLISEEDLn.

In one embodiment the tether or anchor sequence employed is shown in the sequence of SEQ ID NO: 18.

Generally the protein/polypeptide to which the tether or anchor is attached does not comprise a stop codon.

In one embodiment the leader sequence for the protein to be expressed on the cancer cell surface is human, for example the human VH leader sequence (HuVH)(SEQ ID NO. 19) .

In one embodiment the structure of the ORF cassette is as follows:

LS-POLY-TAG-TM_D

wherein

LS is a leader sequence, for example a human leader sequence;

POLY is a polynucleotide encoding polypeptide or proteins of interest, in particular one disclosed herein;

TAG is a tag for example one disclosed herein, such as c-myc, in particular as described herein;

TM_D is a TM domain for example a PDGFR TM domain, also described herein.

When the polypeptide is a scFv then the ORF may be as follows:

LS-VAR_1-LINK-VAR_2-TAG-TM_D

wherein

LS is a leader sequence, for example a human leader sequence;

VAR_1 is a polynucleotide encoding a variable region such as VH region;

LINK is a linker, for example as disclosed herein, such as a linker based on the units of G_4S, in particular the sequence of SEQ ID NO: 20 GGGGSGGGGSGGGGS;

VAR_2 is a polynucleotide encoding a variable region, such as a VL region;

TAG is a tag, for example one disclosed herein, such as c-myc, in particular a sequence described herein;

TM_D is a TM domain for example a PDGFR TM domain, for example a sequence described herein.

The disclosure also extends to embodiments, in particular those described specifically herein, which comprise a tag at the N- or C-termini of the polypeptide chains, such that it resides inside or on the outside of the membrane. Thus a C-termini tag located inside the membrane is advantageous because it is not likely to interfere with the binding or function of the polypeptide.

Alternative methods to employing transmembrane domains for expressing proteins on the surface of the infected cancer cell include approaches employing glycophospholipid anchor (also referred to as a GPI anchor) attached to the C-terminal amino acid of the extracellular protein or

fragment (Low et al 1986, Cross 1987, Low and Saltiel 1988, Ferguson and William 1988). Known glycophospholipid anchors include those from Thy-1, N-CAM and DAF.

In one embodiment a combination of a transmembrane domain and a secretory signal sequence is employed to express a protein encoded by the virus (for example as described herein) on the surface of an infected cancer cell. The present inventors have shown that the proteins encoded are expressed only on cells which are permissive to infection by the oncolytic virus, i.e. cancer cells.

In one embodiment the fragment employed to express the protein on the surface of the infected cancer cell (such as the transmembrane fragment) is selected from the group comprising TM Domain Sequences (Minimal portions) given in SEQ ID NO: 10 to 14.

In one embodiment a transmembrane domain from CD80 or CD86 is employed to express the protein on the surface the cancer cell.

In one embodiment the fragment employed to express the protein on the surface of the infected cancer cell (such as the transmembrane fragment) is selected from about 20 to 25 hydrophobic amino acids which form a transmembrane alpha helix, for example from the proteins including PDGF receptor, insulin-like growth factor receptor, IL-6 receptor, CD28, glycophorin, LDL receptor, influenza HA protein, insulin receptor, Asialoglycoprotein receptor, Transferrin receptor.

In one embodiment the transmembrane domain employed is derived from a G protein-coupled receptor or S antigen from hepatitis B.

In one embodiment a fusion protein comprising a full length extracellular domain of a B7 protein or fragment and also a transmembrane domain derived from a protein other than B7 is arranged such that the B7 protein is located at the terminal end of the fusion protein distal from the cancer cell surface, that is on the outside of the cancer cell facing the extracellular space.

Having the DNA sequence encoding B7 or an active fragment under the control of an endogenous promoter is also advantageous because the protein is expressed in accordance with the virus life cycle as opposed to being constitutively expressed. In the present situation continuous expression under an exogenous promoter, for example a strong promoter like the CMV promoter, may produce more B7 protein than is necessary for a therapeutic effect and result in off-target effects.

In one embodiment oncolytic virus according to present disclosure is an adenovirus, for example a group B adenovirus. In one embodiment the virus according to the present disclosure is a chimeric virus, for example EnAd. In one embodiment the adenovirus is replication competent.

In one embodiment the oncolytic virus is replication capable, for example replication competent.

Replication capable as employed herein is a virus that can replicate in a host cell. In one embodiment replication capable encompasses replication competent and replication selective viruses.

Replication competent as employed herein is intended to mean an oncolytic virus that is capable of replicating in a human cell, such as a cancer cell, without any additional complementation to that required by wild-type viruses, for example without relying on defective cellular machinery.

Replication selective or selective replication as employed herein is intended to mean an oncolytic virus that is able to replicate in cancer cells employing an element which is specific to said cancer cells or upregulated therein, for example defective cellular machinery, such as a p53 mutation, thereby allowing a degree of selectivity over healthy/normal cells.

A "replication capable oncolytic virus" is a replication capable virus which preferentially infects cancer cells. That is, they are tumour selective by infecting tumour cells in preference to non-tumour cells. EnAd is an example of a replication competent virus.

In one embodiment the virus is replication deficient and provided as a viral vector. Viral vectors require a packaging cell or helper to provide a complementary gene to allow replication.

Replication deficient oncolytic viral vector as employed herein refers a replication deficient virus which preferentially infects cancer cells. That is, they are tumour selective by infecting tumour cells in preference to non-tumour cells, for example the viral vector is derived from a replication competent oncolytic virus by deleting a gene essential for replication.

In one embodiment replication deficient oncolytic viral vectors according to the present disclosure

In one embodiment the gene encoding the anti-TCR antibody or binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof) is located between the stop codon and polyA recognition site of the adenoviral gene L5 and the stop codon and polyA recognition site of the gene E4.

In one embodiment gene encoding the anti-TCR antibody or binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof) is located between about bp 29356 and about 29357 of the EnAd genome, for example as shown in SEQ ID NO: 21, or a position equivalent thereto. The skilled person will understand that the absolute numerical value of the location can change based on how the numbering is allocated.

The CD3 antibody component facilitates i*n vivo* stimulation of T cells, for example T cells in the cancer cell environment, to focus on cancerous cells.

Immunomodulator as employed herein means a modulator of immune response. Immunomodulators function in adjustment of the immune response to a desired level, as in immunopotentiation, immunosuppression, or induction of immunologic tolerance.

In one embodiment the immunomodulator is an antibody or binding fragment (in particular an inhibitor antibody or binding fragment) specific to a target selected from the group comprising CTLA-4, PD-1, PD-L1, PD-L2, VISTA, B7-H3, B7-H4, HVEM, ILT-2, ILT-3, ILT-4, TIM-3, LAG-3, BTLA, LIGHT or CD160, for example CTLA-4, PD-1, PD-L1 and PD-L2.

In one embodiment the immunomodulator is an antibody or binding fragment (in particular an inhibitor antibody or binding fragment) specific to a target selected from the group comprising CD16, CD25, CD33, CD332, CD127, CD31, CD43, CD44, CD162, CD301a, CD301b and Galectin-3.

In one embodiment the immunomodulator is an antibody or binding fragment specific to a target selected from the group comprising: FLT-3, FLT-3 ligand, TLRs, TLR ligands, CCR7, CD1a, CD1c, CD11b, CD11c, CD80, CD83, CD86, CD123, CD172a, CD205, CD207, CD209, CD273, CD281,

CD283, CD286, CD289, CD287, CXCR4, GITR Ligand, IFN-α2, IL-12, IL-23, ILT1, ILT2, ILT3, ILT4, ILT5, ILT7, TSLP Receptor, CD141, CD303, CADM1, CLEC9a, XCR1 and CD304.

In one embodiment the immunomodulator is an antibody or binding fragment specific to a target selected from the group comprising OX40, OX40 ligand, CD27, CD28, CD30, CD40, CD40 ligand, CD70, CD137, GITR, 4-1BB, ICOS and ICOS ligand, for example CD40 and CD40 ligand.

In one embodiment the immunomodulator is a membrane-associated protein ligand for immune cell surface receptors selected from the group comprising CTLA-4, PD-1, PD-L1, PD-L2, VISTA, B7-H3, B7-H4, HVEM, ILT-2, ILT-3, ILT-4, TIM-3, LAG-3, BTLA, LIGHT or CD160, for example CTLA-4, PD-1, PD-L1 and PD-L2.

In one embodiment the immunomodulator is a membrane-associated protein ligand for immune cell surface receptors selected from the group comprising CD16, CD25, CD33, CD332, CD127, CD31, CD43, CD44, CD162, CD301a, CD301b and Galectin-3.

In one embodiment the immunomodulator is a membrane-associated protein ligand for immune cell surface receptors selected from the group comprising: FLT-3, FLT-3 ligand, TLRs, TLR ligands, CCR7, CD1a, CD1c, CD11b, CD11c, CD80, CD83, CD86, CD123, CD172a, CD205, CD207, CD209, CD273, CD281, CD283, CD286, CD289, CD287, CXCR4, GITR Ligand, IFN-α2, IL-12, IL-23, ILT1, ILT2, ILT3, ILT4, ILT5, ILT7, TSLP Receptor, CD141, CD303, CADM1, CLEC9a, XCR1 and CD304.

In one embodiment the immunomodulator is a membrane-associated protein ligand for immune cell surface selected from the group comprising OX40, OX40 ligand, CD27, CD28, CD30, CD40, CD40 ligand, CD70, CD137, GITR, 4-1BB, ICOS and ICOS ligand, for example CD40 and CD40 ligand.

In one embodiment the immunomodulator is an antibody or binding fragment specific to a target selected from the group comprising IL-1α, IL-1β, IL-6, IL-9, IL-12, IL-13, IL-17, IL-18, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-33, IL-35, interleukin-2 (IL-2), IL-4, IL-5, IL-7, IL-10, IL-15, IL-21, IL-25, IL-1RA, IFNα, IFNβ, IFNγ, TNFα, TGFβ, lymphotoxin α (LTA) and GM-CSF.

In one embodiment the oncolytic adenovirus according to the present disclosure has a formula **(I)**:

$$5'ITR\text{-}B_1\text{-}B_A\text{-}B_2\text{-}B_X\text{-}B_B\text{-}B_Y\text{-}B_3\text{-}3'ITR \quad \textbf{(I)}$$

wherein:

B_1 is a bond or comprises: E1A, E1B or E1A-E1B;

B_A is E2B-L1-L2-L3-E2A-L4;

B_2 is a bond or comprises E3 or a transgene, for example under an endogenous or exogenous promoter;

B_X is a bond or a DNA sequence comprising: a restriction site, one or more transgenes or both;

B_B comprises L5;

B_Y comprises a transgene encoding a B7 protein or an active fragment thereof; and

B_3 is a bond or comprises E4.

In one embodiment the oncolytic virus has a formula **(Ia)**:

$$5'ITR\text{-}B_1\text{-}B_A\text{-}B_2\text{-}B_B\text{-}B_Y\text{-}B_3\text{-}3'ITR \qquad \textbf{(Ia)}$$

wherein:

B_1 is a bond or comprises: E1A, E1B or E1A-E1B;

B_A is E2B-L1-L2-L3-E2A-L4;

B_2 is a bond or comprises E3;

B_B comprises L5;

B_Y comprises a transgene encoding a B7 protein or an active fragment thereof; and

B_3 is a bond or comprises E4.

In one embodiment the virus genome in constructs of formula (**I**) and/or (**Ia**) is from Ad11 or EnAd, in particular EnAd.

In one embodiment the transgene encoding the anti-TCR antibody or binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof), is under the control of an endogenous promoter, for example the major late promoter.

In one embodiment B_Y comprises a transgene cassette, said cassette comprising a transgene encoding a B7 protein or fragment thereof and a regulatory element, such as combination of regulatory elements.

In one embodiment the regulatory element is splice acceptor sequence.

In one embodiment the regulatory element is a Kozak sequence.

In one embodiment, for example where the transgene encodes a polycistronic RNA molecule, the regulatory element is an IRES sequence.

In one embodiment the regulatory sequence is a high efficiency self-cleavable peptide sequence such as P2A, T2A, F2A, E2A.

In one embodiment the regulatory sequence is a polyA tail.

In one embodiment there are at least two regulatory sequences, for example a splice acceptor and a Kozak sequence or a splice acceptor and a polyA tail, or a splice acceptor and an IRES sequence, or a splice acceptor and a P2A sequence.

In one embodiment there are at least three regulator sequences, for example a splice acceptor sequence, a Kozak sequence and polyA tail, or a splice acceptor sequence an IRES or 2A sequence and a polyA tail; or a splice acceptor sequence, Kozak sequence and an IRES or 2A sequence.

In one embodiment there are at least four regulatory sequences, for example a splice acceptor sequence, a Kozak sequence, an IRES or 2A sequence and a polyA tail, in particular from L5 to E4 in the order splice acceptor sequence, Kozak sequence, IRES or 2A sequence and a polyA tail.

In on embodiment the transgene encodes a polycistronic RNA molecule comprising both an IRES and a 2A regulatory sequence

In one embodiment a non-adenovirus encodes a B7 protein is independently selected from B7-1, B7-2, B7-DC, B7-H1, B7-H2, B7-H3, B7-H4, B7-H5, B7-H6, B7-H7, active fragments of the same, and combinations thereof. In one embodiment the B7 protein is B7-1 (CD80), B7-2 (CD86)

or an active fragment of any of the same and combinations thereof, in particular B7-1 or an active fragment thereof.

In one embodiment the B7 fragment comprises or consists of a transmembrane domain from a B7 protein in particular one described herein, such as B7-1. This domain is thought to contribute expression on the cell surface.

In one embodiment the cytoplasmic domain of the B7 protein is present. In one embodiment the cytoplasmic domain is absent. The absence of the cytoplasmic domain may reduce or eliminate intracellular signaling to the cancer cell, which is relevant to one or more embodiments discussed below.

The elements in a fragment or full length B7 protein may be from the same or different B7 proteins. Thus in one embodiment the B7 fragment or protein is chimeric.

In one embodiment the virus of the present disclosure encodes multiple proteins for expression on the surface of the infected cancer cell, for example a non-adenovirus encodes at least one is a B7 protein or an active fragment thereof, for example two, three, four or more different proteins are encoded, in particular two or three proteins are encoded by the virus for expression on the cancer cell surface or secretion into the extracellular space.

In one embodiment a B7 protein or active fragment is encoded by the non-adenovirus of the present disclosure for expression on the surface of the cancer cell and a soluble form, which is released or secreted from the cell, of the same B7 protein or a different B7 protein (including active fragments) is also encoded by the virus.

In one embodiment at least two different B7 proteins or active fragments are encoded by a non-adenovirus of the present disclosure.

In one embodiment the multiple proteins may be encoded to be expressed as separate proteins which are independently processed and expressed in the cancer cell membrane. The independence of the proteins on the surface of the cancer cell may make a positive contribution to the immune activation. Whilst not wishing to be bound by theory, lipid packing can influence the fluidity (i.e. the viscosity) of the lipid bilayer in the membrane of the cancer cell. Viscosity of the membrane can affect the rotation and orientation of proteins and other bio-molecules within the membrane, thereby affecting the functions of these molecules. Thus when the proteins encoded by the virus are located as individual and separate proteins within the membrane of the infected cancer cell, the fluidity of the lipid bilayer allows independent movement of the molecules which may be a particularly suitable format similar to a natural format that is conducive to biological function.

In one embodiment the independently processed and expressed proteins are located (anchored) in different locations, such as physically separate locations, in the cancer cell membrane.

In one embodiment one or more proteins (for example all the proteins) encoded by the virus and expressed on the surface of the infected cancer cell are not fusion proteins.

Thus in one embodiment the virus according to the present disclosure comprises DNA sequences encoding said multiple proteins for expression, for example on the surface or the infected cancer cell.

Thus in one embodiment the virus according to the present disclosure comprises two or more transgenes, in the same or different locations in the virus genome. When located at the same position in the virus genome the multiple proteins will still be expressed independently at the surface of the cancer cell.

In one embodiment the multiple proteins (including fusion proteins) are encoded in different locations in the virus genome, for example in E3, B_X and/or B_Y and are expressed separately on the surface of the infected cancer cell.

In one embodiment the multiple proteins (including fusion proteins) are encoded in the same location in the virus genome and expressed together on the infected cancer cell surface, for example where the proteins encoded are provided as a fusion protein wherein the fusion protein comprises a B7 protein or an active fragment thereof.

In one embodiment the B7 protein in the fusion protein is a full length protein, in particular a protein described herein, such as B7-1 and/or B7-2, fused or linked to another protein of interest or an active fragment thereof. In one embodiment, the fusion protein comprises a transmembrane from a B7 protein. In one embodiment the B7 is an active fragment excluding the transmembrane domain. In the latter embodiment a transmembrane other than one derived from a B7 protein may be employed to ensure the fusion protein is presented on the surface of the infected cancer cell.

In one embodiment the multiple proteins are encoded in the same location in the virus and are expressed as fusion proteins together on the surface of the infected cancer cell.

When the location in the virus is the same then the genes may, for example be linked by an IRES sequence or a 2A peptide.

In one embodiment the virus according to the present disclosure comprises a "second" transgene and optionally a third transgene (i.e. one or more of said multiple proteins, for example encoding a polypeptide selected from the group comprising a cytokine, a chemokine, a ligand, an antagonistic antibody molecule, and an agonistic antibody molecule.

In one embodiment the additional protein or proteins is/are independently selected from the group comprising an antibody, antibody fragment or protein ligand that binds CD3, CD28, CD80, CD86, 4-1BB, GITR, OX40, CD27, CD40 and combinations in forms suitable for expression on the surface of a cancer cell.

In one embodiment where the first transgene is specific to the alpha and/or beta chain of the TCR the additional protein is an anti-CD3 antibody or binding fragment thereof, for example independently selected from a Muromonab-CD3 (also known as OKT3), otelixizumab (also known as TRX4), teplizumab (also known as hOKT3γ1(Ala-Ala)), or visilizumab.

In one embodiment the anti-TCR antibody is in the form of an antibody fragment (such as an anti-CD3 antibody or binding fragment thereof), for example an scFv form, that is part of a fusion protein with the transmembrane region of another protein, for example the transmembrane domain from the PDGF receptor or from the cell surface form of IgG

In one embodiment an antibody inhibitor (antagonistic antibody) is independently selected from the group comprising an inhibitor of an angiogenesis factor, such as an anti-VEGF antibody molecule, and inhibitor of T cell deactivation factors, such as an anti-CTLA-4, anti-PD1 or anti-

PDL1 antibody molecule. In one embodiment antibody molecule is an agonist independently selected from the group comprising antibodies to CD40, GITR, OX40, CD27 and 4-1BB.

In one embodiment the additional transgene encodes a cytokine, or soluble variant thereof selected from the group comprising IL-2, IFN-alpha, IFN-beta, IFN-gamma, GM-CSF, IL-15, IL-12 and fms-related tyrosine kinase 3 ligand (FLT3L). Advantageously, one or more of this group of proteins expressed by the virus, in particular as a free protein secreted from the cancer cell, may be particularly suitable for stimulating an immune response *in vivo* to the cancer cell.

In one embodiment the additional transgene encodes a chemokine, selected from the group comprising MIP1-alpha, RANTES, IL-8, CCL5, CCL17, CCL20, CCL22, CXCL9, CXCL10, CXCL11, CXCL13, CXCL12, CCL2, CCL19 and CCL21. Advantageously, one or more of this group of proteins is expressed by the virus as a free protein which may be secreted from the cancer cell may be particularly suitable for attracting immune cells and stimulating an immune response to the cancer cell *in vivo.*

In one embodiment the additional transgene encodes a ligand specific for a chemokine receptor, selected from the group comprising CXCR2, CCR2, CCR4, CCR5, CCR6, CCR7, CCR8, CXCR3, CXCR4, CXCR5 and CRTH2.

In one embodiment in addition to at least the anti-TCR antibody or binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof) expressed on the surface of the infected cancer cell, one or more molecules are also expressed on the surface and/or secreted.

Thus in one embodiment the non-adenovirus encodes B7-1, B7-2 or an active fragment of any one of the same or a combination thereof.

Thus in one embodiment the non-adenovirus encodes B7-1, B7-2 or an active fragment of any one of the same or a combination thereof for expression on the surface of the infected cancer cell and an anti-TCR (agonist, such as CD3 agonist) antibody or antibody fragment (such as a scFv) also for expression on the cancer cell surface, in particular where the proteins are expressed as individual proteins on the cell surface.

Thus in one embodiment the non-adenovirus encodes B7-1, B7-2 or an active fragment of any one of the same or a combination thereof for expression on the surface of the infected cancer cell and an anti-VEGF (antagonist) antibody also for expression on the cancer cell surface or for release from the cancer cell, for example by secretion or after lysis/death of the infected cancer cell.

Thus in one embodiment the non-adenovirus encodes B7-1, B7-2 or an active fragment of any one of the same or a combination thereof for expression on the surface of the infected cancer cell and an antibody, antibody fragment or protein ligand that binds CD3, CD28, CD80, CD86, 4-1BB, GITR, OX40, CD27, CD40 also for expression on the cancer cell surface or for release from the cancer cell, for example by secretion or after lysis/death of the infected cancer cell.

Thus in one embodiment the non-adenovirus encodes B7-1, B7-2 or an active fragment of any one of the same or a combination thereof for expression on the surface of the infected cancer cell and a cytokine selected from IL-2, IFN-alpha, IFN-beta, IFN-gamma, GM-CSF, IL-15, IL-12, and FLT3L, for example for release from the cancer cell, in particular by secretion or after cell lysis/death of the infected cancer cell.

Thus in one embodiment the non-adenovirus encodes B7-1, B7-2 or an active fragment of any one of the same or a combination thereof for expression on the surface of the infected cancer cell and a chemokine selected from MIP1-alpha, RANTES, IL-8, CCL5, CCL17, CCL20, CCL22, CXCL9, CXCL10, CXCL11, CXCL13, CXCL12, CCL2, CCL19, CCL21, for example for release from the cancer cell, in particular by secretion or after cell lysis/death of the infected cancer cell.

Thus in one embodiment the non-adenovirus encodes B7-1, B7-2 or an active fragment of any one of the same or a combination thereof for expression on the surface of the infected cancer cell and an anti-TCR (agonist, such as CD3 agonist) antibody or antibody fragment (such as a ScFv) also for expression on the cancer cell surface (in particular where the proteins are expressed as individual proteins on the cell surface) and further encodes a cytokine or chemokine selected from IL-2, IFN-alpha, IFN-gamma, GM-CSF, IL-15, IL-12, FLT3L, MIP1-alpha, RANTES, IL-8, CCL5, CCL17, CCL20, CCL22, CXCL9, CXCL10, CXCL11, CXCL13, CXCL12, CCL2, CCL19, CCL21 for example for release from the cancer cell, in particular by secretion or after cell lysis/death of the infected cancer cell.

Thus in one embodiment the non-adenovirus encodes B7-1, B7-2 or an active fragment of any one of the same or a combination thereof for expression on the surface of the infected cancer cell and an anti-TCR (agonist) antibody or antibody fragment (such as a ScFv) also for expression on the cancer cell surface (in particular where the proteins are expressed as individual proteins on the cell surface) and further encodes an antibody, antibody fragment or protein ligand that binds CD28, CD80, CD86, 4-1BB, GITR, OX40, CD27, CD40 or an anti-VEGF (antagonist) antibody also for expression on the cancer cell surface or for release from the cancer cell, for example by secretion or after lysis/death of the infected cancer cell.

Thus in one embodiment the non-adenovirus encodes B7-1, B7-2 or an active fragment of any one of the same or a combination thereof for expression on the surface of the infected cancer cell and two different cytokines or chemokines selected from IL-2, IFN-alpha, IFN-beta, IFN-gamma, GM-CSF, IL-15, and IL-12, FLT3L, MIP1-alpha, RANTES, IL-8, CCL5, CCL17, CCL20, CCL22, CXCL9, CXCL10, CXCL11, CXCL13, CXCL12, CCL2, CCL19, CCL21, for example for release from the cancer cell, in particular by secretion of after cell lysis/death of the infected cancer cell.

Thus in one embodiment the non-adenovirus encodes B7-1, B7-2 or an active fragment of any one of the same or a combination thereof for expression on the surface of the infected cancer cell and an anti-TCR (agonist, such as CD3 agonist) antibody or antibody fragment (such as a scFv) also for expression on the cancer cell surface (in particular where the proteins are expressed as individual proteins on the cell surface) and further encodes a cytokine selected from IL-2, IFN-alpha, IFN-gamma, GM-CSF, IL-15, and IL-12, and or a chemokine selected from RANTES (CCL5), MIP1α (LD78α (CCL3) or LD78β (CCL3L1) isoforms), MIP1β which can be released from the cancer cell, in particular by secretion before and after cell lysis/death of the infected cancer cell.

In one embodiment which in particular may be combined with any of the embodiments above the virus further encodes an anti-PD-1 antibody (an antagonist).

In one embodiment the protein or proteins encoded in the transgene cassette for cell membrane expression may also comprise a peptide linker or spacer between the transmembrane domain and the extracellular ligand binding domain. Such linkers or spacers may add flexibility to

the cell surface expressed protein that enhances the ability of the protein to interact with its target molecule on an adjacent cell. Such linkers or spacers may also be designed or selected to promote dimerisation or trimerisation of the proteins at the cell surface, via disulphide bond formation or protein-protein interactions. For example the hinge regions of immunoglobulin molecules or CD8 may be employed to enhance both flexibility and dimerisation

In one embodiment the protein or proteins encoded in the transgene cassette may also comprise a peptide tag. The peptide tag may include c-myc, poly-histidine, V5 or FLAG tags and can be located on the N-terminus or C-terminus of the polypeptide, either intracellularly or extracellularly, or may be encoded within the protein for example in an extracellular loop or between the transmembrane domain and the extracellular domain. Peptide tags can be used as spacers or linkers between different protein domains, for example the transmembrane and the extracellular domain, and be used for detection or purification of the protein.

In one embodiment the one or more additional transgenes (other than the gene encoding the B7 protein or fragment thereof) is under the control of an exogenous or endogenous promoter, for example an endogenous promoter. In one embodiment a transgene in the E3 region (B_2) is under control of an exogenous promoter.

In one embodiment the one or more additional transgenes genes are between the E3 region and the fibre L5 in the adenovirus genome, for example at a position B_X in the construct of formula (**I**), in particular under the control of an exogenous promoter. In one embodiment a transgene in B_X is under the control of an exogenous.

In one embodiment the one or more additional transgenes genes are between the E4 region and the fibre L5 in the adenovirus genome, for example at a position B_Y in the construct of formula (**I**) or (**Ia**), in particular under the control of an endogenous promoter, such as the major late promoter. This may be in addition to the gene encoding the anti-TCR antibody or binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof) encoded in the region B_Y.

In one embodiment there is provided a composition comprising an oncolytic virus or viral vector according to the present disclosure, for example a pharmaceutical composition, in particular comprising a pharmaceutically acceptable excipient, such as a diluent or carrier.

In one embodiment there is provided an oncolytic virus or viral vector according to the present disclosure or a composition comprising the same, for use in treatment, in particular for use in the treatment of cancer.

In one embodiment there is provided a method of treating a patient in need thereof comprising administering a therapeutically effective amount of an oncolytic virus or viral vector according to the present disclosure or a composition, such as a pharmaceutical composition comprising the same.

In one embodiment there is provided use of an oncolytic virus or viral vector according to the present disclosure or a composition comprising the same for the manufacture of a medicament for the treatment of cancer, in particular carcinomas, for example colorectal, lung, bladder, renal, pancreatic, hepatic, head and neck, breast or ovarian cancer.

In one embodiment there is provided a polynucleotide comprising genomic sequence of at least 50% of a virus according to the present disclosure (for example 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%) and comprising a sequence encoding an anti-TCR antibody or binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof), for example a sequence disclosed herein. In one embodiment the polynucleotide sequence is in the form of a plasmid.

In one embodiment there is provided a host cell, for example a mammalian cell, such as a HEK293 cell or a derivative thereof, comprising an oncolytic virus or viral vector according to the present disclosure or a polynucleotide sequence according to the present disclosure.

In one embodiment there is provided a process for preparing an oncolytic virus or viral vector according to the present disclosure comprising a step of inserting a polynucleotide encoding an anti-TCR antibody or binding fragment thereof (such as an anti-CD3 antibody or binding fragment thereof) into an oncolytic virus or viral vector.

In one embodiment there is provided a process of replicating a virus or viral according to the present disclosure comprising the step of culture host cells in the presence of the virus under conditions suitable for replication. Generally the method will comprise a further step of harvesting the virus, for example from the supernatant or after lysis of the host cells.

Transgene as employed herein refers to a gene that has been inserted into the genome sequence of the virus or viral vector, wherein the gene is unnatural to the virus (exogenous) or not normally found in that particular location in the virus. Examples of transgenes are given herein. Transgene as employed herein also includes a functional fragment of the gene that is a portion of the gene which when inserted is suitable to perform the function or most of the function of the full-length gene, for example 50% of the function or more. In one embodiment the gene encodes a protein. In one embodiment the virus or vector according to the present disclosure does not contain more than 4 transgenes, which encode a protein or proteins. In one embodiment the virus of vector contains, 3, 2 or 1 transgene that encode a protein or proteins.

Transgene and coding sequence are used interchangeably herein in the context of inserts into the viral genome, unless the context indicates otherwise. Coding sequence as employed herein means, for example a DNA sequence encoding a functional RNA, peptide, polypeptide or protein. Typically the coding sequence is cDNA for the transgene that encodes the functional RNA, peptide, polypeptide or protein of interest. Functional RNA, peptides, polypeptide and proteins of interest are described below.

In one embodiment transgene as employed herein refers to a segment of DNA containing a gene or cDNA sequence that has been isolated from one organism and is introduced into a different organism i.e. the virus of the present disclosure. In one embodiment this non-native segment of DNA will generally retain the ability to produce functional RNA, peptide, polypeptide or protein. Transgenes employed may for example encode a chimeric protein or a fusion protein.

Clearly the virus genome contains coding sequences of DNA. Endogenous (naturally occurring genes) in the genomic sequence of the virus are not considered a transgene, within the context of the present specification unless then have been modified by recombinant techniques such as that they are in a non-natural location or in a non-natural environment.

Thus in one embodiment the transgene inserted encodes a human or humanised protein, polypeptide or peptide.

Fusion protein as employed herein refers to at least two proteins or fragments or a combination of at least one protein and at least one fragment fused directly or attached to each other, for example by a linker. In one embodiment fusion proteins of the present disclosure do not comprise a B7 protein or active fragment thereof. Fusion proteins comprising B7 fragments or protein and additional proteins are not referred to as chimeric proteins herein. Only proteins containing fragments from different B7 proteins are referred to as chimeric herein.

The activity of a given protein fragment may be analysed in a relevant *in vitro* assay, for example using full-length protein as a comparator.

A chimeric fragment as employed herein refers a fragment comprising a sequence from two or more proteins from different origins.

B7 proteins include B7-1(also known as CD80 uniprot number P33681), B7-2 (also known as CD86 uniprot number P42081). These proteins bind CD28 and CTLA-4.

In one embodiment CD80 has the sequence shown ins SEQ ID NO: 111 of the associated sequence listing.

Other B7 proteins include B7-DC (also known as PDCD1LG2 and PD-L2 uniprot number Q9BQ51), B7-H1 (also known as PD-L1 and CD274 uniprot number Q9NZQ7). Both these proteins bind PD-1.

Programmed death-ligand 1 (PD-L1) is a 40kDa type 1 transmembrane protein that has been speculated to play a major role in suppressing the immune system. It appears that upregulation of PD-L1 may allow cancers to evade the host immune system. An analysis of 196 tumor specimens from patients with renal cell carcinoma found that high tumor expression of PD-L1 was associated with increased tumor aggressiveness and a 4.5-fold increased risk of death. Ovarian cancer patients with higher expression of PD-L1 had a significantly poorer prognosis than those with lower expression. PD-L1 expression correlated inversely with intraepithelial CD8+ T-lymphocyte count, suggesting that PD-L1 on tumor cells may suppress antitumor CD8+ T cells. The effect might be tumor type dependent; a study on patients with non-small cell lung cancer showed that greater PD-L1 protein and mRNA expression is associated with increased local lymphocytic infiltrate and longer survival. A number of anti-PDL1 antibodies have been shown to be of interest for treating several cancers in clinical trials.

In one embodiment at least the cytoplasmic (intracellular domain of B7-DC and/or B7-H1 is deleted or non-functional. Whilst not wishing to be bound by theory there is evidence to suggest that removal of the intracellular domain reduces the cancer cells resistance to lysis Blood 2008, April 1; 111(7) 3635-3643.

In one embodiment only the transmembrane domain fragment of B7-DC and/or B7-H1 is employed. In one embodiment the following proteins are not provided as full-length proteins B7-DC and B7-H1.

Other B7 proteins include B7-H2 (also known as ICOSLG, B7RP1, CD275 uniprot number O75144) which binds ICOS, B7-H3 (also known as CD276 uniprot number Q5ZPR3), B7-H4 (also known as VTCN1 uniprot number Q727D3), B7-H5 (also known as VISTA, Platelet receptor Gi24,

SISP1), B7-H6 (also known as NCR3LG1, NR3L1) which binds NKp30, B7-H7 (also known as HHLA2) which binds CD28H.

In one embodiment the fragment only comprises the transmembrane domain of any one B7-H2, B7-H3, B7-H4, B7-H5, B7-H6 and B7-H7.

Individual proteins include single proteins, that is proteins or active fragments thereof that are not part of a fusion protein (including chimeric proteins), and also fusion proteins. In one embodiment the individual proteins are single proteins (including active fragments thereof).

GPI anchor as employed herein refers to is a glycolipid that can be attached to the C-terminus of a protein during posttranslational modification. It is composed of a phosphatidylinositol group linked through a carbohydrate-containing linker (glucosamine and mannose glycosidically bound to the inositol residue) and via an ethanolamine phosphate (EtNP) bridge to the C-terminal amino acid of a mature protein. The two fatty acids within the hydrophobic phosphatidyl-inositol group anchor the protein to the cell membrane.

Glypiated (GPI-linked) proteins contain a signal peptide, thus directing them into the endoplasmic reticulum (ER). The C-terminus is composed of hydrophobic amino acids that stay inserted in the ER membrane. The hydrophobic end is then cleaved off and replaced by the GPI-anchor. As the protein processes through the secretory pathway, it is transferred via vesicles to the Golgi apparatus and finally to the extracellular space where it remains attached to the exterior leaflet of the cell membrane. Since the glypiation is the sole means of attachment of such proteins to the membrane, cleavage of the group by phospholipases will result in controlled release of the protein from the membrane. The latter mechanism is used *in vitro*; i.e., the membrane proteins released from the membranes in the enzymatic assay are glypiated protein.

Phospholipase C (PLC) is an enzyme that is known to cleave the phospho-glycerol bond found in GPI-anchored proteins. Treatment with PLC will cause release of GPI-linked proteins from the outer cell membrane. The T-cell marker Thy-1 and acetylcholinesterase, as well as both intestinal and placental alkaline phosphatases, are known to be GPI-linked and are released by treatment with PLC. GPI-linked proteins are thought to be preferentially located in lipid rafts, suggesting a high level of organization within plasma membrane microdomains.

A review of GPI anchors written by Ferguson, Kinoshita and Hart is available in Chapter 11 of Essentials of Glycobiology 2nd Edition.

Virus as employed herein refers to an oncolytic replication competent virus (or adenovirus) or a replication deficient viral vector (adenoviral vector) unless the context indicates otherwise.

In one embodiment the adenovirus is a human adenovirus. “Adenovirus”, “serotype” or adenoviral serotype” as employed herein refers to any adenovirus that can be assigned to any of the over 50 currently known adenoviral serotypes, which are classified into subgroups A-F, and further extends to any, as yet, unidentified or unclassified adenoviral serotypes. See, for example, Strauss, "Adenovirus infections in humans," in The Adenoviruses, Ginsberg, ea., Plenum Press, New York, NY, pp. 451-596 (1984) and Shenk, "Adenoviridae: The Viruses and Their Replication," in Fields Virology, Vol.2, Fourth Edition, Knipe, 35ea., Lippincott Williams & Wilkins, pp. 2265-2267 (2001), as shown in **Table 2.**

SubGroup	Adenoviral Serotype
A	12, 18, 31
B	3, 7, 11, 14, 16, 21, 34, 35,51
C	1, 2, 5, 6
D	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33,36-39,42-49,
E	4
F	40,41

In one embodiment the adenovirus is a subgroup B, for example independently selected from the group comprising or consisting of: Ad3, Ad7, Ad11, Ad14, Ad16, Ad21, Ad34 and Ad51, such as Ad11, in particular Ad11p (the Slobitski strain). In one embodiment the adenovirus of the invention has the capsid, such as the hexon and/or fibre of a subgroup B adenovirus, such as Ad11, in particular Ad11p. In one embodiment the adenovirus is Ad11 or has the fibre and/or hexon and/or penton of Ad11, such as Ad11p.

In one embodiment it is not a group A virus.

Enadenotucirev (EnAd) is a chimeric oncolytic adenovirus, formerly known as EnAd (WO2005/118825), with fibre, penton and hexon from Ad11p, hence it is a subgroup B virus. It has a chimeric E2B region, which comprises DNA from Ad11p and Ad3. Almost all of the E3 region and part of the E4 region is deleted in EnAd. Therefore, it has significant space in the genome to accommodate additional genetic material whilst remaining viable. Furthermore, because EnAd is a subgroup B adenovirus, pre-existing immunity in humans is less common than, for example, Ad5. Other examples of chimeric oncolytic viruses with Ad11 fibre, penton and hexon include OvAd1 and OvAd2 (see WO2006/060314).

EnAd seems to preferentially infect tumour cells, replicates rapidly in these cells and causes cell lysis. This, in turn, can generate inflammatory immune responses thereby stimulating the body to also fight the cancer. Part of the success of EnAd is hypothesised to be related to the fast replication of the virus *in vivo*.

Importantly, it has been demonstrated clinically that EnAd can be administered systemically (e.g. by intravenous or intraperitoneal injection or infusion) and then subsequently selectively infect and express proteins within tumour cells. This property of EnAd, which may be shared by Ad11p and other group B adenoviruses in particular those expressing the capsid proteins of Ad11p (such as those described herein), makes it possible to express proteins on the surface of cancer cells without having to directly inject the transgenes into the tumour which is not feasible for many cancers.

A derivative of Ad11 virus as employed herein refers to a virus with at least the capsid of Ad11.

Whilst EnAd selectively lyses tumour cells, it may be possible to introduce further beneficial properties, for example increasing the therapeutic activity of the virus or reducing side-effects of the virus by arming it with transgenes, such as a transgene which encodes a cell signalling protein or an antibody, or a transgene which encodes an entity which stimulates a cell signalling protein(s).

Advantageously arming a virus, with DNA encoding certain proteins that can be expressed inside the cancer cell, may enable the body's own defenses to be employed to combat tumour cells more effectively, for example by making the cells more visible to the immune system or by delivering a therapeutic gene/protein preferentially to target tumour cells.

In one embodiment the oncolytic virus or viral of the present disclosure stimulates the patient's immune system to fight the tumor, for example

In one embodiment the oncolytic virus has a fibre, hexon and penton proteins from the same serotype, for example Ad11, in particular Ad11p, for example found at positions 30812-31789, 18254-21100 and 13682-15367 of the genomic sequence of the latter wherein the nucleotide positions are relative to Genbank ID 217307399 (accession number: GC689208).

In one embodiment the adenovirus is enadenotucirev (also known as EnAd and formerly as ColoAd1). Enadenotucirev as employed herein refers the chimeric adenovirus of SEQ ID NO: 21. It is a replication competent oncolytic chimeric adenovirus which has enhanced therapeutic properties compared to wild type adenoviruses (see WO2005/118825). EnAd has a chimeric E2B region, which features DNA from Ad11p and Ad3, and deletions in E3/E4. The structural changes in enadenotucirev result in a genome that is approximately 3.5kb smaller than Ad11p thereby providing additional "space" for the insertion of transgenes.

Linkers suitable for use in fusion proteins of the present disclosure include:

- hinge linker sequences shown in **SEQ ID NO: 22 to 30** (see associated sequence listing);
- flexible linker sequences GS and also the sequences shown in **SEQ ID NO: 31 to 70** (see associated sequence listing);
- linker sequences shown in SEQ ID NO: 73 to 86

Examples of rigid linkers include the peptide sequences GAPAPAAPAPA (SEQ ID NO: 71), PPPP (SEQ ID NO: 72) and PPP.

Definitions Relevant to Formula (I) and (Ia)

A bond refers to a covalent bond connecting the one DNA sequence to another DNA sequence, for example connecting one section of the virus genome to another. Thus when a variable in formula (**I**) and (**Ia**) herein represents a bond the feature or element represented by the bond is absent i.e. deleted.

As the structure of adenoviruses is, in general, similar the elements below are discussed in terms of the structural elements and the commonly used nomenclature referring thereto, which are known to the skilled person. When an element is referred to herein then we refer to the DNA sequence encoding the element or a DNA sequence encoding the same structural protein of the element in an adenovirus. The latter is relevant because of the redundancy of the DNA code. The viruses' preference for codon usage may need to be considered for optimised results.

Any structural element from an adenovirus employed in the viruses of the present disclosure may comprise or consist of the natural sequence or may have similarity over the given length of at least 95%, such as 96%, 97%, 98%, 99% or 100%. The original sequence may be modified to omit 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the genetic material. The skilled

person is aware that when making changes the reading frames of the virus must be not disrupted such that the expression of structural proteins is disrupted.

In one embodiment the given element is a full-length sequence i.e. the full-length gene.

In one embodiment the given element is less than a full-length and retains the same or corresponding function as the full-length sequence.

In one embodiment for a given element which is optional in the constructs of the present disclosure, the DNA sequence may be less than a full-length and have no functionality, for example the E3 region may be totally or partly deleted.

The structural genes encoding structural or functional proteins of the adenovirus are generally linked by non-coding regions of DNA. Thus there is some flexibility about where to "cut" the genomic sequence of the structural element of interest (especially non-coding regions thereof) for the purpose of inserting a transgene into the viruses of the present disclosure. Thus for the purposes of the present specification, the element will be considered a structural element of reference to the extent that it is fit for purpose and does not encode extraneous material. Thus, if appropriate the gene will be associated with suitable non-coding regions, for example as found in the natural structure of the virus.

Thus in one embodiment an insert, such as DNA encoding a restriction site and/or transgene, is inserted into a non-coding region of genomic virus DNA, such as an intron or intergenic sequence. Having said this some non-coding regions of adenovirus may have a function, for example in alternative splicing, transcription regulation or translation regulation, and this may need to be taken into consideration.

The sites identified herein, that are associated with the L5 region, are suitable for accommodating a variety of DNA sequences encoding complex entities such as RNAi, cytokines, single chain or multimeric proteins, such as antibodies.

Gene as employed herein refers to coding and any non-coding sequences associated therewith, for example introns and associated exons. In one embodiment a gene comprises or consists of only essential structural components, for example coding region.

Below follows a discussion relating to specific structural elements of adenoviruses.

The Inverted Terminal Repeat (ITR) sequences are common to all known adenoviruses and were so named because of their symmetry, and are the viral chromosome origins of replication. Another property of these sequences is their ability to form a hairpin.

The 5'ITR as employed herein refers to part or all of an ITR from the 5' end of an adenovirus, which retains the function of the ITR when incorporated into an adenovirus in an appropriate location. In one embodiment the 5'ITR comprises or consists of the sequence from about 1bp to 138bp of SEQ ID NO: 21 or a sequence 90, 95, 96, 97, 98 or 99% identical thereto along the whole length, in particular the sequence consisting of from about 1bp to 138bp of SEQ ID NO: 21.

The 3'ITR as employed herein refers to part or all of an ITR from 3' end of an adenovirus which retains the function of the ITR when incorporated into an adenovirus in an appropriate location. In one embodiment the 3'ITR comprises or consists of the sequence from about 32189bp to 32326bp of SEQ ID NO: 21 or a sequence 90, 95, 96, 97, 98 or 99% identical thereto along the

whole length, in particular the sequence consisting of from about 32189bp to 32326bp of SEQ ID NO: 21.

B1 as employed herein refers to the DNA sequence encoding: part or all of an E1A from an adenovirus, part or all of the E1B region of an adenovirus, and independently part or all of E1A and E1B region of an adenovirus.

When B1 is a bond then E1A and E1B sequences will be omitted from the virus. In one embodiment B1 is a bond and thus the virus is a vector.

In one embodiment B1 further comprises a transgene. It is known in the art that the E1 region can accommodate a transgene which may be inserted in a disruptive way into the E1 region (i.e. in the "middle" of the sequence) or part or all of the E1 region may be deleted to provide more room to accommodate genetic material.

E1A as employed herein refers to the DNA sequence encoding part or all of an adenovirus E1A region. The latter here is referring to the polypeptide/protein E1A. It may be mutated such that the protein encoded by the E1A gene has conservative or non-conservative amino acid changes, such that it has: the same function as wild-type (i.e. the corresponding non-mutated protein); increased function in comparison to wild-type protein; decreased function, such as no function in comparison to wild-type protein; or has a new function in comparison to wild-type protein or a combination of the same as appropriate.

E1B as employed herein refers to the DNA sequence encoding part or all of an adenovirus E1B region (i.e. polypeptide or protein), it may be mutated such that the protein encoded by the E1B gene/region has conservative or non-conservative amino acid changes, such that it has: the same function as wild-type (i.e. the corresponding non-mutated protein); increased function in comparison to wild-type protein; decreased function, such as no function in comparison to wild-type protein; or has a new function in comparison to wild-type protein or a combination of the same as appropriate.

Thus B1 can be modified or unmodified relative to a wild-type E1 region, such as a wild-type E1A and/or E1B. The skilled person can easily identify whether E1A and/or E1B are present or (part) deleted or mutated.

Wild-type as employed herein refers to a known adenovirus. A known adenovirus is one that has been identified and named, regardless of whether the sequence is available.

In one embodiment B1 has the sequence from 139bp to 3932bp of SEQ ID NO: 21.

B_A as employed herein refers to the DNA sequence encoding the E2B-L1-L2-L3-E2A-L4 regions including any non-coding sequences, as appropriate (in particular corresponding to the natural sequence from an adenovirus). Generally this sequence will not comprise a transgene. In one embodiment the sequence is substantially similar or identical to a contiguous sequence from a known adenovirus, for example a serotype shown in Table 1, in particular a group B virus, for example Ad3, Ad7, Ad11, Ad14, Ad16, Ad21, Ad34, Ad35, Ad51 or a combination thereof, such as Ad3, Ad11 or a combination thereof. In one embodiment is E2B-L1-L2-L3-E2A-L4 refers to comprising these elements and other structural elements associated with the region, for example BA will generally include the sequence encoding the protein IV2a, for example as follows: IV2A IV2a-E2B-L1-L2-L3-E2A-L4.

In one embodiment the E2B region is chimeric. That is, comprises DNA sequences from two or more different adenoviral serotypes, for example from Ad3 and Ad11, such as Ad11p. In one embodiment the E2B region has the sequence from 5068bp to 10355bp of SEQ ID NO: 21 or a sequence 95%, 96%, 97%, 98% or 99% identical thereto over the whole length.

In one embodiment the E2B in component B_A comprises the sequences shown in SEQ ID NO: 87 (which corresponds to SEQ ID NO: 3 disclosed in WO2005/118825).

In one embodiment BA has the sequence from 3933bp to 27184bp of SEQ ID NO: 21.

E3 as employed herein refers to the DNA sequence encoding part or all of an adenovirus E3 region (i.e. protein/polypeptide), it may be mutated such that the protein encoded by the E3 gene has conservative or non-conservative amino acid changes, such that it has the same function as wild-type (the corresponding unmutated protein); increased function in comparison to wild-type protein; decreased function, such as no function in comparison to wild-type protein or has a new function in comparison to wild-type protein or a combination of the same, as appropriate.

In one embodiment the E3 region is form an adenovirus serotype given in Table 1 or a combination thereof, in particular a group B serotype, for example Ad3, Ad7, Ad11 (in particular Ad11p), Ad14, Ad16, Ad21, Ad34, Ad35, Ad51 or a combination thereof, such as Ad3, Ad11 (in particular Ad11p) or a combination thereof.

In one embodiment the E3 region is partially deleted, for example is 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5% deleted.

In one embodiment B_2 is a bond, wherein the DNA encoding the E3 region is absent.

In one embodiment the DNA encoding the E3 region can be replaced or interrupted by a transgene. As employed herein “E3 region replaced by a transgene as employed herein includes part or all of the E3 region is replaced with a transgene.

In one embodiment the B_2 region comprises the sequence from 27185bp to 28165bp of SEQ ID NO: 21.

In one embodiment B_2 consists of the sequence from 27185bp to 28165bp of SEQ ID NO: 21.

B_X as employed herein refers to the DNA sequence in the vicinity of the 5’ end of the L5 gene in BB. In the vicinity of or proximal to the 5’ end of the L5 gene as employed herein refers to: adjacent (contiguous) to the 5’ end of the L5 gene or a non-coding region inherently associated herewith i.e. abutting or contiguous to the 5’ prime end of the L5 gene or a non-coding region inherently associated therewith. Alternatively, in the vicinity of or proximal to may refer to being close the L5 gene, such that there are no coding sequences between the BX region and the 5’ end of L5 gene.

Thus in one embodiment B_X is joined directly to a base of L5 which represents, for example the start of a coding sequence of the L5 gene.

Thus in one embodiment B_X is joined directly to a base of L5 which represents, for example the start of a non-coding sequence, or joined directly to a non-coding region naturally associated with L5. A non-coding region naturally associated L5 as employed herein refers to part of all of a non-coding regions which is part of the L5 gene or contiguous therewith but not part of another gene.

In one embodiment B_X comprises the sequence of SEQ ID NO: 88. This sequence is an artificial non-coding sequence wherein a DNA sequence, for example comprising a transgene (or transgene cassette), a restriction site or a combination thereof may be inserted therein. This sequence is advantageous because it acts as a buffer in that allows some flexibility on the exact location of the transgene whilst minimising the disruptive effects on virus stability and viability.

The insert(s) can occur anywhere within SEQ ID NO: 88 from the 5' end, the 3' end or at any point between bp 1 to 201, for example between base pairs 1/2, 2/3, 3/4, 4/5, 5/6, 6/7, 7/8, 8/9, 9/10, 10/11, 11/12, 12/13, 13/14, 14/15, 15/16, 16/17, 17/18, 18/19, 19/20, 20/21, 21/22, 22/23, 23/24, 24/25, 25/26, 26/27, 27/28, 28/29, 29/30, 30/31, 31/32, 32/33, 33/34, 34/35, 35/36, 36/37, 37/38, 38/39, 39/40, 40/41, 41/42, 42/43, 43/44, 44/45, 45/46, 46/47, 47/48, 48/49, 49/50, 50/51, 51/52, 52/53, 53/54, 54/55, 55/56, 56/57, 57/58, 58/59, 59/60, 60/61, 61/62, 62/63, 63/64, 64/65, 65/66, 66/67, 67/68, 68/69, 69/70, 70/71, 71/72, 72/73, 73/74, 74/75, 75/76, 76/77, 77/78, 78/79, 79/80, 80/81, 81/82, 82/83, 83/84, 84/85, 85/86, 86/87, 87/88, 88/89, 89/90, 90/91, 91/92, 92/93, 93/94, 94/95, 95/96, 96/97, 97/98, 98/99, 99/100, 100/101, 101/102, 102/103, 103/104, 104/105, 105/106, 106/107, 107/108, 108/109, 109/110, 110/111, 111/112, 112/113, 113/114, 114/115, 115/116, 116/117, 117/118, 118/119, 119/120, 120/121, 121/122, 122/123, 123/124, 124/125, 125/126, 126/127, 127/128, 128/129, 129/130, 130/131, 131/132, 132/133, 133/134, 134/135, 135/136, 136/137, 137/138, 138/139, 139/140, 140/141, 141/142, 142/143, 143/144, 144/145, 145/146, 146/147, 147/148, 148/149, 150/151, 151/152, 152/153, 153/154, 154/155, 155/156, 156/157, 157/158, 158/159, 159/160, 160/161, 161/162, 162/163, 163/164, 164/165, 165/166, 166/167, 167/168, 168/169, 169/170, 170/171, 171/172, 172/173, 173/174, 174/175, 175/176, 176/177, 177/178, 178/179, 179/180, 180/181, 181/182, 182/183, 183/184, 184/185, 185/186, 186/187, 187/188, 189/190, 190/191, 191/192, 192/193, 193/194, 194/195, 195/196, 196/197, 197/198, 198/199, 199/200 or 200/201.

In one embodiment B_X comprises SEQ ID NO: 88 with a DNA sequence inserted between bp 27 & bp 28 or a place corresponding to between positions 28192bp and 28193bp of SEQ ID NO: 21.

In one embodiment B_X has the sequence from 28166bp to 28366bp of SEQ ID NO: 21. In one embodiment B_X is a bond.

B_B as employed herein refers to the DNA sequence encoding the L5 region. As employed herein the L5 region refers to the DNA sequence containing the gene encoding the fibre polypeptide/protein, as appropriate in the context. The fibre gene/region encodes the fibre protein which is a major capsid component of adenoviruses. The fibre functions in receptor recognition and contributes to the adenovirus' ability to selectively bind and infect cells.

In viruses of the present disclosure the fibre can be from any adenovirus serotype and adenoviruses which are chimeric as result of changing the fibre for one of a different serotype are known. In one embodiment the fibre is from a group B virus, in particular Ad11, such as Ad11p.

DNA sequence in relation to B_Y as employed herein refers to the DNA sequence in the vicinity of the 3' end of the L5 gene of B_B. In the vicinity of or proximal to the 3' end of the L5 gene as employed herein refers to: adjacent (contiguous) to the 3' end of the L5 gene or a non-coding region inherently associated therewith i.e. abutting or contiguous to the 3' prime end of the L5

gene or a non-coding region inherently associated therewith (i.e. all or part of an non-coding sequence endogenous to L5). Alternatively, in the vicinity of or proximal to may refer to being close the L5 gene, such that there are no coding sequences between the B_Y region and the 3' end of the L5 gene.

Thus in one embodiment B_Y is joined directly to a base of L5 which represents the "end" of a coding sequence.

Thus in one embodiment B_Y is joined directly to a base of L5 which represents the "end" of a non-coding sequence, or joined directly to a non-coding region naturally associated with L5.

Inherently and naturally are used interchangeably herein. In one embodiment B_Y comprises the sequence of SEQ ID NO: 89. This sequence is a non-coding sequence wherein a DNA sequence, for example comprising a transgene (or transgene cassette), a restriction site or a combination thereof may be inserted. This sequence is advantageous because it acts a buffer in that allows some flexibility on the exact location of the transgene whilst minimising the disruptive effects on virus stability and viability.

The insert(s) can occur anywhere within SEQ ID NO: 88 from the 5' end, the 3' end or at any point between bp 1 to 35, for example between base pairs 1/2, 2/3, 3/4, 4/5, 5/6, 6/7, 7/8, 8/9, 9/10, 10/11, 11/12, 12/13, 13/14, 14/15, 15/16, 16/17, 17/18, 18/19, 19/20, 20/21, 21/22, 22/23, 23/24, 24/25, 25/26, 26/27, 27/28, 28/29, 29/30, 30/31, 31/32, 32/33, 33/34, or 34/35.

In one embodiment B_Y comprises SEQ ID NO: 89 with a DNA sequence inserted between positions bp 12 and 13 or a place corresponding to 29356bp and 29357bp in SEQ ID NO: 21. In one embodiment the insert is a restriction site insert. In one embodiment the restriction site insert comprises one or two restriction sites. In one embodiment the restriction site is a 19bp restriction site insert comprising 2 restriction sites. In one embodiment the restriction site insert is a 9bp restriction site insert comprising 1 restriction site. In one embodiment the restriction site insert comprises one or two restriction sites and at least one transgene, for example one or two or three transgenes, such as one or two transgenes. In one embodiment the restriction site is a 19bp restriction site insert comprising 2 restriction sites and at least one transgene, for example one or two transgenes. In one embodiment the restriction site insert is a 9bp restriction site insert comprising 1 restriction site and at least one transgene, for example one or two transgenes. In one embodiment two restriction sites sandwich one or more, such as two transgenes (for example in a transgene cassette). In one embodiment when BY comprises two restrictions sites the said restriction sites are different from each other. In one embodiment said one or more restrictions sites in B_Y are non-naturally occurring (such as unique) in the particular adenovirus genome into which they have been inserted. In one embodiment said one or more restrictions sites in BY are different to other restrictions sites located elsewhere in the adenovirus genome, for example different to naturally occurring restrictions sites or restriction sites introduced into other parts of the genome, such as BX. Thus in one embodiment the restriction site or sites allow the DNA in the section to be cut specifically.

In one embodiment B_Y has the sequence from 29345bp to 29379bp of SEQ ID NO: 21. In one embodiment B_Y is a bond.

In one embodiment the insert is after bp 12 in SEQ ID NO: 89.

In one embodiment the insert is at about position 29356bp of SEQ ID NO: 21.

In one embodiment the insert is a transgene cassette comprising one or more transgenes, for example 1, 2 or 3, such as 1 or 2.

E4 as employed herein refers to the DNA sequence encoding part or all of an adenovirus E4 region (i.e. polypeptide/protein region), which may be mutated such that the protein encoded by the E4 gene has conservative or non-conservative amino acid changes, and has the same function as wild-type (the corresponding non-mutated protein); increased function in comparison to wild-type protein; decreased function, such as no function in comparison to wild-type protein or has a new function in comparison to wild-type protein or a combination of the same as appropriate.

In one embodiment the E4 region is partially deleted, for example is 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10% or 5% deleted. In one embodiment the E4 region has the sequence from 32188bp to 29380bp of SEQ ID NO: 21.

In one embodiment E4 is present except for the E4orf4 region which is deleted.

In one embodiment B_3 is a bond, i.e. wherein E4 is absent.

In one embodiment B_3 has the sequence consisting of from 32188bp to 29380bp of SEQ ID NO: 21.

As employed herein number ranges are inclusive of the end points.

The skilled person will appreciate that the elements in the formulas herein, such as formula (**I**), (**Ia**) are contiguous and may embody non-coding DNA sequences as well as the genes and coding DNA sequences (structural features) mentioned herein. In one or more embodiments the formulas of the present disclosure are attempting to describe a naturally occurring sequence in the adenovirus genome. In this context it will be clear to the skilled person that the formula is referring to the major elements characterising the relevant section of genome and is not intended to be an exhaustive description of the genomic stretch of DNA.

E1A, E1B, E3 and E4 as employed herein each independently refer to the wild-type and equivalents thereof, mutated or partially deleted forms of each region as described herein, in particular a wild-type sequence from a known adenovirus.

"Insert" as employed herein refers to a DNA sequence that is incorporated either at the 5' end, the 3' end or within a given DNA sequence reference segment such that it interrupts the reference sequence. The latter is a reference sequence employed as a reference point relative to which the insert is located. In the context of the present disclosure inserts generally occur within either SEQ ID NO: 88 or SEQ ID NO: 89. An insert can be either a restriction site insert, a transgene cassette or both. When the sequence is interrupted the virus will still comprise the original sequence, but generally it will be as two fragments sandwiching the insert.

In one embodiment the transgene or transgene cassette does not comprise a non-biased inserting transposon, such as a TN7 transposon or part thereof. Tn7 transposon as employed herein refers to a non-biased insertion transposon as described in WO2008/080003.

In one embodiment the transgene or transgene cassette further comprises a regulatory element or sequence.

Other Regulatory Sequences

"Regulator of gene expression" (or regulator/regulatory element) as employed herein refers to a genetic feature, such as a promoter, enhancer or a splice acceptor sequence that plays a role in gene expression, typically by initiating or enhancing transcription or translation.

"Splice acceptor sequence", "splice acceptor" or "splice site" as employed herein refers to a regulatory sequence determining when an mRNA molecule will be recognised by small nuclear ribonucleoproteins of the spliceosome complex. Once assembled the spliceosome catalyses splicing between the splice acceptor site of the mRNA molecule to an upstream splice donor site producing a mature mRNA molecule that can be translated to produce a single polypeptide or protein.

Different sized splice acceptor sequences may be employed in the present invention and these can be described as short splice acceptor (small), splice acceptor (medium) and branched splice acceptor (large).

SSA as employed herein means a short splice acceptor, typically comprising just the splice site, for example 4 bp. SA as employed herein means a splice acceptor, typically comprising the short splice acceptor and the polypyrimidine tract, for example 16 bp. bSA as employed herein means a branched splice acceptor, typically comprising the short splice acceptor, polypyrimidine tract and the branch point, for example 26 bp.

In one embodiment the splice acceptor employed in the constructs of the disclosure are CAGG or SEQ ID NO: 90 or 91. In one embodiment the SSA has the nucleotide sequence CAGG. In one embodiment the SA has the nucleotide sequence of SEQ ID NO: 90. In one embodiment the SA has the nucleotide sequence of SEQ ID NO: 91.

In one embodiment the splice site is immediately proceeded (i.e. followed in a 5' to 3' direction) by a consensus Kozak sequence comprising CCACC. In one embodiment the splice site and the Kozak sequence are interspersed by up to 100 or less bp. In one embodiment the Kozak sequence has the nucleotide sequence of CCACC.

Typically, when under the control of an endogenous or exogenous promoter (such as an endogenous promoter), the coding sequence will be immediately preceded by a Kozak sequence. The start of the coding region is indicated by the initiation codon (AUG), for example is in the context of the sequence (gcc)gccRcc**AUG**g [SEQ ID NO: 92] the start of the start of the coding sequences is indicated by the bases in bold. A lower case letter denotes common bases at this position (which can nevertheless vary) and upper case letters indicate highly-conserved bases, *i.e.* the 'AUGG' sequence is constant or rarely, if ever, changes; 'R' indicates that a purine (adenine or guanine) is usually observed at this position and the sequence in brackets (gcc) is of uncertain significance. Thus in one embodiment the initiation codon AUG is incorporated into a Kozak sequence.

Internal Ribosome Entry DNA Sequence as employed herein refers to a DNA sequence encoding an Internal Ribosome Entry Sequence (IRES). IRES as employed herein means a nucleotide sequence that allows for initiation of translation a messenger RNA (mRNA) sequence, including initiation starting within an mRNA sequence. This is particularly useful when the cassette encodes polycistronic mRNA. Using an IRES results in a polycistronic mRNA that is

translated into multiple individual proteins or peptides. In one embodiment the Internal Ribosome Entry DNA sequence has the nucleotide sequence of SEQ ID NO: 93. In one embodiment a particular IRES is only used once in the genome. This may have benefits with respect to stability of the genome.

"High self-cleavage efficiency 2A peptide" or "2A peptide" as employed herein refers to a peptide which is efficiently cleaved following translation. Suitable 2A peptides include P2A, F2A, E2A and T2A. The present inventors have noted that once a specific DNA sequence encoding a given 2A peptide is used once, the same specific DNA sequence may not be used a second time. However, redundancy in the DNA code may be utilised to generate a DNA sequence that is translated into the same 2A peptide. Using 2A peptides is particularly useful when the cassette encodes polycistronic mRNA. Using 2A peptides results in a single polypeptide chain being translated which is modified post-translation to generate multiple individual proteins or peptides.

In one embodiment the encoded P2A peptide employed has the amino acid sequence of SEQ ID NO: 94. In one embodiment the encoded F2A peptide employed has the amino acid sequence of SEQ ID NO: 95. In one embodiment the encoded E2A peptide employed has the amino acid sequence of SEQ ID NO: 96. In one embodiment the encoded T2A peptide employed has the amino acid sequence of SEQ ID NO: 97.

In one embodiment an mRNA or each mRNA encoded by transgene is/are comprise a polyadenylation signal sequence, such as typically at the end of an mRNA sequence. Thus one embodiment the transgene or the transgene cassette comprises at least one sequence encoding a polyadenylation signal sequence.

"PolyA", "Polyadenylation signal" or "polyadenylation sequence" as employed herein means a DNA sequence, usually containing an AATAAA site, that once transcribed can be recognised by a multiprotein complex that cleaves and polyadenylates the nascent mRNA molecule.

In one embodiment the construct does not include a polyadenylation sequence. In one embodiment the regulator of gene expression is a splice acceptor sequence.

In one embodiment the sequence encoding a protein/polypeptide/peptide, such as an antibody or antibody fragment further comprises a polyadenylation signal.

In one embodiment there is provided a virus or construct with a sequence disclosed herein.

Antibodies

Antibody molecule as employed herein includes any construct comprising a full length antibody or a binding fragment thereof, multispecific antibody formats. Thus the antibody molecules of the present invention include a complete antibody molecule having full length heavy and light chains or a fragment thereof and may be, but are not limited to Fab, modified Fab, Fab', modified Fab', F(ab')2, Fv, Fab-Fv, Fab-dsFv, single domain antibodies (e.g. VH or VL or VHH), scFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, Nature Biotech. 23(9):1126-1136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). The methods for creating and manufacturing these antibody fragments are well known in the art (see for example Verma et al., 1998, Journal of Immunological Methods, 216, 165-181). Other antibody

fragments for use in the present invention include the Fab and Fab' fragments described in International patent applications WO2005/003169, WO2005/003170 and WO2005/003171. Multi-valent antibodies may comprise multiple specificities e.g. bispecific or may be monospecific (see for example WO 92/22853 and WO05/113605). Bispecific and multispecific antibody variants are especially considered in this example since the aim is to neutralise two independent target proteins:

Unless the context indicates otherwise antibody will generally refer to a full length antibody.

Antibody binding fragment as employed herein refers to less than a full-length antibody, which retains specificity for the target antigen. Antibody binding fragments may include a Fab, Fab', modified Fab', F(ab')2, Fv, Fab-Fv, Fab-dsFv, single domain antibodies (e.g. VH or VL or VHH), scFv, ds-scFv.

In one embodiment antibodies or binding fragments thereof employed in the technology of the present disclosure are monoclonal.

Monoclonal antibodies for use in the present disclosure may be prepared by any method known in the art such as the hybridoma technique (Kohler & Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today, 4:72) and the EBV-hybridoma technique (Cole et al., Monoclonal Antibodies and Cancer Therapy, pp77-96, Alan R Liss, Inc., 1985).

Antibodies for use in the invention may also be generated using single lymphocyte antibody methods by cloning and expressing immunoglobulin variable region cDNAs generated from single lymphocytes selected for the production of specific antibodies by, for example, the methods described by Babcook, J. et al., 1996, Proc. Natl. Acad. Sci. USA 93(15):7843-7848l; WO92/02551; WO2004/051268 and International Patent Application number WO2004/106377.

Binding domains employed in antibody molecules of the present disclosure may be humanised.

Humanised antibodies (which include CDR-grafted antibodies) are antibody molecules having one or more complementarity determining regions (CDRs) from a non-human species and a framework region from a human immunoglobulin molecule (see, e.g. US 5,585,089; WO91/09967). It will be appreciated that it may only be necessary to transfer the specificity determining residues of the CDRs rather than the entire CDR (see for example, Kashmiri et al., 2005, Methods, 36, 25-34). Humanised antibodies may optionally further comprise one or more framework residues derived from the non-human species from which the CDRs were derived.

When the CDRs or specificity determining residues are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which the CDRs are derived, including mouse, primate and human framework regions. Suitably, the humanised antibody according to the present invention has a variable domain comprising human acceptor framework regions as well as one or more of the CDRs provided herein.

Examples of human frameworks which can be used in the present invention are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (Kabat et al., supra). For example, KOL and NEWM can be used for the heavy chain, REI can be used for the light chain and EU, LAY and POM can be used for

both the heavy chain and the light chain. Alternatively, human germline sequences may be used; these are available at: http://vbase.mrc-cpe.cam.ac.uk/

In a humanised antibody of the present invention, the acceptor heavy and light chains do not necessarily need to be derived from the same antibody and may, if desired, comprise composite chains having framework regions derived from different chains.

Also, in a humanised antibody of the present invention, the framework regions need not have exactly the same sequence as those of the acceptor antibody. For instance, unusual residues may be changed to more frequently-occurring residues for that acceptor chain class or type. Alternatively, selected residues in the acceptor framework regions may be changed so that they correspond to the residue found at the same position in the donor antibody (see Reichmann et al., 1998, Nature, 332, 323-324). Such changes should be kept to the minimum necessary to recover the affinity of the donor antibody. A protocol for selecting residues in the acceptor framework regions which may need to be changed is set forth in WO 91/09967.

The constant region domains of the antibody molecule of the present invention, if present, may be selected having regard to the proposed function of the antibody molecule, and in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when the antibody molecule is intended for therapeutic uses and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simply neutralising or agonising an antigen. It will be appreciated that sequence variants of these constant region domains may also be used. For example IgG4 molecules in which the serine at position 241 has been changed to proline as described in Angal et al., Molecular Immunology, 1993, 30 (1), 105-108 may be used. It will also be understood by one skilled in the art that antibodies may undergo a variety of posttranslational modifications. The type and extent of these modifications often depends on the host cell line used to express the antibody as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, RJ. Journal of Chromatography 705:129-134, 1995).

Formulations

The present disclosure relates also extends to a pharmaceutical formulation of a virus as described herein.

In one embodiment there is provided a liquid parenteral formulation, for example for infusion or injection, of a replication capable oncolytic virus or replication deficient viral vector according to the present disclosure wherein the formulation provides a dose in the range of $1x10^{10}$ to $1x10^{14}$ viral particles per volume of dose.

Parenteral formulation means a formulation designed not to be delivered through the GI tract. Typical parenteral delivery routes include injection, implantation or infusion. In one embodiment the formulation is provided in a form for bolus delivery.

In one embodiment the parenteral formulation is in the form of an injection. Injection includes intravenous, subcutaneous, intra-tumoral or intramuscular injection. Injection as employed herein means the insertion of liquid into the body via a syringe. In one embodiment the method of the present disclosure does not involve intra-tumoral injection.

In one embodiment the parenteral formulation is in the form of an infusion.

Infusion as employed herein means the administration of fluids at a slower rate by drip, infusion pump, syringe driver or equivalent device. In one embodiment the infusion is administered over a period in the range of 1.5 minutes to 120 minutes, such as about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 15, 16, 17, 18, 19 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 65, 80, 85, 90, 95, 100, 105, 110 or 115 minutes.

In one embodiment one dose of the formulation less than 100mls, for example 30mls, such as administered by a syringe driver.

In one embodiment the injection is administered as a slow injection, for example over a period of 1.5 to 30 minutes.

In one embodiment the formulation is for intravenous (i.v.) administration. This route is particularly effective for delivery of oncolytic virus because it allows rapid access to the majority of the organs and tissue and is particular useful for the treatment of metastases, for example established metastases especially those located in highly vascularised regions such as the liver and lungs.

Therapeutic formulations typically will be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other parenteral formulation suitable for administration to a human and may be formulated as a pre-filled device such as a syringe or vial, particular as a single dose.

The formulation will generally comprise a pharmaceutically acceptable diluent or carrier, for example a non-toxic, isotonic carrier that is compatible with the virus, and in which the virus is stable for the requisite period of time.

The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a dispersant or surfactant such as lecithin or a non-ionic surfactant such as polysorbate 80 or 40. In dispersions the maintenance of the required particle size may be assisted by the presence of a surfactant. Examples of isotonic agents include sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition.

In one embodiment parenteral formulations employed may comprise one or more of the following a buffer, for example 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, a phosphate buffer and/or a Tris buffer, a sugar for example dextrose, mannose, sucrose or similar, a salt such as sodium chloride, magnesium chloride or potassium chloride, a detergent such as a non-ionic surfactant such as brij, PS-80, PS-40 or similar. The formulation may also comprise a preservative

such as EDTA or ethanol or a combination of EDTA and ethanol, which are thought to prevent one or more pathways of possible degradation.

In one embodiment the formulation will comprise purified oncolytic virus according to the present disclosure, for example $1x10^{10}$ to $1x10^{14}$ viral particles per dose, such as $1x10^{10}$ to $1x10^{12}$ viral particles per dose. In one embodiment the concentration of virus in the formulation is in the range 2×10^{8} to 2×10^{14} vp/mL, such as 2×10^{12} vp/ml.

In one embodiment the parenteral formulation comprises glycerol.

In one embodiment the formulation comprises oncolytic adenovirus as described herein, HEPES (N-2-hydroxyethylpiperazine-N´-2-ethanesulfonic acid), glycerol and buffer.

In one embodiment the parenteral formulation consists of virus of the disclosure, HEPES for example 5mM, glycerol for example 5-20% (v/v), hydrochloric acid, for example to adjust the pH into the range 7-8 and water for injection.

In one embodiment 0.7 mL of virus of the disclosure at a concentration of 2×10^{12} vp/mL is formulated in 5 mM HEPES, 20% glycerol with a final pH of 7.8.

A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Publishing Company, N.J. 1991).

In one embodiment the formulation is provided as a formulation for topical administrations including inhalation.

Suitable inhalable preparations include inhalable powders, metering aerosols containing propellant gases or inhalable solutions free from propellant gases. Inhalable powders according to the disclosure will generally contain a virus as described herein with a physiologically acceptable excipient.

These inhalable powders may include monosaccharides (e.g. glucose or arabinose), disaccharides (e.g. lactose, saccharose, maltose), oligo- and polysaccharides (e.g. dextranes), polyalcohols (e.g. sorbitol, mannitol, xylitol), salts (e.g. sodium chloride, calcium carbonate) or mixtures of these with one another. Mono- or disaccharides are suitably used, the use of lactose or glucose, particularly but not exclusively in the form of their hydrates.

Particles for deposition in the lung require a particle size less than 10 microns, such as 1-9 microns for example from 0.1 to 5 µm, in particular from 1 to 5 µm. The particle size of the carrying the virus is of primary importance and thus in one embodiment the virus according to the present disclosure may be adsorbed or absorbed onto a particle, such as a lactose particle of the given size.

The propellant gases which can be used to prepare the inhalable aerosols are known in the art. Suitable propellant gases are selected from among hydrocarbons such as n-propane, n-butane or isobutane and halohydrocarbons such as chlorinated and/or fluorinated derivatives of methane, ethane, propane, butane, cyclopropane or cyclobutane. The above-mentioned propellant gases may be used on their own or in mixtures thereof.

Particularly suitable propellant gases are halogenated alkane derivatives selected from among TG 11, TG 12, TG 134a and TG227. Of the abovementioned halogenated hydrocarbons, TG134a (1,1,1,2-tetrafluoroethane) and TG227 (1,1,1,2,3,3,3-heptafluoropropane) and mixtures thereof are particularly suitable.

The propellant gas-containing inhalable aerosols may also contain other ingredients, such as co-solvents, stabilisers, surface-active agents (surfactants), antioxidants, lubricants and means for adjusting the pH. All these ingredients are known in the art.

The propellant gas-containing inhalable aerosols according to the invention may contain up to 5 % by weight of active substance. Aerosols according to the invention contain, for example, 0.002 to 5 % by weight, 0.01 to 3 % by weight, 0.015 to 2 % by weight, 0.1 to 2 % by weight, 0.5 to 2 % by weight or 0.5 to 1 % by weight of active ingredient.

Alternatively topical administrations to the lung may also be by administration of a liquid solution or suspension formulation, for example employing a device such as a nebulizer, for example, a nebulizer connected to a compressor (e.g., the Pari LC-Jet Plus(R) nebulizer connected to a Pari Master(R) compressor manufactured by Pari Respiratory Equipment, Inc., Richmond, Va.).

The virus of the invention can be delivered dispersed in a solvent, e.g. in the form of a solution or a suspension, for example as already described above for parenteral formulations. It can be suspended in an appropriate physiological solution, e.g., saline or other pharmacologically acceptable solvent or a buffered solution. Buffered solutions known in the art may contain 0.05 mg to 0.15 mg disodium edetate, 8.0 mg to 9.0 mg NaCl, 0.15 mg to 0.25 mg polysorbate, 0.25 mg to 0.30 mg anhydrous citric acid, and 0.45 mg to 0.55 mg sodium citrate per 1 ml of water so as to achieve a pH of about 4.0 to 5.0.

The therapeutic suspensions or solution formulations can also contain one or more excipients. Excipients are well known in the art and include buffers (e.g., citrate buffer, phosphate buffer, acetate buffer and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (e.g., serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. Solutions or suspensions can be encapsulated in liposomes or biodegradable microspheres. The formulation will generally be provided in a substantially sterile form employing sterile manufacture processes.

This may include production and sterilization by filtration of the buffered solvent/solution used for the formulation, aseptic suspension of the antibody in the sterile buffered solvent solution and dispensing of the formulation into sterile receptacles by methods familiar to those of ordinary skill in the art.

Nebulisable formulation according to the present disclosure may be provided, for example, as single dose units (e.g., sealed plastic containers or vials) packed in foil envelopes. Each vial contains a unit dose in a volume, e.g., 2 mL, of solvent/solution buffer.

Treatment

In a further aspect the present disclosure extends to a virus or viral vector or a formulation thereof as described herein for use in treatment, in particular for the treatment of cancer.

In one embodiment the method of treatment is for use in the treatment of a tumour.

Tumour as employed herein is intended to refer to an abnormal mass of tissue that results from excessive cell division that is uncontrolled and progressive, also called a neoplasm. Tumours may be either benign (not cancerous) or malignant. Tumour encompasses all forms of cancer and metastases.

In one embodiment the tumour is a solid tumour. The solid tumour may be localised or metastasised.

In one embodiment the tumour is of epithelial origin.

In one embodiment the tumour is a malignancy, such as colorectal cancer, hepatoma, prostate cancer, pancreatic cancer, breast cancer, ovarian cancer, thyroid cancer, renal cancer, bladder cancer, head and neck cancer or lung cancer.

In one embodiment the tumour is a colorectal malignancy.

Malignancy as employed herein means cancerous cells.

In one embodiment the oncolytic virus or viral vector is employed in the treatment or prevention of metastasis.

In one embodiment the method or formulation herein is employed in the treatment of drug resistant cancers.

In one embodiment the virus or viral vector is administered in combination with the administration of a further cancer treatment or therapy.

In one embodiment there is provided a virus, viral vector or formulation according to the present disclosure for use in the manufacture of a medicament for the treatment of cancer, for example a cancer described above.

In a further aspect there is provide a method of treating cancer comprising administering a therapeutically effective amount of a virus or formulation according to the present disclosure to a patient in need thereof, for example a human patient.

In one embodiment the oncolytic virus or formulation herein is administered in combination with another therapy.

"In combination" as employed herein is intended to encompass where the oncolytic virus or viral vector is administered before, concurrently and/or post cancer treatment or therapy.

Cancer therapy includes surgery, radiation therapy, targeted therapy and/or chemotherapy.

Cancer treatment as employed herein refers to treatment with a therapeutic compound or biological agent, for example an antibody intended to treat the cancer and/or maintenance therapy thereof.

In one embodiment the cancer treatment is selected from any other anti-cancer therapy including a chemotherapeutic agent, a targeted anticancer agent, radiotherapy, radio-isotope therapy or any combination thereof.

In one embodiment the virus or viral vector of the present disclosure ay be used as a pre-treatment to the therapy, such as a surgery (neoadjuvant therapy), to shrink the tumour, to treat metastasis and/or prevent metastasis or further metastasis. The oncolytic virus or viral vector may be used after the therapy, such as a surgery (adjuvant therapy), to treat metastasis and/or prevent metastasis or further metastasis.

Concurrently as employed herein is the administration of the additional cancer treatment at the same time or approximately the same time as the oncolytic virus or viral vector formulation. The treatment may be contained within the same formulation or administered as a separate formulation.

In one embodiment the virus or viral vector is administered in combination with the administration of a chemotherapeutic agent.

Chemotherapeutic agent as employed herein is intended to refer to specific antineoplastic chemical agents or drugs that are selectively destructive to malignant cells and tissues. For example, alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, and other antitumour agents. Other examples of chemotherapy include doxorubicin, 5-fluorouracil (5-FU), paclitaxel, capecitabine, irinotecan, and platins such as cisplatin and oxaliplatin. The preferred dose may be chosen by the practitioner based on the nature of the cancer being treated.

In one embodiment the therapeutic agent is ganciclovir, which may assist in controlling immune responses and/or tumour vascularisation.

In one embodiment the therapeutic agent is a check-point inhibitor, such as PD-1 inhibitor, a PD-L1 inhibitor, in particular wherein the inhibitor is a monoclonal antibody or antibody fragment.

In one embodiment one or more therapies employed in the method herein are metronomic, that is a continuous or frequent treatment with low doses of anticancer drugs, often given concomitant with other methods of therapy.

Subgroup B oncolytic adenoviruses, in particular Ad11 and those derived therefrom such as EnAd may be particularly synergistic with chemotherapeutics because they seem to have a mechanism of action that is largely independent of apoptosis, killing cancer cells by a predominantly necrolytic mechanism. Moreover, the immunosuppression that occurs during chemotherapy may allow the oncolytic virus to function with greater efficiency.

Therapeutic dose as employed herein refers to the amount of virus or viral vector that is suitable for achieving the intended therapeutic effect when employed in a suitable treatment regimen, for example ameliorates symptoms or conditions of a disease. A dose may be considered a therapeutic dose in the treatment of cancer or metastases when the number of viral particles may be sufficient to result in the following: tumour or metastatic growth is slowed or stopped, or the tumour or metastasis is found to shrink in size, and/or the life span of the patient is extended. Suitable therapeutic doses are generally a balance between therapeutic effect and tolerable toxicity, for example where the side-effect and toxicity are tolerable given the benefit achieved by the therapy.

In one embodiment there is provided systemically administering multiple doses of a parenteral formulation of an oncolytic adenovirus according to the present disclosure in a single treatment cycle, for example wherein the total dose given in each administration is in the range of $1x10^{10}$ to $1x10^{14}$ viral particles per dose.

In one embodiment one or more doses (for example each dose) of virus or viral vector is administered such that the rate of viral particle delivery is in the range of $2x10^{10}$ particles per minute to $2x10^{12}$ particles per minute.

In one embodiment a virus or therapeutic construct according to the present disclosure (including a formulation comprising same) is administered weekly, for example one week 1 the dose is administered on day 1, 3, 5, followed by one dose each subsequent week.

In one embodiment a virus or therapeutic construct according to the present disclosure (including a formulation comprising same) is administered bi-weekly or tri-weekly, for example is administered in week 1 one on days 1, 3 and 5, and on week 2 or 3 is also administered on days 1, 3 and 5 thereof. This dosing regimen may be repeated as many times as appropriate.

In one embodiment a virus or therapeutic construct according to the present disclosure (including a formulation comprising same) is administered monthly.

In one embodiment the viruses, viral vectors and constructs of the present disclosure are prepared by recombinant techniques. The skilled person will appreciate that the armed virus or viral vector genome can be manufactured by other technical means, including entirely synthesising the genome or a plasmid comprising part of all of the genome. The skilled person will appreciate that in the event of synthesising the genome the region of insertion may not comprise the restriction site nucleotides as the latter are artefacts following insertion of genes using cloning methods.

In one embodiment the armed virus or viral vector genome is entirely synthetically manufactured,

The disclosure herein further extends to a virus of formula (I) or a subformula thereof, obtained or obtainable from inserting a transgene or transgene cassette.

"Is" as employed herein means comprising.

In the context of this specification "comprising" is to be interpreted as "including".

Embodiments of the invention comprising certain features/elements are also intended to extend to alternative embodiments "consisting" or "consisting essentially" of the relevant elements/features.

Where technically appropriate, embodiments of the invention may be combined. Technical references such as patents and applications are incorporated herein by reference.

Any embodiments specifically and explicitly recited herein may form the basis of a disclaimer either alone or in combination with one or more further embodiments.

The present invention is further described by way of illustration only in the following examples.

EXAMPLES

Example 1: Production of EnAd viruses expressing the T cell activating antigen CD80 and a membrane-anchored single chain Fv fragment antibody to the ε chain of the human CD3 complex (CD3ε)

The plasmid pEnAd2.4 was used to generate the plasmids pNG-348 by direct insertion of a cassette encoding the human T cell activating antigen CD80 (SEQ ID NO 98) and a membrane-anchored chimeric form of the single chain Fv anti-human CD3e (SEQ ID NO 4). The pNG-348 cassette contains; a 5' short splice acceptor sequence (CAGG); membrane-anchored anti-human CD3e ScFv cDNA; a high efficiency self-cleavable P2A peptide sequence (SEQ ID NO. 94); human CD80 cDNA sequence and a 3' polyadenylation sequence (SEQ ID NO. 99. A Schematic of the inserted transgene cassette is shown in Figure 2C. Construction of the plasmid is confirmed by DNA sequencing.

Virus Production and characterisation

The plasmid pNG-348 is linearised by restriction digest with the enzyme AscI to produce the virus genome NG-348 (SEQ ID NO: 100). The virus NG-348 is amplified and purified according to methods given below.

Digested DNA was purified by phenol/chloroform extraction and precipitated for 16hrs, -20^{0}C in 300µl >95% molecular biology grade ethanol and 10µl 3M Sodium Acetate. The precipitated DNA was pelleted by centrifuging at 14000rpm, 5 mins and was washed in 500µl 70% ethanol, before centrifuging again, 14000rpm, 5mins. The clean DNA pellet was air dried, resuspended in 500µl OptiMEM containing 15µl lipofectamine transfection reagent and incubated for 30 mins, RT. The transfection mixture was then added drop wise to a T-25 flask containing 293 cells grown to 70% confluency. After incubation of the cells with the transfection mix for 2hrs at 37^{0}C, 5% CO_2 4mls of cell media (DMEM high glucose with glutamine supplemented with 2% FBS) was added to the cells and the flasks was incubated 37^{0}C, 5% CO_2.

The transfected 293 cells were monitored every 24hrs and were supplemented with additional media every 48-72hrs. The production of virus was monitored by observation of a significant cytopathic effect (CPE) in the cell monolayer. Once extensive CPE was observed the virus was harvested from 293 cells by three freeze-thaw cycles. The harvested viruses were used to re-infect 293 cells in order to amplify the virus stocks. Viable virus production during amplification was confirmed by observation of significant CPE in the cell monolayer. Once CPE was observed the virus was harvested from 293 cells by three freeze-thaw cycles. The amplified stock was used for further amplification before the virus was purified by double caesium chloride banding to produce a NG-330 virus stock.

Example 2: Production of EnAd viruses expressing the T cell activating antigen CD80 and a membrane-anchored single chain Fv fragment antibody to the ε chain of the human CD3 complex (CD3ε)

The plasmid pEnAd2.4 was used to generate the plasmids pNG-348A by direct insertion of a cassette encoding the human T cell activating antigen CD80 (SEQ ID NO 98) and a membrane-anchored chimeric form of the single chain Fv anti-human CD3e with a C-terminal V5 tag (SEQ ID NO: 5). The pNG-348 cassette contains; a 5' short splice acceptor sequence (CAGG); membrane-anchored anti-human CD3e ScFv cDNA; a C-terminal V5 tag (SEQ ID NO: 101); a high efficiency self-cleavable P2A peptide sequence (SEQ ID NO: 94); human CD80 cDNA sequence and a 3' polyadenylation sequence (SEQ ID NO: 99). A Schematic of the NG-348A transgene cassettes is shown in Figure 26A. Construction of the plasmid is confirmed by DNA sequencing.

Virus Production and characterisation

The plasmid pNG-348A is linearised by restriction digest with the enzyme AscI to produce the virus genome NG-348A (SEQ ID NO: 102). The virus NG-348A is amplified and purified according to methods detailed in Example 1.

Example 3: Production of EnAd viruses expressing a membrane-anchored single chain Fv fragment antibody to the ε chain of the human CD3 complex (CD3ε)

The plasmid pEnAd2.4 was used to generate the plasmids pNG-420 and pNG-420A by direct insertion of a cassettes encoding a membrane-anchored chimeric form of the single chain Fv anti-human CD3e with a C-terminal V5 tag (SEQ ID NO: 5) or without a V5 tag (SEQ ID NO: 4). The pNG-420 cassette contains; a 5' short splice acceptor sequence (CAGG); membrane-anchored anti-human CD3e ScFv cDNA and a 3' polyadenylation sequence (SEQ ID NO: 99). The pNG-420A cassette contains; a 5' short splice acceptor sequence (CAGG); membrane-anchored anti-human CD3e ScFv cDNA; a C-terminal V5 tag (SEQ ID NO: 101) and a 3' polyadenylation sequence (SEQ ID NO: 99). Schematics of the NG-420 and NG-420A transgene cassettes are shown in Figure 3B and 3C. Construction of each plasmid is confirmed by DNA sequencing.

Virus Production and characterisation

The plasmids pNG-420 and pNG-420A are linearised by restriction digest with the enzyme AscI to produce the virus genomes NG-420 (SEQ ID NO: 103) and NG-420A (SEQ ID NO: 104). The viruses NG-420 and NG-420A are amplified and purified according to methods detailed in Example 1.

Example 4: Oncolytic activity and infectivity of NG-347 and NG-348 viruses in colon carcinoma cells

Virus oncolytic potency

HT-29 colon carcinoma cells were seeded in 96 well plates at a cell density of 2.5e4 cells/well. Plates were incubated for 4 hrs, 37^{0}C, 5% CO_2, before cells were either infected with EnAd, NG-347 or NG-348 virus particles at an infection density range of 100-0.39 particles per cell (ppc). HT-29 cell viability was assessed using Cell Titre 96 MTS Reagent (Promega: G3581) 72 hrs post infection. Quantification of the % cell survival at each infection density demonstrated that NG-348 oncolytic potency was comparable to EnAd (Figure 43).

Viral particle infectivity

HT-29 colon carcinoma cells were seeded in 12 well plates at a cell density of 4e5 cells/well. Plates were incubated for 24 hrs, 37^{0}C, 5% CO_2, before cells were either infected with EnAd, NG-347 or NG-348 virus particles at an infection density range of 1.6e7-2e6 vp/mL. Infection of HT-29 cells was detected by antibody staining of the virus protein hexon. Stained cells were quantified by manual counting of 6 fields of view per well, across 6 replicate wells for each dilution tested. The particle to infectivity ratio (P:I) was calculated for each virus from the viral titre and demonstrated NG-348 has similar infectivity ratios to EnAd reference controls (Figure 43 Table).

Example 5: Cell surface expression of the T cell activating antigen, CD80, in NG-347 and NG-348 infected carcinoma cell lines

CD80 transgene expression (assessed by flow cytometry) was compared in NG-348 and EnAd treated colon carcinoma cell line, DLD-1 or lung carcinoma cell line, A549. A549 or DLD-1 carcinoma cell lines were seeded in 12 well plates at cell densities of 7.5e5 cells/well. Plates were incubated for 18 hrs, 37^{0}C, 5% CO_2, before cells were either infected with, 10 EnAd, NG-348 virus particles per cell (ppc) or were left uninfected. CD80 protein expression was compared on the surface of A549 or DLD-1 cells at 24, 48, 72 or 96hrs post-infection. At each time point cells were harvested and stained according to methods detailed below.

For CD80 cell surface expression, cells were then either incubated at 5^0C for 1hr with buffer, mouse isotype control antibody conjugated to Cy5 or anti-human CD80 antibody conjugated to Cy5 (clone 2D10). All samples were also co-stained with Zombie Aqua live/dead to differentiate viable cells. Samples were washed 3 times with 1% BSA/PBS before analysis by flow cytometry (FACS, Attune) for cell viability and CD80 protein expression on the cell surface. In keeping with the IFNα expression data, CD80 expression could only be detected on HT-29 cells, with no detectable expression on either the fibroblast or bronchial epithelial cell lines.

Cells were analysed for cell viability and CD80 protein expression at the cell surface by flow cytometry. Analysis of CD80 expression at 72hrs post infection in A549 cells showed CD80 could be detected on the surface of >95% of NG-348 treated cells (Figure 4A and 4B). At 96hrs post infection the virus treatments had lysed the majority of A549 cells therefore FACs analysis was not carried out. For DLD-1 cells expression could be detected on >50% of cells by 96hrs post-treatment with NG-348 (Figure 5A and 5B). Staining was not detected on EnAd or untreated controls.

Example 6: T cell activation and degranulation mediated by NG-348 infected carcinoma cell lines

A549 lung carcinoma cells, either infected with NG-348 or EnAd virus particles or left uninfected, were co-cultured with T cells isolated from human PBMC donors. The selectivity of expression of NG-348 virus encoded CD80 was assessed on the surface of both A549 and T cells by flow cytometry. T cell activation was assessed by analysing cell surface activation markers (by Flow cytometry), CD107a cell surface expression as a marker for degranulation (by Flow cytometry) and secretion of stimulatory cytokines, IL-2 and IFNγ (by ELISA).

A549 cells were seeded into 12 well plates at a density of 5e5 cells/well. Plates were incubated for 18 hrs, 37^0C, 5% CO_2, before cells were either infected with 10 EnAd or NG-348 virus particles per cell (ppc) or were left uninfected. At 48hrs post-infection $CD3^+$ T cells, isolated by negative selection from PBMCs (MACs) were added to the A549 cell monolayers at a ratio of 8 T cells: 1 tumour cell. The co-culture was carried out for 16hrs, after which point cellular supernatants were collected for ELISA analysis and tumour cells and T cells harvested for Flow cytometry analysis.

Culture media containing non-adherent cells was removed from co-culture wells and centrifuged (300xg). The supernatant was carefully removed, diluted 1 in 2 with PBS 5% BSA and stored for ELISA analysis. The adherent cell monolayers were washed once with PBS and then detached using trypsin. The trypsin was inactivated using complete media and the cells were added to the cell pellets that had been collected from the culture supernatants. The cells were centrifuged (300xg), the supernatant discarded and the cell pellet washed in 200µL of PBS. The cells were centrifuged again then resuspended in 50µL of FACs buffer (5% BSA PBS) containing Live/Dead Aqua (Life tech) for 15 minutes at RT. The cells were washed once in FACs buffer before staining with panels of directly conjugated antibodies: anti-CD3 conjugated to BV605; anti-CD25 conjugated to BV421; anti-CD107a conjugated to FITC; anti-EpCam conjugated to PE or anti-CD4 conjugated to PE; and either anti-CD80 conjugated to PE/Cy5 or anti-HLA-DR conjugated to

PE/Cy5. A sample of cells from each co-culture condition was also stained with relevant isotype control antibodies. All staining was carried out in FACs buffer in a total volume of 50µL/well for 15 minutes, 4°C. Cells were then washed with FACs buffer (200µL) before resuspension in 200µL of FACs buffer and analysis by Flow cytometry (Attune).

Selective expression of CD80

Similar to results shown in example 14, CD80 expression was detectable at the surface of >80% of NG-348 infected EpCam$^+$ A549 cells but not EnAd infected or uninfected control cells (Figure 6). In contrast CD3$^+$ T cells showed no detectable expression of CD80 at the cell surface indicating, at least under these experimental conditions, transgene expression is selective for tumour cells in the co-culture.

Upregulation of T cell activation markers

Flow cytometry analysis of T cell activation was assessed by expression of the T cell activation markers CD25 and HLA-DR on live, CD3$^+$, single cells. These data showed that both the number of T cells expressing CD25 (Figure 7A and 7B) and the average level of CD25 expression on the T cell surface (Figure 7C) were significantly higher for T cells cultured with NG-348 infected A549 cells than EnAd or uninfected controls. Specifically, there was no difference in T cell activation status when comparing untreated controls to EnAd (26.9 % ± 3.4% and 25.3 ± 3.5% of T cells expressing CD25, respectively) whereas CD25 was upregulated on the majority of cells co-cultured with NG-348 (83.2 ± 1.5%). CD25 expression was also analysed on CD4 and CD8 T cell subsets by gating the CD3$^+$ T cells based on their expression of CD4. These analyses showed that CD25 expression is significantly upregulated on both CD4$^+$ and CD4$^-$ T cell subsets in NG-348 treated co-cultures compared to EnAd and uninfected controls (Figure 8).

In contrast to CD25 the number of cells expressing HLA-DR was low, <5%, for all conditions tested (Figure 9A). This is likely due to the early time point after co-culture at which flow cytometry analysis was carried out. However, there was a slight but significant increase in the mean fluorescence intensity of HLA-DR staining CD3$^+$HLA-DR$^+$ cells from NG-348 treated co-cultures compared to controls (Figure 9B).

Stimulation of T cell degranulation

Analysis of CD107a expression on the surface of live, CD3$^+$ T cells showed a significant increase in the number of T cells which had degranulated and were therefore stained with CD107a, when A549 cells were infected with NG-348 (8.3% ± 1.7% of cells) compared to either EnAd (0.6% ± 0.2% of cells) or untreated controls (0.1% ± 0.02% of cells) (Figure 10). Similar to CD25 upregulation, both CD4$^+$ and CD4$^-$ T cell subsets showed significantly increased CD107a expression compared to EnAd or A549 controls (Figure 11).

Secretion of the stimulatory cytokines IL-2 and IFNγ

For detection of IL-2 or IFNγ expression, co-culture supernatants were diluted into 5% BSA/PBS assay buffer (in a range of 1:100 to 1:1000) and ELISA was carried out using the Human IL-2 Ready Set go Kit (Affymetrix) or Human IFN gamma Ready set go kit (Affymetrix) according to the manufacturer's protocol.

The concentrations of secreted IL-2 or IFNγ were determined by interpolating from the standard curves. Expression of IL-2 could only be detected in the supernatants of co-cultures using NG-348 infected A549 cells and was not detectable in either the EnAd, or untreated controls (Figure 12A). Expression of IFNγ could also be detected, at very high levels (>300ng/mL) in supernatants of co-cultures from NG-348 infected A549 cells, which was significantly higher that either EnAd or untreated controls (Figure 12B).

Example 7: T cell activation of CD4 and CD8 T cells can be independently mediated by NG-348 infected carcinoma cell lines

A549 lung carcinoma cells infected with NG-348 or EnAd virus particles or left uninfected, were co-cultured with either $CD4^+$ T cells or $CD8^+$ T cells isolated from human PBMC donors. T cell activation was assessed by the secretion of the stimulatory cytokine IFNγ into culture supernatants.

A549 cells were seeded and infected with NG-348 or EnAd virus particles or left uninfected according to the methods detailed in Example 14. 48hrs post infection $CD4^+$ T cells or $CD8^+$ T cells isolated by negative selection from a PBMC donor were added to the A549 cell monolayer at a ratio of 8 T cells to 1 tumour cells. After 16hrs of co-culture supernatants were harvested and assessed for IFNγ according to the methods detailed.

Analysis of IFNα expression by ELISA

Supernatants of HT-29 or A549 cell lines infected for 24, 48 or 72 hrs with 10ppc of EnAd or NG-343 or left uninfected were analysed for expression of secreted IFNα by ELISA.

Culture supernatants were removed from each well and centrifuged for 5 mins, 1200rpm to remove cell debris. Supernatants were diluted into 5% BSA/PBS assay buffer (1:2 or 1:50 or 1:100) and ELISA was carried out using the Verikine Human IFN alpha Kit (Pbl assay science) according to the manufacturer's protocol.

The concentrations of secreted IFNα were determined by interpolating from the standard curves. IFNα expression which increased in the cellular supernatants over the course of infection was detected in both HT-29 and A549 cells lines

For $CD4^+$ T cells Expression of IFNγ was only detected in supernatants of co-cultures from NG-348 infected A549 cells and was not detectable in either the EnAd or untreated controls (Figure 13A).

For $CD8^+$ T cells expression of IFNγ was detected at significantly higher levels for NG-348 infected A549 cells than for EnAd or untreated controls (Figure 13B), demonstrating that both CD8 and CD4 cells can be activated to secret IFNγ by NG-348 virus activity in tumour cell lines.

Example 8

A549 human lung carcinoma cells and MRC5 human fibroblast cells were cultured with EnAd, NG-347 or NG-348 viruses (at 10ppc) to compare virus genome replication, virus hexon and transgene expression by these cell types. After 72 hours culture, cells were either stained for FACS analyses of surface markers or supernatants and cell lysates prepared for virus genome replication (qPCR) or mRNA (RT-qPCR) analyses of hexon or transgene expression.

Virus genome replication and hexon mRNA expression for the two transgene bearing viruses, NG-347 and NG-348 were equivalent to those for the parental virus, EnAd (Figure 14). For NG-348 (Figure 15), CD80 and anti-human CD3-ScFv transgene mRNA expression levels were high with A549 tumour cells, with only a low level signal for the non-tumour MRC5 cells. CD80 protein expression on the surface of cells assessed by FACS was detected on the majority of NG-348 treated A549 cells but was not detectable on MRC5 cells, with no CD80 detected on either cell type left untreated or treated with EnAd. Similarly, CD80 transgene mRNA and protein expression following NG-347 treatment was selectively detected in A549 tumour cells not MRC5 cells (Fig 16). For EnAd and NG-347 treated cell cultures, levels of MIP1α and IFNα mRNA in cell lysates and secreted proteins in supernatants were measured by RT-qPCR and specific ELISAs, respectively. Data (Figure 17) show selective expression of both transgenes by A549 tumour cells, with no detectable MIP1α chemokine or IFNα cytokine in MRC5 supernatants.

Example 9

The selectivity/activity of EnAd, NG-347 and NG-348 viruses with human T-cells was evaluated by culturing isolated $CD3^+$ T cells for 3 days with either 500 ppc or 5000 ppc of each virus. Selectivity/activity was assessed by a) flow cytometry analysis of T cells stained with antibodies targeting CD69, CD4, CD80, CD25 and CD3, b) ELISA analysis of human MIP1α, IFNα and IFNγ protein secretion, c) qPCR analysis of virus replication and d) RT-qPCR analysis of gene expression As shown in Figure 18, T-cells were not supportive of virus genome replication for any of the viruses tested with only background signals in the virus hexon RT-qPCR assay. A549 tumour cells supported high levels of hexon mRNA expression. RT-qPCR analyses for transgene mRNA expression by T-cells showed only background signals (<1 copy/cell) for CD80 by both NG-347 and NG-348, and a similar lack of significant expression of anti-CD3-ScFv mRNA by NG-348, despite the high virus exposure (5000ppc). High levels of expression of both transgenes were detected with treated (10ppc) A549 tumour cells (Figures 19 & 20). Expression of IFNα and MIP1α transgene mRNA was also selectively detected by NG-347 (not EnAd) treated A549 tumour cells (at 10ppc) and not by T-cells treated with 5000ppc (Figure 21). In addition, CD80 cell surface protein expression was only detectable with A549 cells not T-cells for both NG-347 and NG-348 (Figures 19 & 20). EnAd treatment did not lead to CD80 expression by either cell type, and A549 cell death (as assessed by dye uptake) was similarly high for all three viruses; a low level of non-specific T-cell death was induced by all viruses due to the very high levels of virus particles used in the experiment (Figures 19 & 20). Similar transgene mRNA and protein expression data were obtained when viruses were used at 500ppc (data not shown).

In the absence of tumour cells, purified human T-cells were not activated to upregulate activation markers CD25 or CD69 when cultured with any of the viruses (Table 6).

Table 6. Lack of expression of activation markers CD25 and CD69 by purified human $CD3^+$ T-cells treated with 5000 ppc of different viruses

	Untreated	EnAd	NG-347	NG-348
CD25+ CD4 T-cells	30.7	24.6	23.4	23.3
CD69+ CD4 T-cells	0.1	0.4	0.3	0.7
CD25+ CD8 T-cells	5.9	4.7	4.1	4.1
CD69+ CD8 T-cells	0.5	1.0	0.9	1.3

Example 10

A similar virus selectivity experiment to that described in Example 9 was carried out using unseparated human PBMCs rather than purified T-cells, including making the same activity assessments. As with human T-cells in example 9, the data from this study collectively demonstrate lack of virus replication and transgene expression by human PBMCs. Figures 12-14 show data using 5000 ppc of EnAd, NG-347 or NG-348, but similar data was generated using 500 ppc (not shown). Figure 12 shows virus genome replication and hexon mRNA expression and Figures 13 & 14 show transgene mRNA expression. Assay backgrounds were set according to signals generated in the assay with the respective virus spiked into culture media and then processed in the same way as for the cell lysate samples. There was no detectable expression of CD80 transgene on CD3+ T-cells or CD40+ cells (primarily B-cells) in these PBMC cultures with any of the viruses (not shown).

NG-347 and NG-348 virus particle-mediated activation of innate immune cells (monocytes, DCs) in the PBMC cultures were similar to those of EnAd, as shown in Figures 15 and 16 for downregulation of CD14 expression and upregulation of HLA-DR and endogenous cell surface CD80, as well as secretion of MIP1α and IFNα (note that despite NG-347 encoding both of these molecules in its genome there was no increase in production levels over those for EnAd and NG-348 which do not encode the transgenes).

Example 11

This example is similar in design to experiments in examples 9 and 22 10 in these studies, the human PBMCs or purified T-cells were co-cultured with virus pre-treated (48 hours) A549 tumour cells or MRC5 fibroblasts. A549 or MRC5 cells were treated with 10ppc of EnAd, NG-347, NG-348 or left untreated (UTC) and cultured for 48 hours to allow sufficient time for virus replication and any transgene expression. PBMCs or T-cells were then added to the cultures and left for 24 or 48 hours to evaluate the ability of virus treated cells to activate T-cells.

Figure 17 shows virus genome replication data showing comparable replication of the three viruses in PBMC or T-cell co-cultures with both cell types, replication levels being high with A549 tumour cells and low with MRC5 fibroblasts.

T-cell activation as measured by upregulation of CD25 surface expression and CD8 effector T-cell degranulation, as measured by upregulation of CD107a on the cell surface, and IFNγ production measured by intracellular cytokine staining were all selectively stimulated by NG-348 treated A549 cells compared to EnAd, with no stimulation mediated with MRC co-cultures (Table 7).

Table 7. Flow cytometry analyses of activation of human CD3+ T-cells in T-cell and PBMC co-cultures with viruses

Cells	Treatment	%CD25+	%CD8+CD107a+	%IFN γ
A549 + T-cells	Untreated	37.5	0.1	0.1
A549 + T-cells	EnAd	38.4	0.1	0.2
A549 + T-cells	NG-348	**88.2**	**17.9**	**12.0**
MRC5 + T-cells	Untreated	38.8	0.3	0.4
MRC5 + T-cells	EnAd	38.9	0.2	0.4
MRC5 + T-cells	NG-348	39.1	0.3	0.3
A549 + PBMCs	Untreated	28.3	ND	ND
A549 + PBMCs	EnAd	29.4	ND	ND
A549 + PBMCs	NG-348	73.7	ND	ND
MRC5 + PBMCs	Untreated	23.0	ND	ND
MRC5 + PBMCs	EnAd	23.3	ND	ND
MRC5 + PBMCs	NG-348	21.7	ND	ND

ND = Not determined

IFNγ secretion into co-culture supernatants was also quantified by ELISA. The data (Figure 28) similarly demonstrate selective activation of T-cells co-cultured with NG-348 treated A549 tumour cells not MRC5 fibroblasts, with either purified T-cells or PBMCs used in the assays.

Ability of NG-347 to activate T-cells was also assessed by measuring CD69 levels on T-cells from co-cultures of either purified T-cells or PBMCs with A549 tumour cells or MRC5 fibroblasts. As shown in Table 8, a small enhancement in CD69 positive T-cells was seen with NG-347 treatment of A549 tumour cells compared to EnAd, which itself leads to upregulation of this early activation marker. These effects were not seen in MRC5 co-cultures. No CD80 expression was detected on the T-cells (not shown).

Table 8. CD69 expression on T-cells from NG-347 or EnAd treated cocultures

Cells	Treatment	%CD69+
A549 + T-cells	Untreated	2.1
A549 + T-cells	EnAd	18.7
A549 + T-cells	NG-348	**35.0**
MRC5 + T-cells	Untreated	3.8
MRC5 + T-cells	EnAd	3.6
MRC5 + T-cells	NG-348	4.4
A549 + PBMCs	Untreated	1.2
A549 + PBMCs	EnAd	19.1
A549 + PBMCs	NG-348	**28.7**
MRC5 + PBMCs	Untreated	2.6
MRC5 + PBMCs	EnAd	2.7
MRC5 + PBMCs	NG-348	3.9

In a separate experiment, A549 cells treated with NG-347 and co-cultured with human CD3+ T-cells led to upregulation of CD69 activation marker on the T-cells and secretion of IFNγ (see Figures 41 & 42).

Example 12

CD14+ monocytic cells were isolated from PBMCs by antibody coated magnetic bead separation and cultured with human IL-4 and GM-CSF to differentiate them into dendritic cells. After 3 days of culture, the cells were treated with EnAd, NG-347 or NG-348 at 5000 ppc or left untreated. As a positive activation control, some cells were stimulated with LPS. Two days later supernatants were taken for cytokine ELISAs and cells were stained for surface activation marker expression and analysed by flow cytometry. As shown in table 9, all viruses induced upregulation of the costimulatory molecules CD80 and CD86, indicating that this previously identified particle-mediated innate immune cell activation effect was not altered by the transgene incorporation into the genomes of NG-347 and NG-348. All viruses also stimulated secretion of similar levels of MIP1α and IFNα (Figure 29).

Table 9. Particle-mediated activation of human dendritic cells by EnAd, NG-347 and NG-348

DC treatment	%CD80+	%CD86+
Untreated	3.0	10.4
EnAd	81.6	99.3
NG-347	82.1	99.4
NG-348	62.5	99.5
LPS positive control	97.5	98.5

Example 13

In a set of experiments, JurkatDual cells were used in co-cultures with tumour cells as a T-cell activation reporter assay for assessing functionality of transgene expression by NG-347, NG-348 and NG-420 viruses, with EnAd serving as a negative control. JurkatDual cells stably express two different reporter genes: an NFκB reporter gene producing a secreted form of luciferase which is responsive to signalling via the T-cell receptor complex and an IFNα-responsive secreted alkaline phosphatase (SEAP) reporter gene. A549 cells were pre-cultured with viruses at `10 ppc for two days, and then JurkatDual cells were added for overnight co-culture (18-24h) and then supernatants collected for assay of luciferase and SEAP activities. As shown in Figure 43, NG-347 infected A549 cells selectively induced SEAP production, which aligns with their production of IFNα (see Figure 7) but did not induce luciferase activity. In contrast, NG-348 which expresses the membrane anti-CD3-ScFv to activate the T-cell receptor complex induced luciferase but not SEAP. In another experiment A549 lung carcinoma cells and HCT-116, HT-29 & DLD colon carcinoma cells were pre-cultured for 48 hours with 10 ppc of EnAd, NG-347, NG-348 or NG-420 viruses before co-culturing with JurkatDual cells overnight, with supernatants tested for levels of luciferase to indicate level of activation induced. As shown in Figure 31, all four tumour cell types cultured with NG-348 or NG-420 viruses, which encode cell surface anti-CD3-ScFv, activated the

JurkatDual cells whereas EnAd and NG-347 did not, with levels of luciferase similar to that of uninfected tumour cell controls (UIC).

In another experiment, A549 or HT-29 tumour cells were pre-cultured with different amounts of either NG-348 or NG-420 before adding the JurkatDual cells and measuring their luciferase secretion. The data in Figure 32 show that activation of the NFκB activity in JurkatDual cells is dependent on the dose of virus used to treat the tumour cells with.

Example 14

The *in vivo* pharmacokinetic, biodistribution and particle-mediated systemic cytokine induction activities of EnAd and NG-348 following IV dosing in immunocompetent CD1 mice were compared, Mice were dosed intravenously with $5x10^9$ particles of either EnAd or NG-348 and bled 2, 10, 30, 60 and 120 minutes post dosing. Whole blood was DNA extracted and analysed by qPCR for levels of virus genome (Figure 33). Clearance of both viruses from the blood followed similar kinetics. Similarly, the induction of MCP-1 cytokine response (a measure of particle-mediated activation of innate immune such as liver Kupffer cells) was also similar for both viruses, as were the tissue biodistribution patterns (Figure 33).

Example 15

CB17 SCID mice were implanted subcutaneously with HCT116 cells and injected intratumourally (IT) or intravenously (IV) with EnAd, NG-347 or NG-348 viruses ($5x10^9$ virus particles), or control, once tumours were greater than $70mm^3$. For the IV dosed mice, blood samples were taken from three mice from each group 3, 15 and 30 minutes after IV dosing, DNA extracted and the level of virus genomes in the blood assessed by qPCR (pharmacokinetics [PK] analysis). Data (Figure 34) show that NG-347 and NG-348 have similar PK to EnAd (and to each other). After 6 hours, tumours, livers, lungs and spleens were resected from 3 mice from each group. Homogenised tissues were DNA extracted and analysed for level of virus genomes by qPCR (biodistribution analysis). Data (Figure 35A) show similar tissue biodistribution for the three viruses. After 7 days or 14-21 days, tumours were excised from three mice from each group and homogenized to produce a tumour lysate which was used to prepare both DNA and RNA. Level of virus genomes in the tumours at the two time points were measured by qPCR analyses of the extracted DNA. Data (Figure 35B) show that tumours from both IV and IT dosed mice have levels of virus genomes higher than the amount of virus dosed, indicating virus replication in the tissue, with IT dosing giving higher genome levels than IV at day 7, but both being similarly high at the 14-21 day timeframe. All three viruses replicated to similar levels.

Similarly, levels of virus hexon mRNA in tumour lysates detected by RT-qPCR were comparable between EnAd, NG-347 and NG-348 at both time points tested (Figures 36 and 37). Similar levels of anti-CD3-ScFv and CD80 mRNA were detected at both time points and both dosing routes for NG-348 treatment, with only assay background readings with EnAd dosing (Figure 37 & 38). MIP1α and IFNα mRNA levels were also selectively detected following NG-347 dosing, either IT or IV (Figure 39).

Levels of CD80 protein encoded by both NG-347 and NG-348, and MIP1a protein encoded by NG-347 were measured in tumour lysates using specific ELISAs. The data in Figure 40 show that following the single IV virus dose, both proteins could also be detected selectively in tumour extracts. Neither protein was detected in blood samples from the same mice.

Example 16

To evaluate the activity and tumour cell dependency of NG-348 virus in vivo, different combination of human PBMCs ($5x10^7$ cells), A549 human tumour cells ($5x10^6$) and either EnAd or NG-348 (at $5x10^9$ ppc) were injected into the peritoneum of immunodeficient SCID-beige mice, with viruses or control (saline) being dosed within 15 minutes after injection of the cells. After 3 days, the peritoneal cavity was lavaged with 5mL of saline and recovered cells were analysed by flow cytometric analyses with a panel of T-cell activation markers (CD25, CD69 and HLA-DR) to assess levels of T-cell activation, following gating on the CD3+ T-cell population. Data from two separate experiments (Table 10) demonstrate that NG-348 selectively leads to human T-cell activation in vivo in a tumour cell dependent manner.

Table 10. *In vivo* activation of human T-cells in A549 tumour bearing mice by NG-348

Group	Virus	Tumour	N	%CD25+	%CD69+	%DR+	%CD25+,CD69+	%CD25+,DR+
Experiment 1								
1	EnAd	Saline	2	1.9 2.3	1.6 3.0	7.7 9.1	0.2 0.5	0.3 0.6
2	EnAd	$5x10^6$ A549 cells	2	4.2 2.9	6.2 5.5	8.4 8.4	0.8 0.3	1.4 0.4
3	NG-348	Saline	1	3.4	2.6	9.2	0.5	0.8
4	NG-348	$5x10^6$ A549 cells	2	35.8, 36.6	50.4 42.2	26.3 19.2	22.4 18.0	16.4 12.2
Experiment 2								
1	Saline	Saline	1	25.6	37.3	14.8	14.1	7.08
2	EnAd	Saline	2	6.5 7.3	17.8 18.2	5.50 6.1	3.58 3.46	1.01 1.49
3	NG-348	Saline	2	10.2 6.5	26.7, 18.3	7.7 6.0	6.73 3.61	2.16 1.44
4	Saline	$5x10^6$ A549 cells	2	28.4 22.7	54.4, 51.1	13.3 15.0	22.3 17.5	8.54 7.72
5	EnAd	$5x10^6$ A549 cells	1	13.2	29.4	5.1	7.84	1.62
6	NG-348	$5x10^6$ A549 cells	3	34.4 29.6 56.4	58.9, 59.2, 85.0	12.5 9.8 17.0	27.2 23.3 52.7	9.07 7.5 14.2

Examples describing virus cloning (HSV-1 and Vaccinia virus) to express CD3scFv-2A-CD80 transgene and evaluation of biological properties

Example 17: Cloning of Herpes Simplex Virus -1 (HSV-1) to express the CD3scFv-2A-CD80 transgene

An oncolytic HSV-1 virus was produced using standard cloning methodologies, with both copies of ICP34.5 deleted, with a transgene cassette inserted into the U_L39 locus encoding the membrane-anchored anti-human CD3e ScFv cDNA; a high efficiency self-cleavable P2A peptide sequence (SEQ ID NO. 94); human CD80 cDNA sequence and a 3' polyadenylation sequence (SEQ ID NO. 99), under control of the CMV immediate early promoter and enhancer (termed CD3scFv-2A-CD80).

Cloning of the recombinant HSV-1 was based on three steps: Initially the CD3scFv-2A-CD80 transgene was cloned into a shuttle plasmid by restriction and ligation. The shuttle vector was then inserted into a bacterial artificial chromosome containing HSV-1 by homologous recombination, virus DNA was isolated and then the virus was rescued by transfection of the DNA into Vero cells. Plaques apparently containing recombinant viruses were selected and used to infect new Vero cells, for amplification. In total four plaques of CD3scFv-2A-CD80 transgene-containing HSV-1 (HSV-X) were obtained (No's 5.1, 8.1, 9.1 & 9.3) and amplified for functional analysis.

Example 18: Transgene expression and T cell activation by HSV-X

(i) Transgene Expression in Vero cells

Vero cells were seeded (150,000 cells /well, 24-well plate) 24 hours prior to infection with HSV-X-5.1, HSV-X-8.1, HSV-X-9.1 and HSV-X-9.3 (100µl from crude virus preparation, titre unknown). As a control, HSV that expresses only GFP, was also tested. Infected cells were harvested 24 h post infection and stained with anti-human CD80-APC conjugated antibody (1:200 dilution). Expression of the GFP reporter and of CD80 was evaluated by Flow cytometry, measuring the geometric mean fluorescence intensity and the percentage of positive cells, for each parameter (see **Figure 46 A-E**). Expression of CD80 was only observed only in cells infected with HSV-X-8.1.

(ii) T cell activation upon infection of Vero cells

Additionally, Vero cells were seeded (50,000 cells /well, 96-well plate) 24 hours prior to infection with HSV-X-5.1, HSV-X-8.1, HSV-X-9.1 and HSV-X-9.3 (20µl from crude virus preparation, titre unknown) or HSV-GFP. Cryopreserved peripheral blood lymphocytes were thawed and added to HSV-X-infected Vero cells (150,000 cells/well), 24 hours post infection. The non-adherent lymphocytes and cellular supernatants were harvested 24 hours later. Supernatant samples were assessed for IFNγ secretion (see example 8 and Figure D4). The T cells were, and stained with anti-human CD69-APC conjugated antibody (1:200 dilution). CD69 expression on lymphocytes was evaluated by flow cytometry, by measuring the geometric mean fluorescence intensity and the percentage of positive cells (see **Figure 46 F-H**). T cell activation was only observed upon stimulation with cells infected with HSV-X-8.1, which confirms functional expression of the transgene.

(iii) Evaluation of Transgene expression in tumour target cells

HSV-X-8.1 was subjected to two rounds of plaque purification, and 8 subclones (1-8) were isolated and amplified. HT-29 colon and SKOV ovarian human tumour cells were seeded in 96 well plates (10,000/well) 24 hours prior to infection with dilutions of HSV-X-8.1 subclones (10^{-2}, 10^{-3}

dilutions, unknown titre). HSV-GFP was included as control. In parallel, cryopreserved peripheral blood lymphocytes were thawed and left to rest over night at 37°C. Selection of T-cells was performed with a negative isolation kit, and purified T-cells were added to HSV-X-infected HT-29 or SKOV cells (50,000/well). The non-adherent T cells were harvested 24 hours later, and stained with anti-human CD69-APC conjugated antibody. T-cell activation was evaluated by flow cytometry (see **Figure 47**. All 8 subclones were able to induce activation of T-cells as assessed by CD69 upregulation in tumour cells. Differences in T cells activation can be attributed to differences in the virus titres of the HSV-X-8.1 subclones, which was observed by differences in GFP expression in infected cells (data not shown).

HT-29 and SKOV tumour cells were also seeded in 24 well plates (200.000/well) infected with HSV-GFP or HSV-x-8.1.8 (subclone 8) at different virus doses (Concentration 1 (C1) = 4^3 TCID50/well, Concentration 2 (C2) = 4^2 TCID50/well). Expression of CD80 and GFP (**Figure 48A-B**) was evaluated by flow cytometry.

In an additional experiment, HT-29 cells were seeded in a 96-well plate (40.000cells/well) 24 hours prior to infection with HSV-GFP or HSV-X at Concentration 1 (C1 = 4^3 TCID50/well). T cells were added as described previously, and harvested 24 hours later. Expression of CD69 was measured by flow cytometry (**Figure 49A-B**), and IFNγ production was measured by ELISA (**Figure 58D**)

(iv) Evaluation of the ability of HSV-X-8.1 to activate T-cell mediated killing of cancer cells

HT-29 and SKOV tumour cells were seeded on 16 well-plates (20,000 cells/well), 24 hours before infection with HSV-X-8.1.8 (Concentration 1 (C1) = 4^3 TCID50/well, Concentration 2 (C2) = 4^2 TCID50/well). Cryopreserved Peripheral blood lymphocytes were thawed and left to rest over night at 37°C. Selection of T-cells was performed with a negative isolation kit, and cells were added to infected tumour cells, 24 hours post infection. T-cell mediated killing was evaluated with XCelligence for a period of 5-6 days (See **Figure 50**). Untreated cells (solid black lines), or those incubated with T cells but no virus (black dotted line), showed healthy growth that reached a high level after about 72h (for both types of cancer cell) and then stabilises. Cells infected with HSV-X-8.1 showed a similar profile (grey solid line) and the virus caused no obvious cell death over the time frame up to 120h. In contrast, addition of T cells to cells infected with HSV-X-8.1 lead to rapid killing of the cancer cells (grey dotted line), showing that expression of the membrane anchored anti-CD3 ScFv and CD80 encoded by the CD3scFv-2A-CD80 transgene can activated T cell-mediated cytotoxicity to the cancer cells. This is most obvious for HT29 cells infected with a higher concentration (C1) of HSV-X-8.1.8 **(Figure 50**), but is also seen in HT29 cells using a lower (C2) virus dose (**Figure 50**) and also in SKOV cells treated at the higher virus dose (**Figure 50**).

Example 19: Cloning of Vaccinia virus to express the CD3scFv-2A-CD80 transgene

The overall cloning strategy for the vaccinia viruses is shown in **Figure V1.**

(i) CD3scFv-2A-CD80 transgene was cloned into the shuttle plasmids (x3) by restriction digest and ligation using the enzyme Xho.

To insert the transgene into the vaccinia shuttle plasmids (ID: 434-MVA-GFP in which transgene expression is under the control of the p7.5 promoter, ID: 1863-MVA-GFP in which transgene

expression is under the control of the mH5 promoter, ID: 1864-MVA-GFP in which transgene expression is under the control of the ssp promoter) the following steps were performed. The three shuttle plasmids were linearised by Xho1 restriction enzyme digest overnight at 37°C, followed by incubation with shrimp alkaline phosphatase (rSAP) for 2 hours at 37°C for plasmid dephosphorylation to prevent recircularisation of the DNA. To heat inactivate the enzymes, the sample was incubated at 65°C for 15 minutes. The products were run on a 1% agarose gel to ensure the plasmids were fully linearised as indicated by a single band of 5727 bp for digested 434-MVA-GFP, of 5559 bp for 1863-MVA-GFP and of 5492 bp for 1864-MVA-GFP.

The transgene and polyA sequences were amplified by PCR from the NG-444-R in pUC57-Kan plasmid (SEQ ID NO: 105) and Xho1 restriction sites were added to the ends of the PCR product using the following primers: forward primer = 5'-atatatctcgagcccaccatgggatggagc-3' and reverse primer = 3'-atatatctcgagcccgggatagctgacgact-5'. The PCR was performed using 100ng of plasmid DNA and Thermo Scientific Phusion Flash High-Fidelity PCR Master Mix under the following cycling conditions: 1 cycle of 98°C for 10s, 30 cycles of 98°C for 1s, 64°C for 5s, 72°C for 30s and 1 cycle of 72°C for 1 minute. The PCR product was cleaned using Monarch PCR & DNA Cleanup Kit from New England Biolabs and was subsequently digested with Xho1 restriction enzyme for 2 hours at 37°C. To heat inactivate the enzymes, the sample was incubated at 65°C for 15 minutes. The digested PCR product was run on a 1% agarose gel and a single band of 2182 bp was observed.

The CD3scFv-2A-CD80 transgene-polyA insert was ligated with the 434-MVA-GFP, 1863-MVA-GFP or 1864-MVA-GFP shuttle vectors (ratio of 3 insert: 1 vector) overnight at 16°C using T4 ligase. The next day 10-beta competent E.coli was transformed with 2µl of the ligation mixture, 100µl of the transformation spread on LB agar plates with ampicillin and incubated overnight at 37°C. Colonies were picked and screened for the insertion of the transgene-pA in the correct orientation by colony PCR (forward primer = 5'-CGTTGAAATGTCCCATCGAG-3' and reverse primer = 3'-AGGTACACGATGCACTGGGTAA-5'). The colony PCR products were run on a 1% agarose gel and those with the correct size band of 2182bp were grown in a maxi prep culture before the plasmid DNA was extracted and purified. The new plasmid constructs (**SEQ ID Nos 106, 107 and 108: 434-MVA-GFP+transgene; 1863-MVA-GFP+transgene; 1864-MVA-GFP+transgene**) were verified by restriction digest using Nhe1 and HindIII enzymes **(Figure 51A-B)** and by Sanger sequencing.

(ii) Production of Vaccinia Virus (Western Reserve or Wyeth) expressing CD3scFv-2A-CD80 transgene

TK -/- cells were seeded in a 6-well plate 24 hours before infection, at a density of 0.6 x 10^6 per well. For infection, serial dilutions (10, 7.5, 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05 and 0.01 µl) of Western Reserve or Wyeth virus were used. Fresh media (DMEM 5% FBS) together with the corresponding virus amount was added to the cells and incubated for 2h. After 2h of vaccinia infection, the cells were transfected with the vaccinia shuttle plasmids (434-MVA-GFP+transgene, 1863-MVA-GFP+transgene or 1864-MVA-GFP+transgene) containing the CD3scFv-2A-CD80 transgene for homologous recombination. Each plasmid (2.5µg) was diluted in 250µl of Opti-MEM media, subsequently mixed with Lipofectamine transfection reagent (also diluted in Opti-MEM) and

incubated for 20min. The transfection complexes were then added dropwise to the cells and incubated for 4-6hours, when fresh 5% FBS DMEM was added to the cells. Cells were harvested after 48-72 hours, and pelleted at 20000xg for 20min at 4°C. The supernatant was discarded, and the cells were resuspended in warm PBS and subjected to 3 freeze-thaw cycles. Subsequently, cell debris was removed by centrifugation at 500g for 2minutes and the supernatant containing the virus was transferred to a new tube and stored at -80°C.

(iii) Amplification of Vaccinia Viruses (Western Reserve or Wyeth) expressing CD3scFv-2A-CD80 transgene

For the amplification of TK-negative recombinant viruses expressing CD3scFv-2A-CD80 transgene, the harvested virus was passaged in TK-/- cells in the presence of BrdU (25 μg/ml or 81.4 μM). In brief, TK -/- cells were seeded in a 6-well plate 24h before infection at a density of 0.6 x 106 per well. Fresh media (DMEM 5% FBS) containing BrdU (25 mg/ml or 81.4 μM) together with the corresponding virus was added to the cells and incubated for 48 - 72 h. This process was repeated three times.

Example 20: Transgene expression and T cell activation by Vaccina Virus expressing CD3scFv-2A-CD80

(i) Evaluation of transgene expression in TK-/- cells

To evaluate if the recombinant Vaccinia Virus could express transgene CD3scFv-2A-CD80, TK-/- cells were seeded in 24-well plates, 24 hours prior to infection with VV-Wyeth-434-CD3scFv-2A-CD80 (Wy-434), VV-Western Reserve-434-CD3scFv-2A-CD80 (WR-434) (100ul of 1:10 or 1:100 dilution from crude virus preparation, titre unknown). As control, the wild type viruses VV-Wyeth wild type (Wy-wt) and VV-Western Reserve-wild type (WR-wt) were also included. Cells were harvested 24 hours after infection, and stained with anti-human CD80-APC conjugated antibody, to evaluate CD80 expression by flow cytometry, measuring the geometric mean fluorescence intensity and the percentage of positive cells, for each parameter (see **Figure 52A** for Western Reserve and **Figure52B** for Wyeth Strains). Additionally, expression of the GFP reporter gene was also evaluated by flow cytometry at two different concentrations (1:10 and 1:100) and GFP was expressed only by the recombinant viruses WR-434 and Wy-434 (**Figure 52 C&D**). Both recombinant viruses were also able to express CD80, unlike the wild type control viruses (**Figure 52 E&F**).

(ii) Activation of T cells exposed to HT-29 and SKOV tumour cells that were pre-infected with the recombinant viruses

HT-29 and SKOV cells were seeded in 96 well plates (10,000cells/well) 24 hours before infection with dilutions of Wy-434, Wy-wt, WR-434 and WR-wt (10^{-1} and 10^{-2} dilutions from crude virus preparations). Cryopreserved peripheral blood lymphocytes were thawed and left to rest over night at 37°C. Selection of T cells was performed with a negative isolation kit, and cells (50,000/well) were added to VV-CD3scFv-2A-CD80-infected HT-29 or SKOV cells. Non-adherent T cells and cellular supernatant samples were harvested 24 hours later. Supernatant samples were assessed for IFNγ secretion (see example 8 and Figure D4). The T cells were stained with anti-human CD69-APC conjugated antibody. T cell activation was evaluated by flow cytometry for CD69 expression (see **Figure 53A** for HT29 cells and **Figure 53B** for SKOV cells). Both CD3scFv-2A-

CD80 transgene-bearing vaccinia viruses led to activation of T-cells as assessed by CD69 upregulation. Evidence of the functional CD80 and/or antiCD3 scFv transgene expression is shown by the ability to activate T cells upon infection of HT-29 and SKOV cells by WR-434 and Wy-434, but not by the wild type viruses. The data are expressed as % positive cells and also as the gMFI (the geometric mean fluorescence of the positive population) and summarised in **Figure 53C-J**).

In an additional experiment, HT-29 cells were seeded in a 96-well plate (40.000cells/well) 24 hours prior to infection with Wy-434, Wy-wt, WR-434 and WR-wt (10^{-1} and 10^{-2} dilutions from crude virus preparations). T cells were added as described previously, and harvested 24 hours later. Expression of CD69 was measured by flow cytometry (**Figure 53K-L**), and IFNγ production was measured by ELISA (**Figure 58D**).

Recombinant viruses expressing CD3scFv-2A-CD80 under different promoters (434, 1863 or 1864, referring to the p7.5, mH5 and ssp promoters, respectively) were also used to infect HT29 or SKOV tumour cells. Cells were seeded in 24-well plates, 24 hours prior to infection with different doses of WR-wt, WR-434, WY-wt and WY-434 (C1 =10ul from crude virus preparations, C2=2ul from crude virus preparations) or WR-1863, WR1864, WY-1863 and WY-1864 (C1 =100ul from crude virus preparations, C2=20ul from crude virus preparations). Expression of CD80 and GFP was measured in infected tumour cells, and CD69 was measured in lymphocytes, as described previously. The results show that all recombinant viruses are able to express CD80 upon infection of HT29 (**Figure 54A**) or SKOV cells (**Figure 54C**) and induce activation of T cells (**Figure 54B, D**).

(iii) **Infection of HT-29 and evaluation of direct virus-mediated killing by XCelligence**

HT-29 and SKOV cells were seeded on 16 well-plates (20,000 cells/well), 24 hours before infection with VV-WY-434-X (Concentration 1 [C1] = 5^{-5} from crude virus preparation, titre unknown; Concentration 2 [C2] =1^{-5} from crude virus preparation, titre unknown). Cryopreserved Peripheral blood lymphocytes were thawed and left to rest over night at 37°C. Selection of T-cells was performed with a negative isolation kit, and cells were added to infected cells, 24 hours post infection. T-cell mediated killing was evaluated with XCelligence for a period of 5-6 days (See **Figure 55**). Untreated cells (solid black lines), or those incubated with T-cells but no virus (black dotted line), show healthy growth that reaches a high level after about 72h (for both types of cancer cell) and then stabilises. Cells infected with VV-WY-434-X show a similar profile (grey solid line) up to 72h, although the virus then shows direct cytotoxicity and progressive cell kill. Addition of T-cells to tumour cells infected with VV-WY-434-X leads to accelerated killing of the cancer cells (grey dotted line), showing that expression of the membrane anchored anti-CD3 ScFv and CD80 encoded by the CD3scFv-2A-CD80 transgene can activated T cell-mediated cytotoxicity to the cancer cells. This is most obvious for HT29 cells infected with a higher concentration (C1) of VV-WY-434-X (**Figure 55A**), but is also seen in HT29 cells using a lower (C2) virus dose (**Figure 55B**). In SKOV cells treated at the higher virus dose the virus alone shows appreciable cytotoxicity, and the presence of T cells appears to slightly delay cytotoxicity but then potentiates it so that SKOV cells are rapidly killed (**Figure 55C**).

Example 21: Production of replication competent adenovirus type 5 virus expressing the T cell activating antigen CD80 and a membrane-anchored single chain Fv fragment antibody to the ε chain of the human CD3 complex (CD3ε)

The plasmid pAd5-348 was generated by two-step assembly. First, a DNA cassette encoding the human T cell activating antigen CD80 (SEQ ID NO 126) and a membrane-anchored chimeric form of the single chain Fv anti-human CD3ε (SEQ ID NO: 127) was assembled into an Ad5 shuttle vector to produce an Ad5-348 precursor vector. Secondly a DNA fragment containing the Ad5 E2B polymerase was inserted back into the Ad5-348 precursor vector to generate the pAd5-348 plasmid. The pAd5-348 plasmid therefore contains the Ad5 genome with the transgene cassette inserted in the deleted E3 region. The transgene cassette contains: a 5'CMV promoter (SEQ ID NO. 109); membrane-anchored anti-human CD3ε ScFv cDNA; a high efficiency self-cleavable P2A peptide sequence (SEQ ID NO: 94); human CD80 cDNA sequence and a 3' polyadenylation sequence (SEQ ID NO: 99). Construction of the plasmid was confirmed by DNA sequencing.

Virus Production and characterisation

The plasmid pAd5-348 was linearised by restriction digest with the enzyme PacI to produce the virus genome Ad5-348 (SEQ ID NO: 110). The Ad5-348 genome (10μg) was transfected into Ad293 cells and then monitored every monitored every 24hrs and supplemented with additional media every 48-72hrs. The production of virus was monitored by observation of a significant cytopathic effect (CPE) in the cell monolayer. Once extensive CPE was observed the Ad5-348 virus was harvested from AD293 cells by four freeze-thaw cycles to produce a viral lysate stock.

Example 22: Oncolytic activity and replication of the Ad5-348Rep virus in colon and lung carcinoma cells

Virus oncolytic activity

HT-29 colon carcinoma cells or A549 lung carcinoma were seeded in 24 well plates at a cell density of 8e5 cells/well and 5e5 cells/well respectively. Plates were incubated for 4 hrs, 37^{0}C, 5% CO_2, before cells were either infected with Ad5 or NG-348 (100ppc) or Ad5-348 virus particles (1 in 50 or 1 in 10 dilution). Cell viability was assessed by observation of CPE in the cell monolayers. NG-348, Ad5-348 and Ad5 all showed significant CPE by 48 hrs post-infection with 100 ppc (NG-348 or Ad5) or 1 in 10 and 1 in 50 dilution of virus stock (Ad5-348) (**Figure 56A**).

Virus Replication assessed by qPCR

HT-29 or A549 cells were either infected with 100ppc NG-348 or Ad5 or a 1 in 10 or 1 in 50 dilution of Ad5-348 viral lysates or were left uninfected. After 24 or 48 virus supernatants were harvested and used for quantification of viral DNA by qPCR. Cell supernatants were collected and clarified by centrifuging for 5 mins, 1200rpm. DNA was extracted from 0.1μL of lysate using the Qiagen Blood and Tissue DNA extraction kit, according to the manufacturer's protocol. A standard curve using NG-348 virus particles (2.5e10-2.5e5vp) was also prepared and extracted using the Qiagen Blood and Tissue Kit. Each extracted sample or standard was analysed by qPCR using a CD80 gene specific primer-probe set. Quantification of the number of detected virus genomes per cell demonstrated that NG-348 and Ad5-348 replicated in both cell lines (**Figures 56B and 56C**). No virus genomes could be detected in uninfected cells (data not shown).

Example 23: Cell surface expression of the T cell activating antigen, CD80, in lung and carcinoma cell lines

CD80 transgene expression (assessed by flow cytometry) was compared in NG-348, Ad5-348, Ad5 and untreated A549 or HT-29 cell lines. A549 cells were seeded in 24 well plates at cell densities

of 5e5 cells/well and HT-29 at cell density of 8e5 cells/well. Plates were incubated for 4 hrs, 37^{0}C, 5% CO_2, before cells were either infected with 100 ppc NG-348 or Ad5 or 1 in 50 dilution of Ad5-348 or were left uninfected. CD80 protein expression was compared on the surface of A549 or HT-29 cells at 24, 48 and 72 hrs post-infection. At each time point cells were harvested and stained according to methods detailed below.

Cells were washed once with PBS before trypsin-EDTA was used to detach the cells and the trypsin was deactivated using 5% FBS DMEM media. The cells were then removed from the well, washed once with 5% FBS FACS buffer, then incubated at 5^{0}C for 15 minutes with either buffer, mouse isotype control antibody conjugated to BV605 or anti-human CD80 antibody conjugated to BV605 (clone 2D10). All samples were also co-stained with Aqua live/dead to differentiate viable cells. Samples were washed twice with 5% FBS/PBS before analysis by flow cytometry (FACS, Attune) for cell viability and CD80 protein expression on the cell surface. Figure 2 shows CD80 expression after infection with 100 ppc NG-348 and Ad5 and 1 in 50 dilutions of Ad5-348. CD80 expression could only be detected on A549 or HT-29 cells infected with NG-348 or Ad5-348 virus. Analysis of CD80 expression at 24, 48 and 72 hrs post infection showed CD80 could be detected on the surface of Ad5-348 and NG-348 treated A549 cells (**Figure 57A**) and HT-29 cells (**Figure 57B**) at all timepoints assayed. By 72hrs post infection transgene expression can be detected in > 50% of HT-29 and >80% of A549 NG-348 or Ad5-348 infected cells.

Example 24: T cell activation and degranulation mediated by NG-348 or Ad5-348 infected carcinoma cell lines

A549 or HT-29 cell lines, either infected with NG-348, Ad5 (100 ppc), Ad5-348 (diluted 1 in 50) or left uninfected, were co-cultured with T cells isolated from human PBMC donors. T cell activation was assessed by analysing cell surface activation marker CD25 (by Flow cytometry), CD107a cell surface expression as a marker for degranulation (by Flow cytometry) and secretion of stimulatory cytokine, IFNγ (by ELISA).

A549 or HT-29 cells were seeded into 24 well plates at a density of 5e5 cells/well and 8e5 cells/well, respectively. Plates were incubated for 4 hrs, 37^{0}C, 5% CO_2, before cells were either infected or were left uninfected. At 48hrs post-infection $CD3^+$ T cells, isolated by negative selection from PBMCs (MACs) were added to the A549 or HT-29 cell monolayers at a ratio of 2 T cells: 1 tumour cell. The co-culture was carried out for 16hrs, after which point cellular supernatants were collected for ELISA analysis and T cells harvested for Flow cytometry analysis.

Culture media containing non-adherent cells was removed from co-culture wells and centrifuged (300xg). The supernatant was carefully removed, diluted 1 in 2 with PBS 5% BSA and stored for ELISA analysis. The adherent cell monolayers were washed once with PBS and then detached using trypsin. The trypsin was inactivated using 10% FBS RPMI media and the cells were added to the cell pellets that had been collected from the culture supernatants. The cells were centrifuged (300xg), the supernatant discarded and the cell pellet washed in 200µL of PBS. The cells were centrifuged again then resuspended in 50µL of PBS containing Live/Dead Aqua (Life tech) for 15 minutes at RT. The cells were washed once in FACs buffer before staining with panels of directly conjugated antibodies: anti-CD3 conjugated to AF700; anti-CD25 conjugated to BV421; anti-CD107a conjugated to FITC. A sample of cells from each co-culture condition was also stained with

relevant isotype control antibodies. All staining was carried out in FACs buffer in a total volume of 50µL/well for 15 minutes, 4°C. Cells were then washed twice with FACs buffer (200µL) before resuspension in 200µL of FACs buffer and analysis by Flow cytometry (Attune).

Upregulation of T cell activation and degranulation markers

Flow cytometry analysis of T cell activation was assessed by expression of the T cell activation marker CD25 and the marker for T cell degranulation, CD107a on live, $CD3^+$, single cells. These data showed that the number of CD4 and CD8 T cells expressing CD25 and CD107a was significantly higher for T cells cultured with NG-348 or Ad5-348 infected A549 or HT-29 cells than Ad5 or uninfected controls. Similar levels of T cell activation were demonstrated with the NG-348 and Ad5-348 viruses (**Figure 58A and B).**

Secretion of the stimulatory cytokine IFNγ

For detection of IFNγ expression, co-culture supernatants from the experiment detailed above were diluted into 5% BSA/PBS assay buffer (in a range of 1:20 to 1:50) and ELISA was carried out using the human IFNγ Quantikine kit (R and D systems) according to the manufacturer's protocol. The concentration of secreted IFNγ was determined by interpolating from the standard curve. Expression of IFNγ could only be detected in the supernatants of co-cultures using NG-348 or Ad5-348 infected A549 or HT-29 cells and was not detectable in either the Ad5 or untreated controls (**Figure 58C**).

In addition, IFNγ expression was assayed by ELISA from co-cultures of HSV and vaccinia virus infected HT-29 cells with T cells, described in examples 2 and 4. ELISA was carried out using the human IFNγ Quantikine kit (R and D systems) according to the manufacturer's protocol. The concentration of secreted IFNγ was determined by interpolating from the standard curve. Expression of IFNγ could only be detected in the supernatants of co-cultures using viruses that expressed the transgene cassette (HSV-X-8.1, WR-434 and Wy-434) infected HT-29 cells and was not detectable in either the HSV.GFP, WR-wt, Wy-wt or untreated controls (**Figure 58D**).

Claims

1. A replication deficient oncolytic viral vector or replication capable oncolytic virus encoding an antibody or a binding fragment thereof to the antigen-specific T-cell receptor complex (TCR) for expression on the surface of a cancer cell.
2. A replication deficient oncolytic viral vector or replication capable oncolytic virus according to claim 1, wherein the antibody or a binding fragment thereof is an anti-CD3 antibody or binding fragment thereof.
3. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to claim 1or 2 wherein the encoded antibody further comprises a transmembrane domain or a GPI anchor, for example wherein the transmembrane domain is selected from a sequence shown in SEQ ID NO: 10 to 14.
4. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of claims 1 to 3, wherein the oncolytic virus is selected from an adenovirus (such as Ad5, Ad3, Ad11, Ad35, Ad11/Ad3 and Ad5/3 or a derivative thereof), herpes simplex virus (such as HSV-1), vesicular stomatitis virus, reovirus, vaccina virus, Seneca valley virus, coxsackievirus, measles, Maraba virus and Newcastle disease virus, for example wherein the oncolytic virus is an adenovirus, such as wherein the oncolytic adenovirus is a group B virus, such as Ad11 or a derivative thereof, in particular wherein the oncolytic adenovirus is EnAd.
5. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of claims 1 to 4, wherein the antibody or binding fragment is selected from the group comprising a full length antibody, a Fab, modified Fab, Fab', modified Fab', F(ab')2, Fv, single domain antibodies, scFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies, ,humabodies, disulfide stabilised forms of any one of the same and epitope-binding fragments thereof, for example wherein the antibody binding fragment is a single chain Fv.
6. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of claims 1 to 6, wherein the oncolytic virus encodes at least one further transgene.
7. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to claim 6, wherein the oncolytic virus encodes at least two further transgenes.
8. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to claim 7 or 8, wherein the further transgene(s) encodes a protein independently selected from the group comprising a cytokine, a chemokine, an antagonistic antibody molecule or binding fragment thereof, an agonistic antibody molecule or binding fragment thereof, and immunomodulator and combinations thereof.
9. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to claim 8, wherein at least one further transgene encodes an antibody molecule or a binding fragment thereof.

10. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to claim 9, wherein the antibody molecule or binding fragment is an inhibitor, for example wherein the inhibitor is an inhibitor of an angiogenesis factor or an inhibitor of a T cell deactivating factor.
11. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to claim 9 or 10, wherein antibody molecule or binding fragment is an agonist, for example wherein the agonist is independently selected from the group comprising an antibody or binding fragment specific to CD40, GITR, OX40, CD27 and 4-1BB.
12. A replication deficient oncolytic viral vector or a replication competent oncolytic virus according to any one of claims 9 to 11, where the immunomodulator is a membrane-associated protein ligand for immune cell surface receptors selected from the group comprising CTLA-4, PD-1, PD-L1, PD-L2, VISTA, B7-H3, B7-H4, HVEM, ILT-2, ILT-3, ILT-4, TIM-3, LAG-3, BTLA, LIGHT or CD160, CD16, CD25, CD33, CD332, CD127, CD31, CD43, CD44, CD162, CD301a, CD301b and Galectin-3, FLT-3, FLT-3 ligand, TLRs, TLR ligands, CCR7, CD1a, CD1c, CD11b, CD11c, CD80, CD83, CD86, CD123, CD172a, CD205, CD207, CD209, CD273, CD281, CD283, CD286, CD289, CD287, CXCR4, GITR Ligand, IFN-α2, IL-12, IL-23, ILT1, ILT2, ILT3, ILT4, ILT5, ILT7, TSLP Receptor, CD141, CD303, CADM1, CLEC9a, XCR1 and CD304, OX40, OX40 ligand, CD27, CD28, CD30, CD40, CD40 ligand, CD70, CD137, GITR, 4-1BB, ICOS and ICOS ligand.
13. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of claims 6 to 12, wherein at least one further transgene encodes a cytokine (including secreted or cell membrane associated molecules).
14. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of claims 7 to 12, wherein a second further transgene encodes a cytokine (including secreted or cell membrane associated molecules).
15. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of claims 8 to 15, wherein the encoded cytokine is selected from TNF alpha super family (TNFRSF includes TNF-alpha, TNF-C, OX40L, CD154, FasL, LIGHT, TL1A, CD70, Siva, CD153, 4-1BB ligand, TRAIL, RANKL, TWEAK, APRIL, BAFF, CAMLG, NGF, BDNF, NT-3, NT-4, GITR ligand, EDA-A, EDA-A2), TGF-beta superfamily, IL-1 family (i.e. IL-1 and IL-8), IL-2 family, IL-10 family, IL-17 family, interferon family, for example wherein the encoded cytokine is independently selected from TNF-alpha, IL-1, IL-8, IL-10, IL-17, interferon, LIGHT, TL1A, Siva, TRAIL, RANKL, TWEAK, APRIL, NGF, BDNF, NT-3, and EDA-A2.
16. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to claim 15, wherein the encoded cytokine is independently selected from TNF-C, OX40L, CD154, FasL, CD70, CD153, 4-1BB ligand, and EDA-A.
17. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of claims 7 to 16, wherein the cytokine is selected from the group comprising IL-2, IFN-alpha, IFN-beta, IFN-gamma, Flt3 ligand, GM-CSF, IL-15, and IL-12.

18. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of claims 7 to 17, wherein at least one further transgene encodes a chemokine.
19. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of claims 7 to 18, wherein the chemokine is selected from the group comprising MIP-1 alpha, RANTES, IL-8, CCL5, CCL17, CCL20, CCL22, CXCL9, CXCL10, CXCL11, CXCL13, CXCL12, CCL2, CCL19 and CCL21, for example wherein the chemokine encoded is MIP-1 alpha.
20. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to claim 19, wherein the virus comprises transgenes encoding a cytokine and chemokine combination selected from i) MIP-1α and Flt3, and ii) MIP-1α and IFNα.
21. A replication deficient oncolytic viral vector or replication competent oncolytic virus according to any one of claims 1 to 20, wherein the anti-CD3 antibody or binding fragment has at least the binding domain comprising a VH and a VL regions from muromonab-CD3 (OKT3), otelixizumab, teplizumab or visilizumab.
22. A method of treating a cancer patient by *in vivo* stimulation of T cells, for example T cells in the cancer cell environment, to focus on cancerous cells by:
administering a therapeutically effective amount of a replication deficient oncolytic viral vector or oncolytic replication capable virus encoding an antibody or a binding fragment thereof to the antigen-specific T-cell receptor complex (TCR),
wherein the virus or viral vector selectively infects said cancerous cells and expresses on the surface of the cell the said encoded anti-TCR antibody or binding fragment.
23. A method of treating a cancer patient according to claim 22 by:
administering a therapeutically effective amount of a replication deficient oncolytic viral vector or oncolytic replication capable virus encoding an anti-CD3 antibody or a binding fragment thereof,
wherein the virus or viral vector selectively infects said cancerous cells and expresses on the surface of the cell the said encoded anti-CD3 antibody or binding fragment.
24. A method of treating a cancer patient comprising administering a therapeutically effective amount of a replication deficient oncolytic viral vector or oncolytic replication capable virus encoding an anti-TCR antibody or binding fragment thereof (such as anti-CD3 antibody or a binding fragment thereof), for expression on the surface of a cancer cell.
25. A method according to any one of claims 22 to 26, wherein the virus is replication competent.
26. A method according to any one of claims 22 to 26, wherein the virus is replication deficient.
27. A method according to any one of claims 22 to 26, wherein the oncolytic virus or viral vector is selected from an adenovirus (such as Ad5, Ad3, Ad11, Ad35, Ad11/Ad3 and Ad5/3 based viruses), herpes simplex virus (such as HSV-1), reovirus, vaccina virus, Seneca valley virus, coxsackie virus, measles virus, Maraba virus and Newcastle disease virus, for example wherein the virus or viral vector is selected from the group comprising Oncorine (H101), Talimogene laparepvec, Reolysin, Pexastimogene devacirepvec (JX-594), Cavatak, Seprehvir,CGTG-102,

MV-NIS, PV701, CL-ONC1, CG0070, and enadenotucirev (EnAd), such as wherein the oncolytic virus or viral vector is an adenovirus, in particular wherein the oncolytic virus or viral vector is a group B virus, for example Ad11 or a derivative thereof, especially wherein the oncolytic virus or viral vector is enadenotucirev.

28. A method according to any one of claims 22 to 27, wherein the antibody or binding fragment is selected from the group comprising a full length antibody, a Fab, modified Fab, Fab', modified Fab', F(ab')2, Fv, single domain antibodies, scFv, bi, tri or tetra-valent antibodies, bis-scFv, diabodies, triabodies, tetrabodies, humabodies, disulfide stabilised forms of any one of the same and epitope-binding fragments thereof, for example wherein the antibody binding fragment is a single chain Fv.
29. A method according to any one of claims 22 to 28, wherein the oncolytic virus or viral vector encodes a further transgene.
30. A method according to claim 29, wherein the further transgene encodes a protein independently selected from the group comprising a cytokine, a chemokine, an antagonistic antibody molecule or fragment thereof, an agonistic antibody molecule or fragment thereof and combinations thereof.
31. A method according to claim 30, wherein the cytokine is selected from the group comprising IL-2, IFN-alpha, IFN-beta, IFN-gamma, Flt3 ligand, GM-CSF, IL-15, IL-12 and combinations thereof.
32. A method according to claim 30 or 31, wherein the chemokine is selected from the group comprising MIP-1 alpha, RANTES, IL-8, CCL5, CCL17, CCL20, CCL22, CXCL9, CXCL10, CXCL11, CXCL13, CXCL12, CCL2, CCL19, CCL21 and combinations thereof.
33. A method according to any one of claims 29 to 32, wherein a cytokine and chemokine combination of MIP-1α and Flt3 or MIP-1α and IFNα is encoded in the virus.
34. A method according to any one of claims 22 to 33, wherein the anti-CD3 antibody or binding fragment has at least the binding domain comprising a VH and a VL regions from muromonab-CD3 (OKT3), otelixizumab, teplizumab or visilizumab.
35. A method according any one of claims 22 to 34, wherein the anti-CD3 antibody or binding fragment thereof comprises a transmembrane domain or a GPI anchor, for example wherein the transmembrane is selected from a sequence shown in SEQ ID NO: 10 to 14.
36. A method according to any one of claims 22 to 35, where a linker sequence is employed between the transmembrane domain and the antibody or binding antibody fragment, such as ScFv (e.g. myc tag sequence, CD80, CD86 membrane proximal sequences, immunoglobulin sequences).

Figure 1

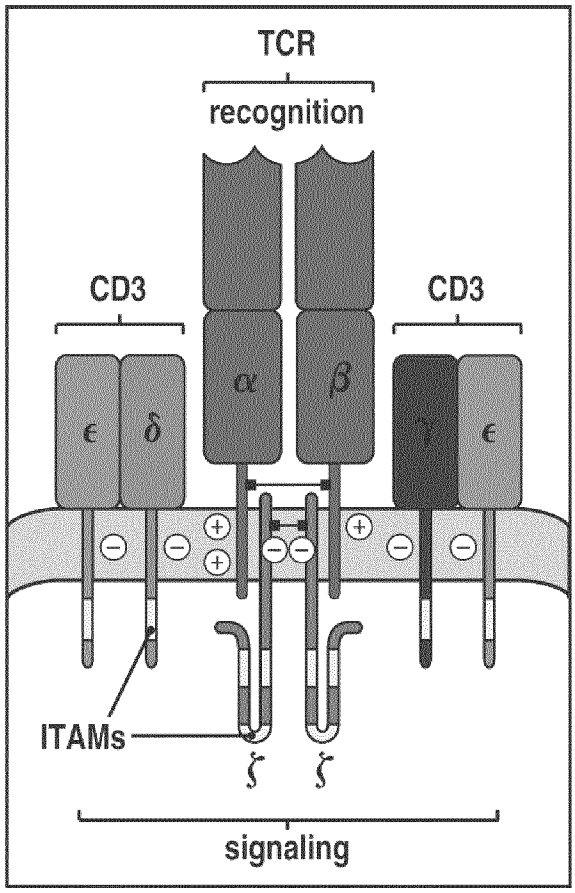


Figure 6-9 Immunobiology, 6/e. (© Garland Science 2005)

Figure 2A

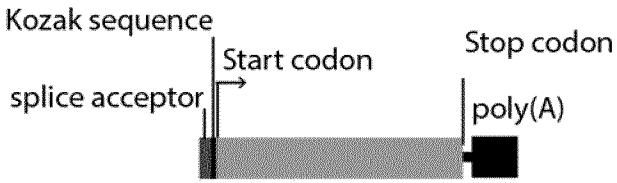


Figure 2B

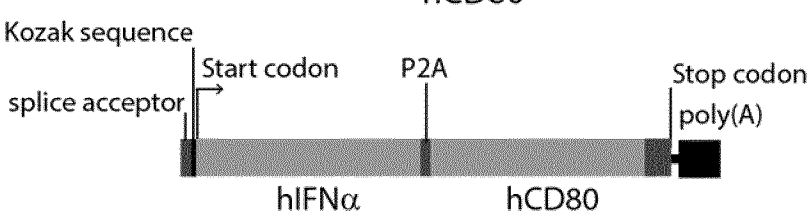
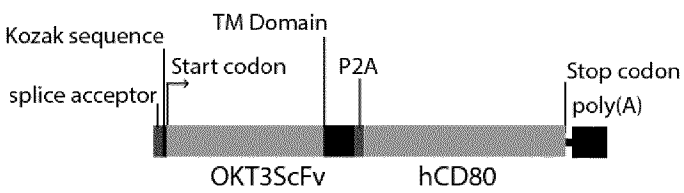


Figure 2C



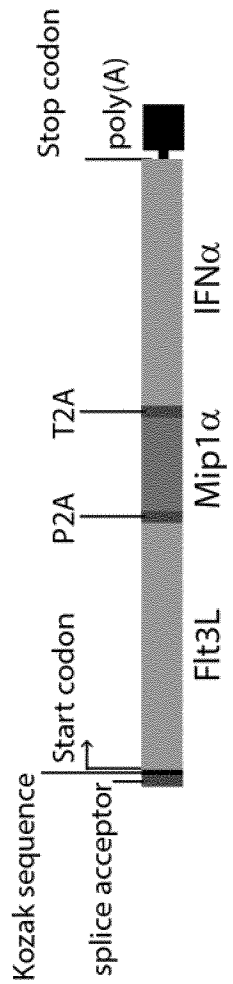


Figure 2D

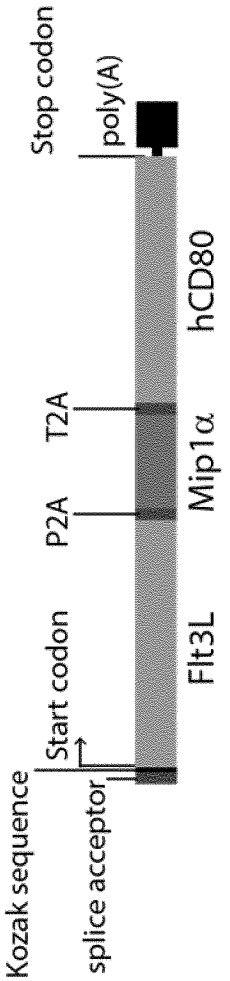


Figure 2E

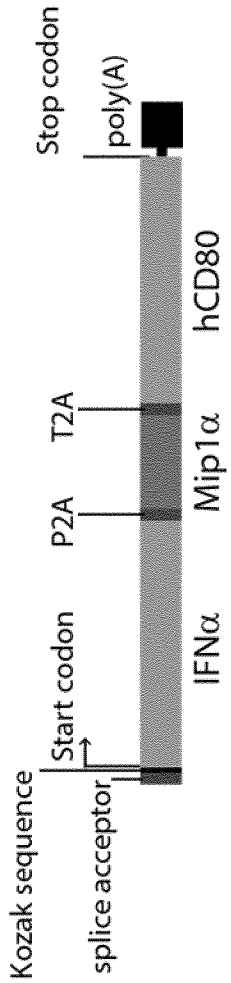


Figure 2F

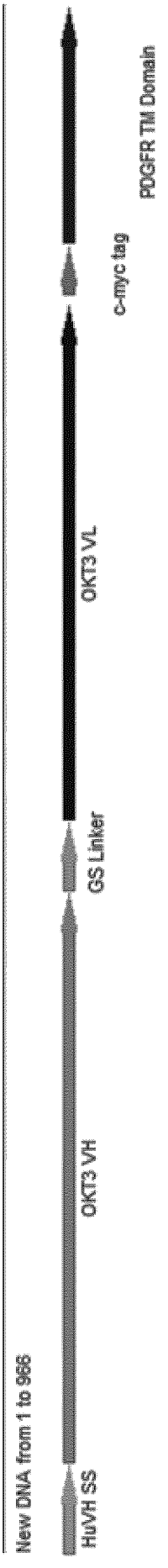


Figure 2G ORF cassette for scFv antibody

Figure 3

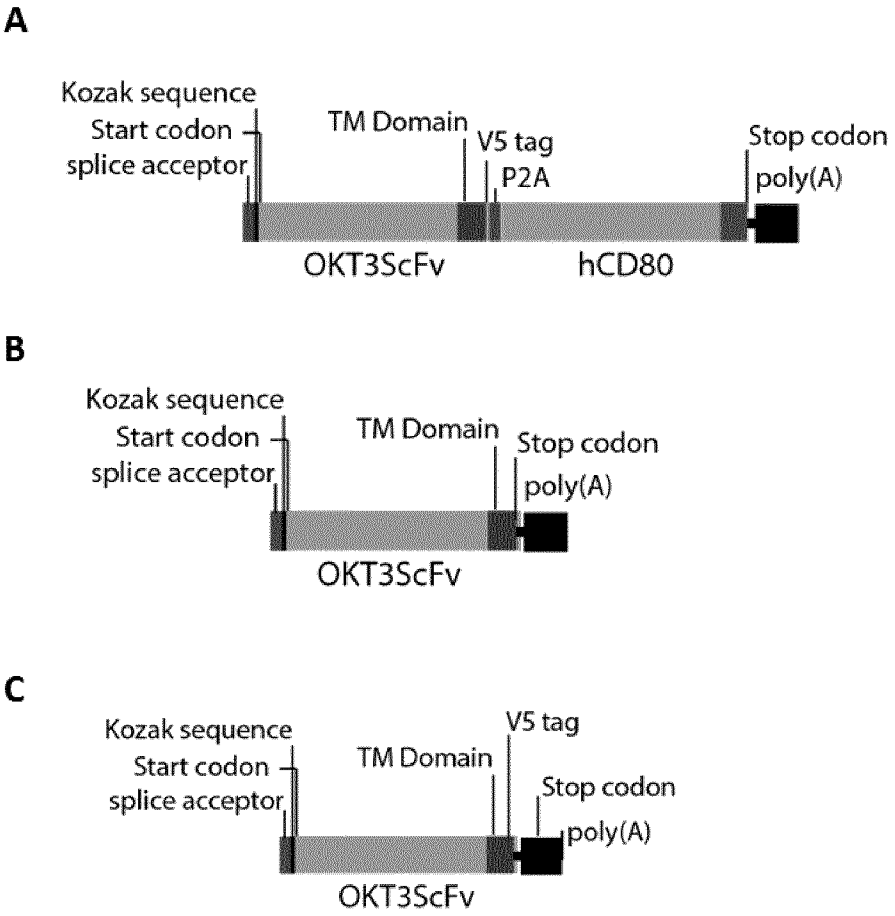

A
Kozak sequence
Start codon
splice acceptor
TM Domain
V5 tag
P2A
Stop codon
poly(A)
OKT3ScFv
hCD80
B
Kozak sequence
Start codon
splice acceptor
TM Domain
Stop codon
poly(A)
OKT3ScFv
C
Kozak sequence
Start codon
splice acceptor
TM Domain
V5 tag
Stop codon
poly(A)
OKT3ScFv

Figure 4A

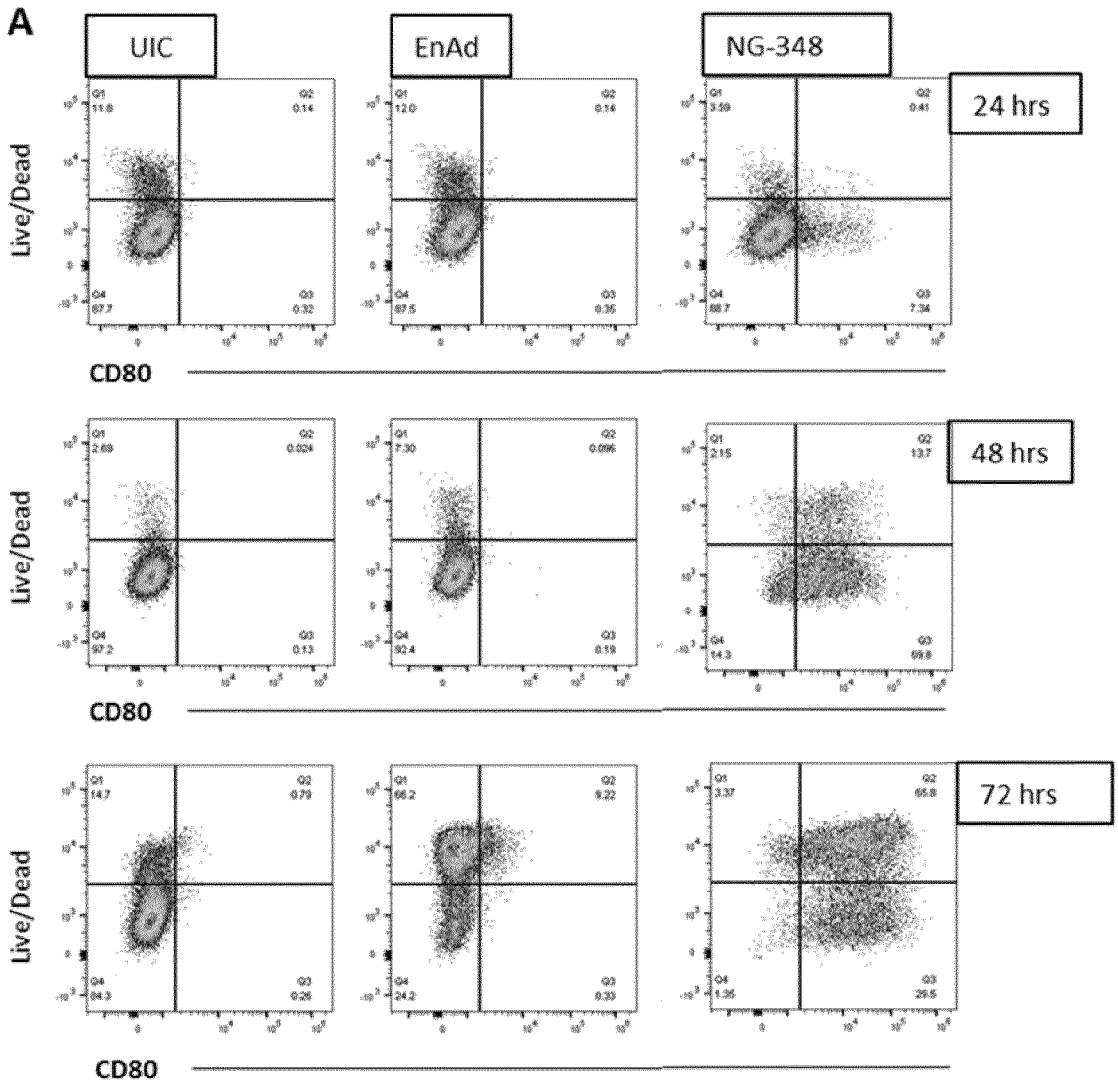
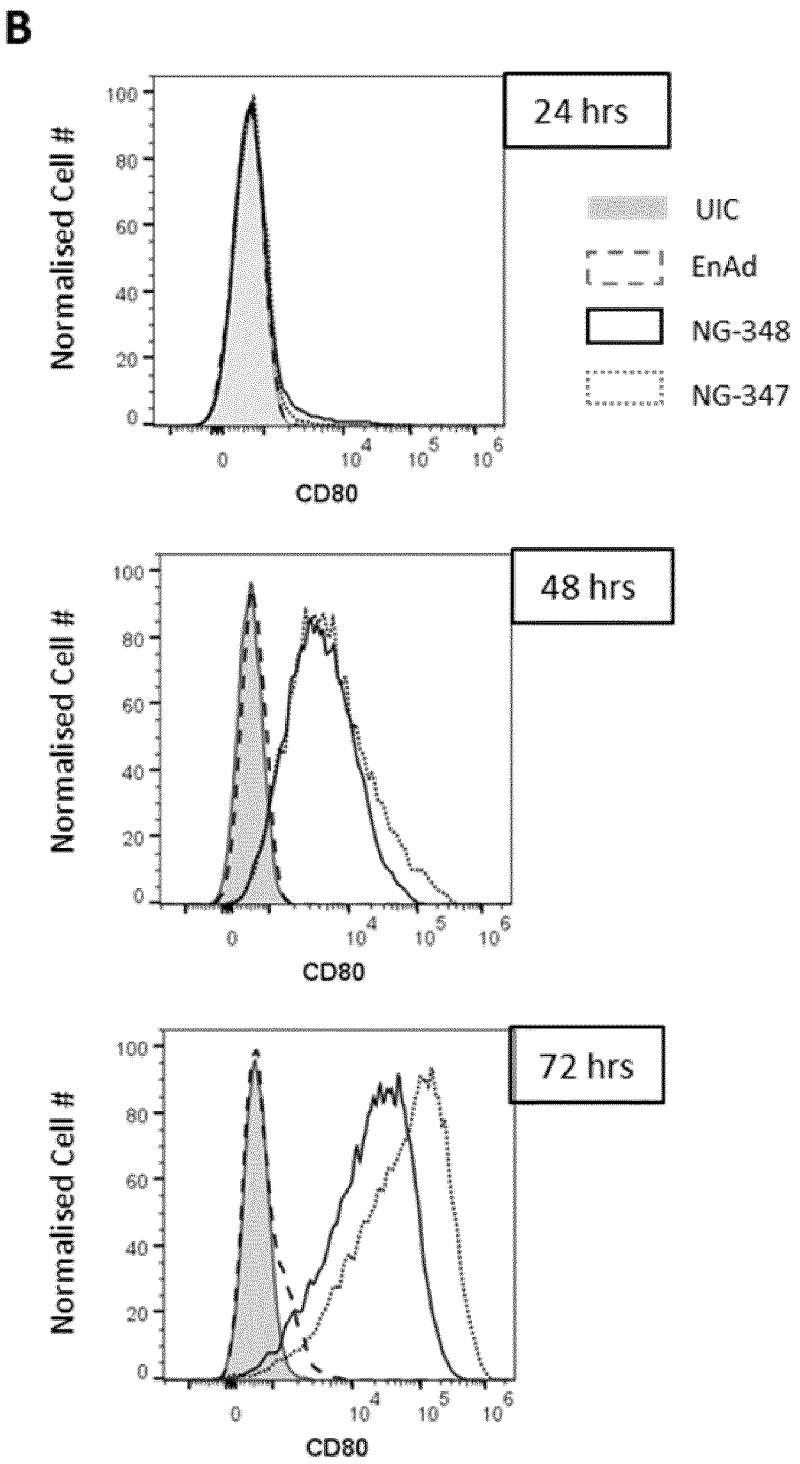


Figure 4B


B
24 hrs
48 hrs
72 hrs
Normalised Cell #
CD80
UIC
EnAd
NG-348
NG-347

P000164_WO

Figure 5A

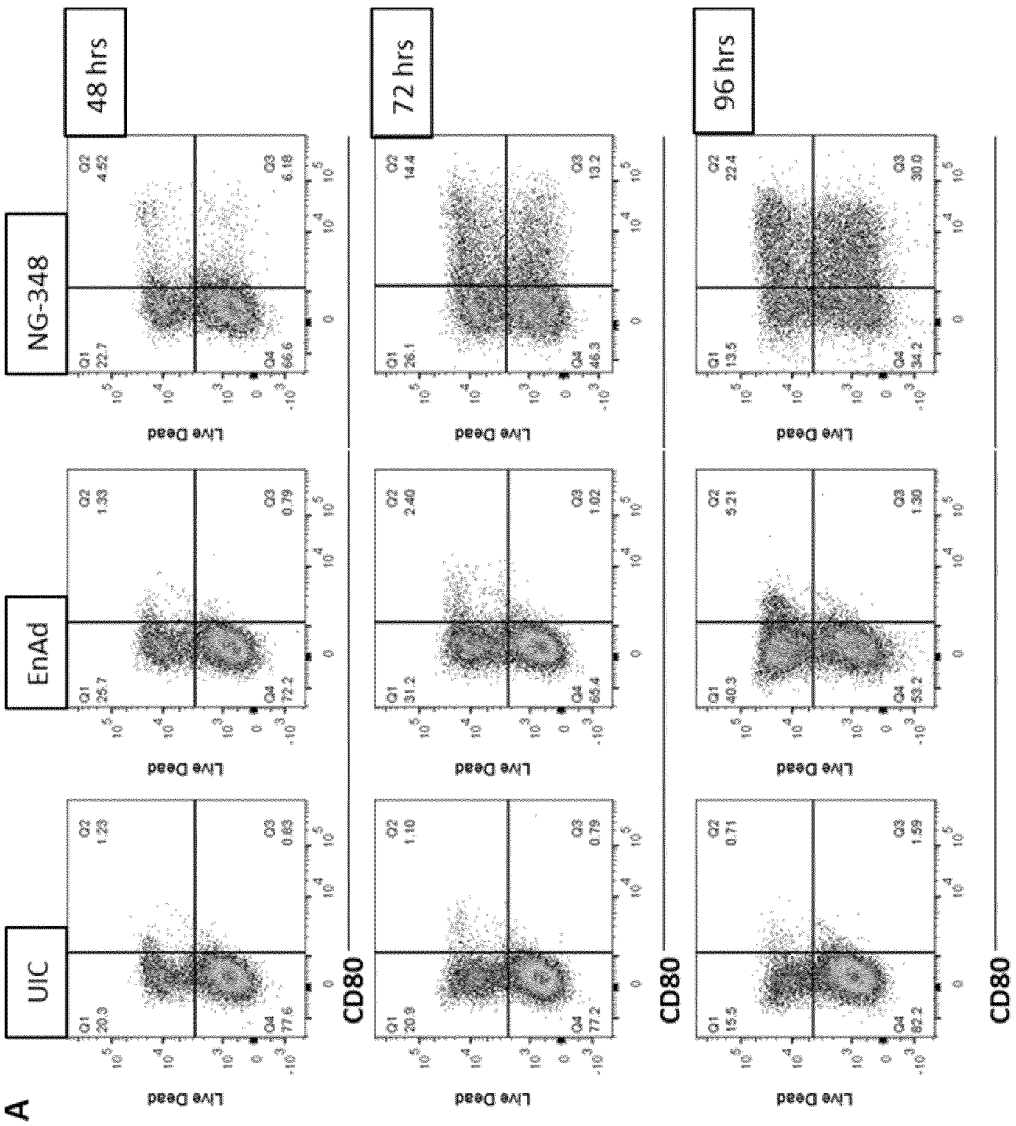
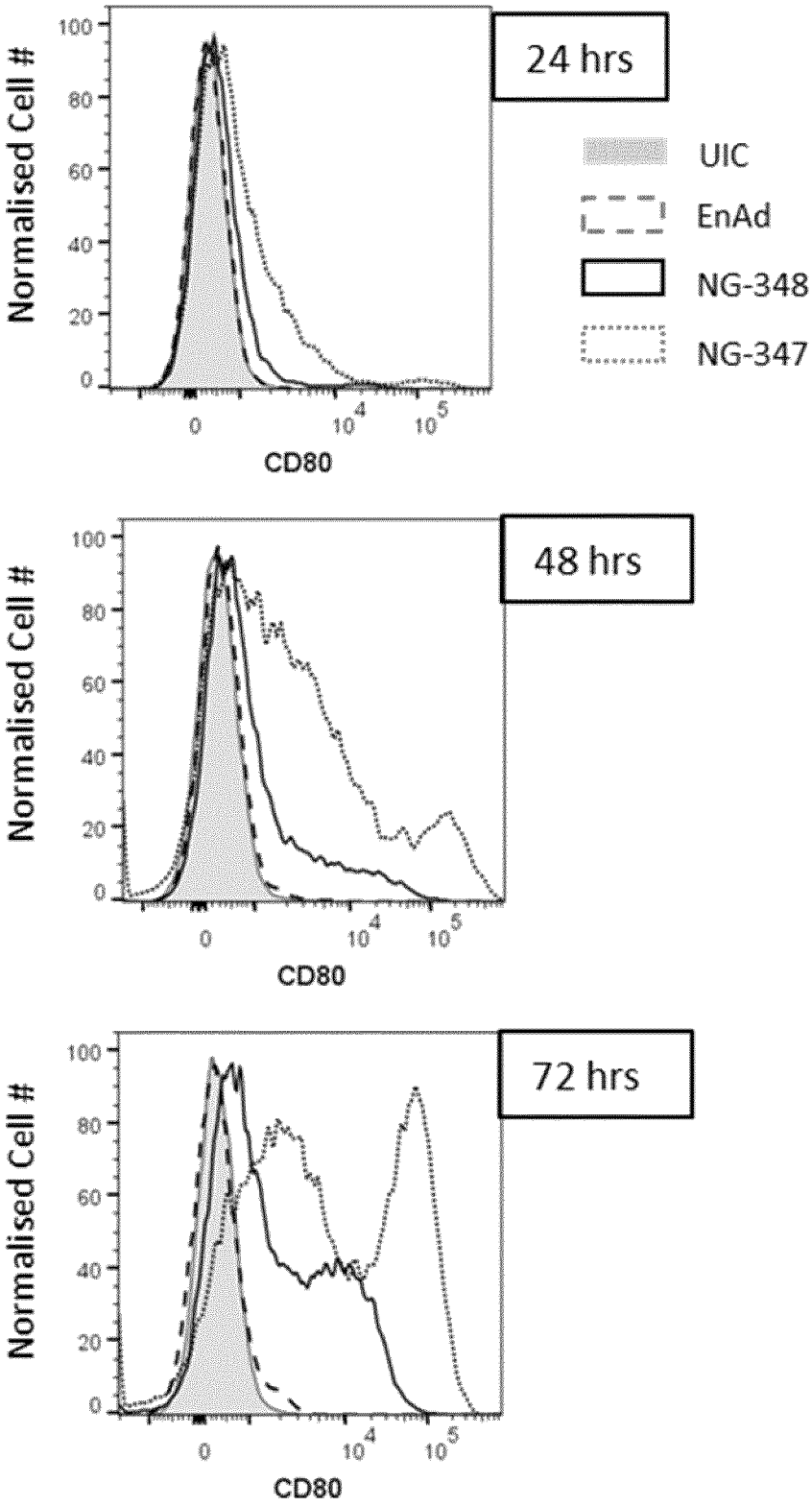


Figure 5B


24 hrs
48 hrs
72 hrs
UIC
EnAd
NG-348
NG-347
Normalised Cell #
CD80
0
20
40
60
80
100
10^4
10^5

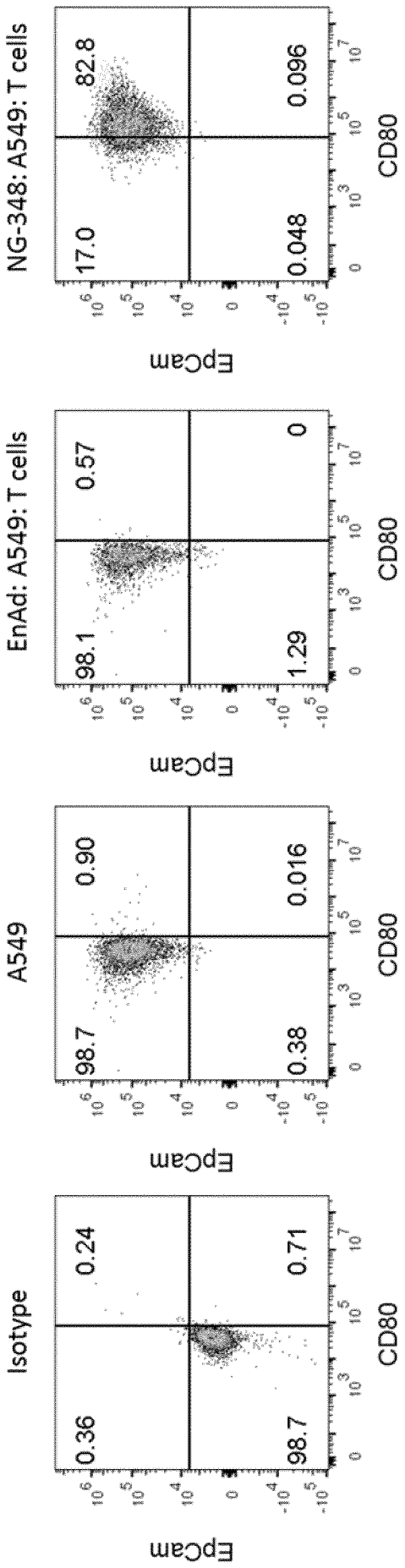
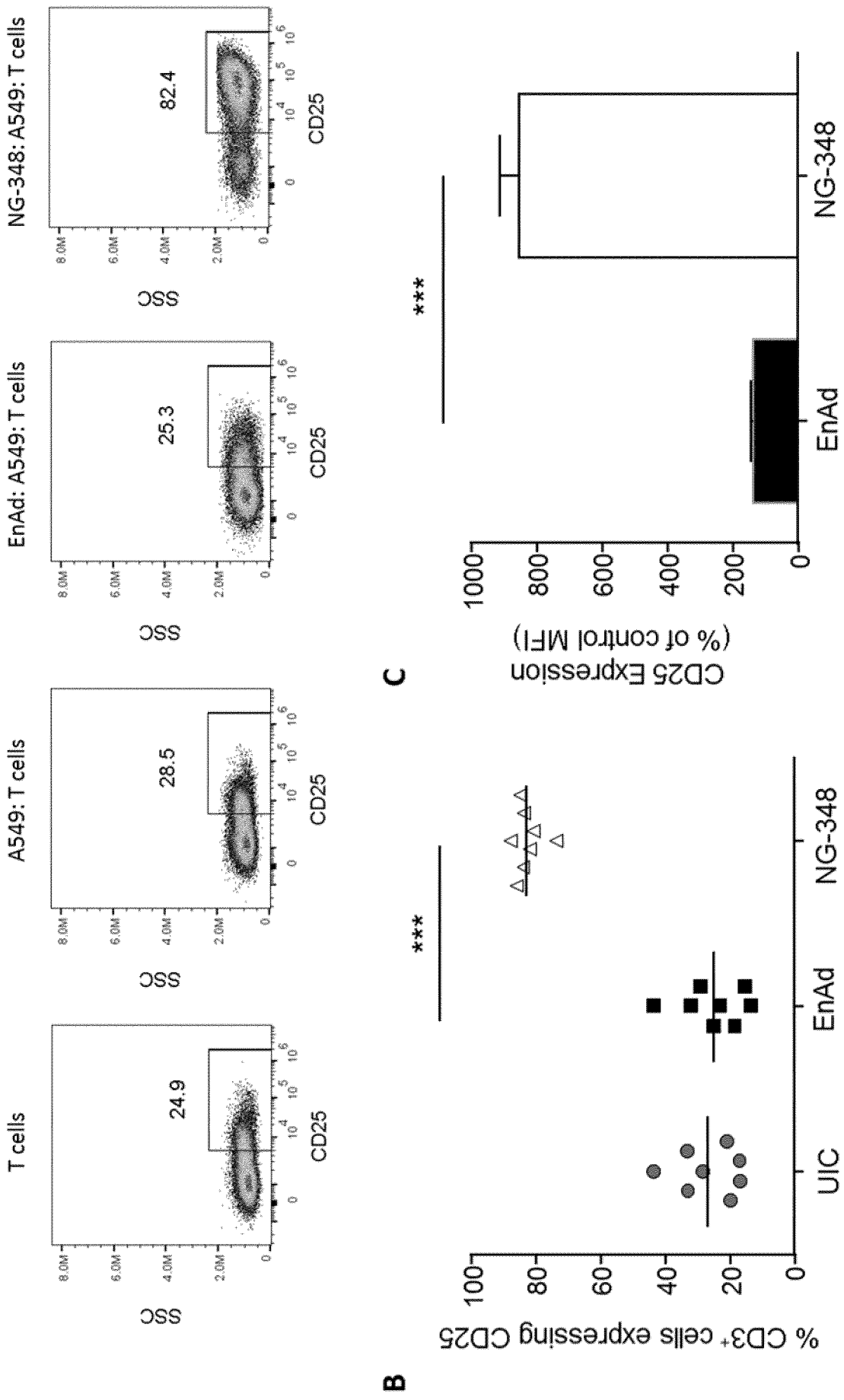

Isotype
0.24
0.36
98.7
0.71
A549
0.90
98.7
0.38
0.016
EnAd: A549: T cells
0.57
98.1
1.29
0
NG-348: A549: T cells
82.8
17.0
0.048
0.096
EpCam
CD80

Figure 6

Figure 7A


T cells
A549: T cells
EnAd: A549: T cells
NG-348: A549: T cells
SSC
CD25
24.9
28.5
25.3
82.4
B
% CD3+ cells expressing CD25
UIC
EnAd
NG-348
***
C
CD25 Expression
(% of control MFI)
***
EnAd
NG-348

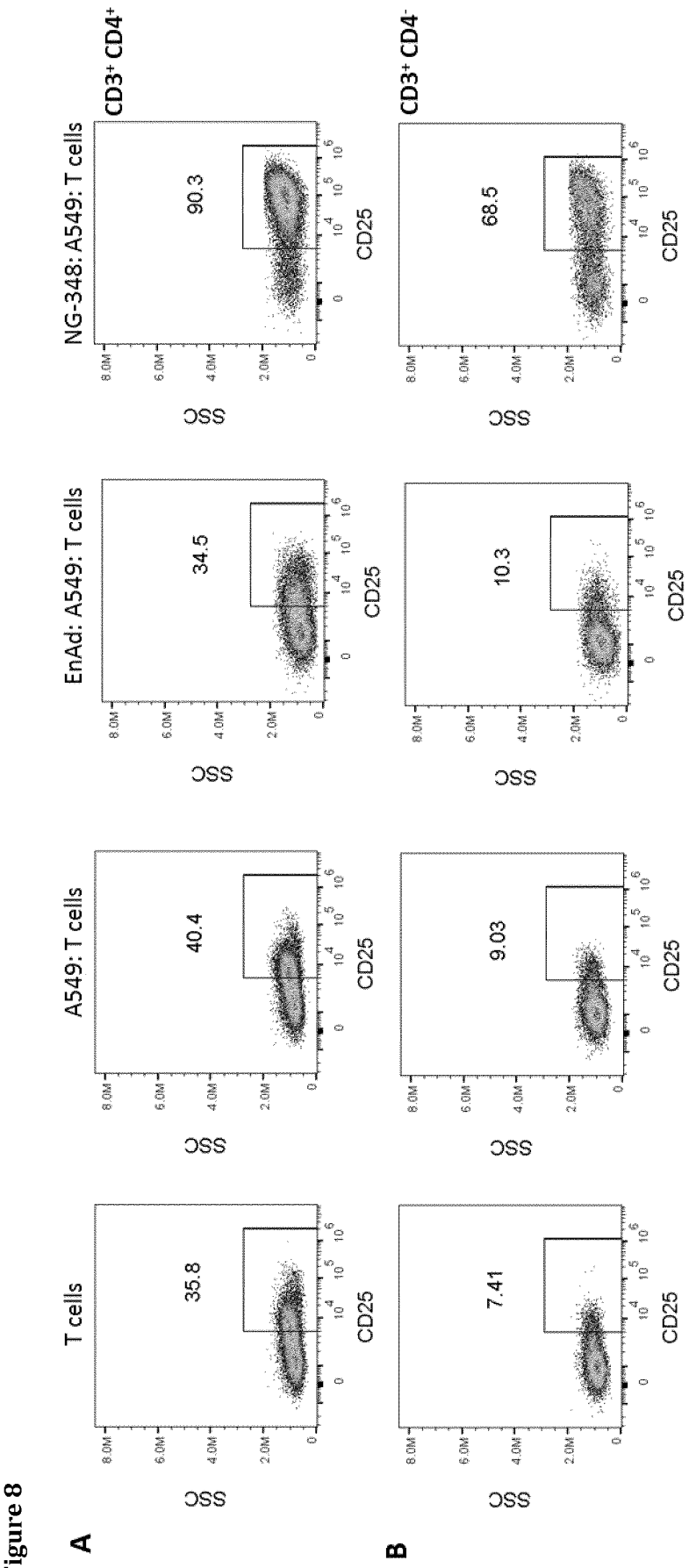

A
B
T cells
A549: T cells
EnAd: A549: T cells
NG-348: A549: T cells
CD3+ CD4+
CD3+ CD4-
35.8
40.4
34.5
90.3
7.41
9.03
10.3
68.5
SSC
CD25

Figure 8

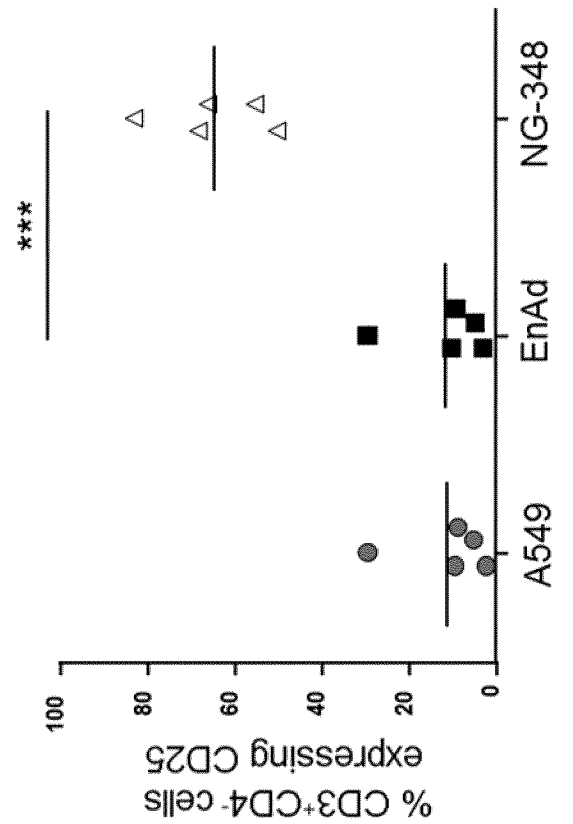
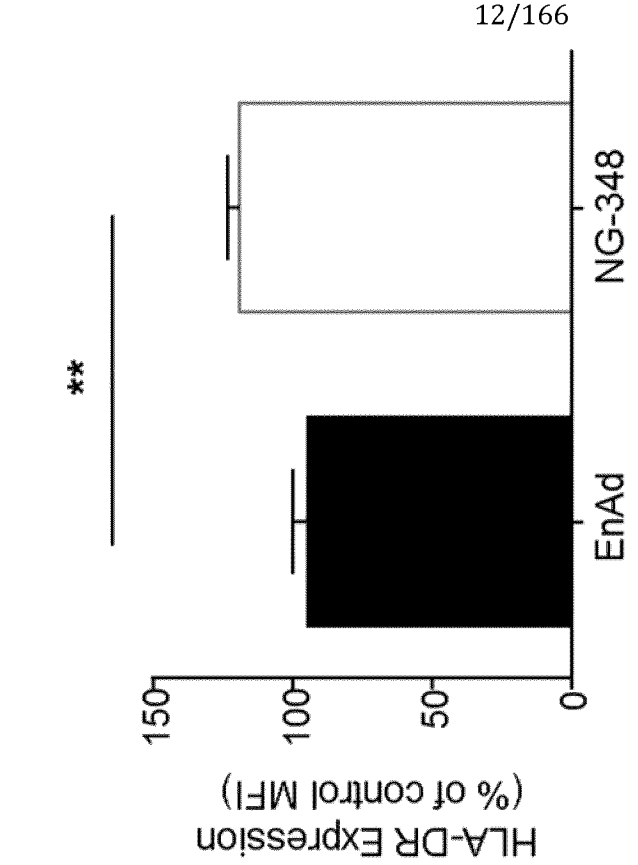

C
% CD3+CD4+ cells expressing CD25
100
80
60
40
20
0
***
A549
EnAd
NG-348
D
% CD3+CD4- cells expressing CD25
100
80
60
40
20
0
***
A549
EnAd
NG-348

Figure 8


B
HLA-DR Expression
(% of control MFI)
150
100
50
0
**
EnAd
NG-348

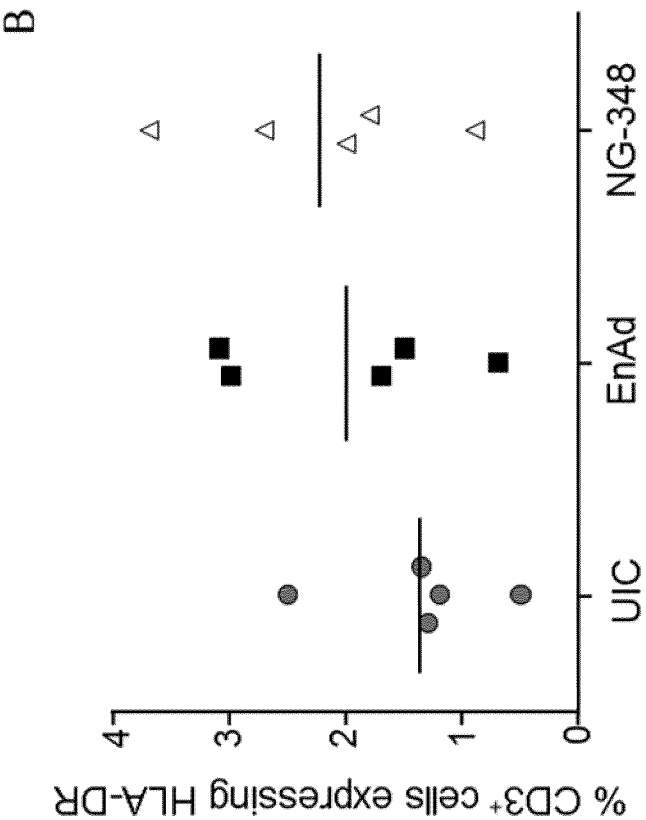
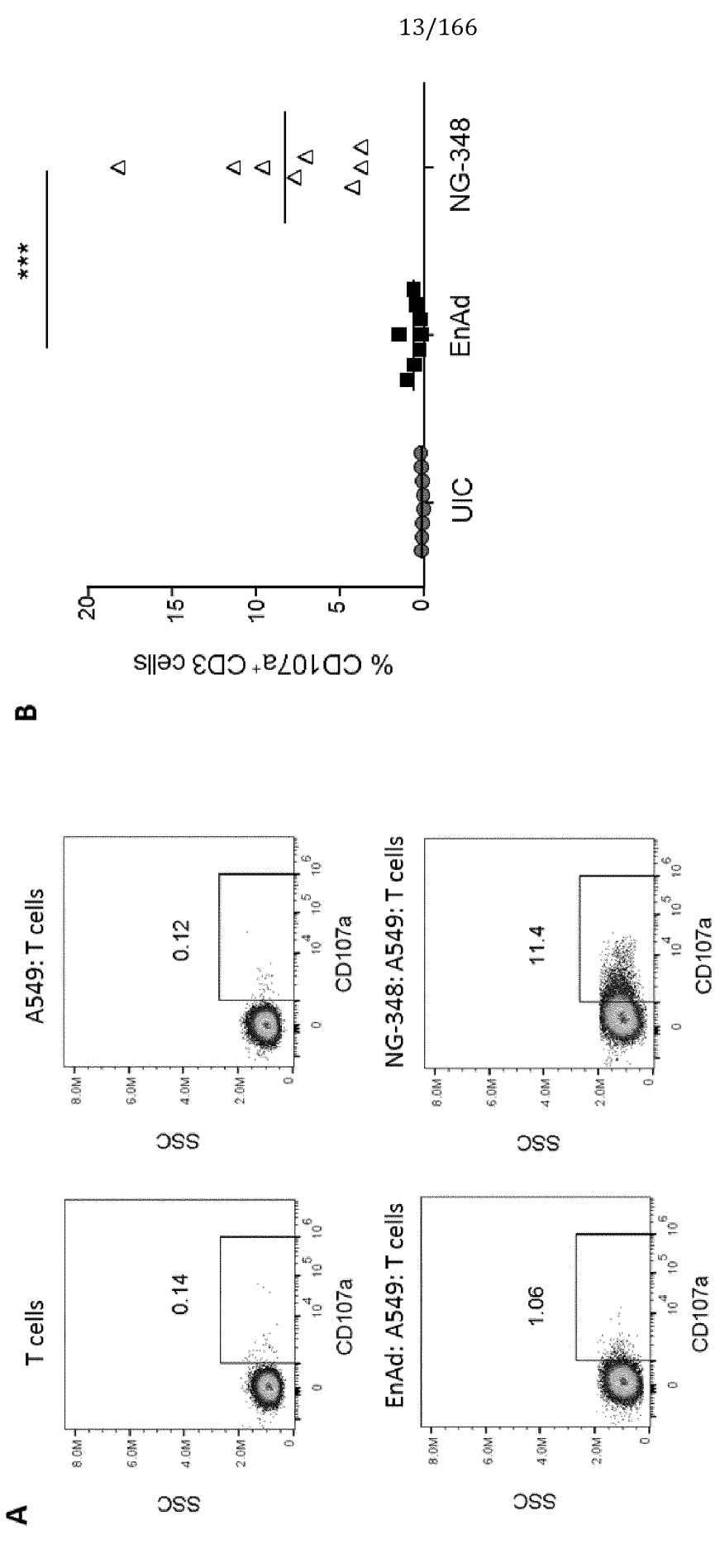

A
% CD3+ cells expressing HLA-DR
4
3
2
1
0
UIC
EnAd
NG-348

Figure 9

Figure 10


A
T cells
0.14
A549: T cells
0.12
EnAd: A549: T cells
1.06
NG-348: A549: T cells
11.4
SSC
CD107a
B
% CD107a+ CD3 cells
UIC
EnAd
NG-348
***

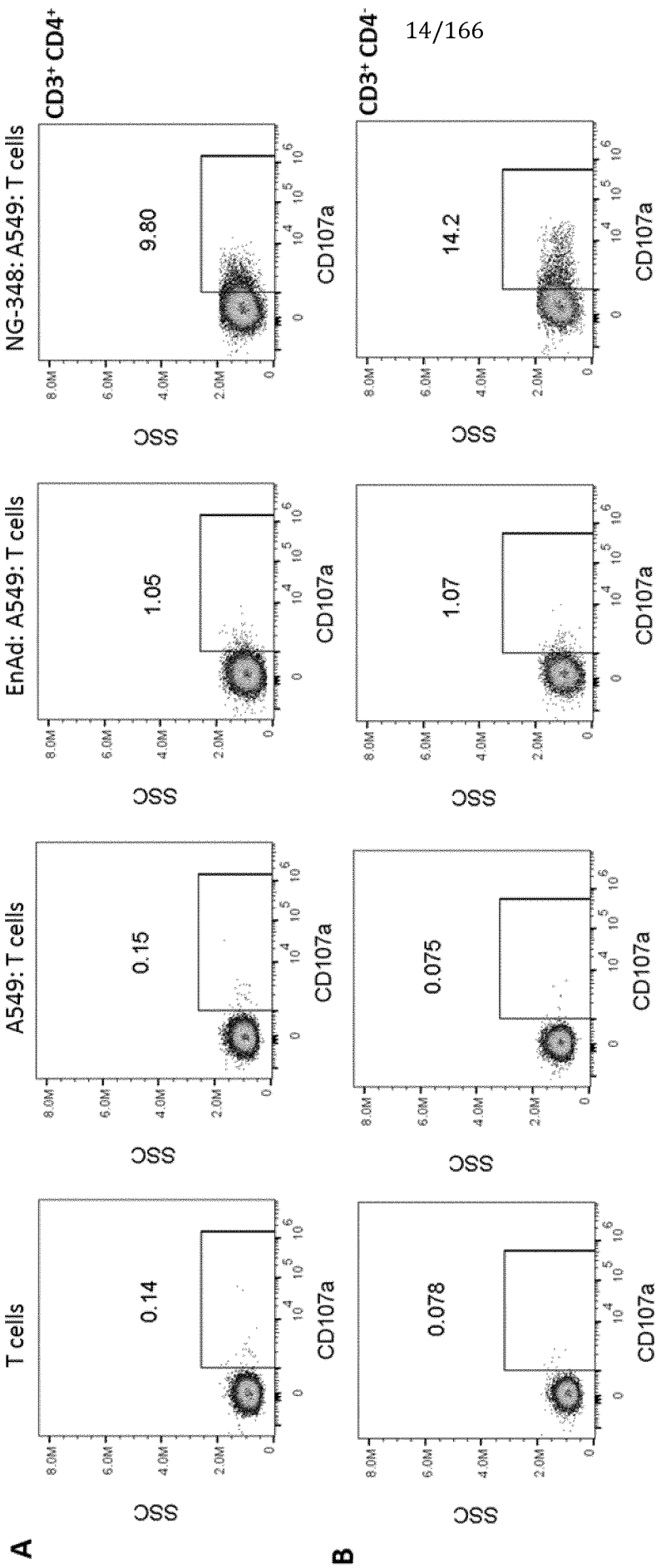

A
B
T cells
A549: T cells
EnAd: A549: T cells
NG-348: A549: T cells
CD3+ CD4+
CD3+ CD4-
0.14
0.15
1.05
9.80
0.078
0.075
1.07
14.2
SSC
CD107a

Figure 11

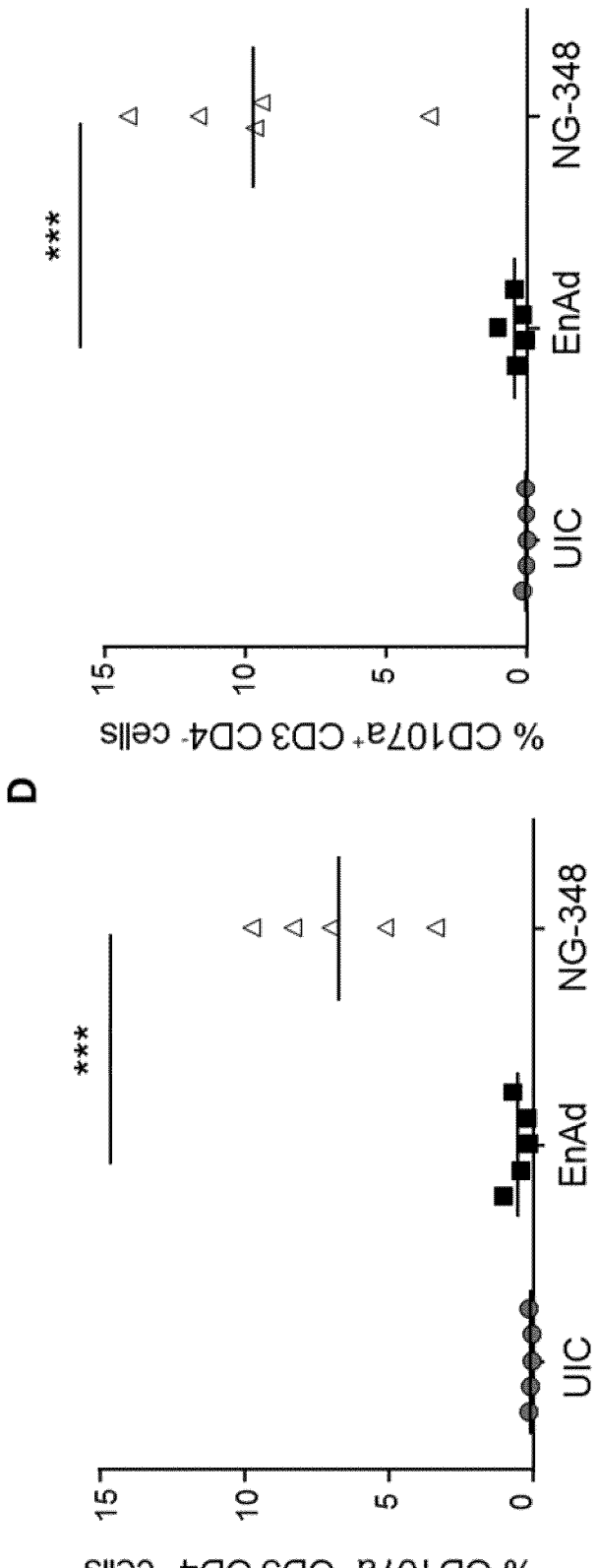
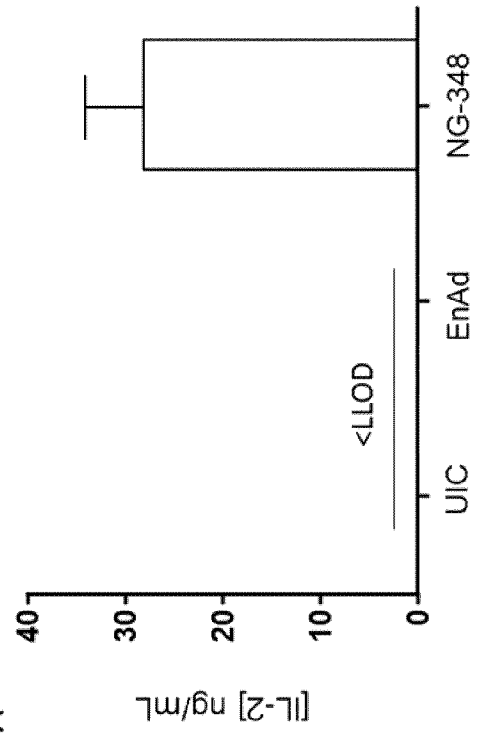

C
% CD107a+ CD3 CD4+ cells
15
10
5
0
***
UIC
EnAd
NG-348
D
% CD107a+ CD3 CD4- cells
15
10
5
0
***
UIC
EnAd
NG-348

Figure 11

Figure 12



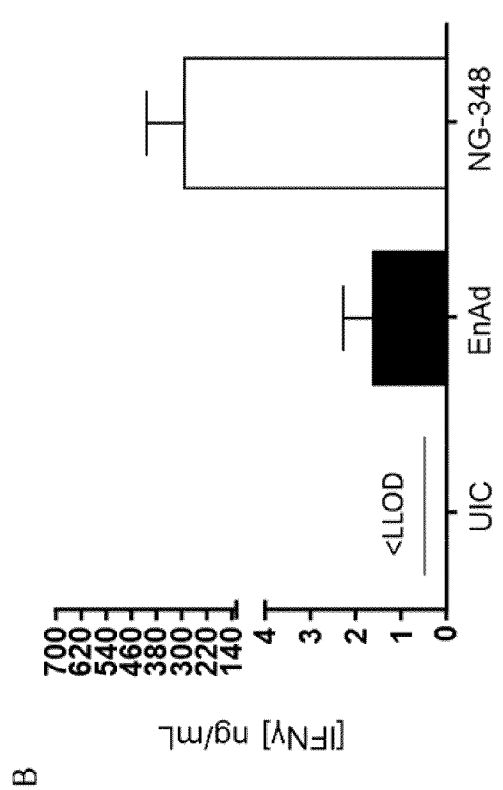
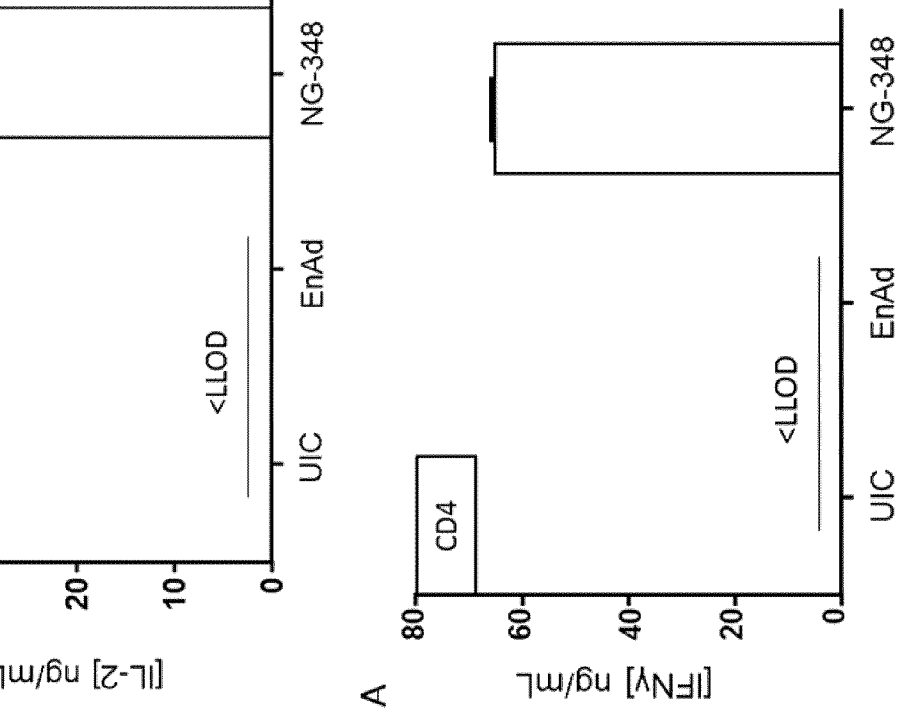
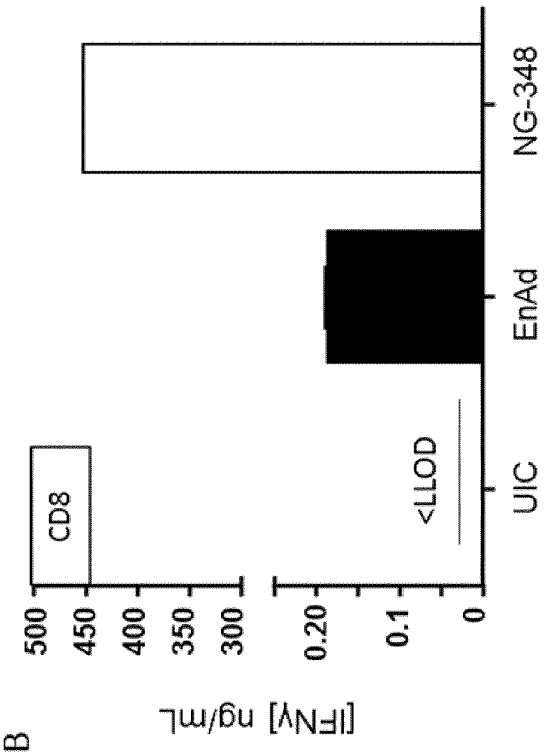


Figure 13





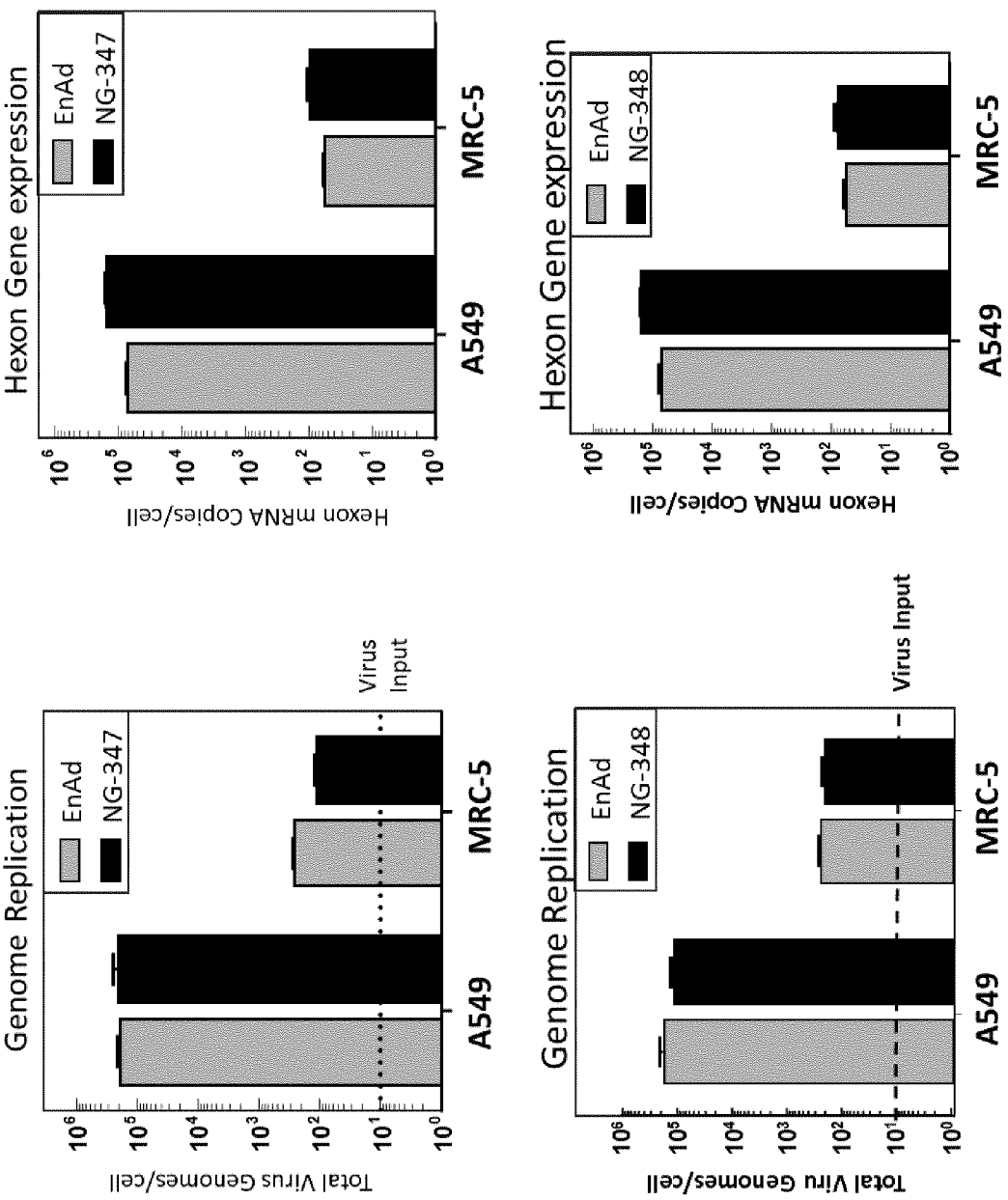

Hexon Gene expression
EnAd
NG-347
Hexon mRNA Copies/cell
A549
MRC-5
Hexon Gene expression
EnAd
NG-348
Hexon mRNA Copies/cell
A549
MRC-5
Genome Replication
EnAd
NG-347
Virus Input
Total Virus Genomes/cell
A549
MRC-5
Genome Replication
EnAd
NG-348
Virus Input
Total Virus Genomes/cell
A549
MRC-5

Figure 14

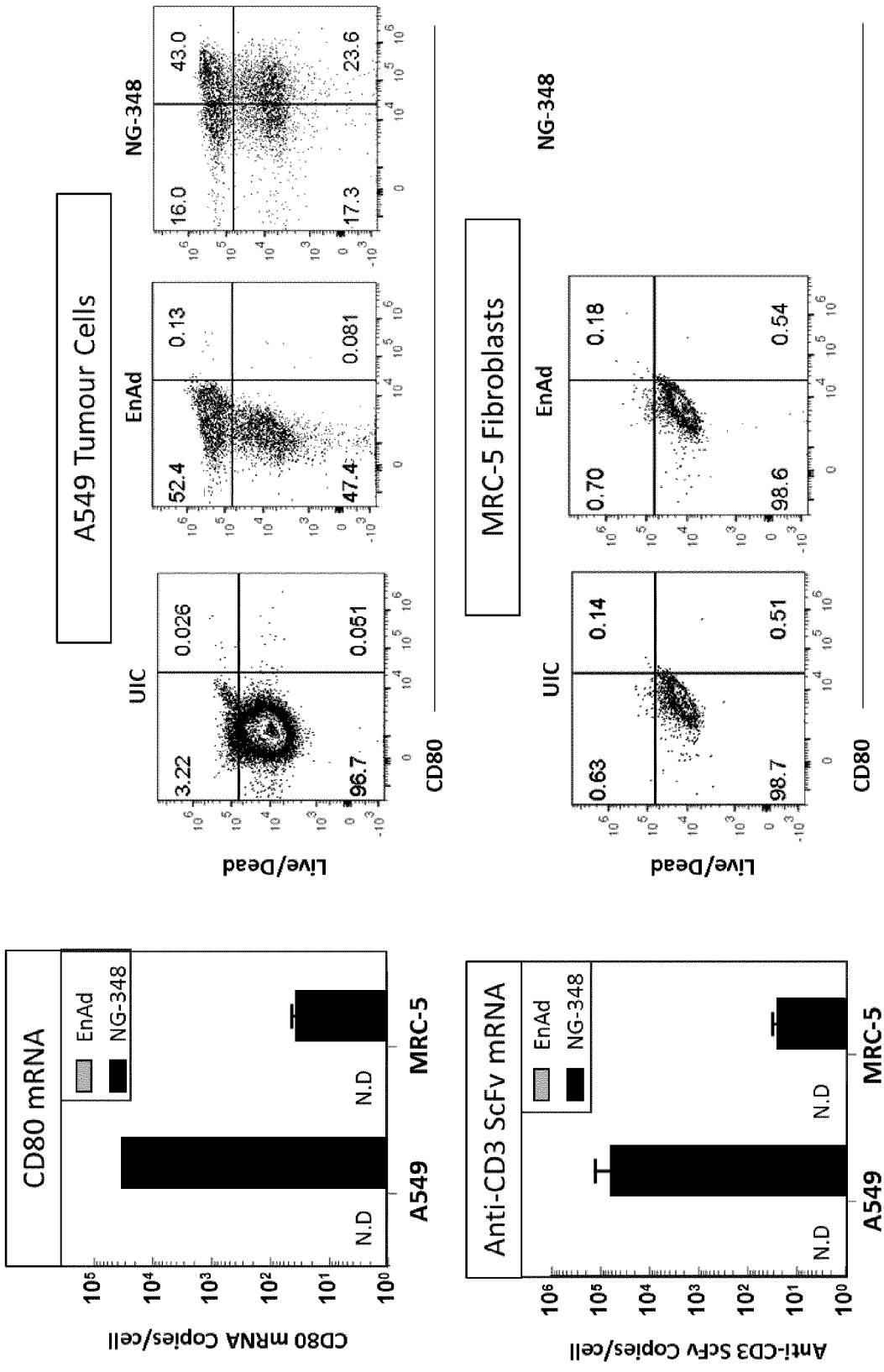

CD80 mRNA
EnAd
NG-348
CD80 mRNA Copies/cell
N.D
N.D
A549
MRC-5
Anti-CD3 ScFv mRNA
EnAd
NG-348
Anti-CD3 ScFv Copies/cell
N.D
N.D
A549
MRC-5
A549 Tumour Cells
UIC
EnAd
NG-348
3.22
0.026
96.7
0.051
52.4
0.13
47.4
0.081
16.0
43.0
17.3
23.6
Live/Dead
CD80
MRC-5 Fibroblasts
UIC
EnAd
NG-348
0.63
0.14
98.7
0.51
0.70
0.18
98.6
0.54
Live/Dead
CD80

Figure 15

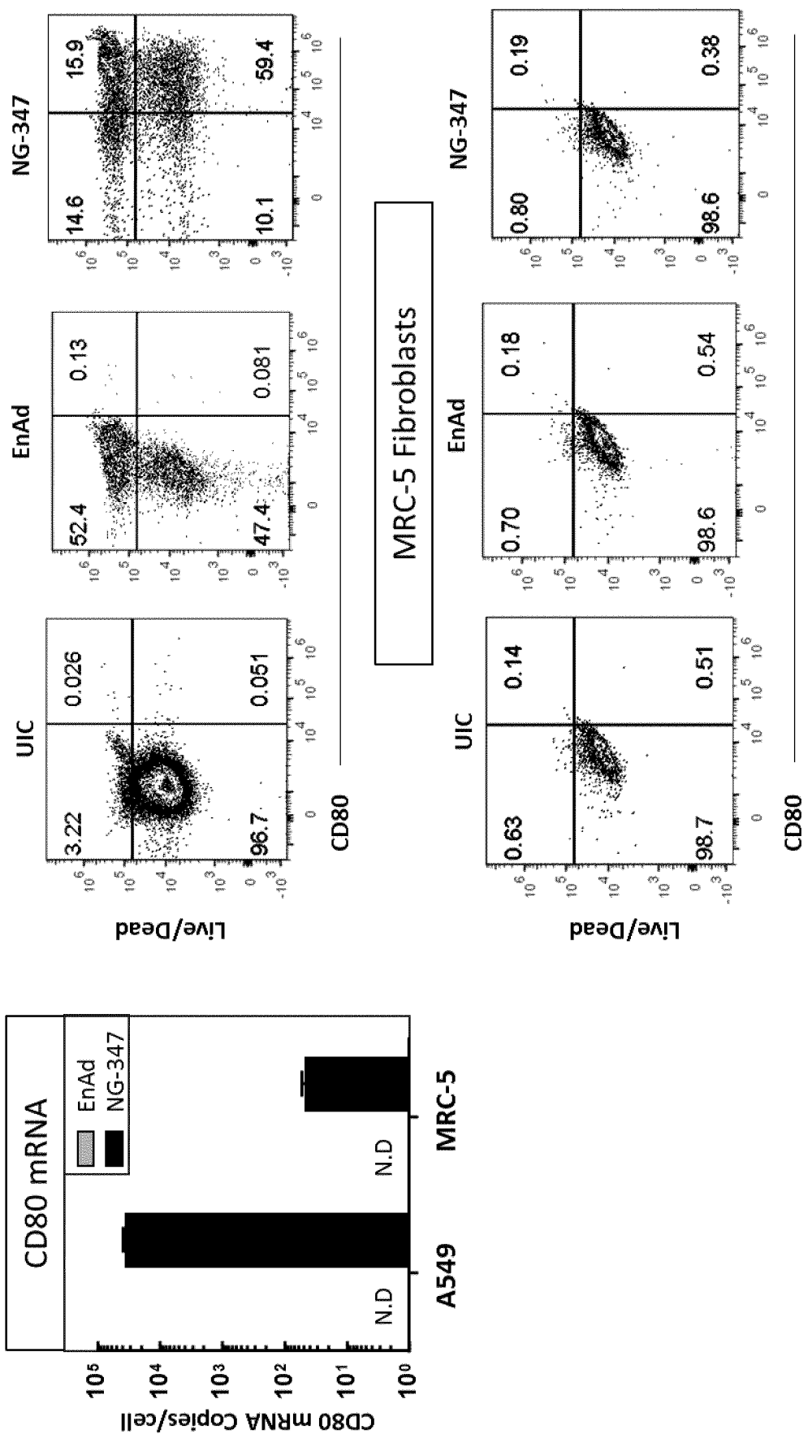

A549 Tumour Cells
MRC-5 Fibroblasts
UIC
EnAd
NG-347
Live/Dead
CD80
3.22
96.7
0.026
0.051
52.4
47.4
0.13
0.081
14.6
10.1
15.9
59.4
0.63
98.7
0.14
0.51
0.70
98.6
0.18
0.54
0.80
98.6
0.19
0.38
CD80 mRNA
EnAd
NG-347
CD80 mRNA Copies/cell
N.D
N.D
A549
MRC-5

Figure 16

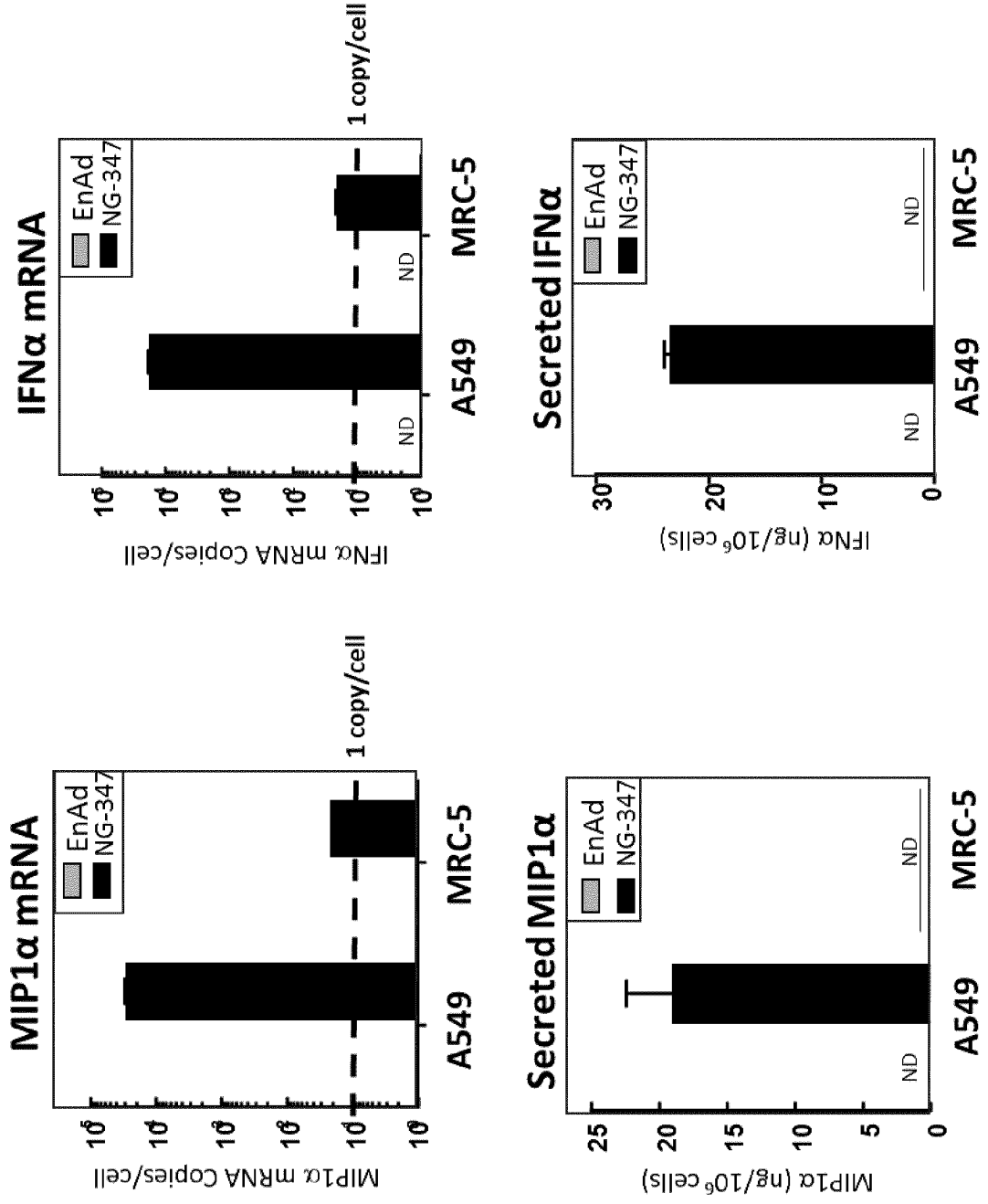
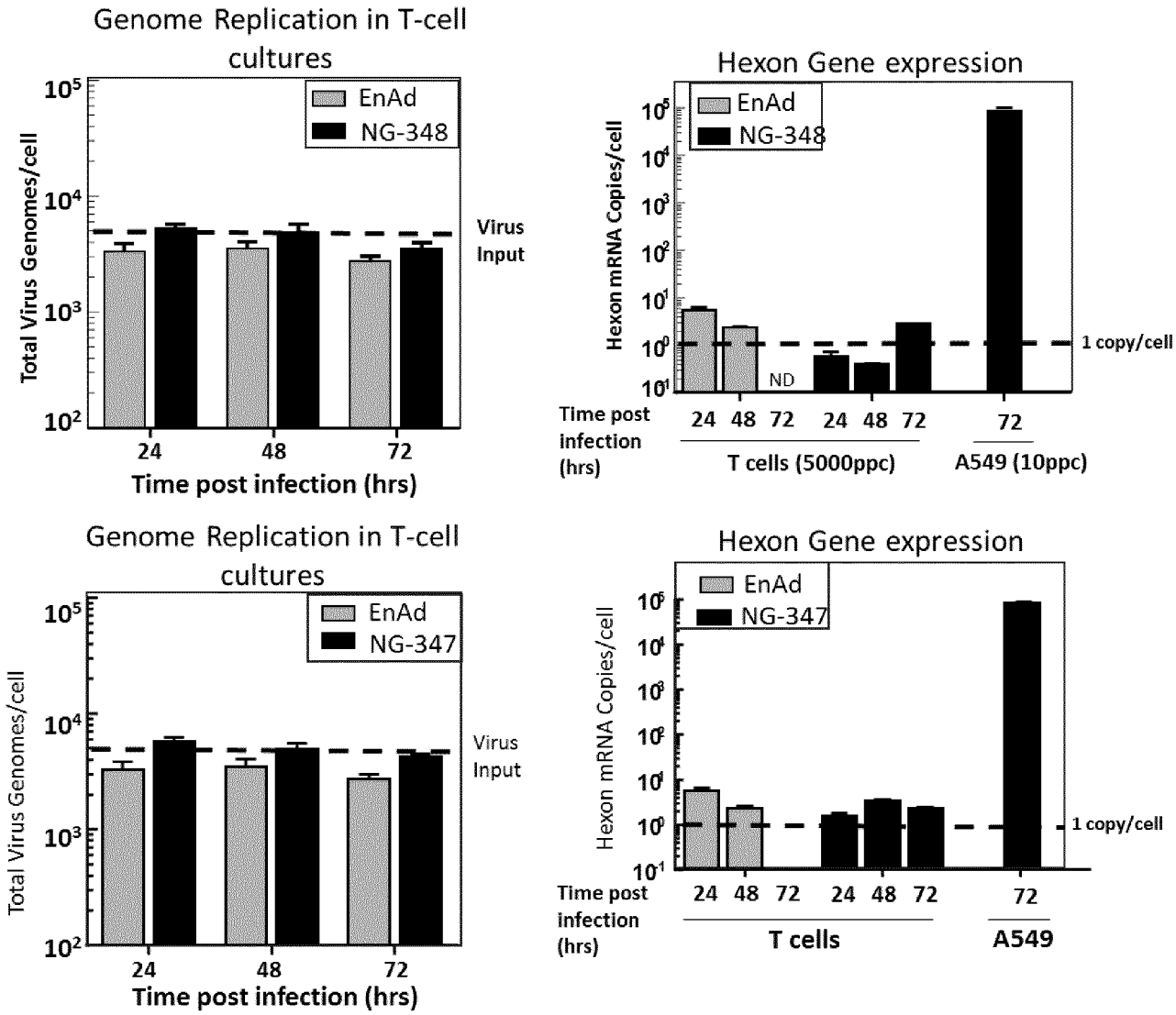

MIP1α mRNA
EnAd
NG-347
1 copy/cell
MIP1α mRNA Copies/cell
A549
MRC-5
Secreted MIP1α
MIP1α (ng/10⁶ cells)
ND
IFNα mRNA
IFNα mRNA Copies/cell
Secreted IFNα
IFNα (ng/10⁶ cells)

Figure 17

Figure 18


Genome Replication in T-cell cultures
EnAd
NG-348
Total Virus Genomes/cell
Virus Input
Time post infection (hrs)
Hexon Gene expression
Hexon mRNA Copies/cell
ND
1 copy/cell
Time post infection (hrs)
T cells (5000ppc)
A549 (10ppc)
Genome Replication in T-cell cultures
EnAd
NG-347
Total Virus Genomes/cell
Virus Input
Time post infection (hrs)
Hexon Gene expression
Hexon mRNA Copies/cell
1 copy/cell
Time post infection (hrs)
T cells
A549

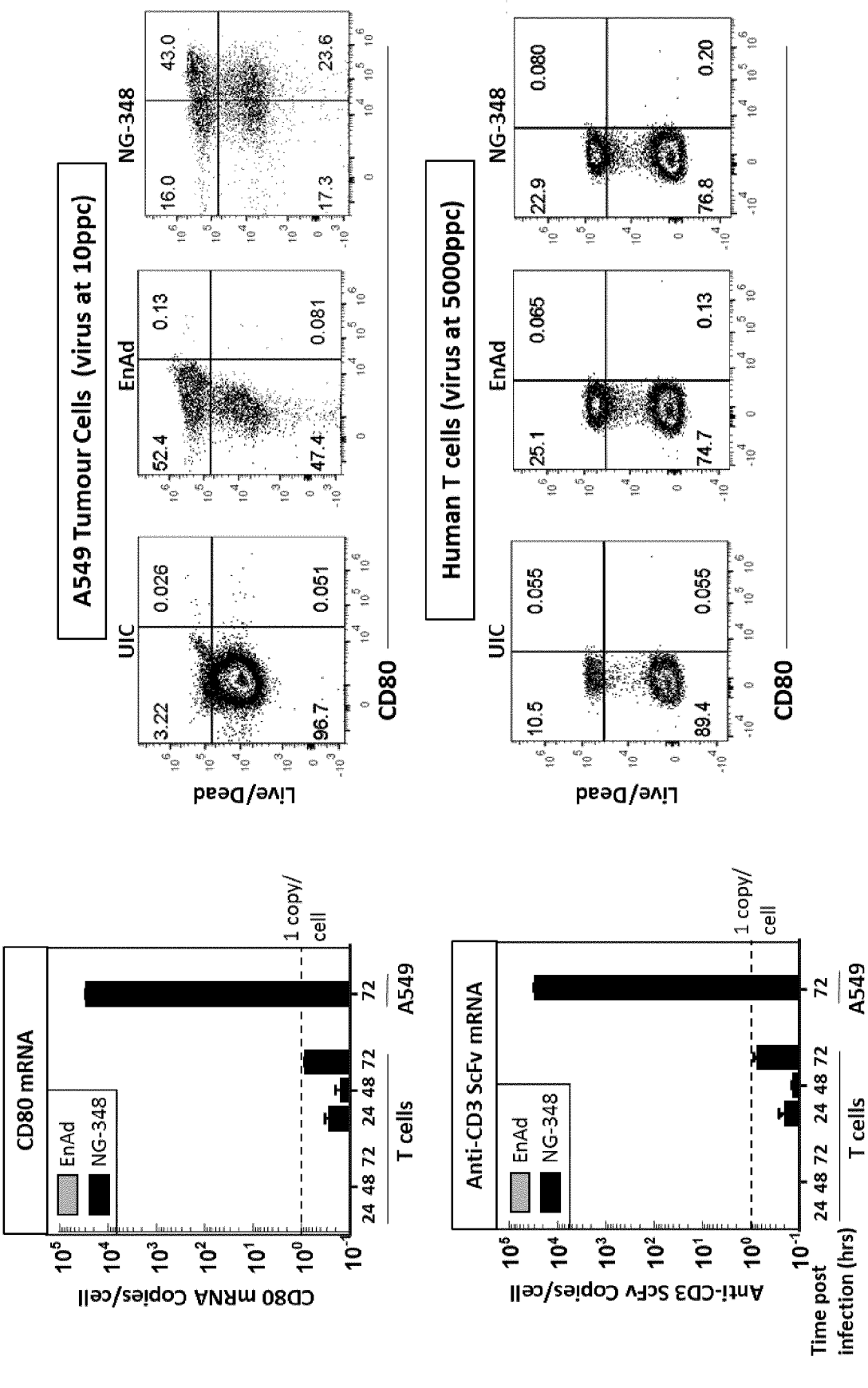

A549 Tumour Cells (virus at 10ppc)
Human T cells (virus at 5000ppc)
UIC
EnAd
NG-348
Live/Dead
CD80
3.22
96.7
0.026
0.051
52.4
47.4
0.13
0.081
16.0
17.3
43.0
23.6
10.5
89.4
0.055
0.055
25.1
74.7
0.065
0.13
22.9
76.8
0.080
0.20
CD80 mRNA
Anti-CD3 ScFv mRNA
CD80 mRNA Copies/cell
Anti-CD3 ScFv Copies/cell
1 copy/cell
Time post infection (hrs)
24 48 72 24 48 72
T cells
72
A549

Figure 19

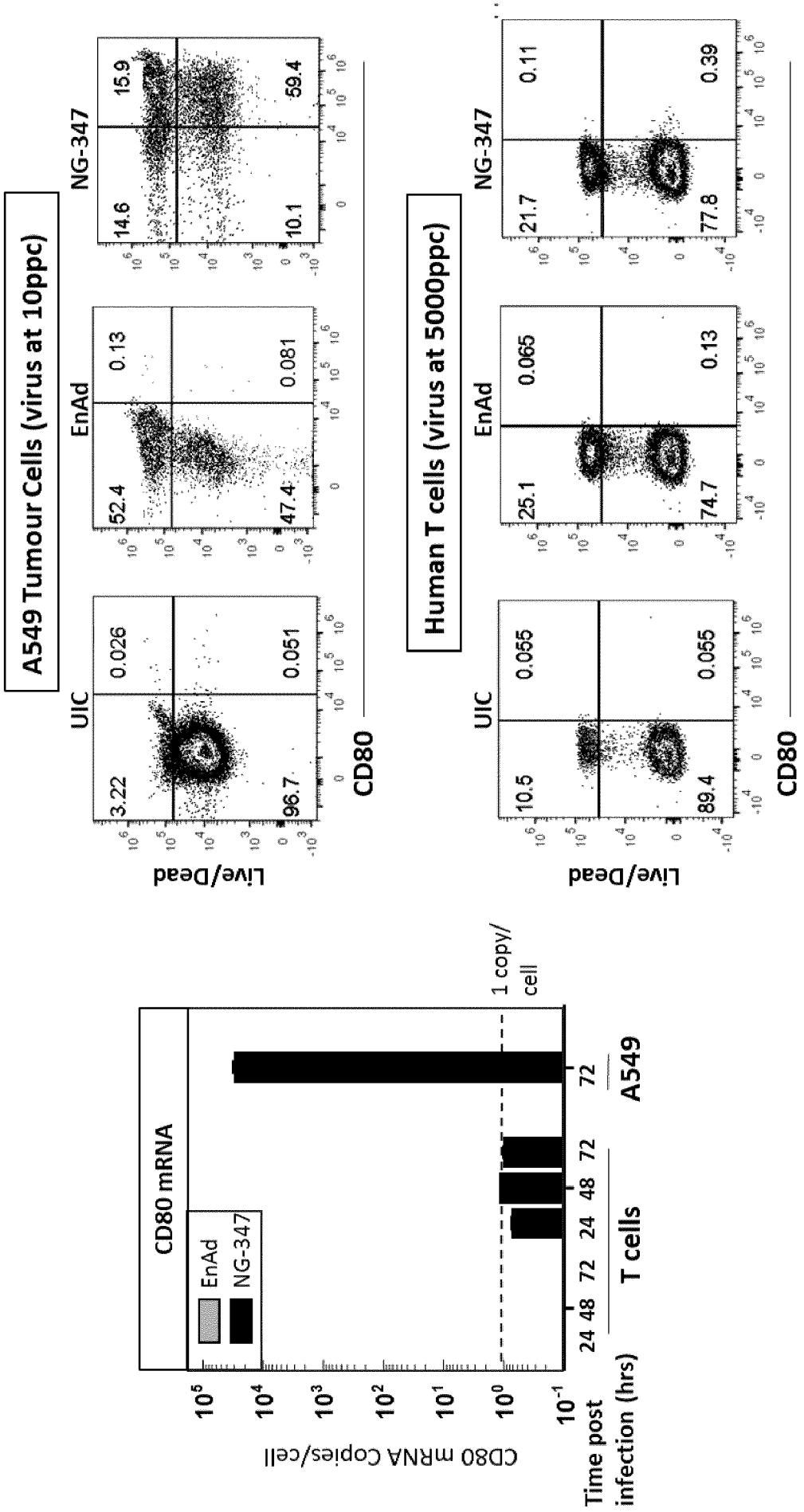
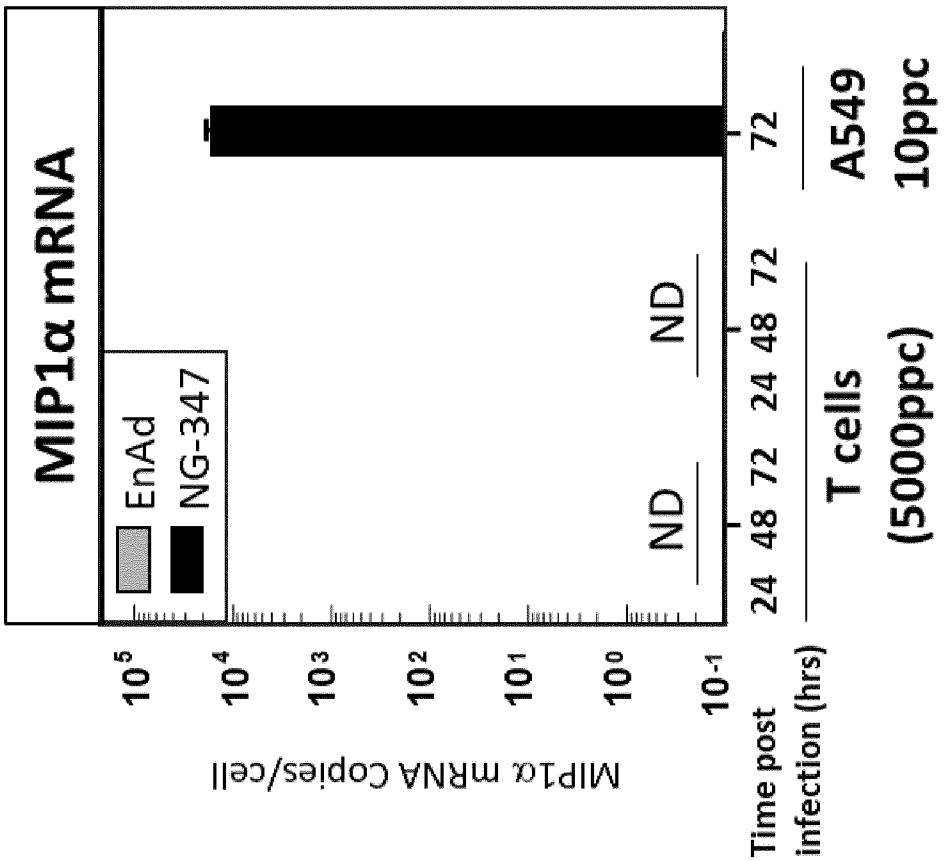

A549 Tumour Cells (virus at 10ppc)
Human T cells (virus at 5000ppc)
UIC
EnAd
NG-347
Live/Dead
CD80
3.22
96.7
0.026
0.051
52.4
47.4
0.13
0.081
14.6
10.1
15.9
59.4
10.5
89.4
0.055
0.055
25.1
74.7
0.065
0.13
21.7
77.8
0.11
0.39
CD80 mRNA
EnAd
NG-347
1 copy/ cell
CD80 mRNA Copies/cell
Time post infection (hrs)
24 48 72 24 48 72
T cells
72
A549

Figure 20


MIP1α mRNA
EnAd
NG-347
MIP1α mRNA Copies/cell
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
ND
ND
24 48 72 24 48 72 72
Time post infection (hrs)
T cells (5000ppc)
A549 10ppc

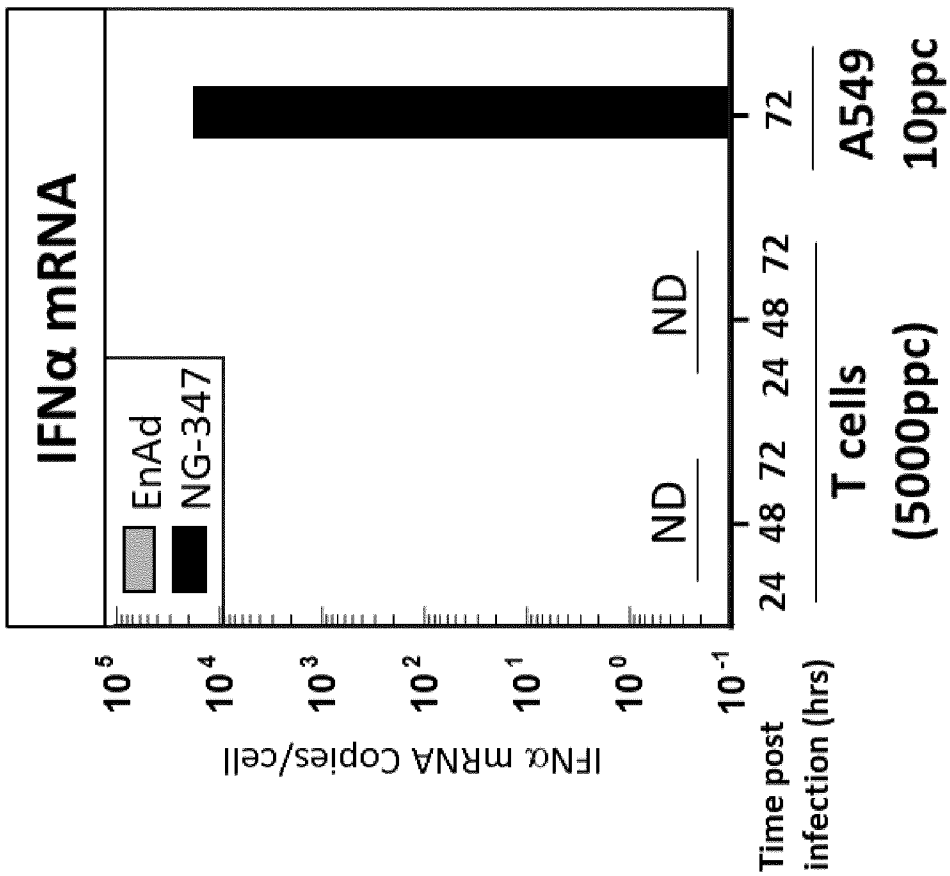

IFNα mRNA
EnAd
NG-347
IFNα mRNA Copies/cell
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
ND
ND
24 48 72 24 48 72 72
Time post infection (hrs)
T cells (5000ppc)
A549 10ppc

Figure 21

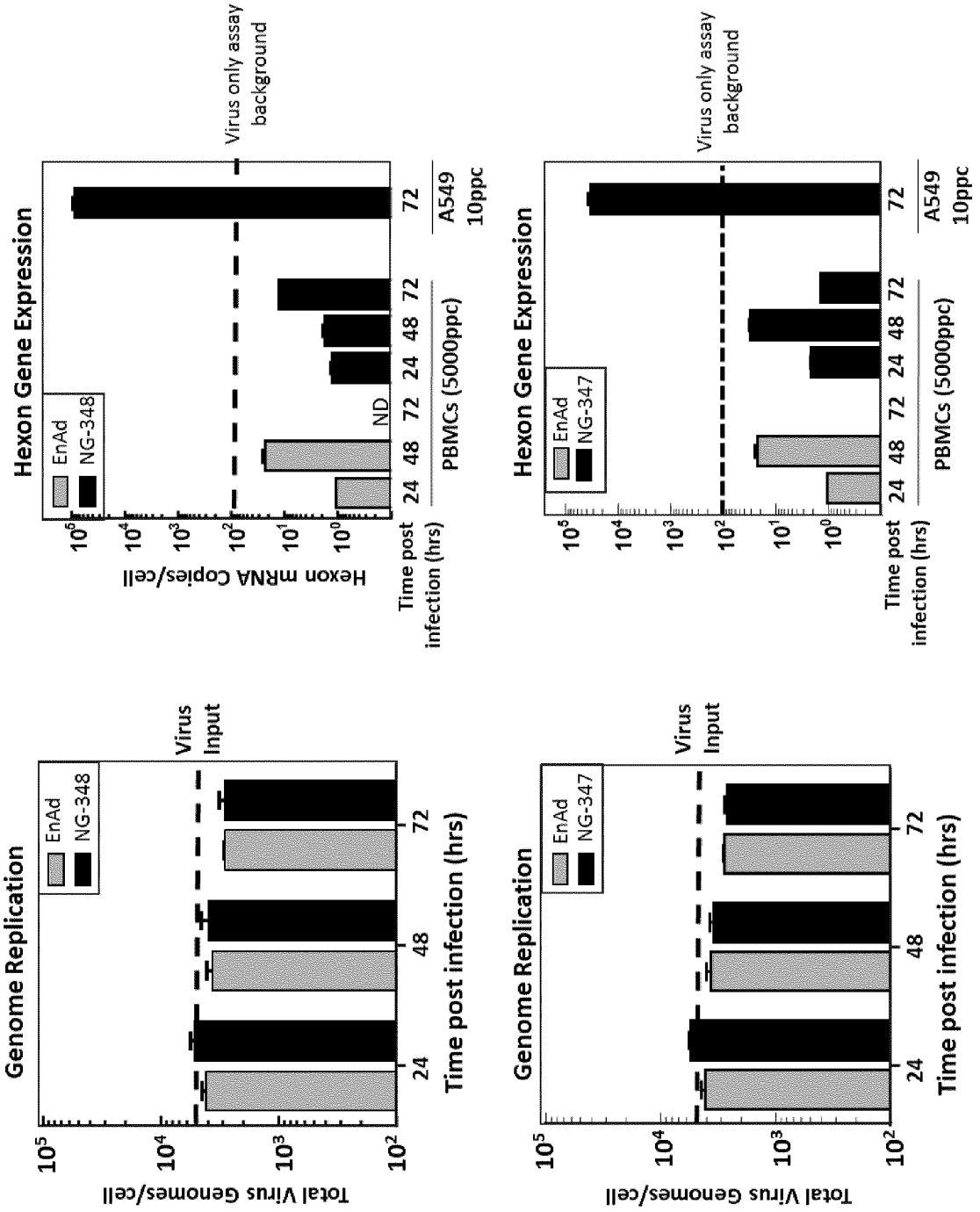

Genome Replication
EnAd
NG-348
Virus Input
Total Virus Genomes/cell
Time post infection (hrs)
Hexon Gene Expression
Virus only assay background
Hexon mRNA Copies/cell
ND
Time post infection (hrs)
PBMCs (5000ppc)
A549 10ppc
NG-347

Figure 22

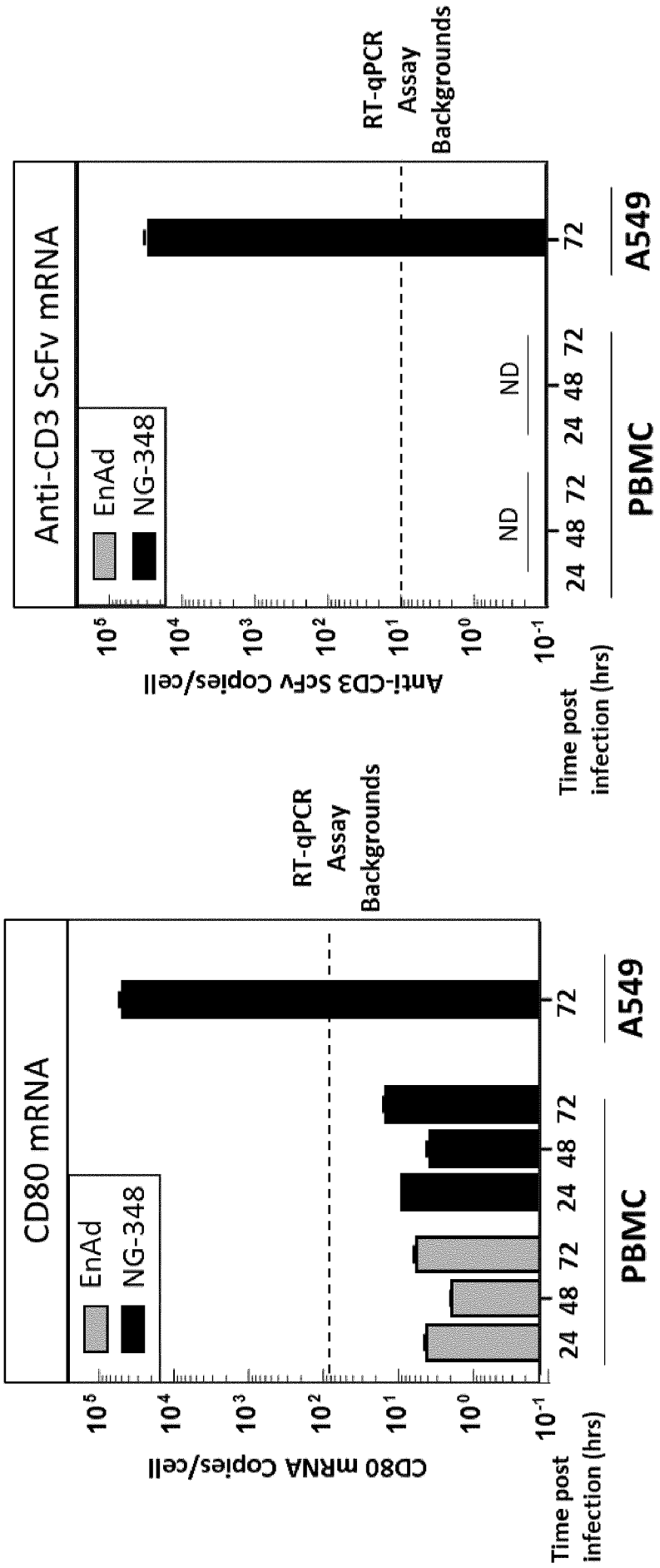
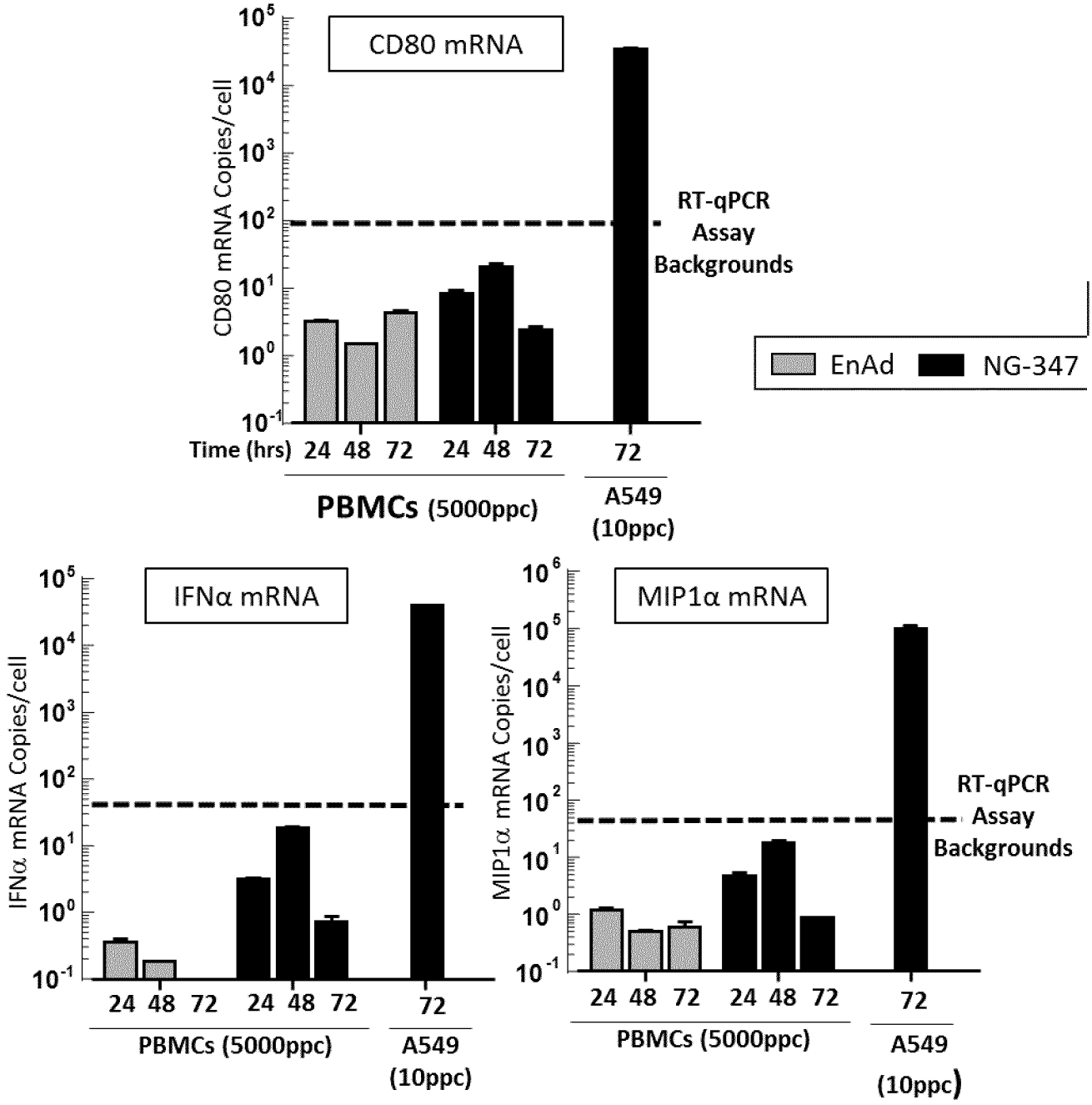

CD80 mRNA
EnAd
NG-348
CD80 mRNA Copies/cell
RT-qPCR Assay Backgrounds
Time post infection (hrs)
24 48 72 24 48 72
PBMC
A549
Anti-CD3 ScFv mRNA
Anti-CD3 ScFv Copies/cell
ND
ND

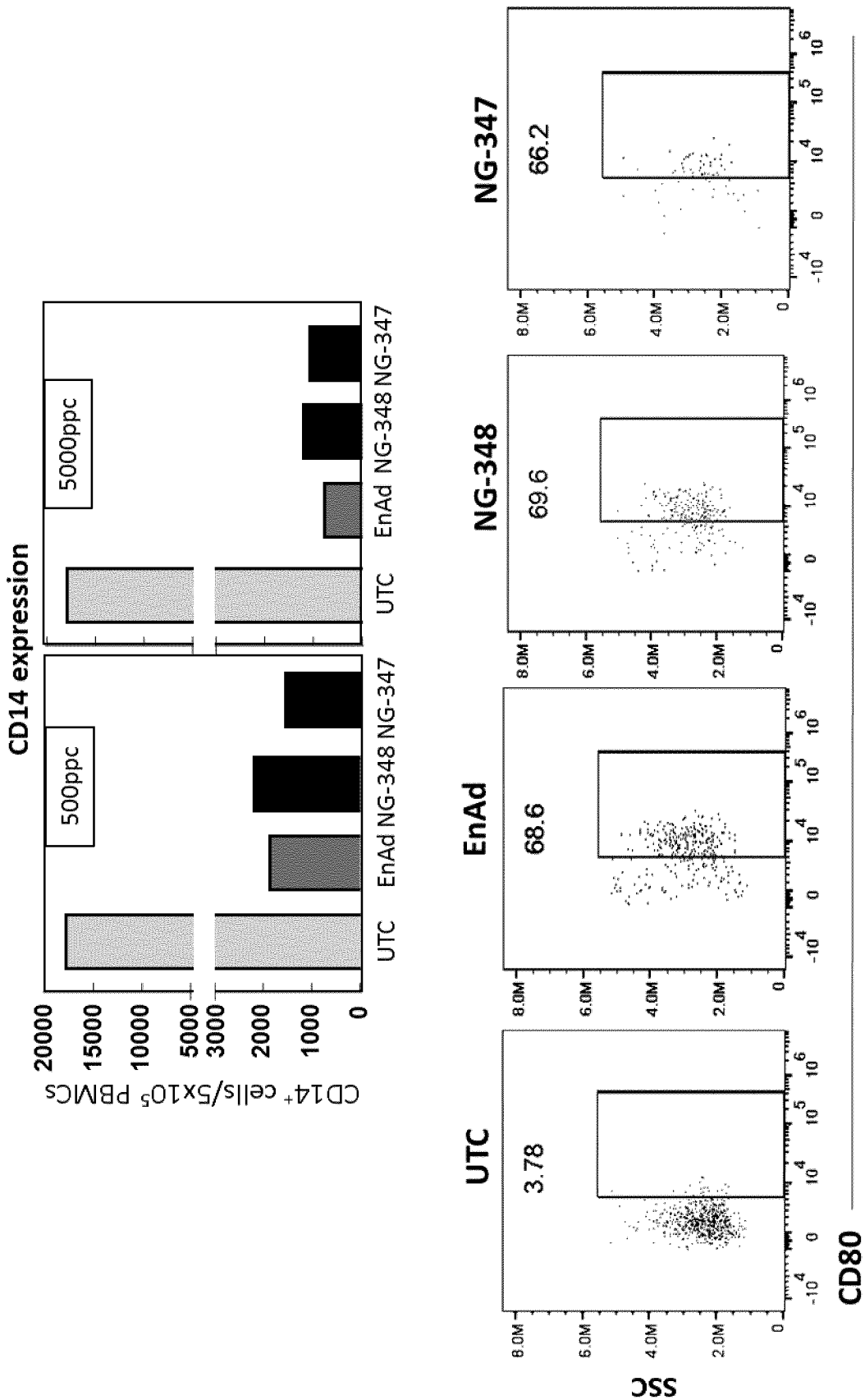
Figure 23

Figure 24


CD80 mRNA
CD80 mRNA Copies/cell
RT-qPCR Assay Backgrounds
EnAd
NG-347
Time (hrs) 24 48 72 24 48 72 72
PBMCs (5000ppc)
A549 (10ppc)
IFNα mRNA
IFNα mRNA Copies/cell
24 48 72 24 48 72 72
PBMCs (5000ppc)
A549 (10ppc)
MIP1α mRNA
MIP1α mRNA Copies/cell
RT-qPCR Assay Backgrounds
24 48 72 24 48 72 72
PBMCs (5000ppc)
A549 (10ppc)

P000164_WO

Figure 25



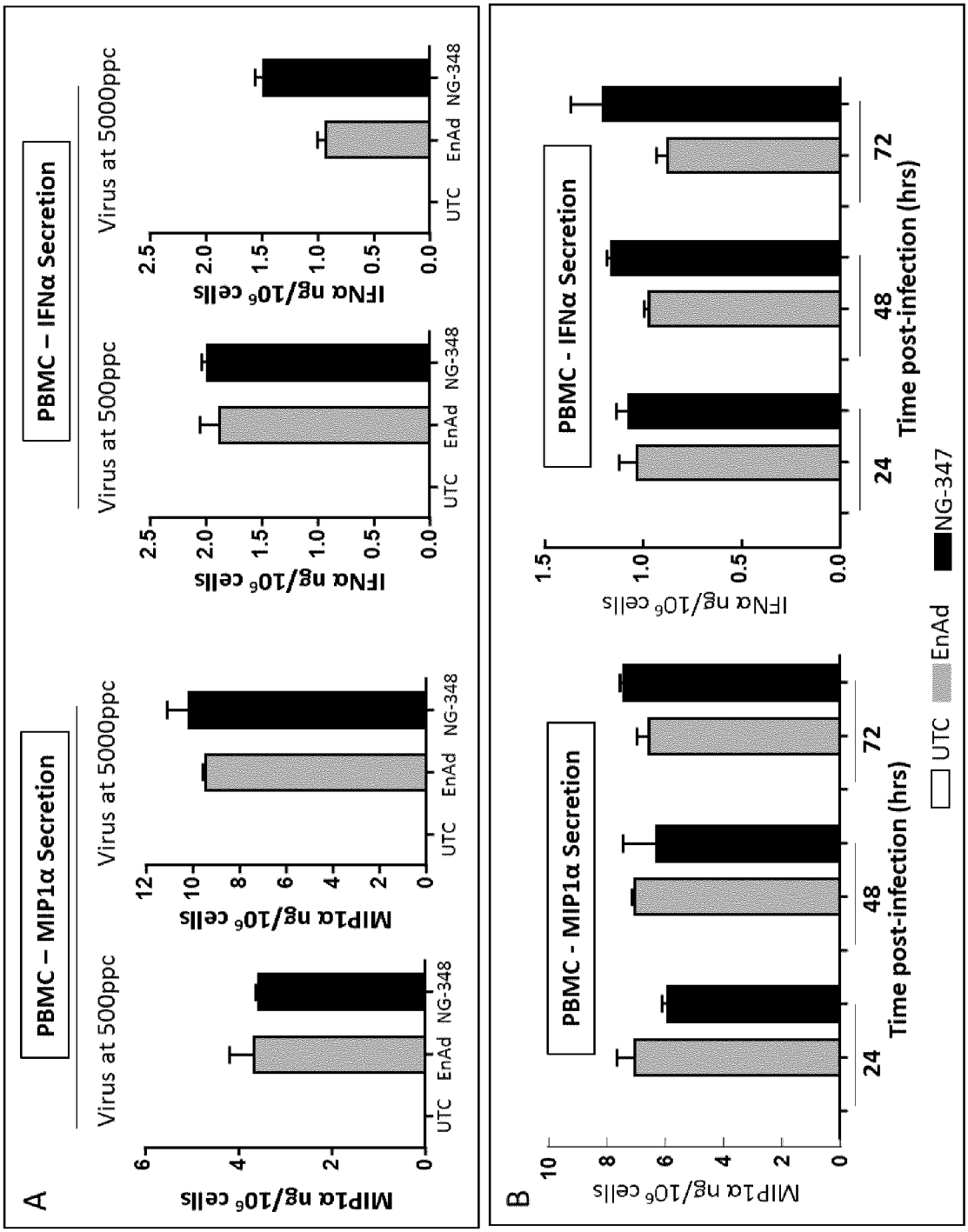

A
PBMC – MIP1α Secretion
PBMC – IFNα Secretion
Virus at 500ppc
Virus at 5000ppc
MIP1α ng/10^6 cells
IFNα ng/10^6 cells
UTC
EnAd
NG-348
B
PBMC - MIP1α Secretion
PBMC - IFNα Secretion
Time post-infection (hrs)
24
48
72
UTC
EnAd
NG-347

Figure 26

Figure 27

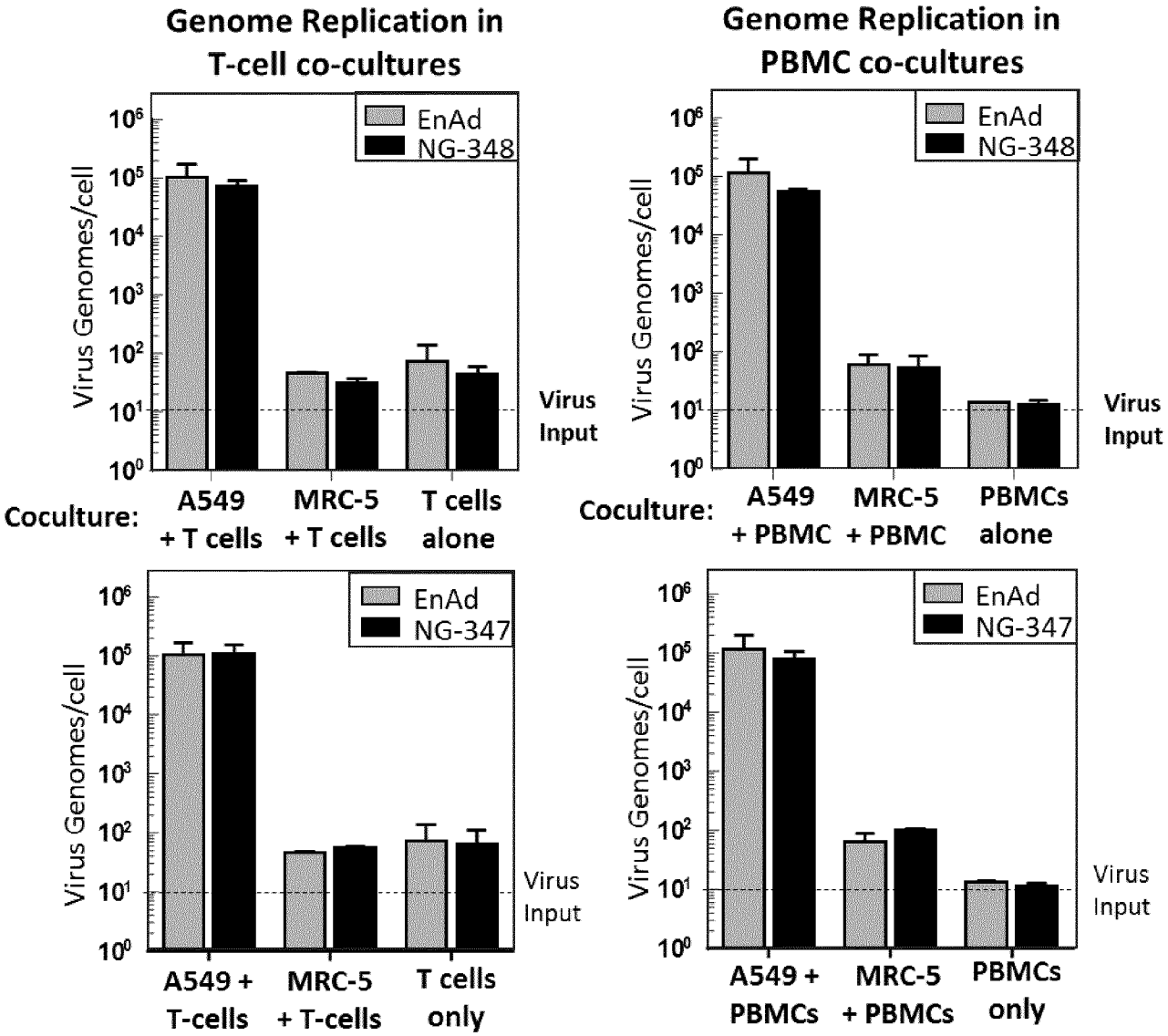

Genome Replication in T-cell co-cultures
Virus Genomes/cell
EnAd
NG-348
Virus Input
Coculture:
A549 + T cells
MRC-5 + T cells
T cells alone
Genome Replication in PBMC co-cultures
Virus Genomes/cell
EnAd
NG-348
Virus Input
Coculture:
A549 + PBMC
MRC-5 + PBMC
PBMCs alone
Virus Genomes/cell
EnAd
NG-347
Virus Input
A549 + T-cells
MRC-5 + T-cells
T cells only
Virus Genomes/cell
EnAd
NG-347
Virus Input
A549 + PBMCs
MRC-5 + PBMCs
PBMCs only

Figure 28

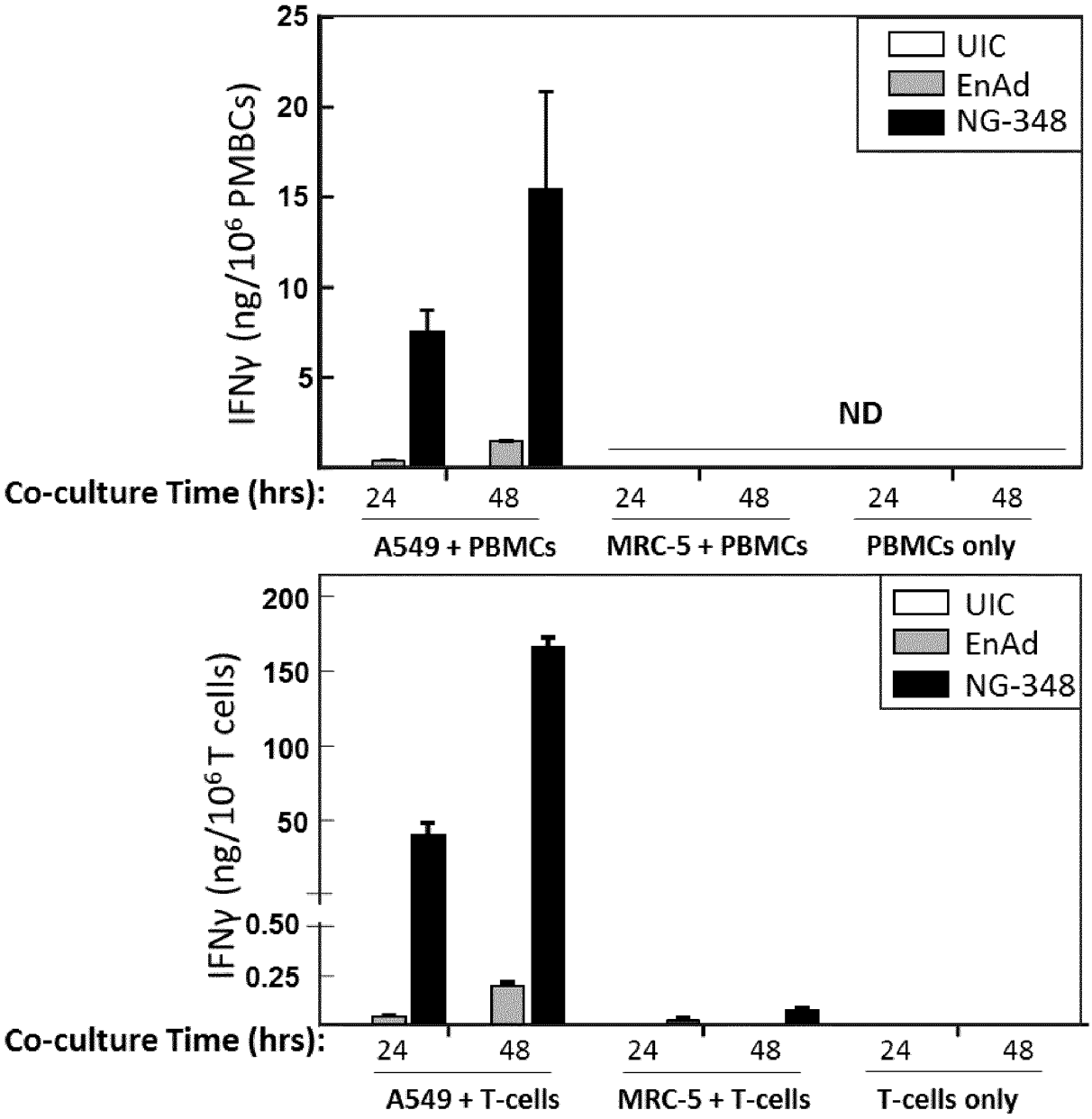

IFNγ (ng/10⁶ PMBCs)
25
20
15
10
5
UIC
EnAd
NG-348
ND
Co-culture Time (hrs):
24
48
24
48
24
48
A549 + PBMCs
MRC-5 + PBMCs
PBMCs only
IFNγ (ng/10⁶ T cells)
200
150
100
50
0.50
0.25
UIC
EnAd
NG-348
Co-culture Time (hrs):
24
48
24
48
24
48
A549 + T-cells
MRC-5 + T-cells
T-cells only

Figure 29

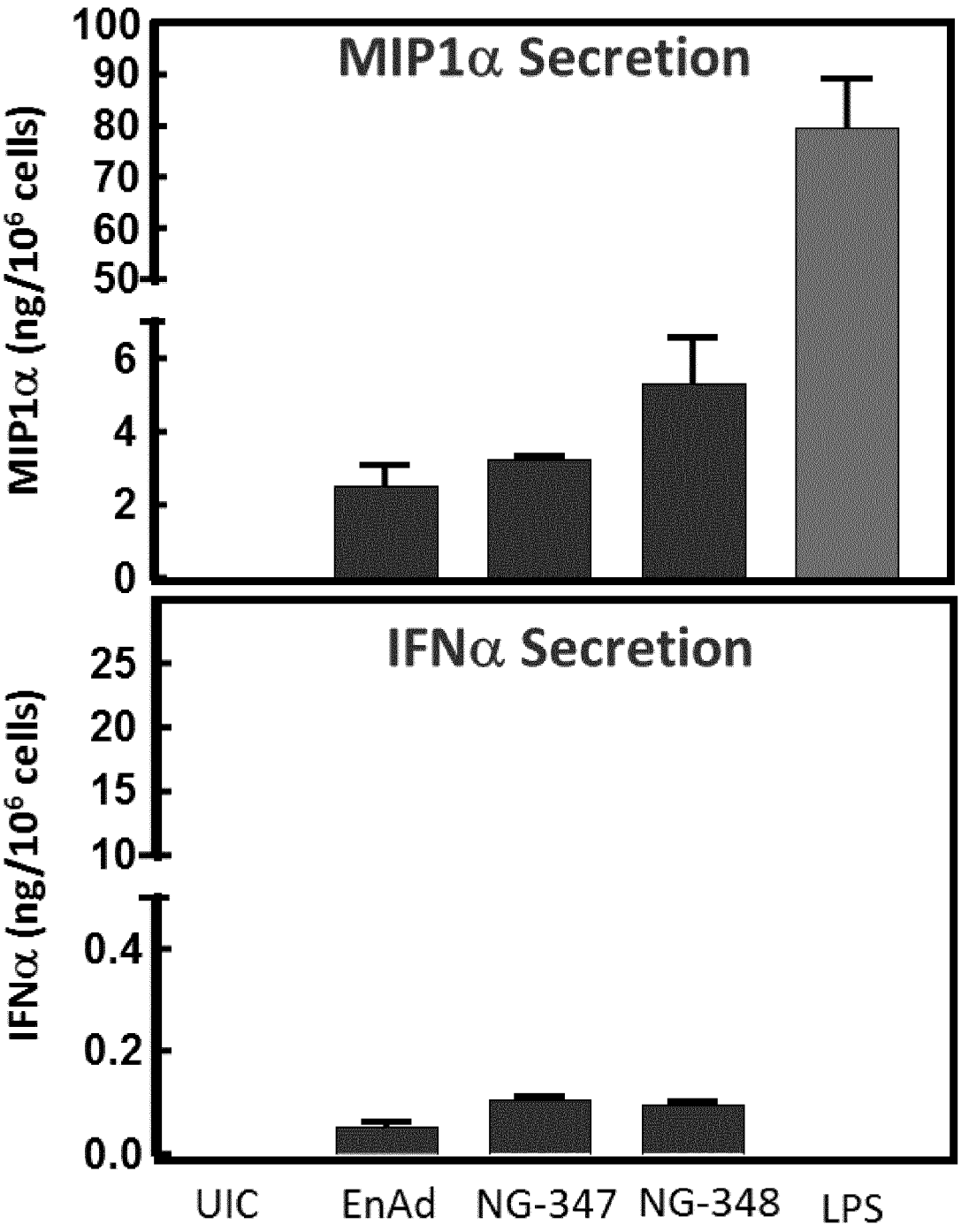
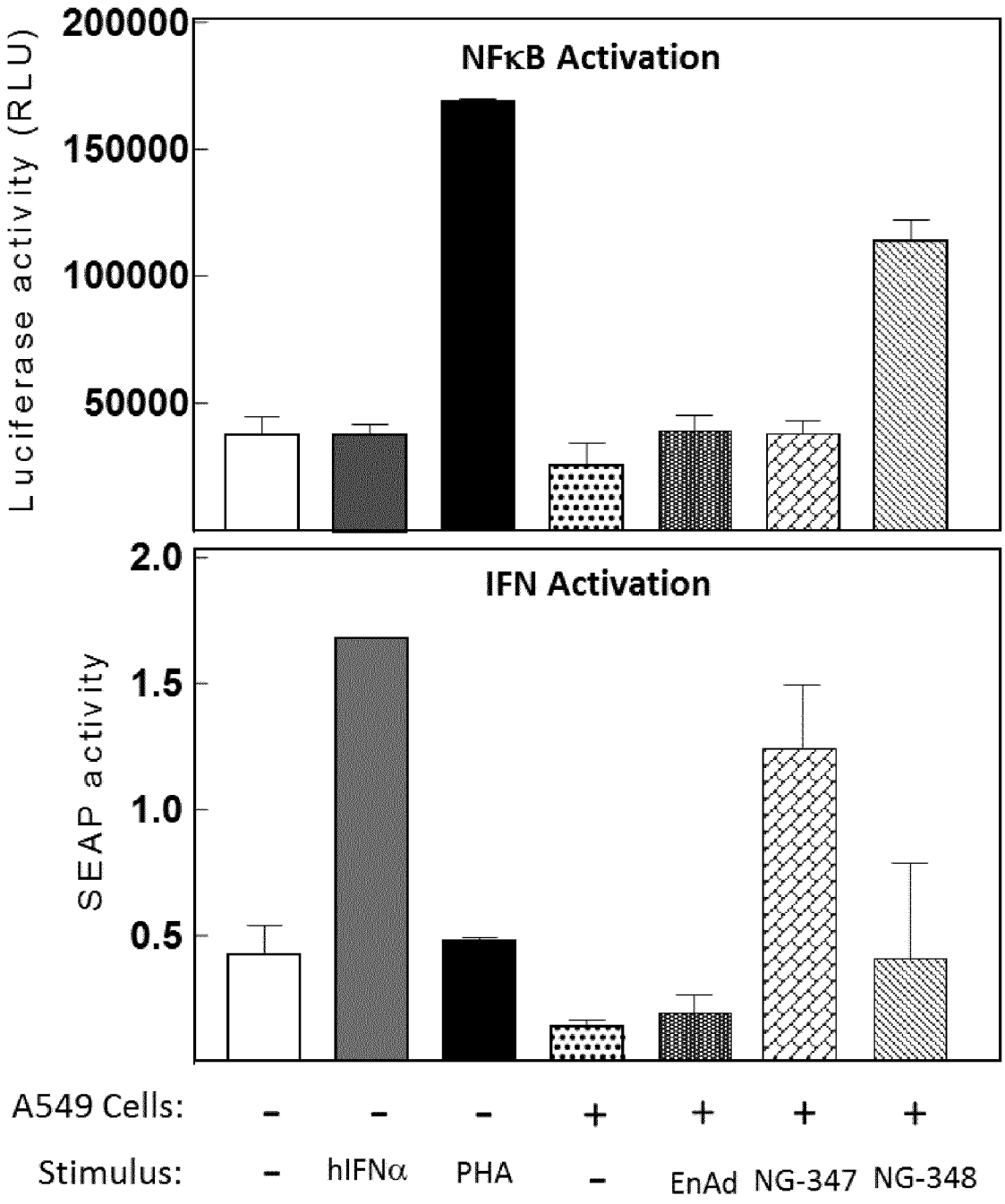

MIP1α Secretion
MIP1α (ng/10^6 cells)
100
90
80
70
60
50
6
4
2
0
IFNα Secretion
IFNα (ng/10^6 cells)
25
20
15
10
0.4
0.2
0.0
UIC
EnAd
NG-347
NG-348
LPS

Figure 30


NFκB Activation
Luciferase activity (RLU)
200000
150000
100000
50000
IFN Activation
SEAP activity
2.0
1.5
1.0
0.5
A549 Cells: - - - + + + +
Stimulus: - hIFNα PHA - EnAd NG-347 NG-348

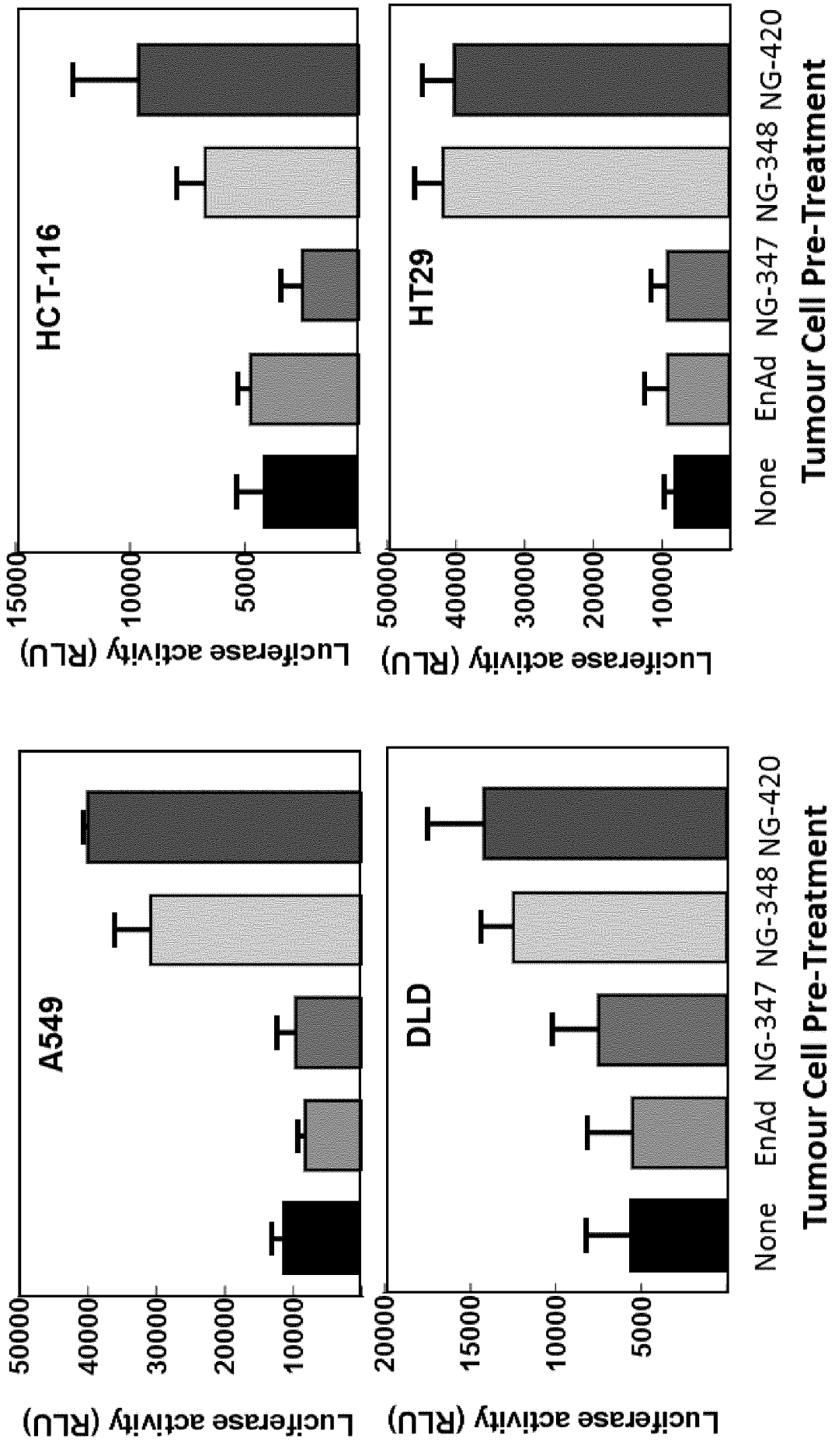

A549
DLD
HCT-116
HT29
Luciferase activity (RLU)
50000
40000
30000
20000
10000
15000
5000
None
EnAd
NG-347
NG-348
NG-420
Tumour Cell Pre-Treatment

Figure 31

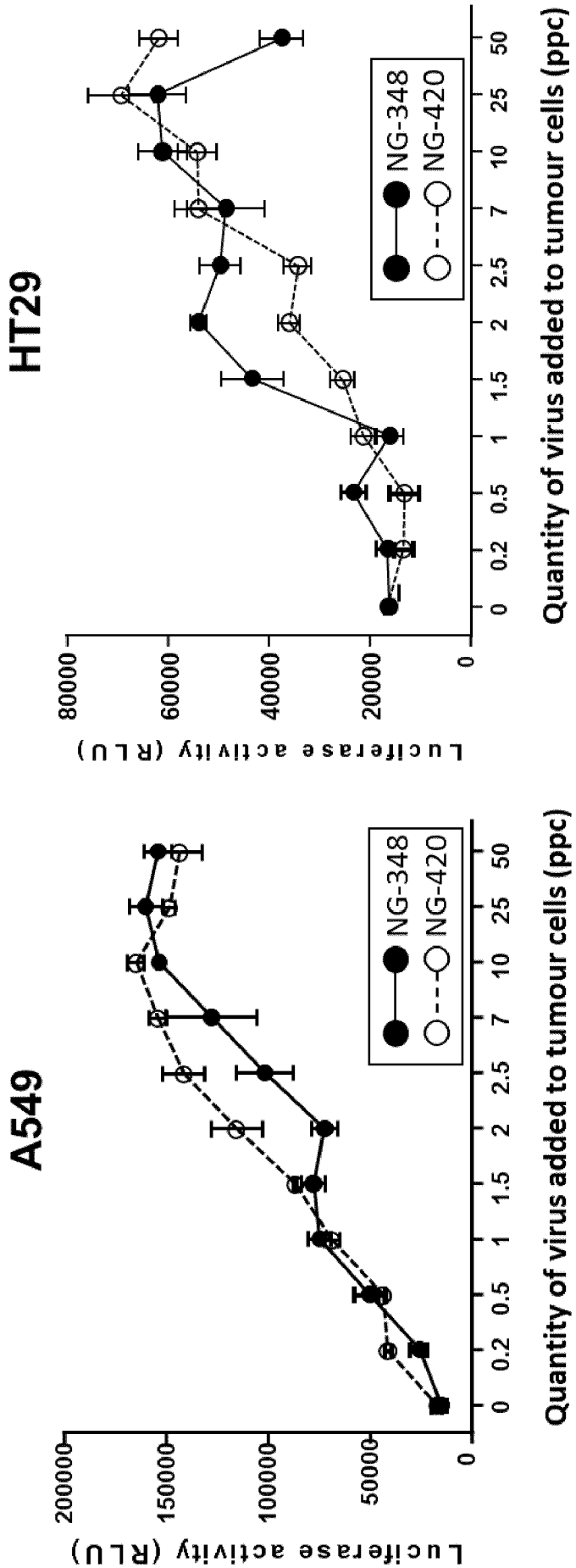

A549
HT29
Luciferase activity (RLU)
Quantity of virus added to tumour cells (ppc)
NG-348
NG-420
0
50000
100000
150000
200000
20000
40000
60000
80000
0
0.2
0.5
1
1.5
2
2.5
7
10
25
50

Figure 32

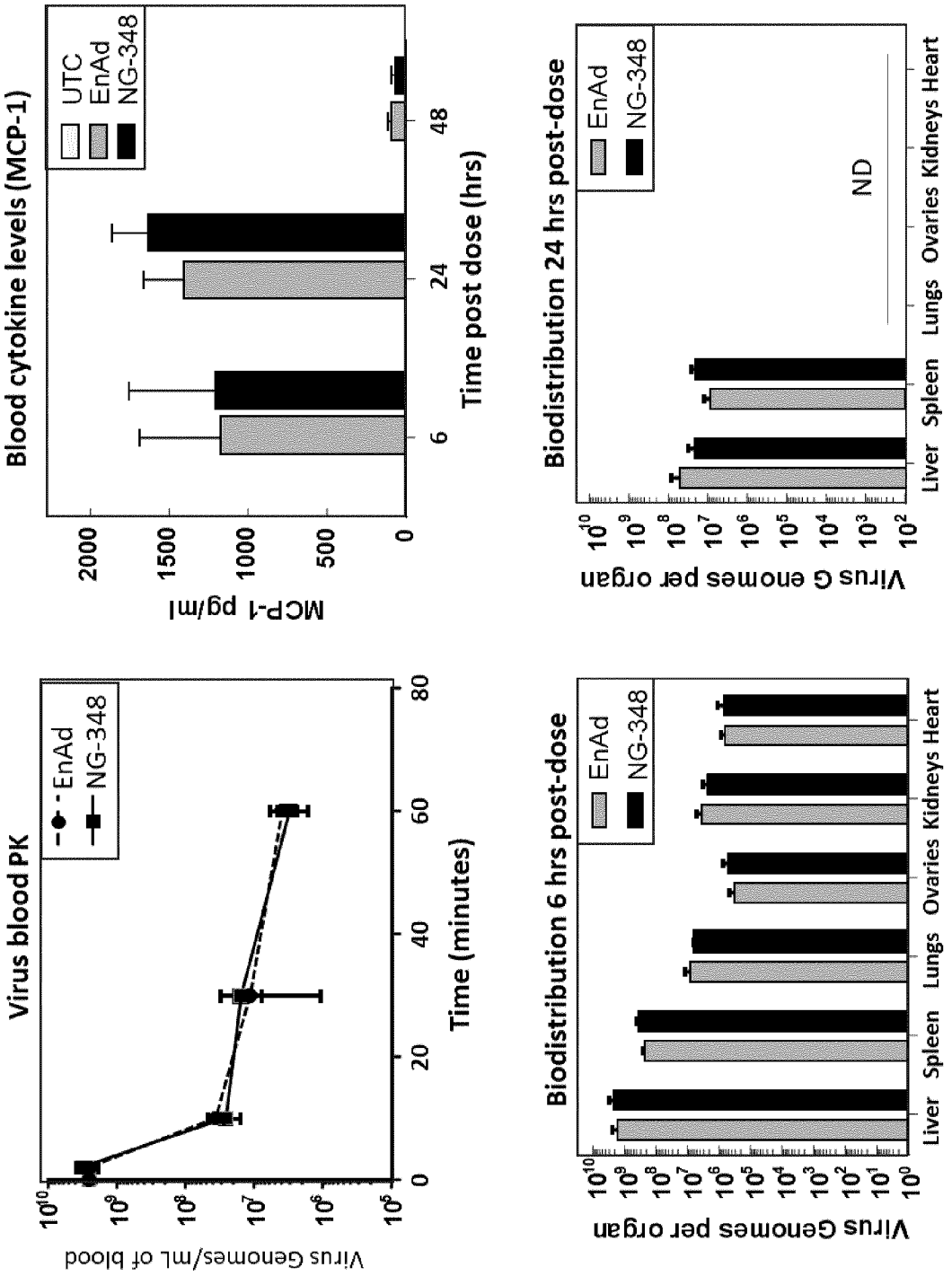

Virus blood PK
EnAd
NG-348
Virus Genomes/mL of blood
Time (minutes)
Blood cytokine levels (MCP-1)
UTC
EnAd
NG-348
MCP-1 pg/ml
Time post dose (hrs)
Biodistribution 6 hrs post-dose
EnAd
NG-348
Virus Genomes per organ
Liver Spleen Lungs Ovaries Kidneys Heart
Biodistribution 24 hrs post-dose
EnAd
NG-348
ND
Virus G enomes per organ
Liver Spleen Lungs Ovaries Kidneys Heart

Figure 33

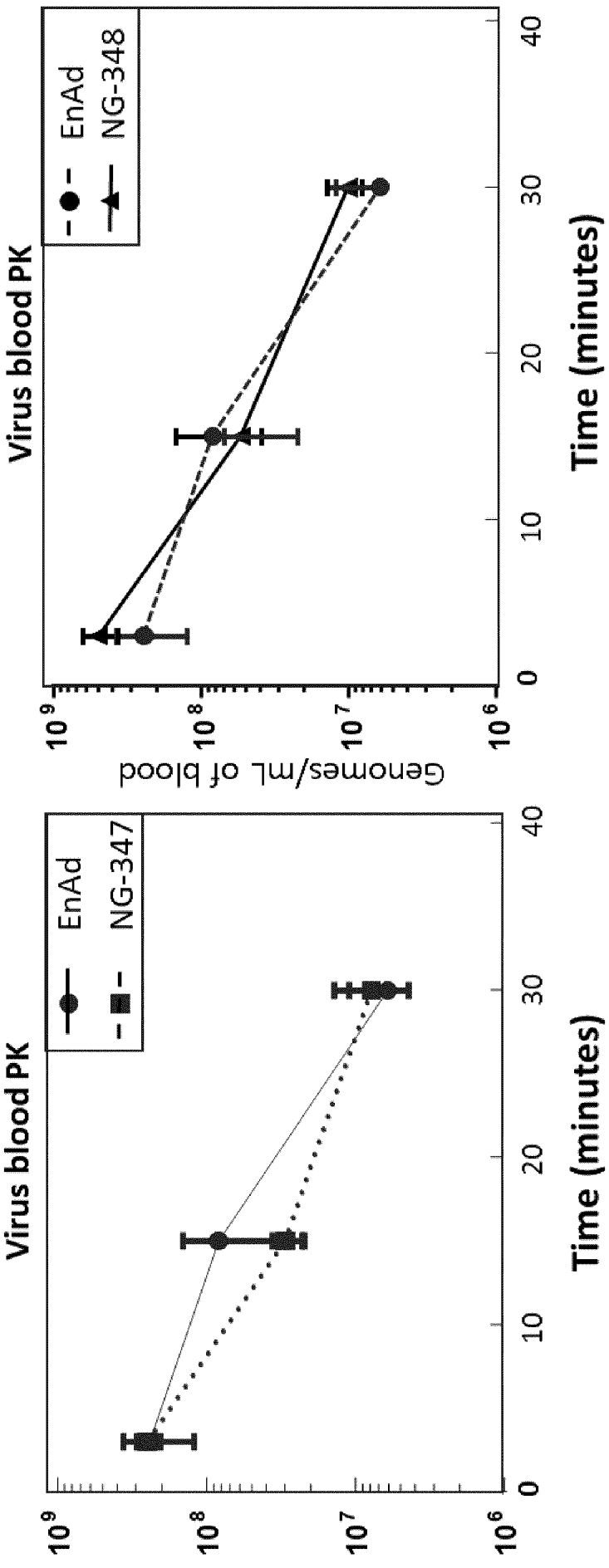

Virus blood PK
EnAd
NG-348
Genomes/mL of blood
Time (minutes)
Virus blood PK
EnAd
NG-347
Time (minutes)

Figure 34

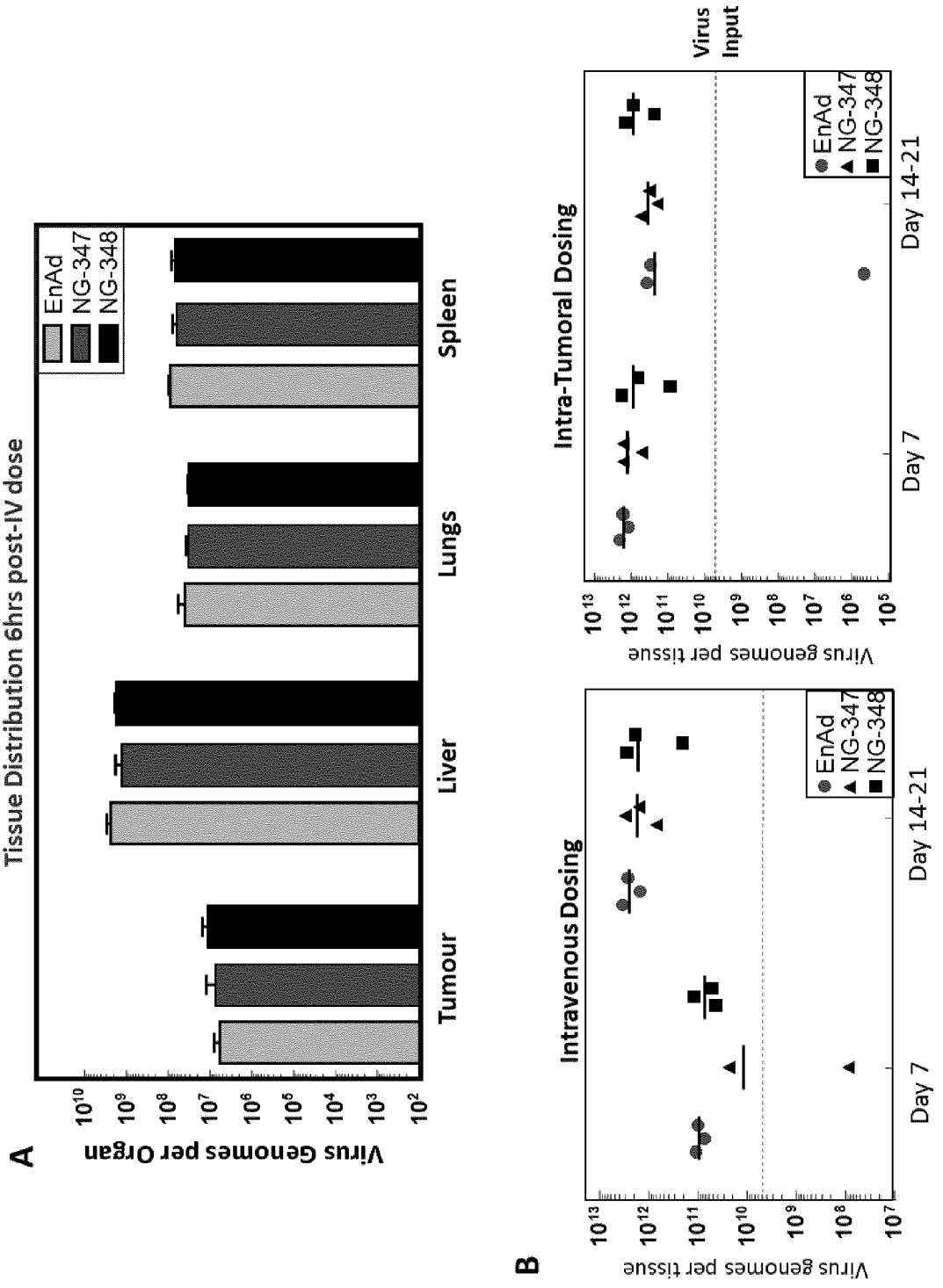

A
Tissue Distribution 6hrs post-IV dose
EnAd
NG-347
NG-348
Virus Genomes per Organ
Tumour
Liver
Lungs
Spleen
B
Intravenous Dosing
Intra-Tumoral Dosing
Virus genomes per tissue
Day 7
Day 14-21
Virus Input

Figure 35

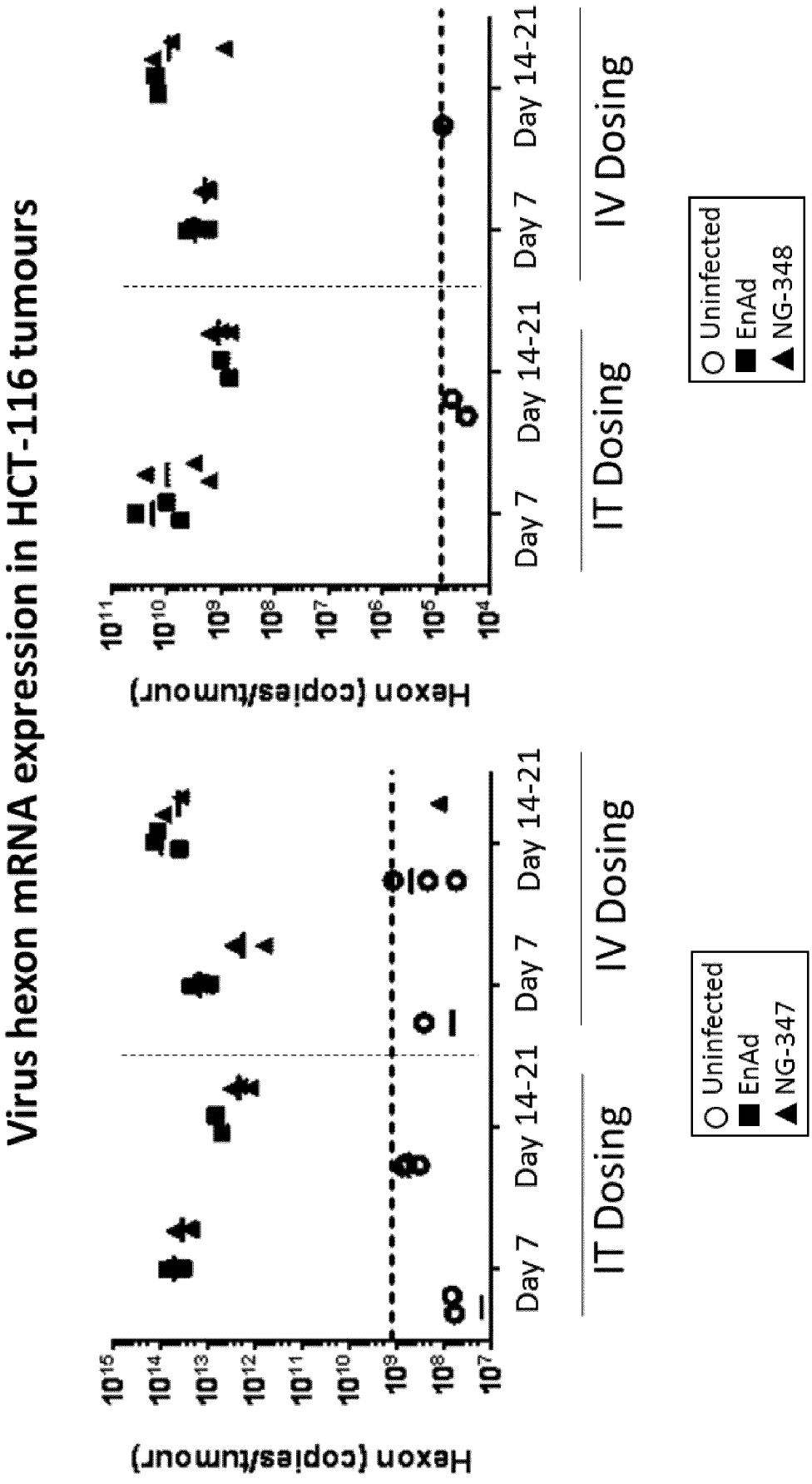

Virus hexon mRNA expression in HCT-116 tumours
Hexon (copies/tumour)
Day 7
Day 14-21
Day 7
Day 14-21
IT Dosing
IV Dosing
Uninfected
EnAd
NG-347
NG-348

Figure 36

Figure 37

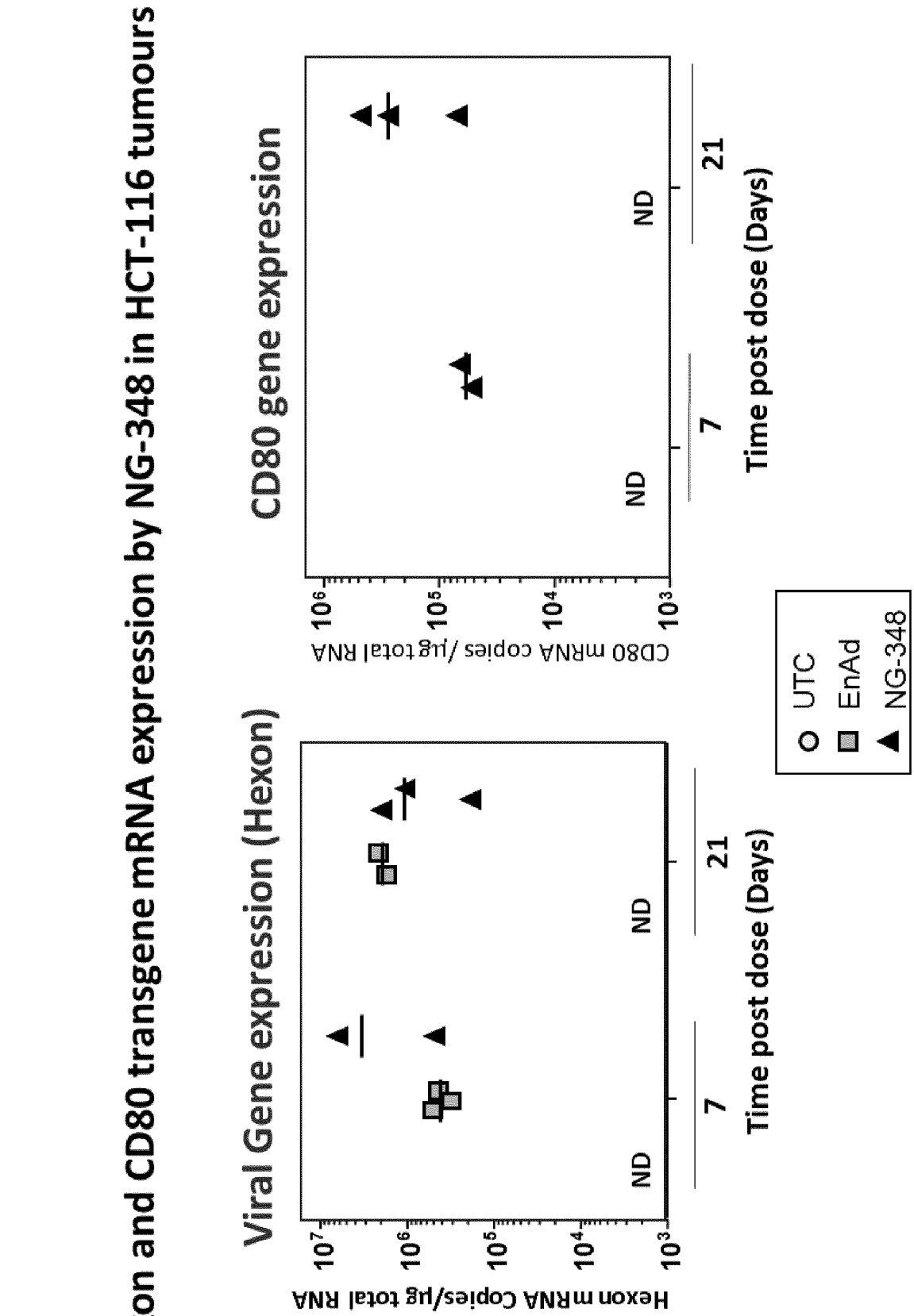
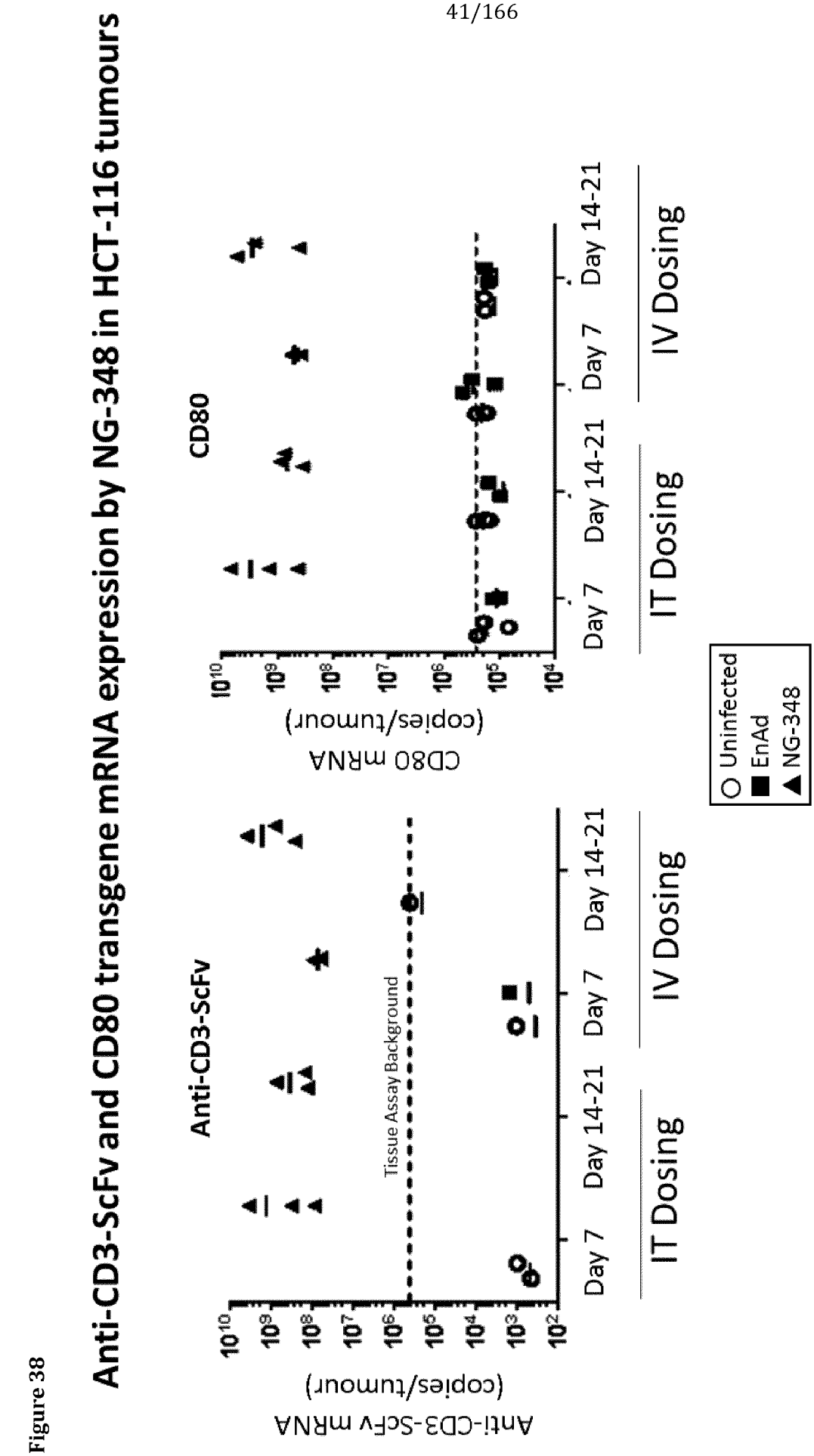

Hexon and CD80 transgene mRNA expression by NG-348 in HCT-116 tumours
Viral Gene expression (Hexon)
Hexon mRNA Copies/µg total RNA
10^7
10^6
10^5
10^4
10^3
ND
ND
7
21
Time post dose (Days)
CD80 gene expression
CD80 mRNA copies /µg total RNA
10^6
10^5
10^4
10^3
ND
ND
7
21
Time post dose (Days)
UTC
EnAd
NG-348

Figure 38


Anti-CD3-ScFv and CD80 transgene mRNA expression by NG-348 in HCT-116 tumours
Anti-CD3-ScFv
Anti-CD3-ScFv mRNA (copies/tumour)
Tissue Assay Background
CD80
CD80 mRNA (copies/tumour)
Day 7
Day 14-21
Day 7
Day 14-21
IT Dosing
IV Dosing
IT Dosing
IV Dosing
Uninfected
EnAd
NG-348

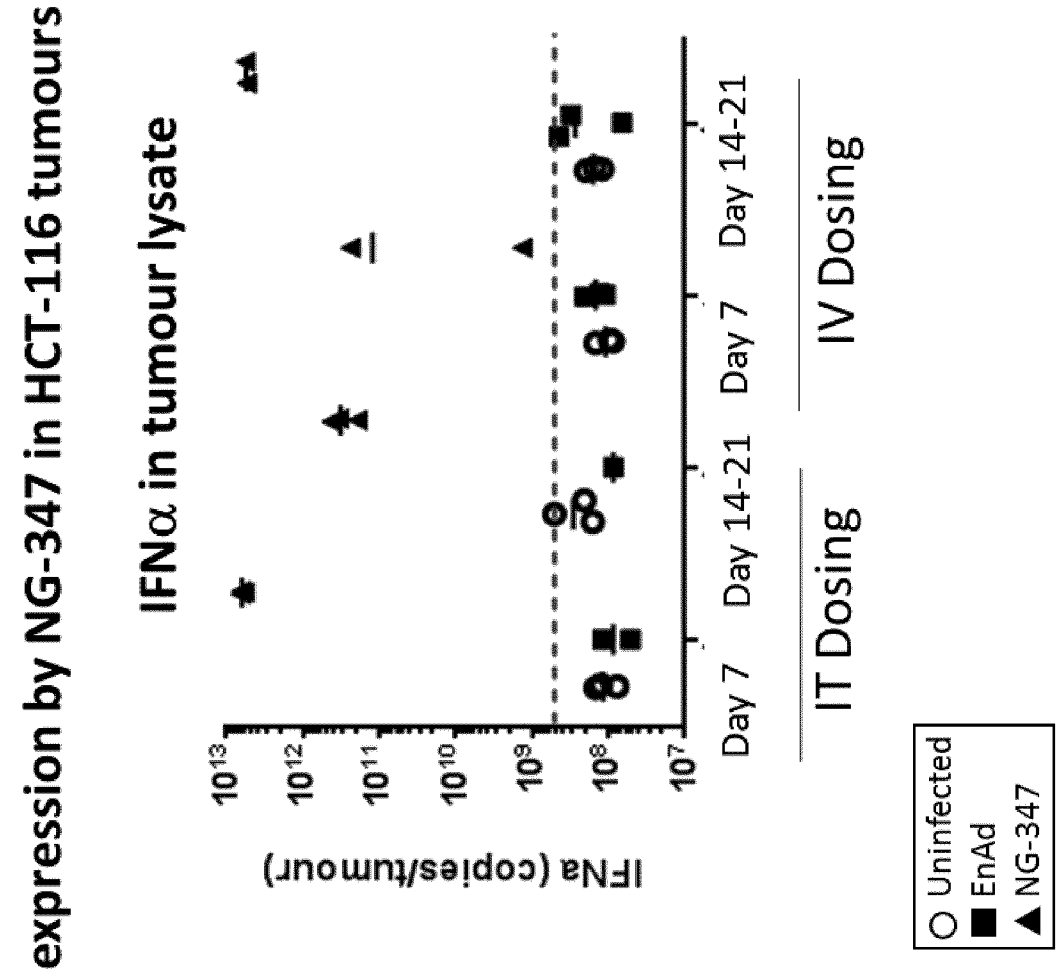

MIP1α and IFNα transgene mRNA expression by NG-347 in HCT-116 tumours
MIP1α in tumour lysate
MIP1a (copies/tumour)
IFNα in tumour lysate
IFNa (copies/tumour)
Day 7
Day 14-21
Day 7
Day 14-21
IT Dosing
IV Dosing
Uninfected
EnAd
NG-347

Figure 39

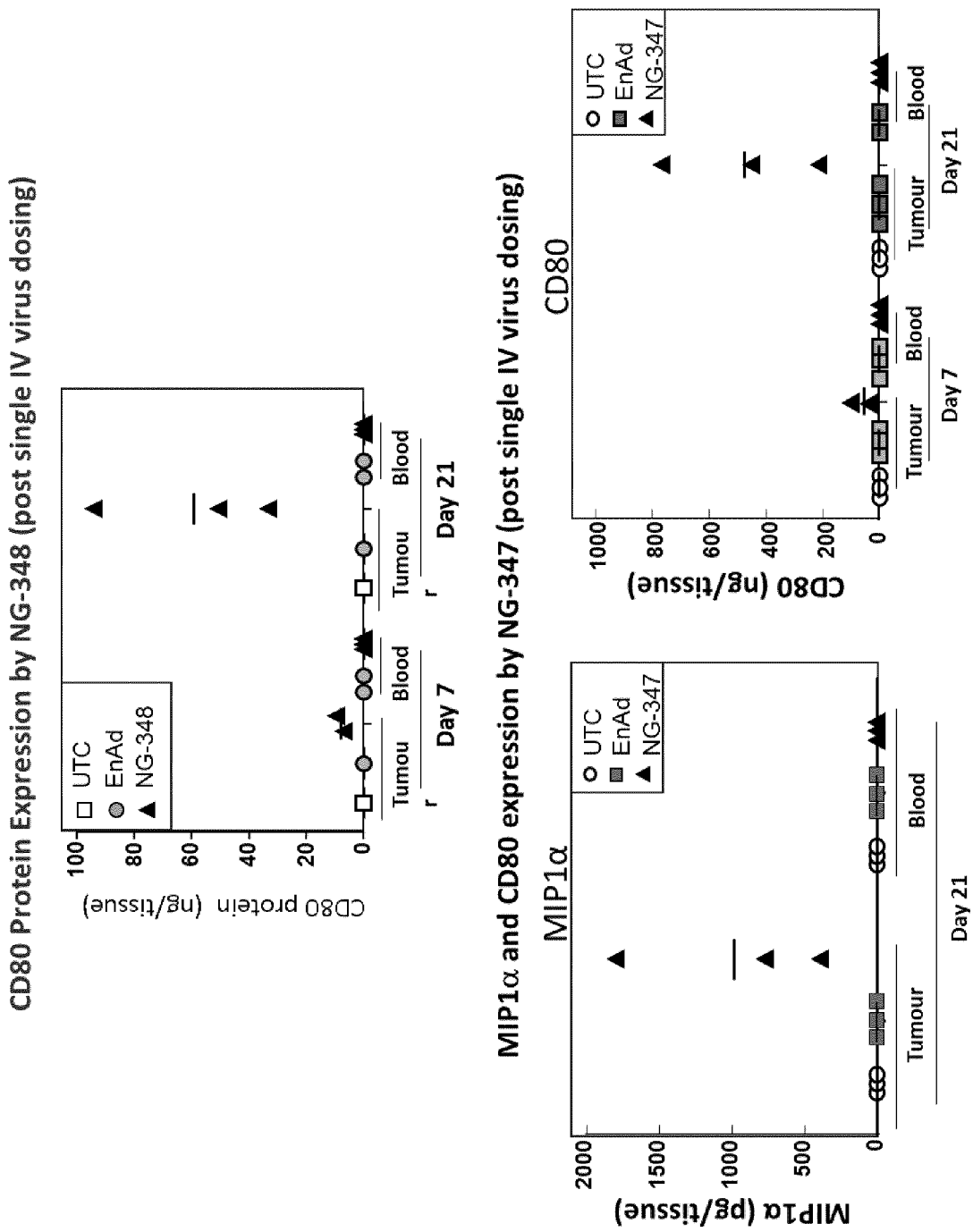

CD80 Protein Expression by NG-348 (post single IV virus dosing)
UTC
EnAd
NG-348
CD80 protein (ng/tissue)
100
80
60
40
20
0
Tumou
Blood
Tumou
Blood
Day 7
Day 21
MIP1α and CD80 expression by NG-347 (post single IV virus dosing)
MIP1α
UTC
EnAd
NG-347
MIP1α (pg/tissue)
2000
1500
1000
500
0
Tumour
Blood
Day 21
CD80
UTC
EnAd
NG-347
CD80 (ng/tissue)
1000
800
600
400
200
0
Tumour
Blood
Tumour
Blood
Day 7
Day 21

Figure 40

Figure 41

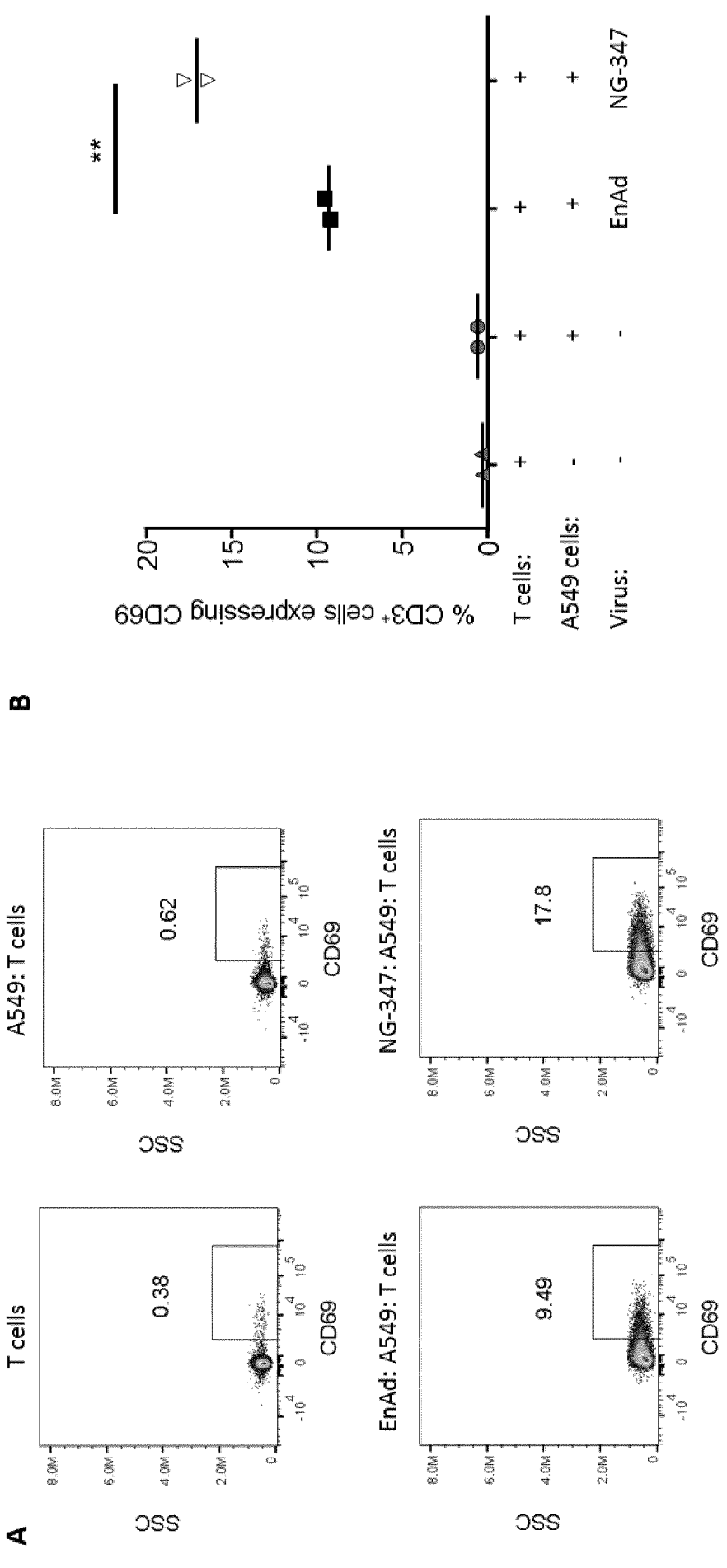
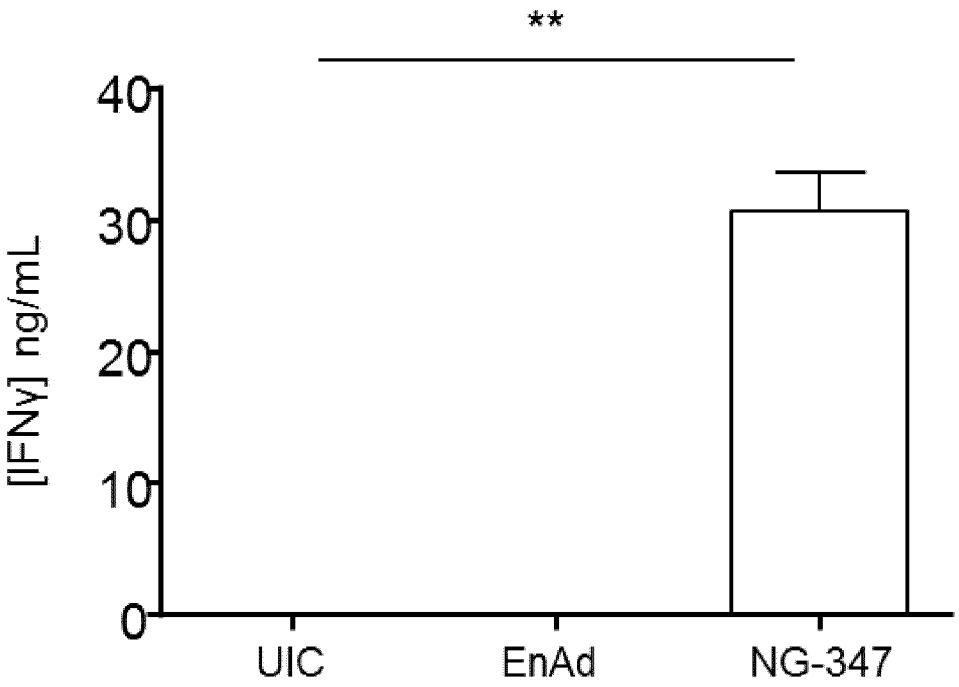

A
T cells
0.38
A549: T cells
0.62
EnAd: A549: T cells
9.49
NG-347: A549: T cells
17.8
SSC
CD69
B
% CD3+ cells expressing CD69
**
T cells:
A549 cells:
Virus:
EnAd
NG-347

Figure 42



VIRUS	TITRE (vp/mL)	INFECTIOUS TITRE (IFU/mL)	Infectivity Ratio (P:I)
EnAd (Ref 1)	3e12	7.83e10	38.3
EnAd (Ref 2)	3e12	9.37e10	32.0
NG-348	6.68e12	3.31e11	20.2

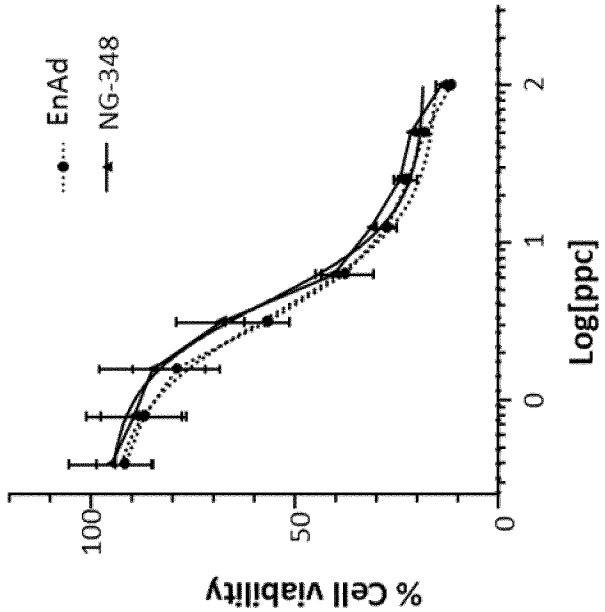


Figure 43

Figure 44

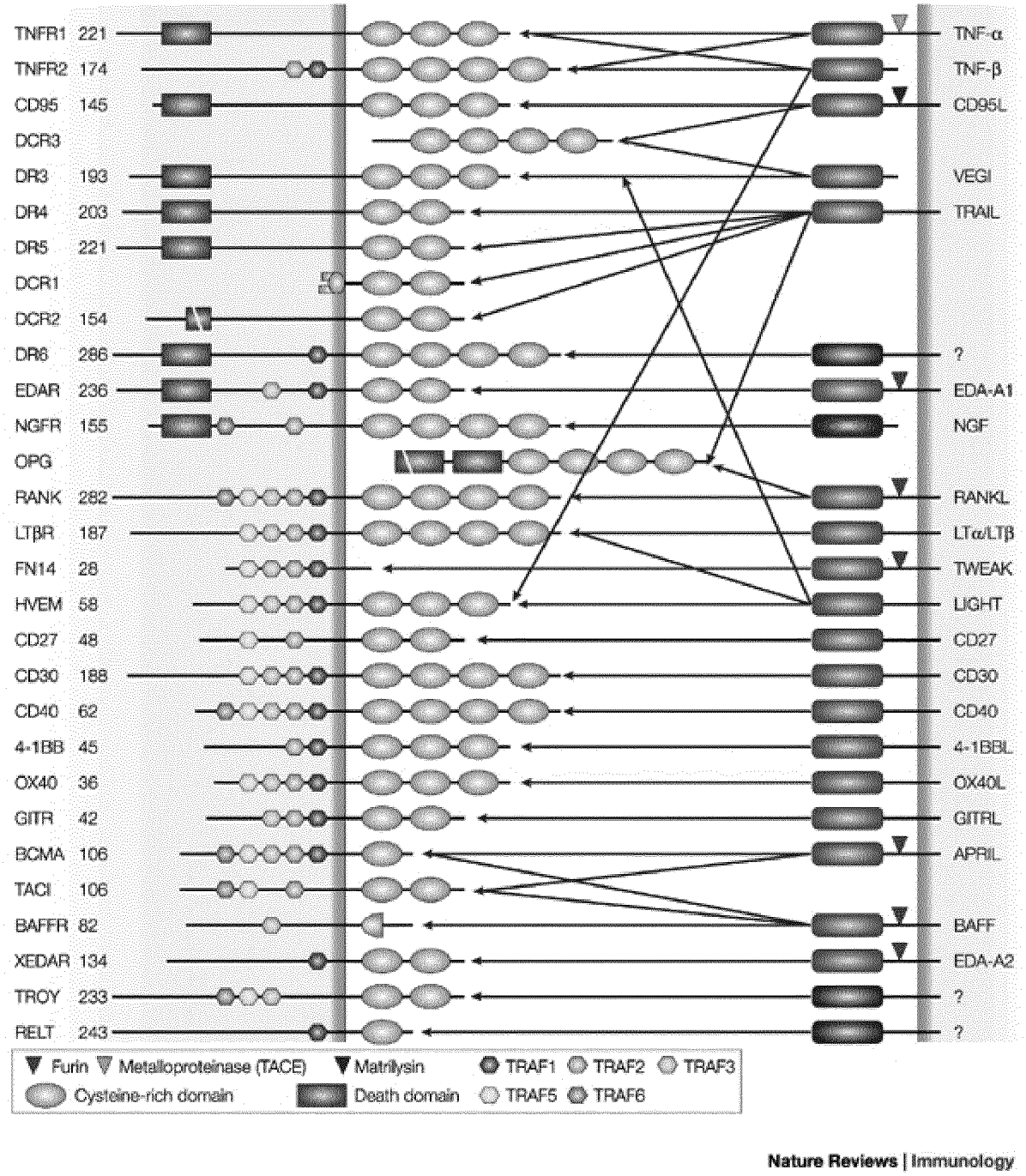
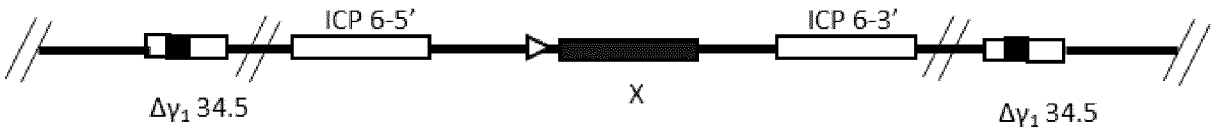


FIGURE 45 RECOMBINANT HSV-1 CONTAINING TRANSGENE X WITH BOTH COPIES OF ICP34.5 DELETED



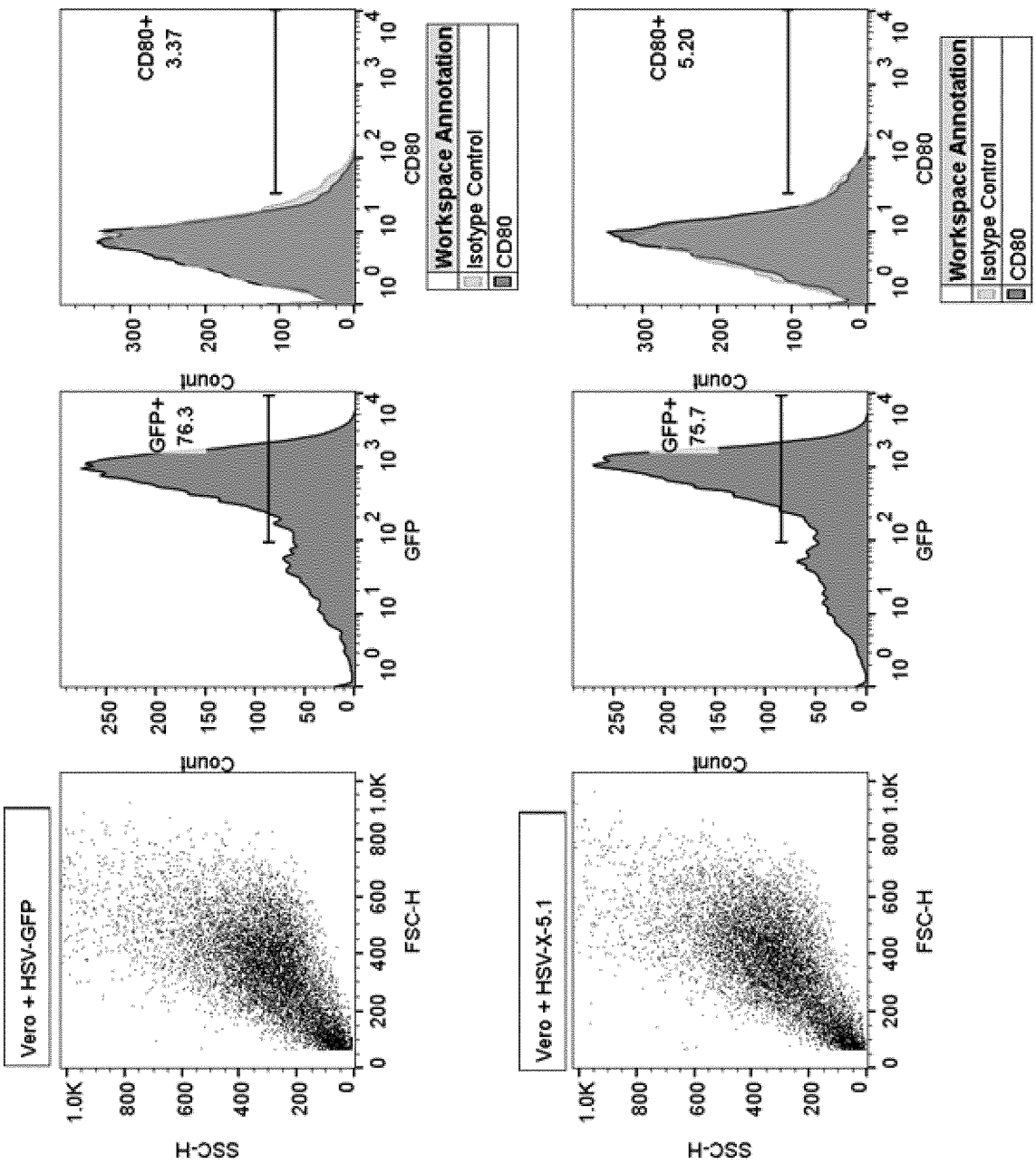

Vero + HSV-GFP
Vero + HSV-X-5.1
SSC-H
FSC-H
Count
GFP
GFP+
76.3
GFP+
75.7
CD80
CD80+
3.37
CD80+
5.20
Workspace Annotation
Isotype Control
CD80

Figure 46A

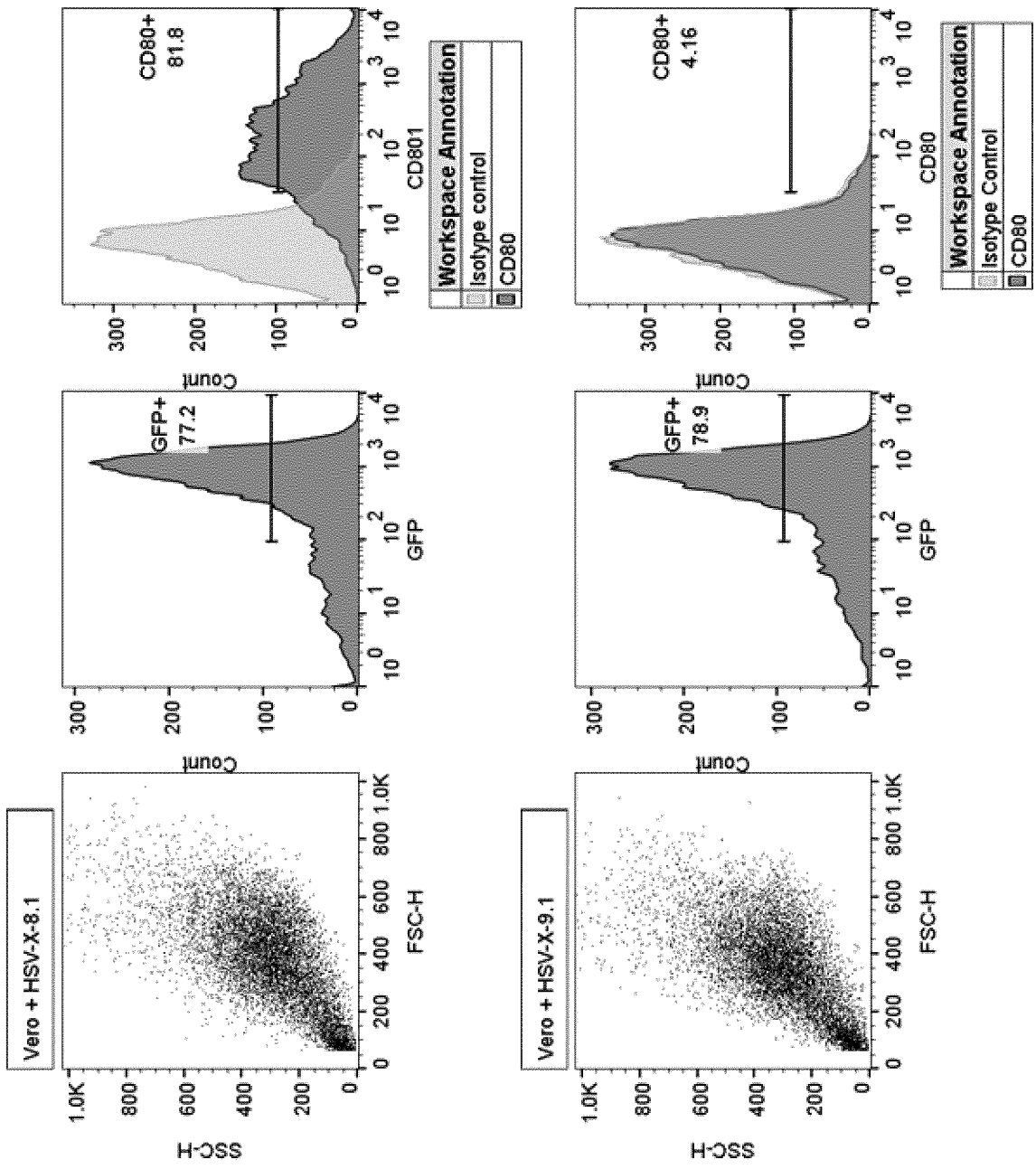
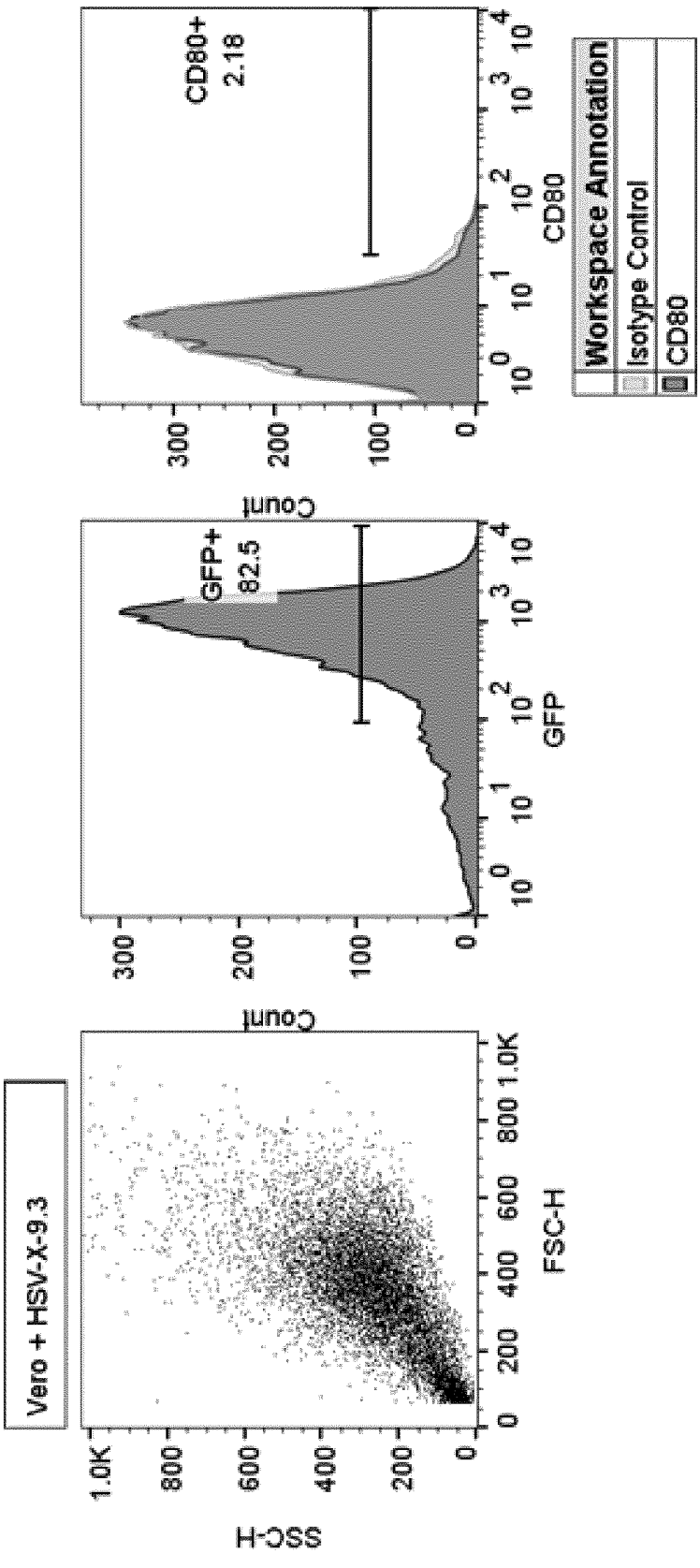


Figure 46A continued

Figure 46A continued



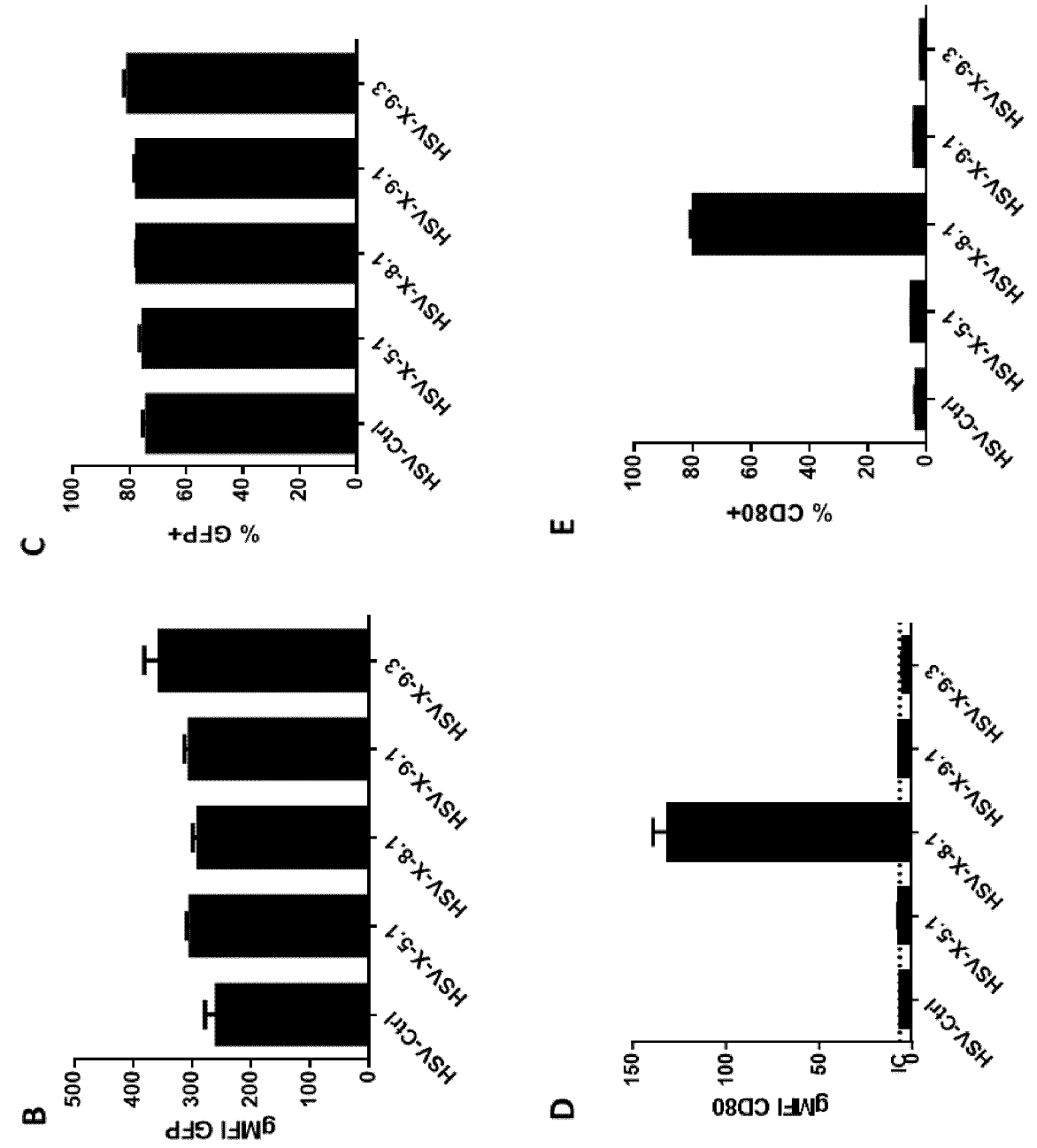

B
gMFI GFP
500
400
300
200
100
0
HSV-Ctrl
HSV-X-5.1
HSV-X-8.1
HSV-X-9.1
HSV-X-9.3
C
% GFP+
100
80
60
40
20
0
HSV-Ctrl
HSV-X-5.1
HSV-X-8.1
HSV-X-9.1
HSV-X-9.3
D
gMFI CD80
150
100
50
0
HSV-Ctrl
HSV-X-5.1
HSV-X-8.1
HSV-X-9.1
HSV-X-9.3
E
% CD80+
100
80
60
40
20
0
HSV-Ctrl
HSV-X-5.1
HSV-X-8.1
HSV-X-9.1
HSV-X-9.3

Figure 46B-E

Figure 46F

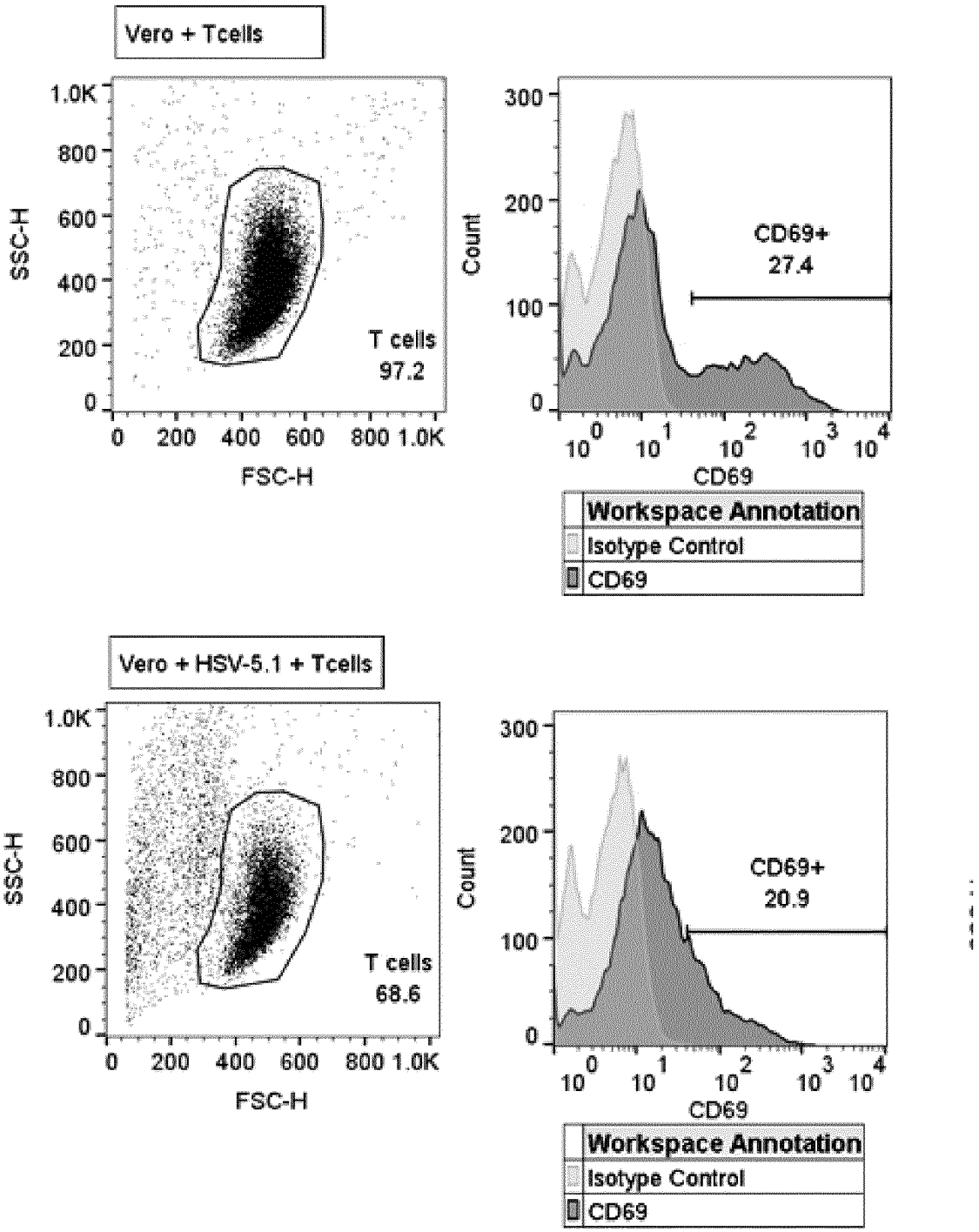

Vero + Tcells
SSC-H
FSC-H
T cells
97.2
Count
CD69+
27.4
CD69
Workspace Annotation
Isotype Control
CD69
Vero + HSV-5.1 + Tcells
SSC-H
FSC-H
T cells
68.6
Count
CD69+
20.9
CD69
Workspace Annotation
Isotype Control
CD69

Figure 46F continued

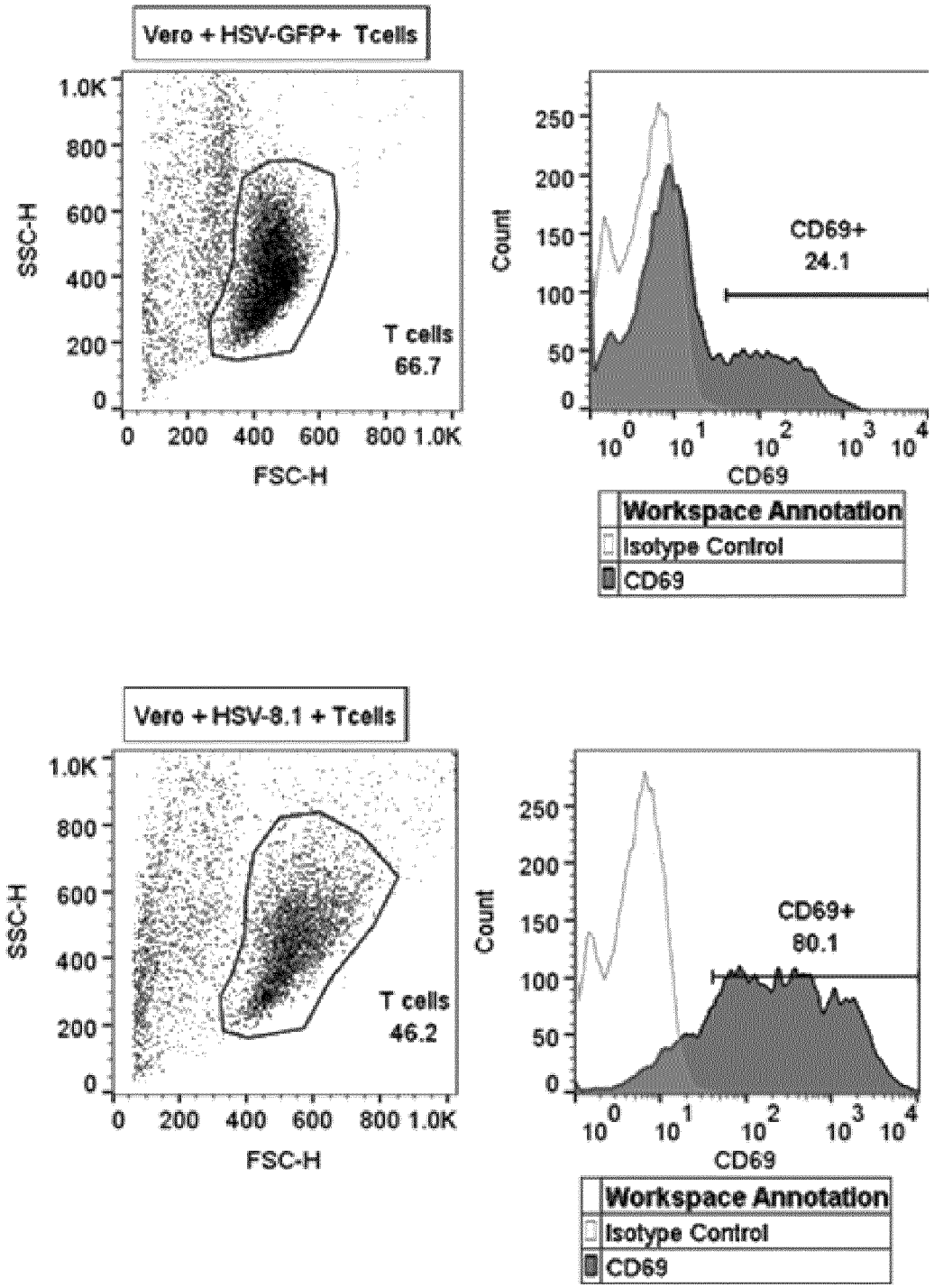


Figure 46F continued

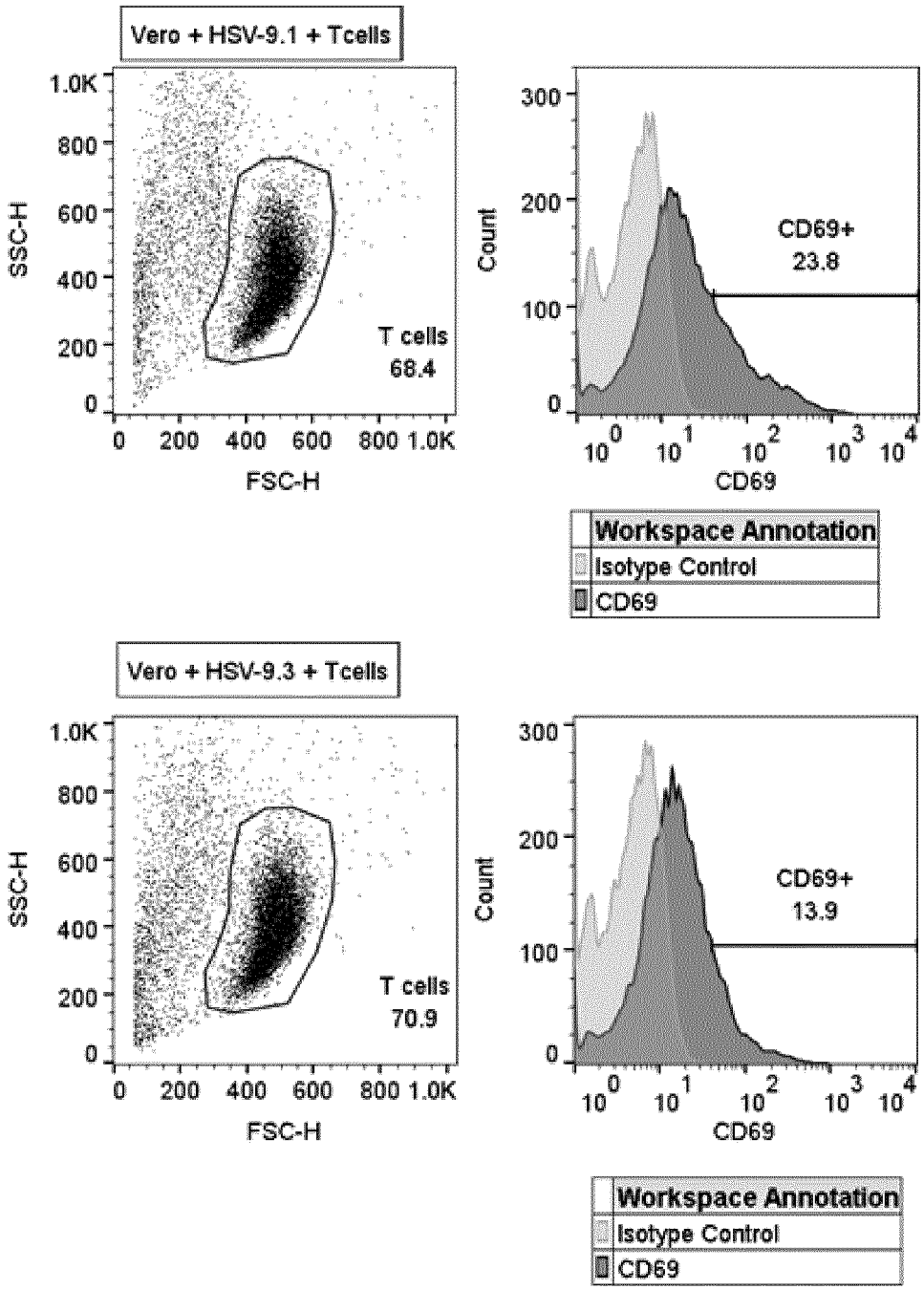


Figure 46G

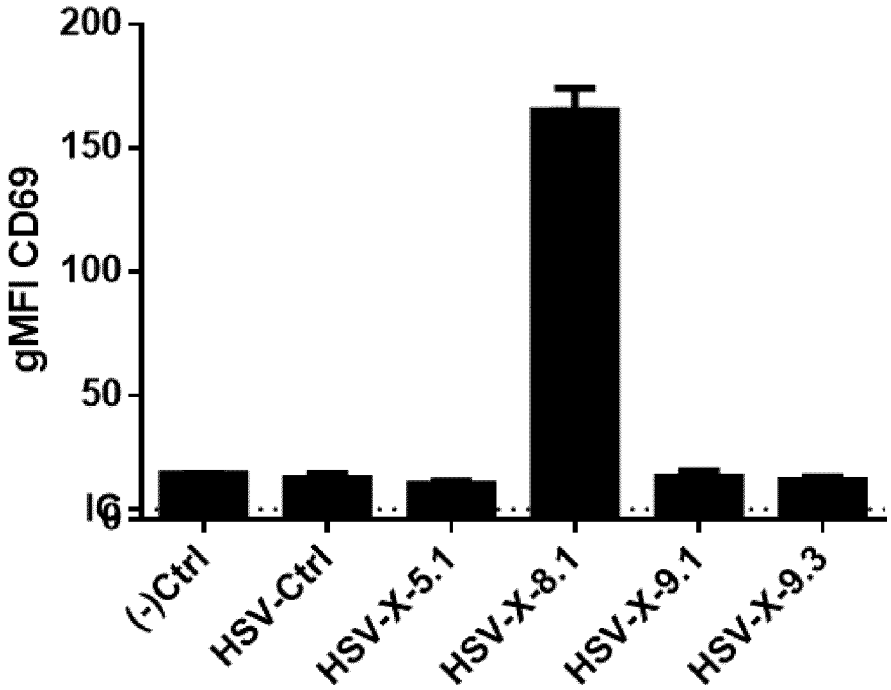
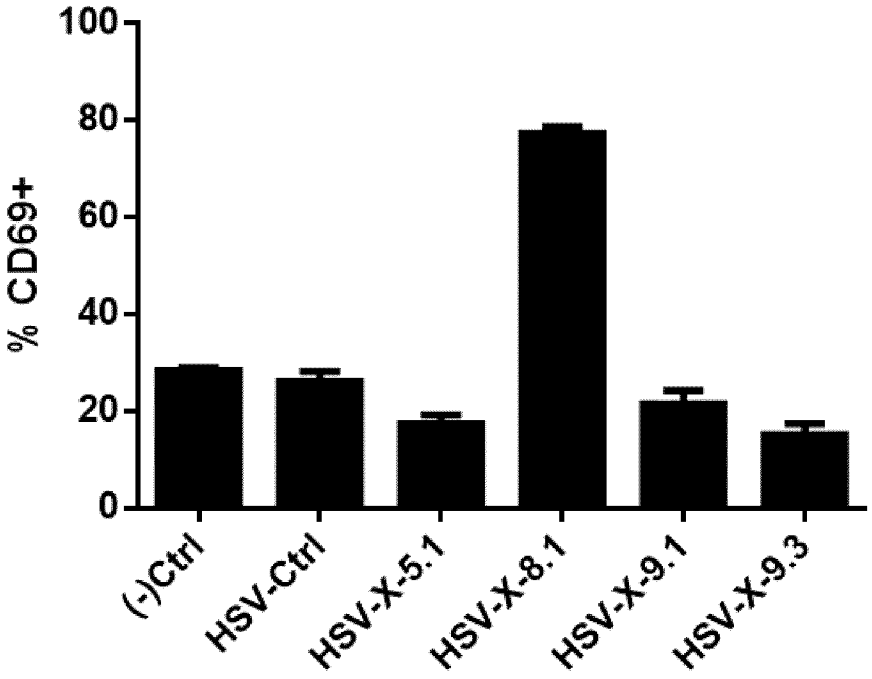


Figure 46H



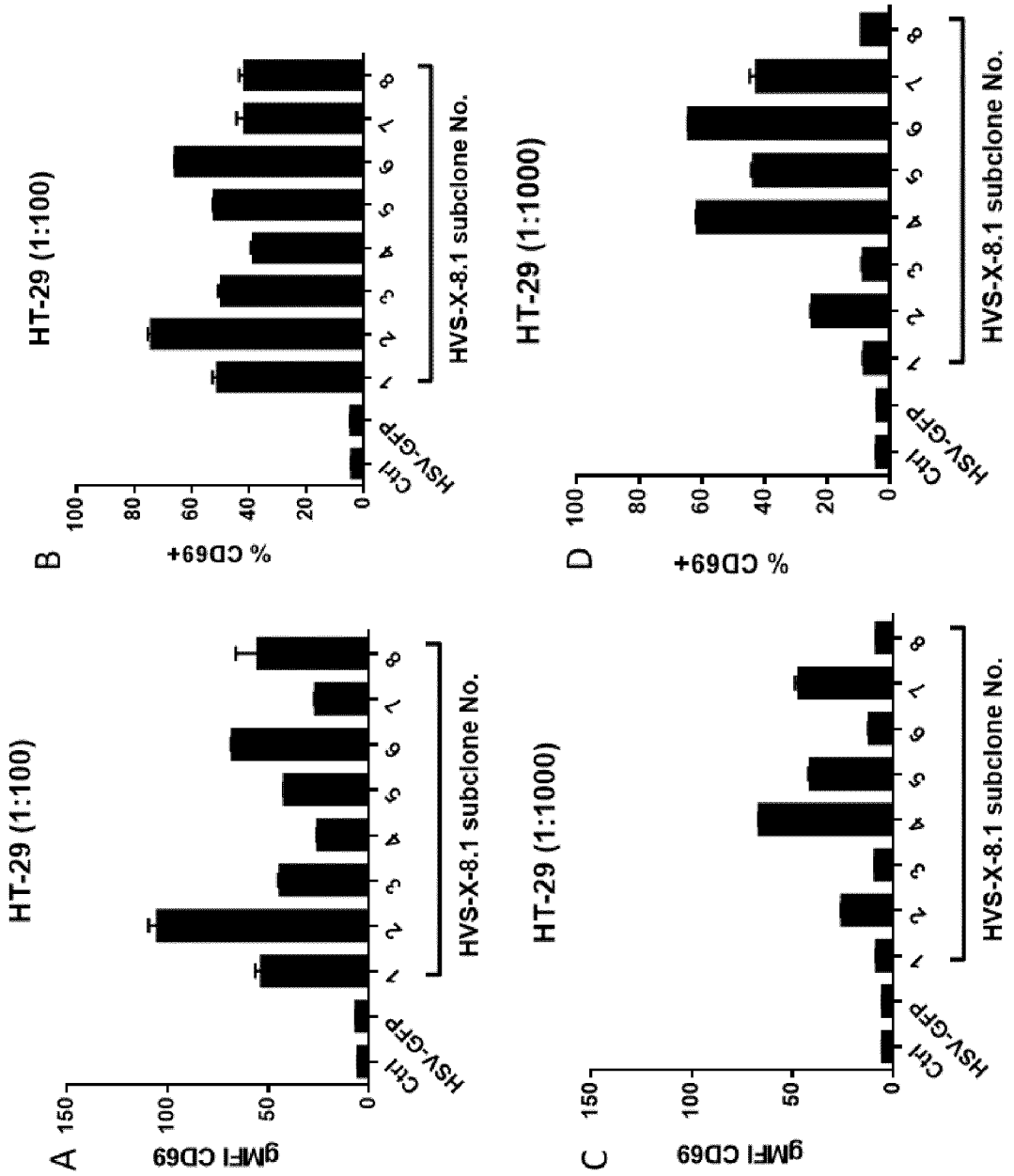

A
gMFI CD69
HT-29 (1:100)
Ctrl
HSV-GFP
HVS-X-8.1 subclone No.
B
% CD69+
HT-29 (1:100)
C
gMFI CD69
HT-29 (1:1000)
D
% CD69+
HT-29 (1:1000)

Figure 47A-D

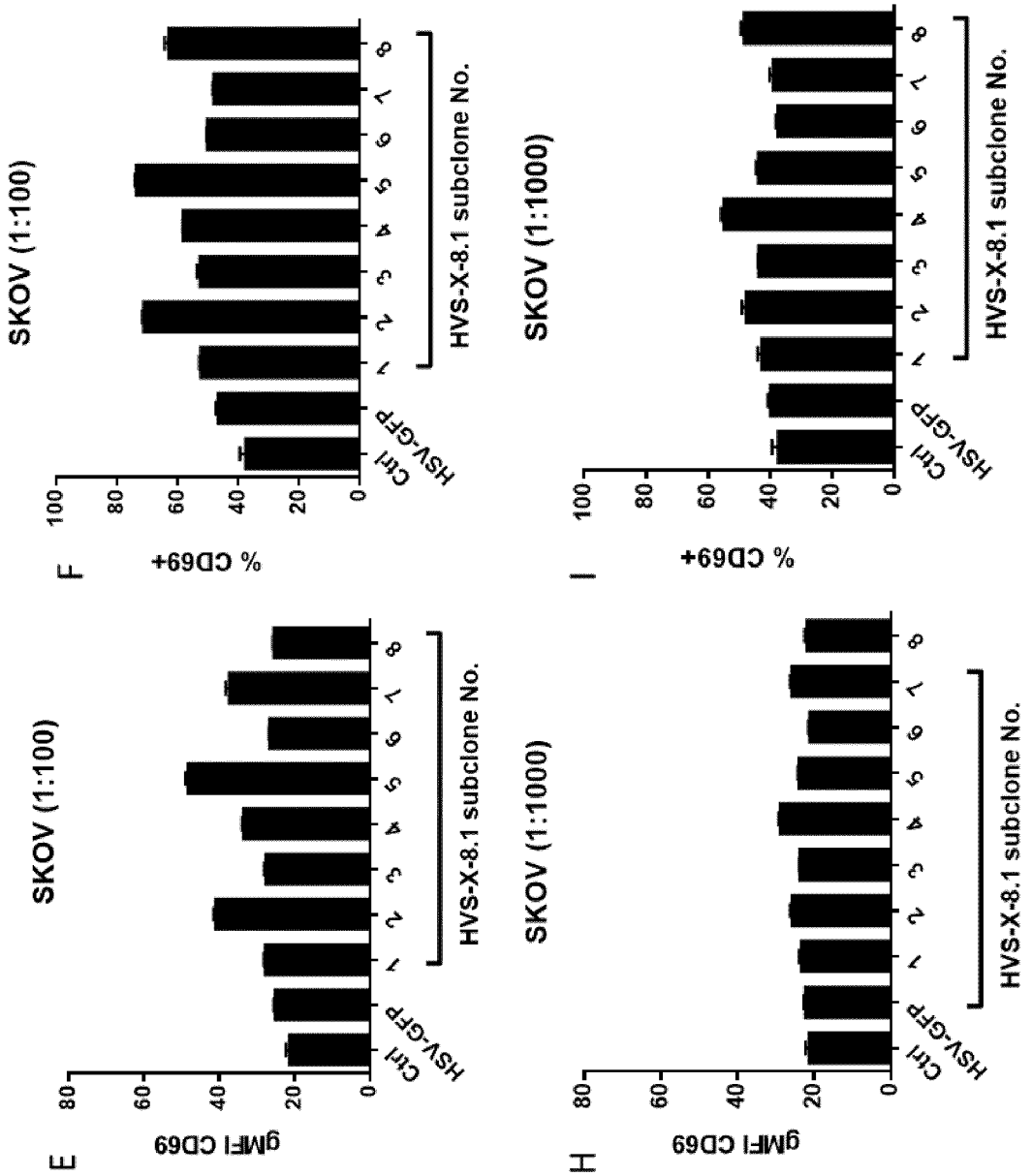

E
SKOV (1:100)
gMFI CD69
0
20
40
60
80
Ctrl
HSV-GFP
1
2
3
4
5
6
7
8
HVS-X-8.1 subclone No.
F
SKOV (1:100)
% CD69+
0
20
40
60
80
100
Ctrl
HSV-GFP
1
2
3
4
5
6
7
8
HVS-X-8.1 subclone No.
H
SKOV (1:1000)
gMFI CD69
0
20
40
60
80
Ctrl
HSV-GFP
1
2
3
4
5
6
7
8
HVS-X-8.1 subclone No.
I
SKOV (1:1000)
% CD69+
0
20
40
60
80
100
Ctrl
HSV-GFP
1
2
3
4
5
6
7
8
HVS-X-8.1 subclone No.

Figure 47E-I

Figure 48A

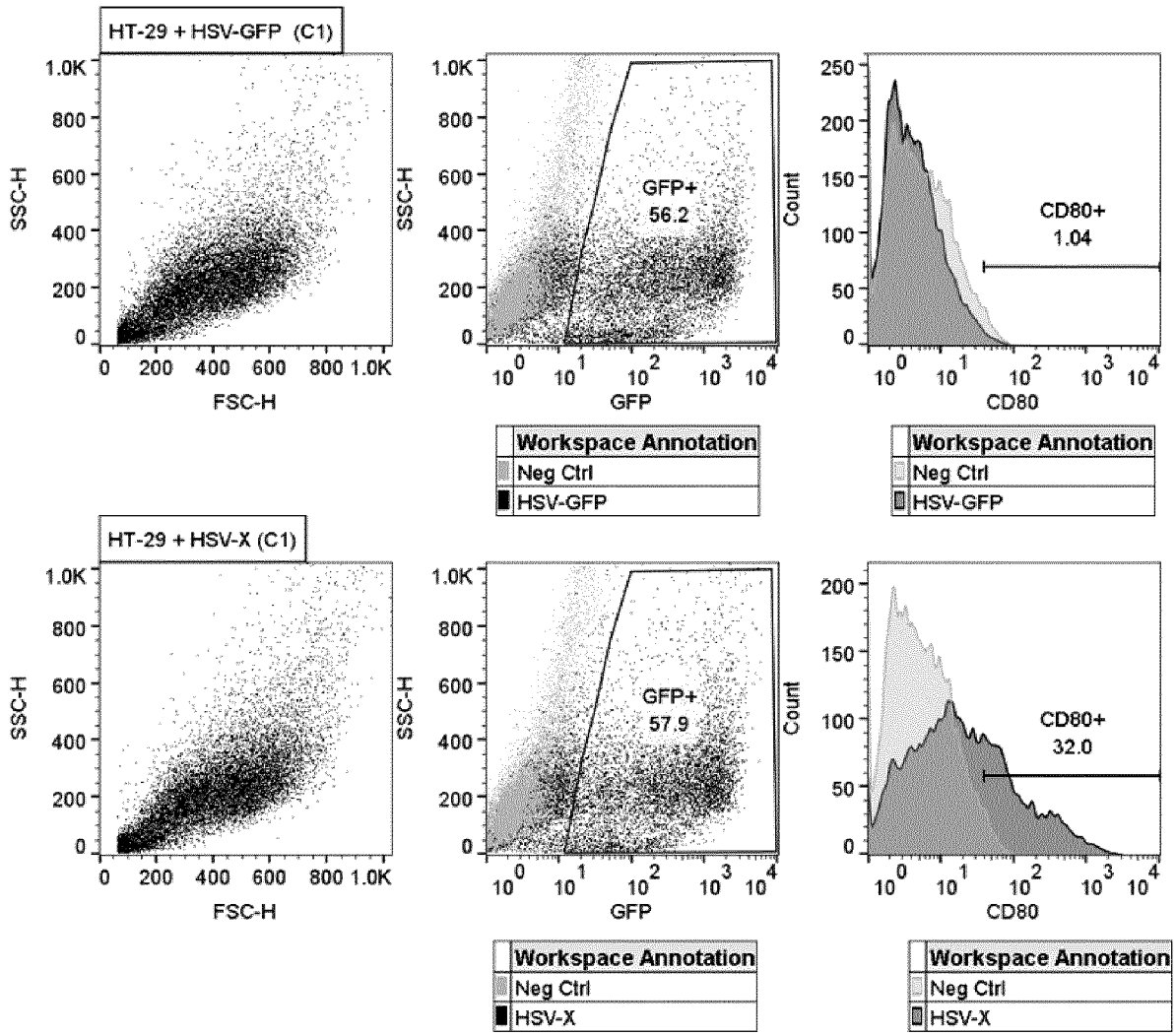
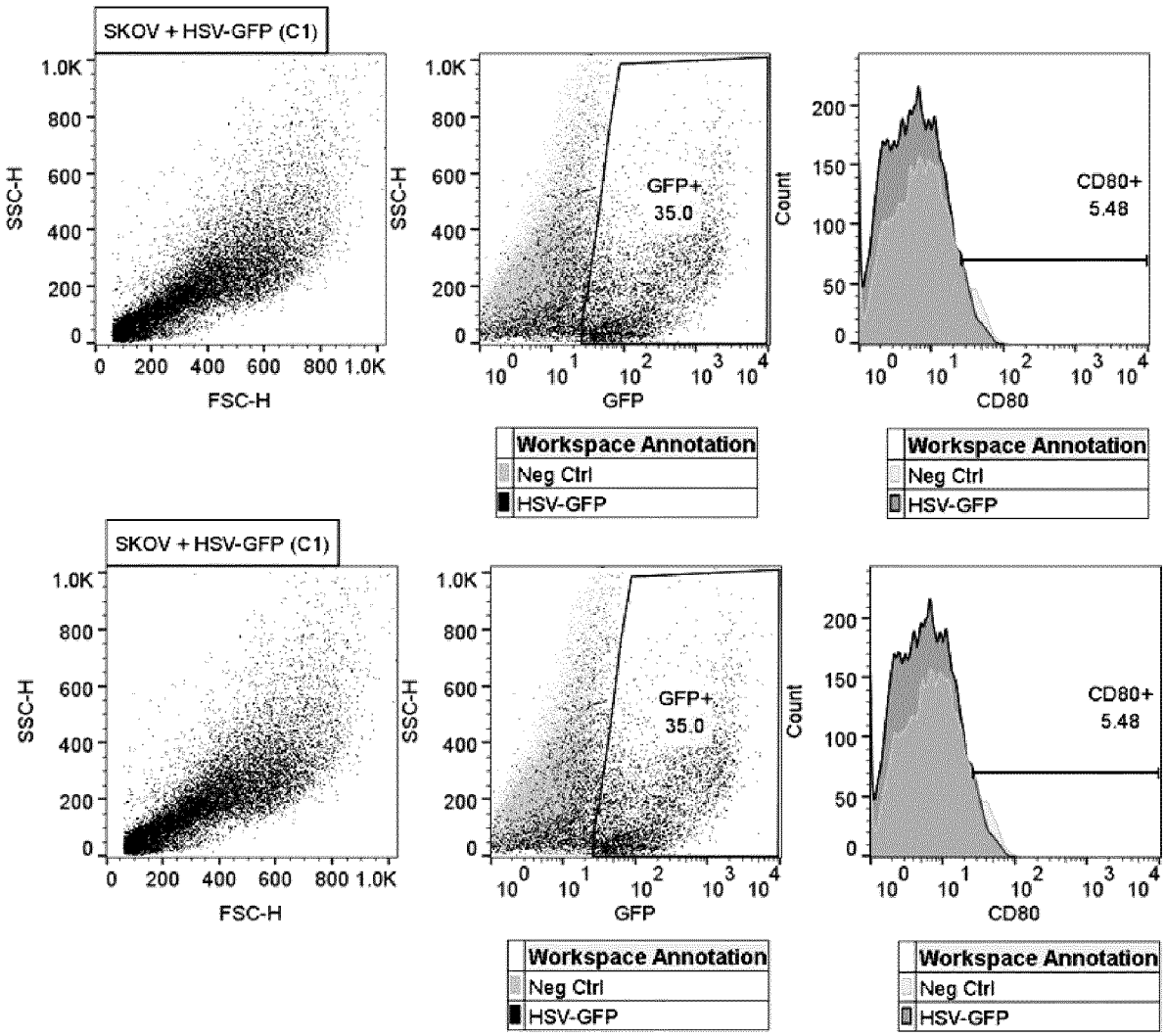

HT-29 + HSV-GFP (C1)
SSC-H
FSC-H
GFP+
56.2
GFP
Count
CD80+
1.04
CD80
Workspace Annotation
Neg Ctrl
HSV-GFP
HT-29 + HSV-X (C1)
GFP+
57.9
CD80+
32.0
HSV-X

Figure 48A continued



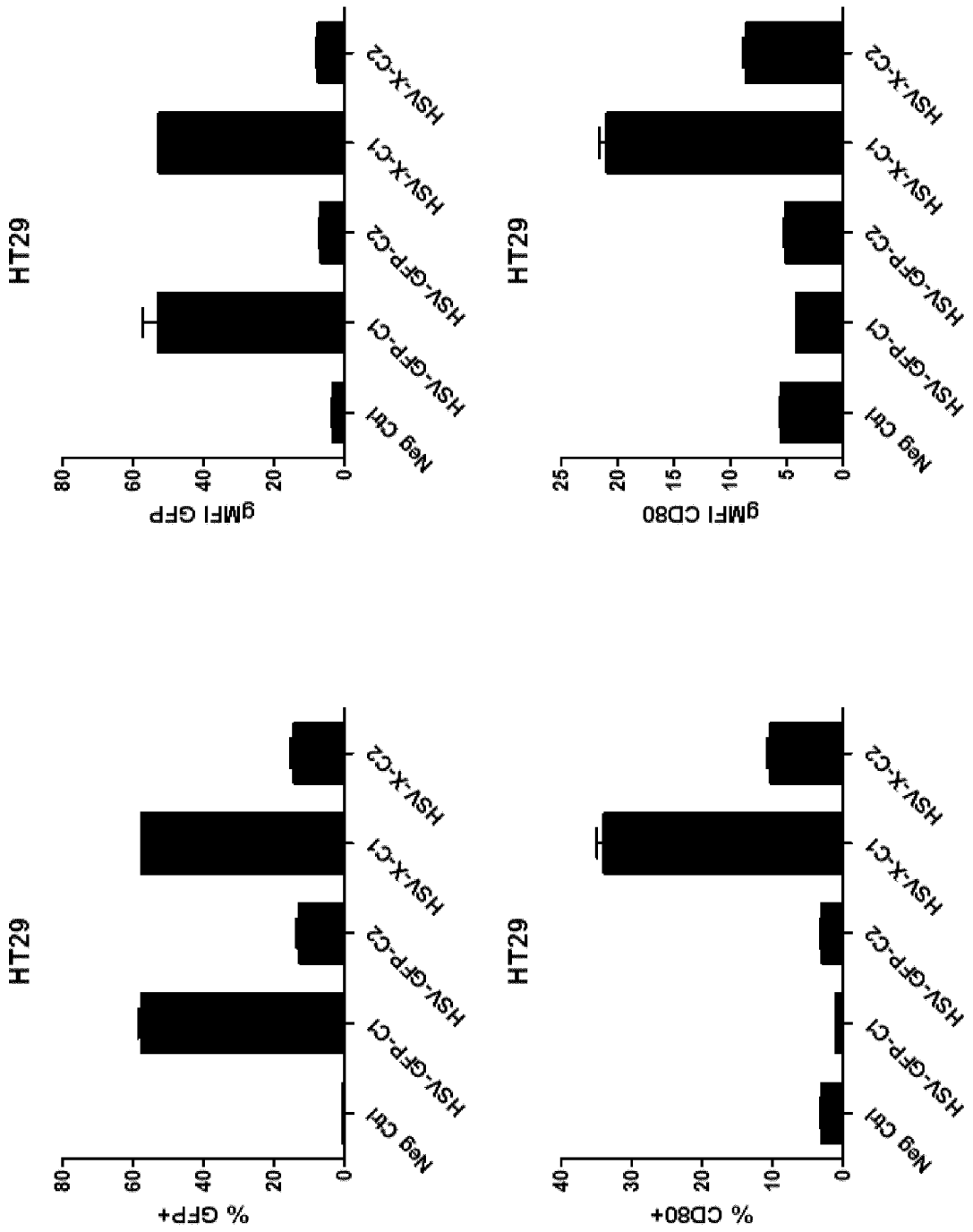

HT29
% GFP+
gMFI GFP
% CD80+
gMFI CD80
Neg Ctrl
HSV-GFP-C1
HSV-GFP-C2
HSV-X-C1
HSV-X-C2

Figure 48B

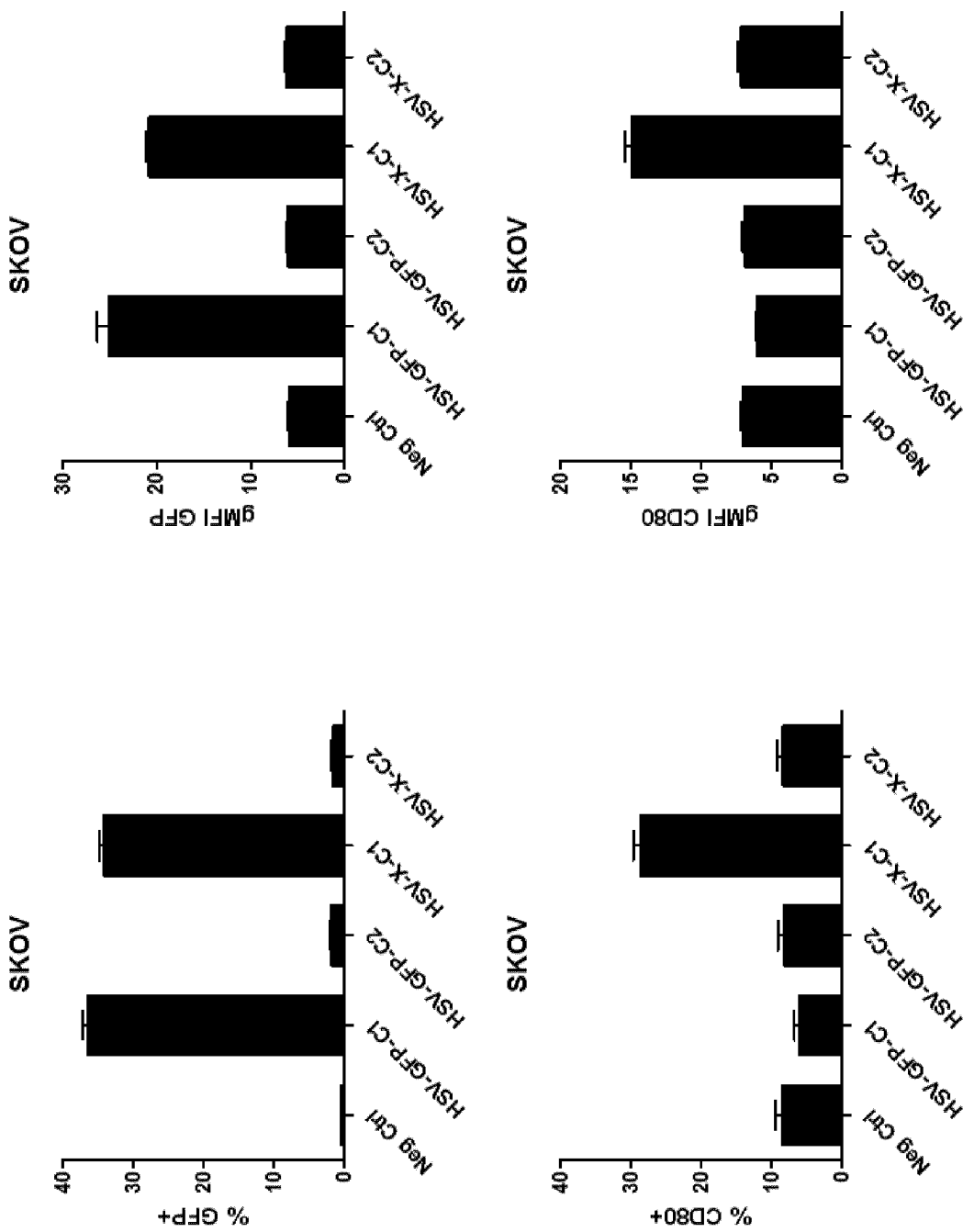


Figure 48B continued

Figure 49A

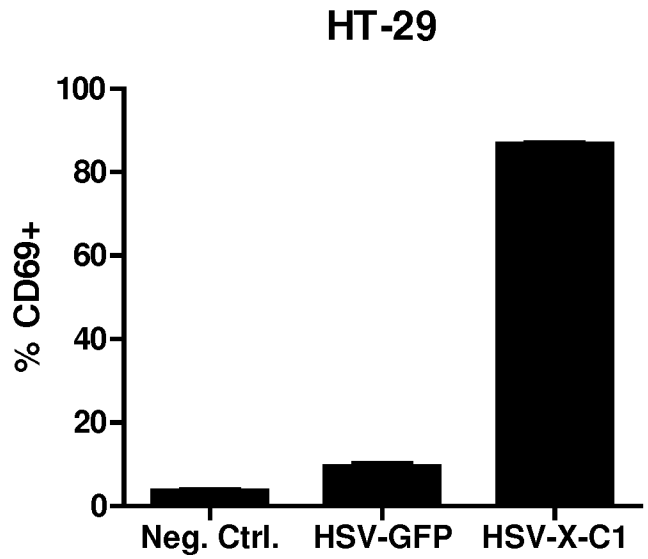


Figure 49B

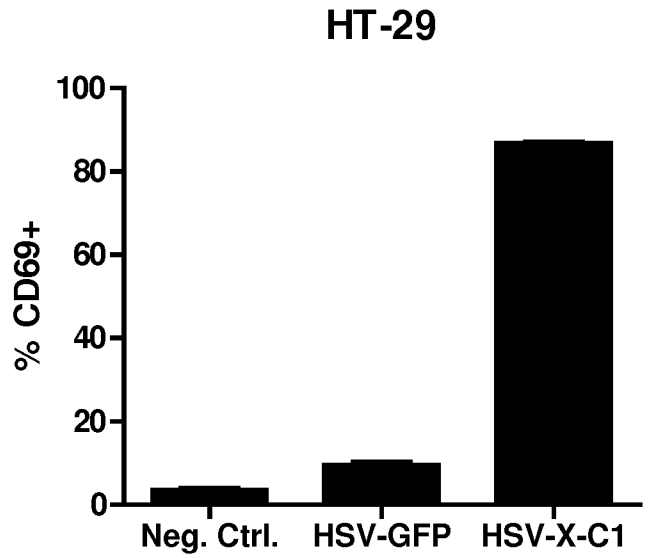
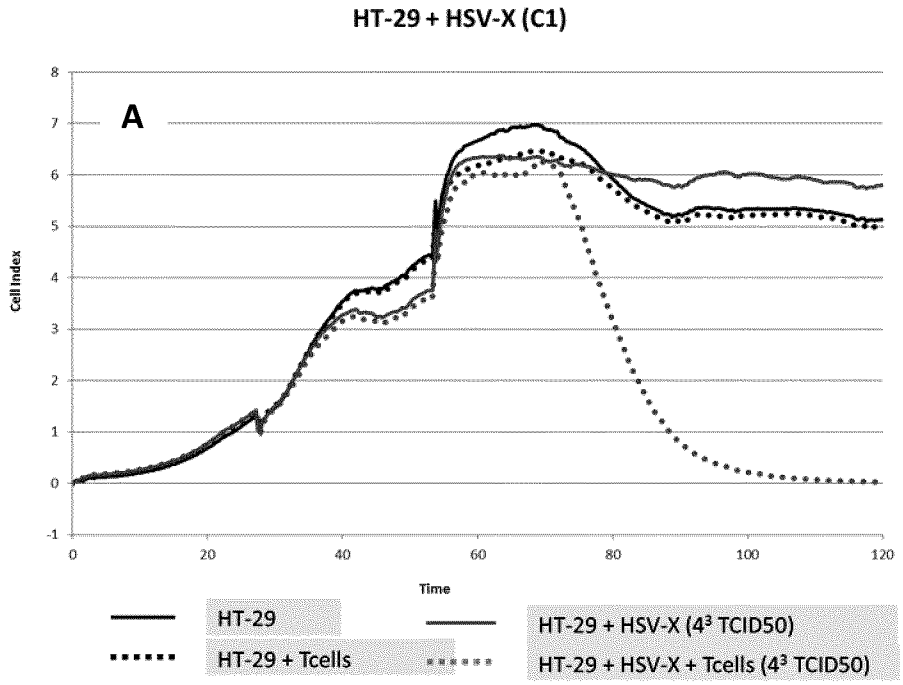


Figure 50


HT-29 + HSV-X (C1)
A
Cell Index
Time
HT-29
HT-29 + Tcells
HT-29 + HSV-X (4³ TCID50)
HT-29 + HSV-X + Tcells (4³ TCID50)

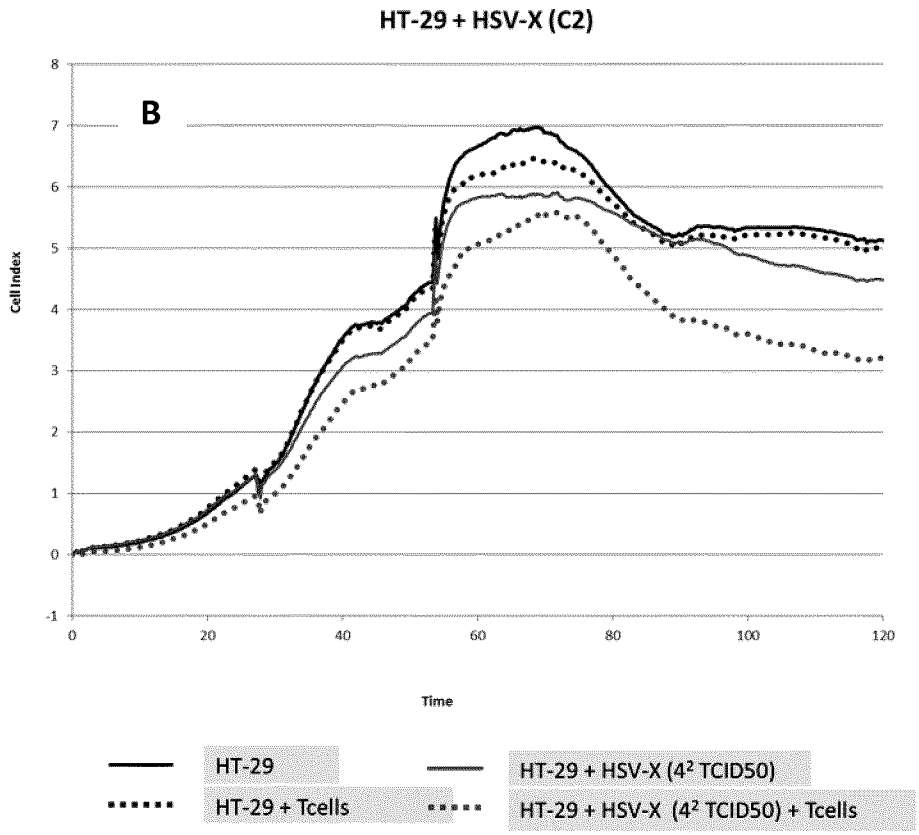

HT-29 + HSV-X (C2)
B
Cell Index
Time
HT-29
HT-29 + Tcells
HT-29 + HSV-X (4² TCID50)
HT-29 + HSV-X (4² TCID50) + Tcells

Figure 50C

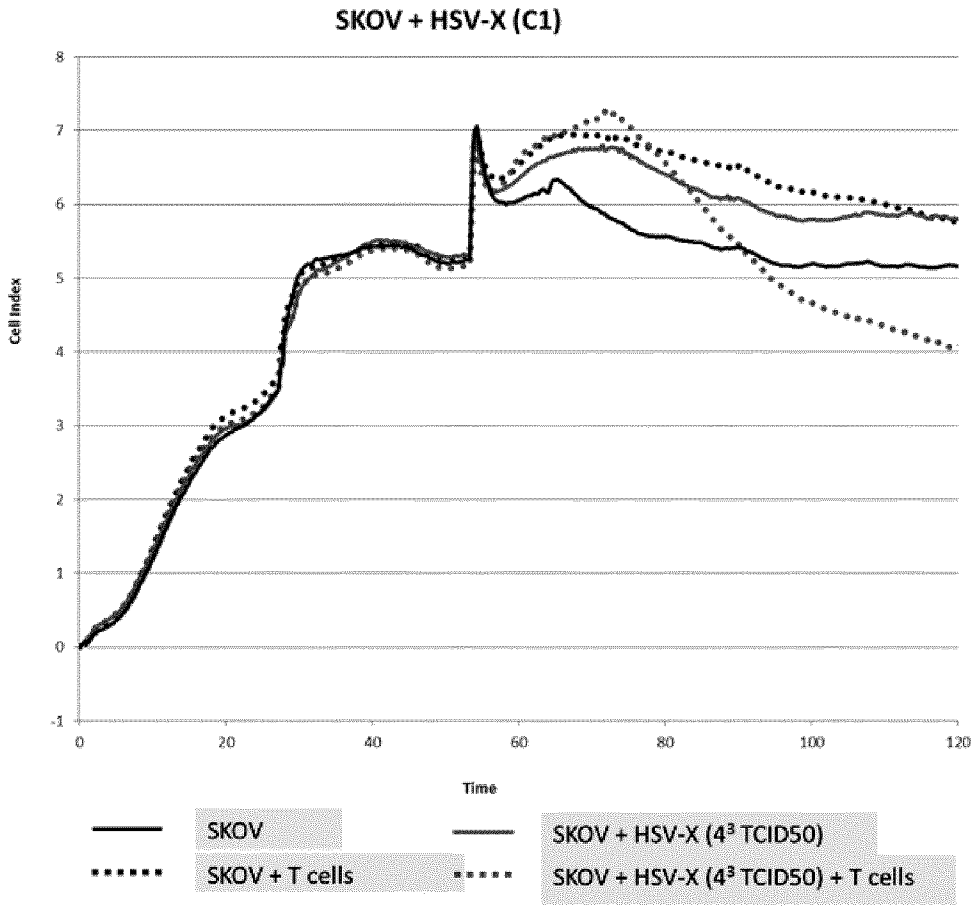

SKOV + HSV-X (C1)
Cell Index
Time
8
7
6
5
4
3
2
1
0
-1
0
20
40
60
80
100
120
SKOV
SKOV + T cells
SKOV + HSV-X (4³ TCID50)
SKOV + HSV-X (4³ TCID50) + T cells

Figure 51A

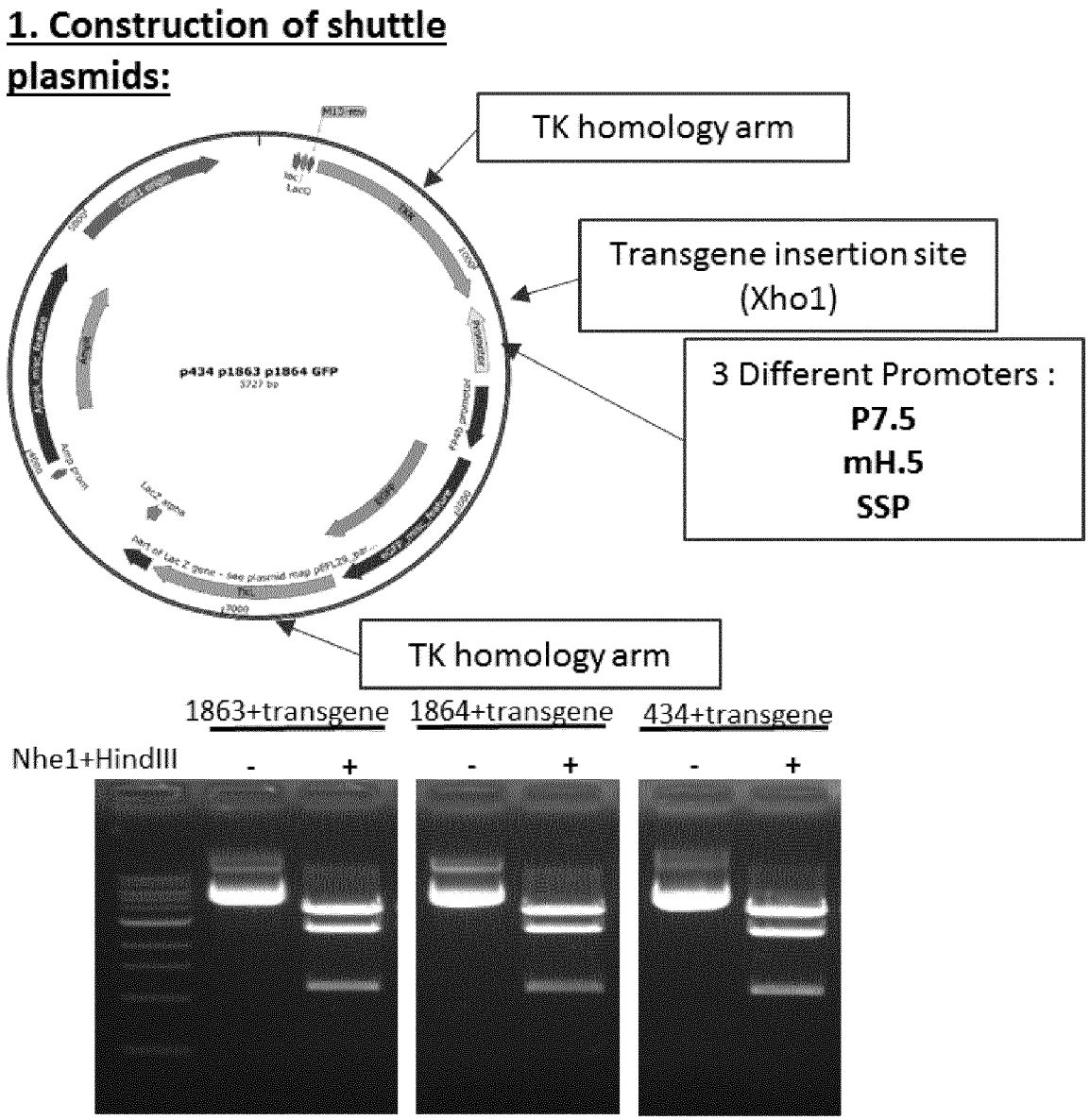
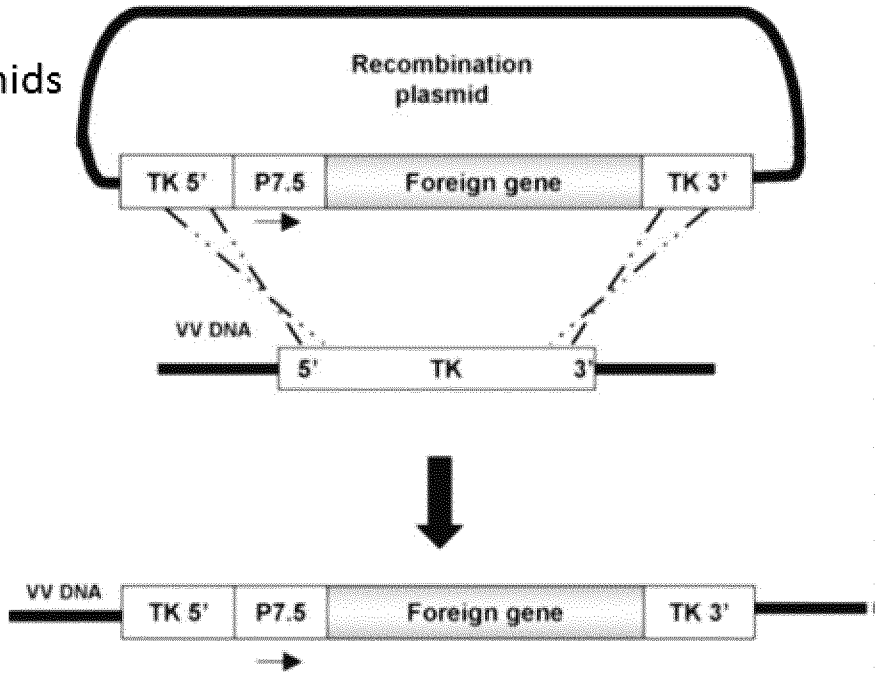

1. Construction of shuttle plasmids:
TK homology arm
Transgene insertion site (Xho1)
3 Different Promoters :
P7.5
mH.5
SSP
TK homology arm
1863+transgene
1864+transgene
434+transgene
Nhe1+HindIII
- + - + - +

Figure 51B

2. Homologous recombination:

- Infect 143 TK-/- cells with Western Reserve or Wyeth strains
- Transfect with shuttle plasmids
- HR in vitro



3. Selection of recombinant viruses:

- Amplify recombinants on 143 TK-/- cells + BRDU
- Isolate single clones by 2x rounds of plaque purification on 143 TK-/- cells + BRDU
- Bulk prep on HEK293 cells

4. Output: 6x viruses

- 3x Wyeth strains with transgene under P7.5/mH.5/SSP promoters
- 3x Western Reserve with transgene under P7.5/mH.5/SSP promoters

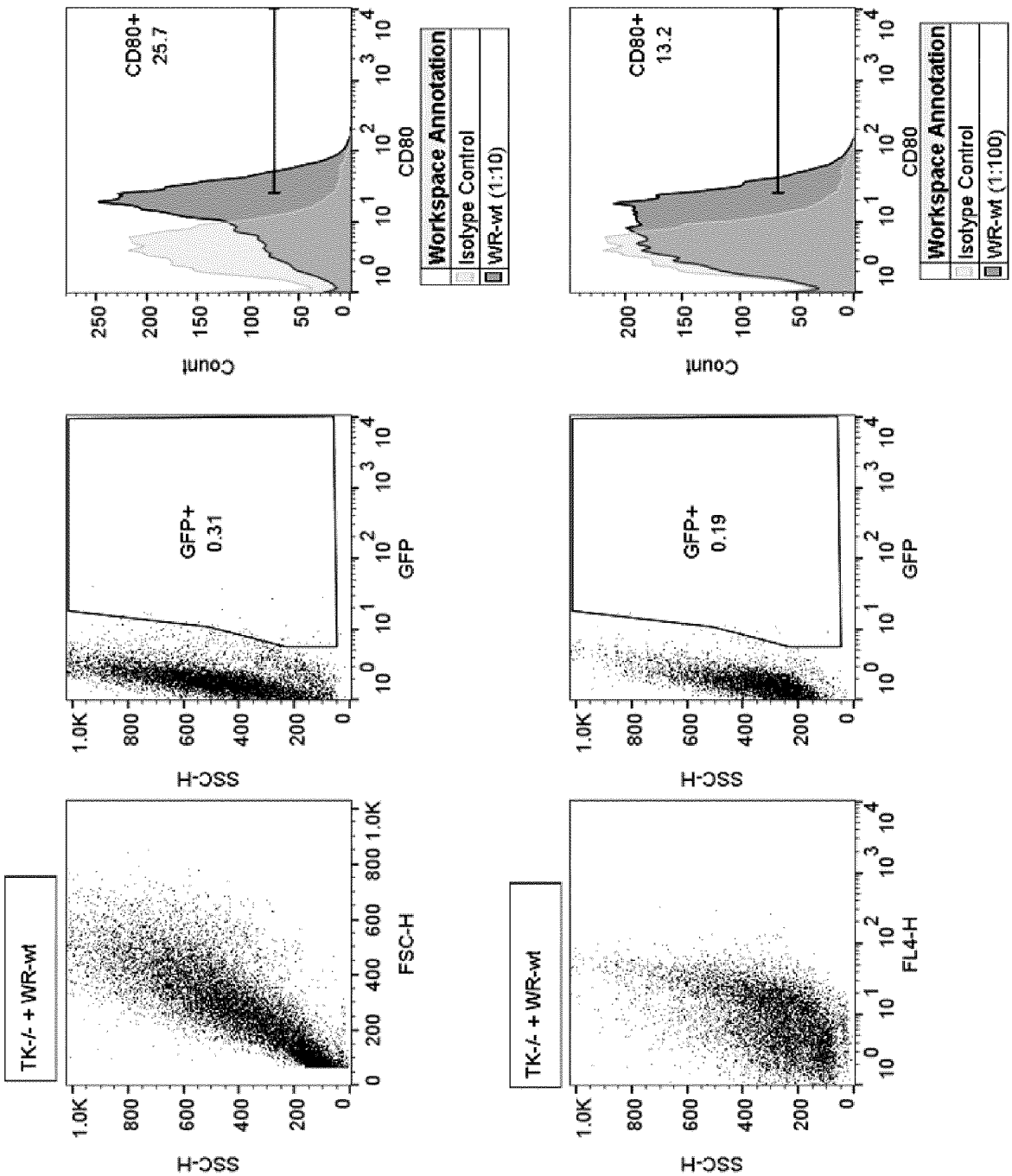

TK-/- + WR-wt
FSC-H
SSC-H
GFP
GFP+
0.31
CD80
CD80+
25.7
Count
Workspace Annotation
Isotype Control
WR-wt (1:10)
TK-/- + WR-wt
FL4-H
SSC-H
GFP
GFP+
0.19
CD80
CD80+
13.2
Count
Workspace Annotation
Isotype Control
WR-wt (1:100)

Figure 52A

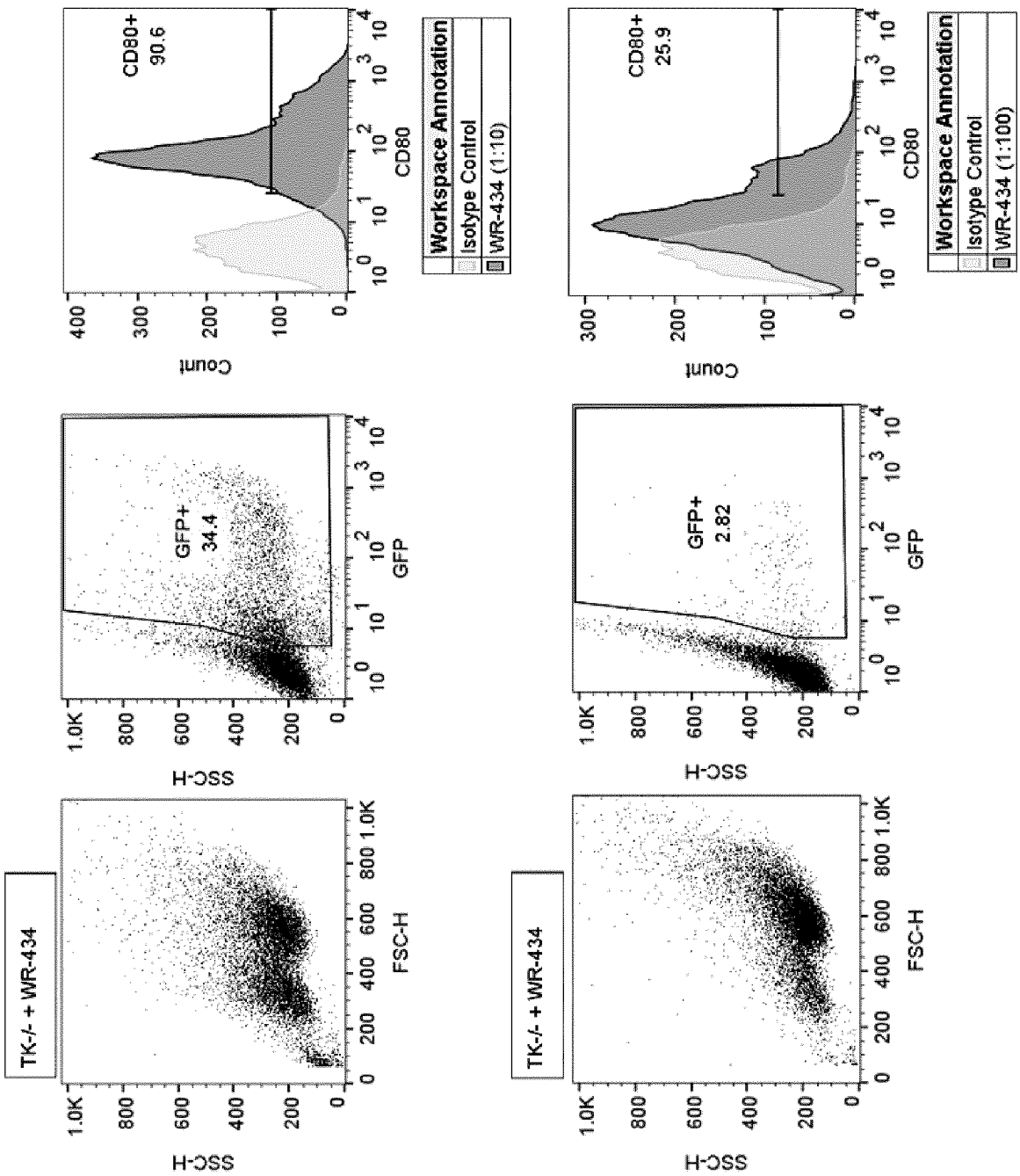


Figure 52A continued

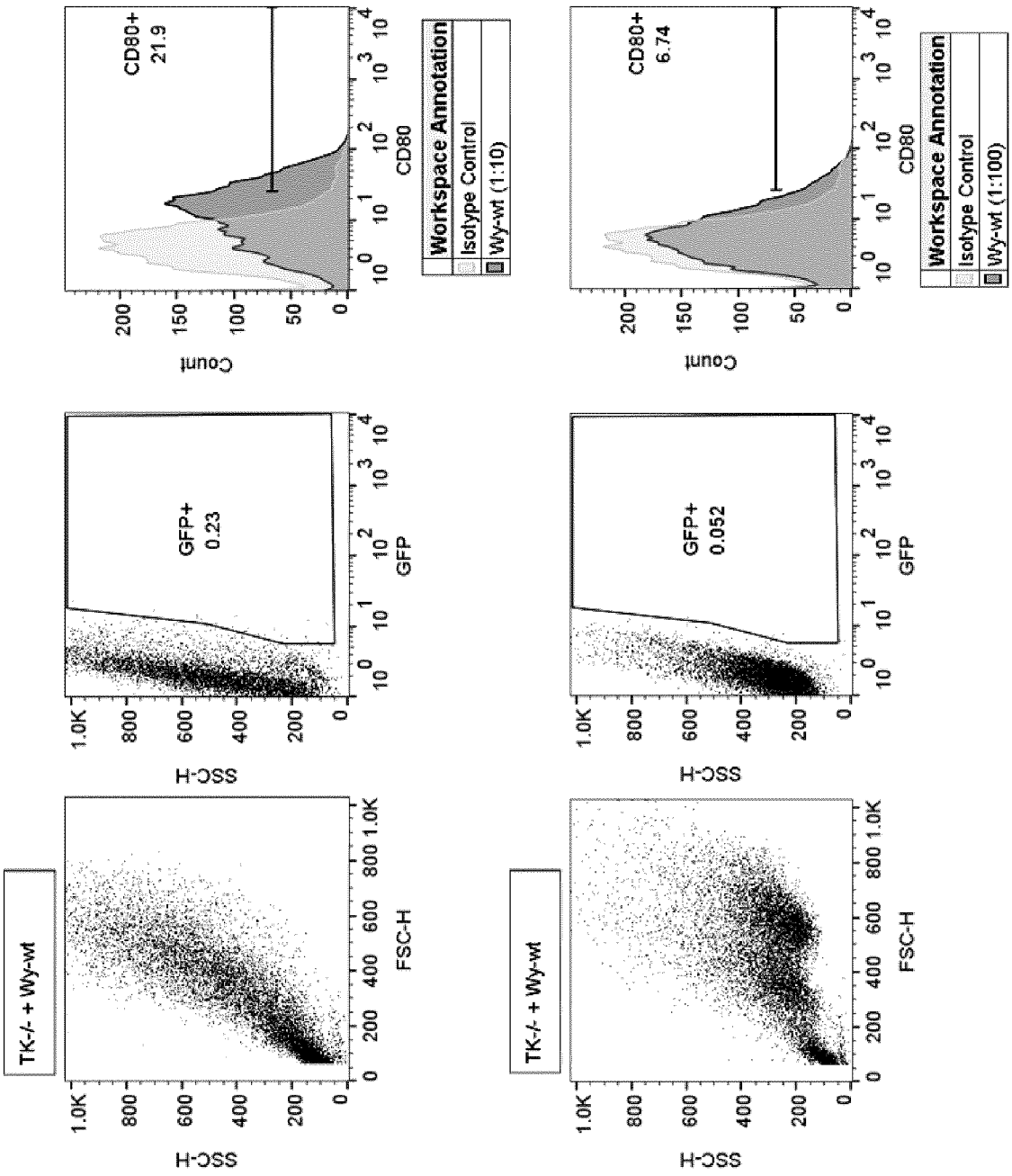

TK-/- + Wy-wt
FSC-H
SSC-H
GFP+
0.23
GFP
CD80+
21.9
CD80
Count
Workspace Annotation
Isotype Control
Wy-wt (1:10)
TK-/- + Wy-wt
FSC-H
SSC-H
GFP+
0.052
GFP
CD80+
6.74
CD80
Count
Workspace Annotation
Isotype Control
Wy-wt (1:100)

Figure 52B

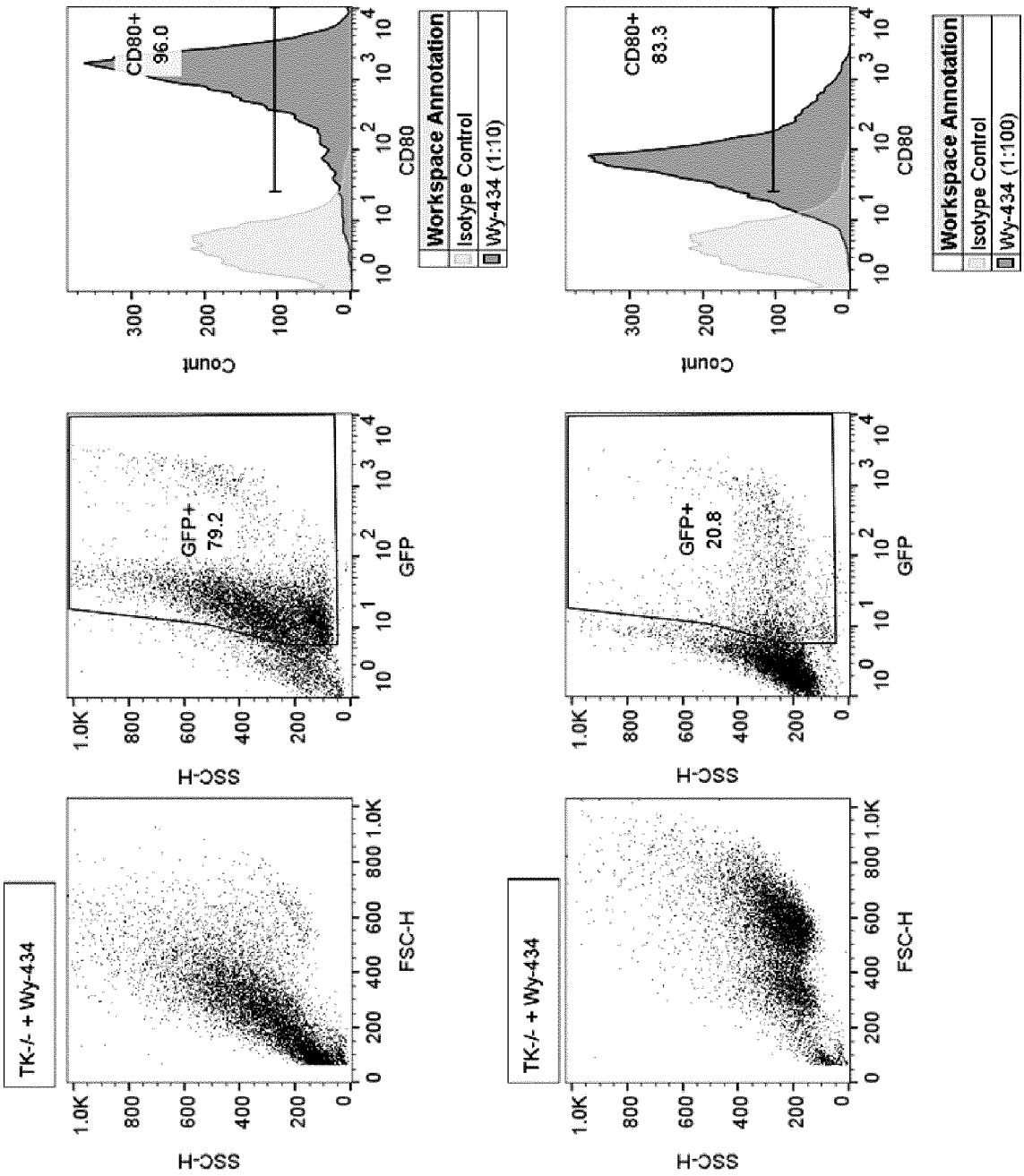


Figure 52B continued

Figure 52C-F

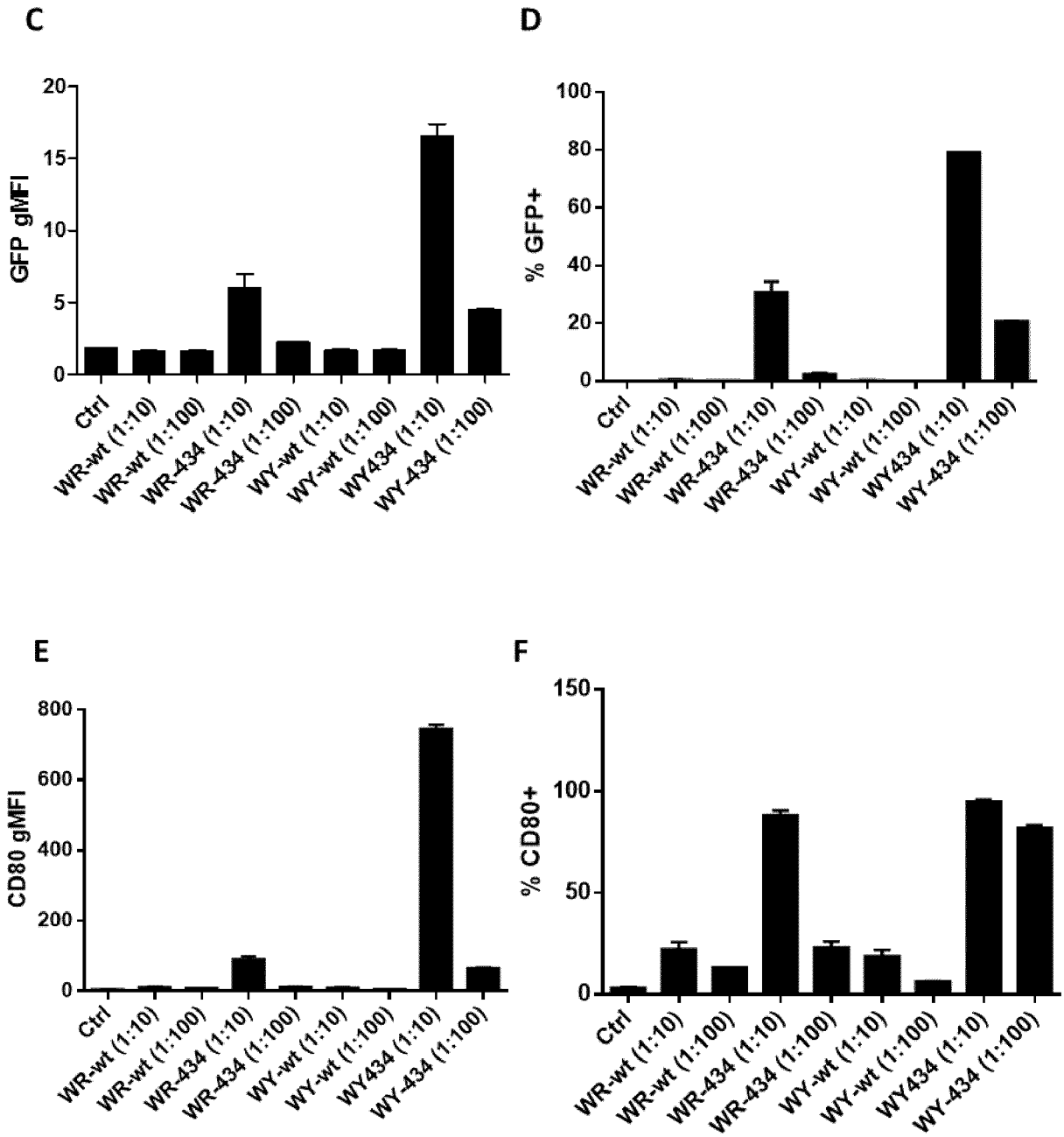

C
GFP gMFI
20
15
10
5
0
Ctrl
WR-wt (1:10)
WR-wt (1:100)
WR-434 (1:10)
WR-434 (1:100)
WY-wt (1:10)
WY-wt (1:100)
WY434 (1:10)
WY-434 (1:100)
D
% GFP+
100
80
60
40
20
0
Ctrl
WR-wt (1:10)
WR-wt (1:100)
WR-434 (1:10)
WR-434 (1:100)
WY-wt (1:10)
WY-wt (1:100)
WY434 (1:10)
WY-434 (1:100)
E
CD80 gMFI
800
600
400
200
0
Ctrl
WR-wt (1:10)
WR-wt (1:100)
WR-434 (1:10)
WR-434 (1:100)
WY-wt (1:10)
WY-wt (1:100)
WY434 (1:10)
WY-434 (1:100)
F
% CD80+
150
100
50
0
Ctrl
WR-wt (1:10)
WR-wt (1:100)
WR-434 (1:10)
WR-434 (1:100)
WY-wt (1:10)
WY-wt (1:100)
WY434 (1:10)
WY-434 (1:100)

Figure 53A Upregulation of CD69 by T cells exposed to SKOV cells infected with wild type viruses (left side) or transgene-expressing viruses (right side)

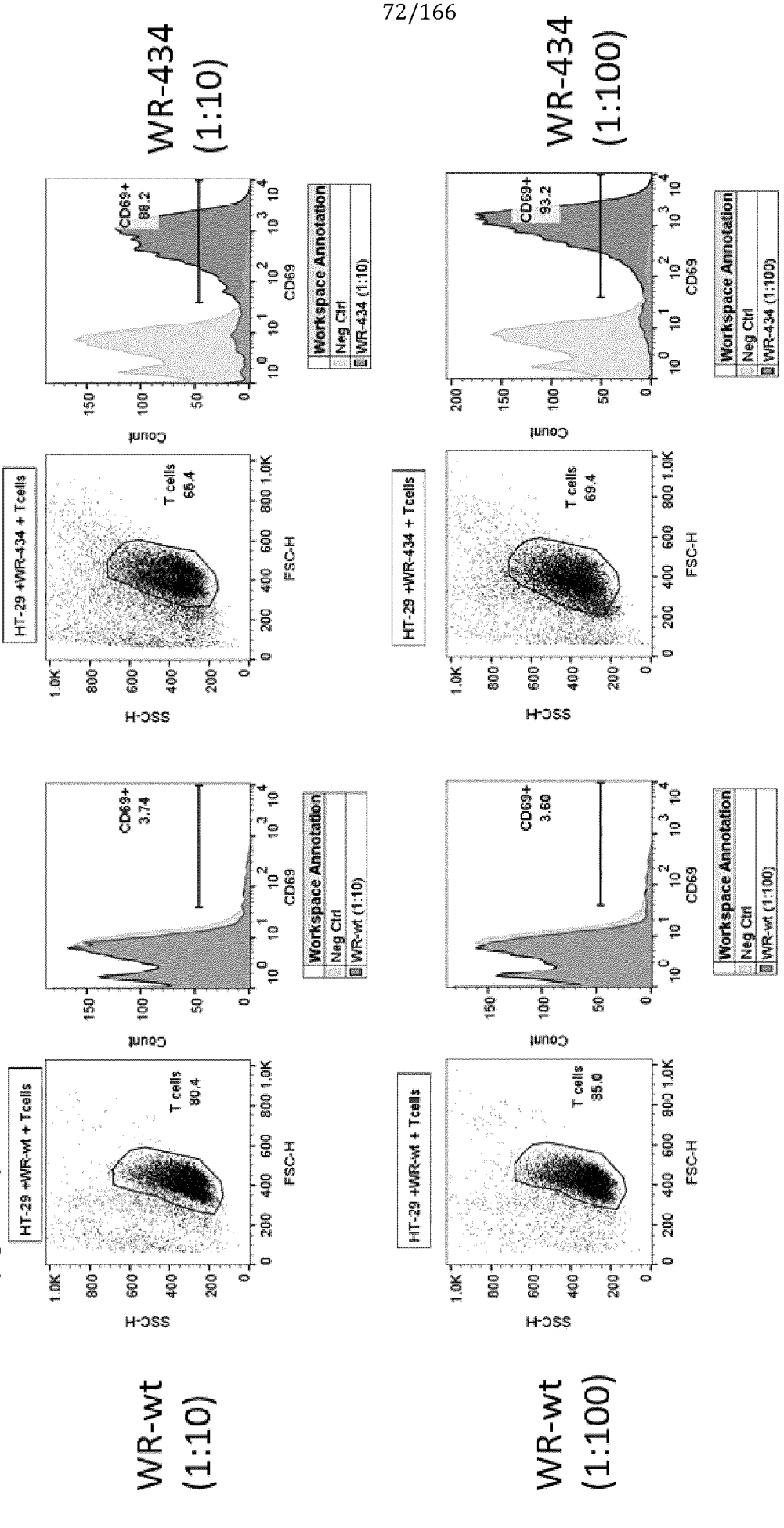


Figure 53A continued

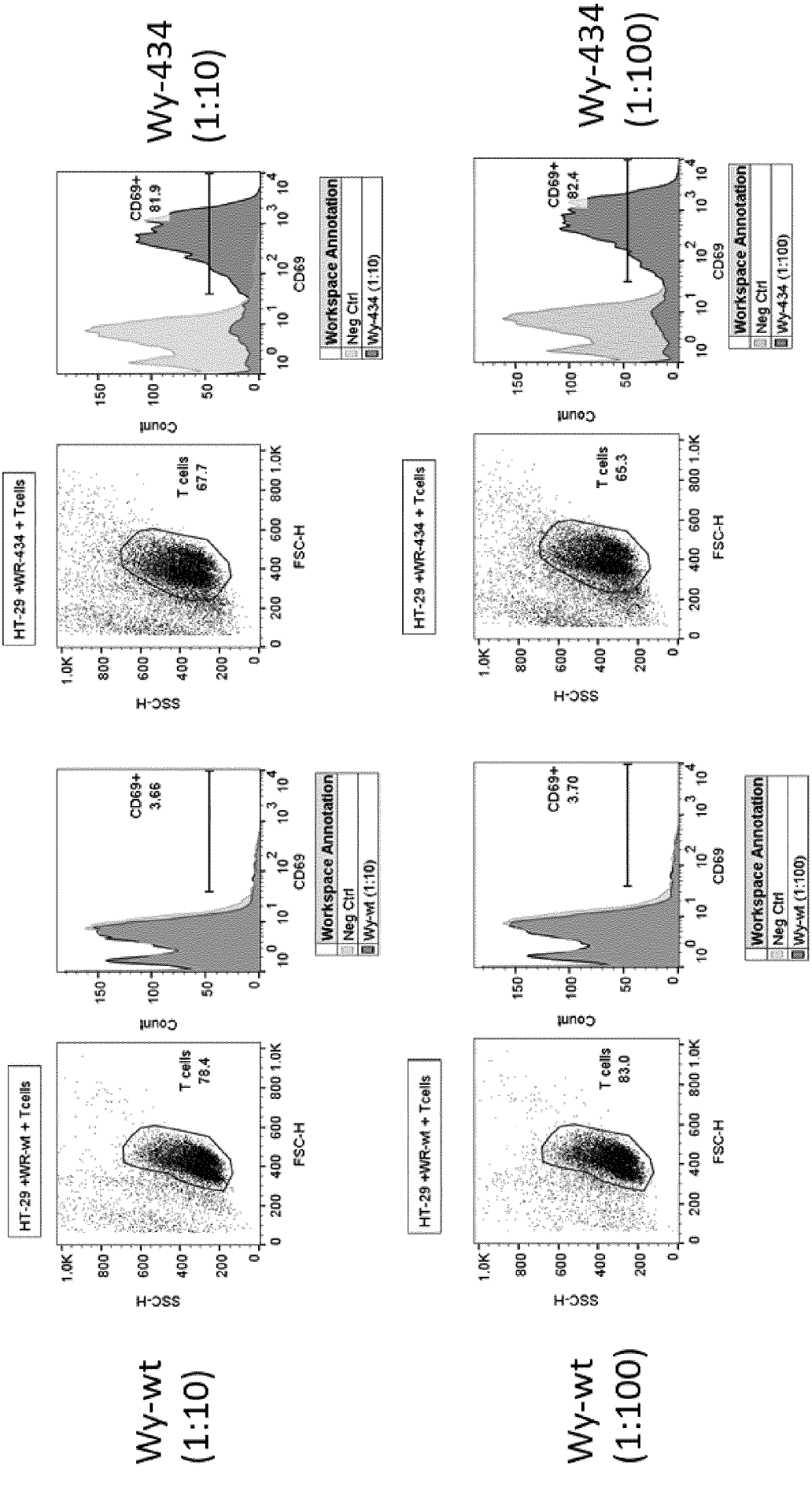
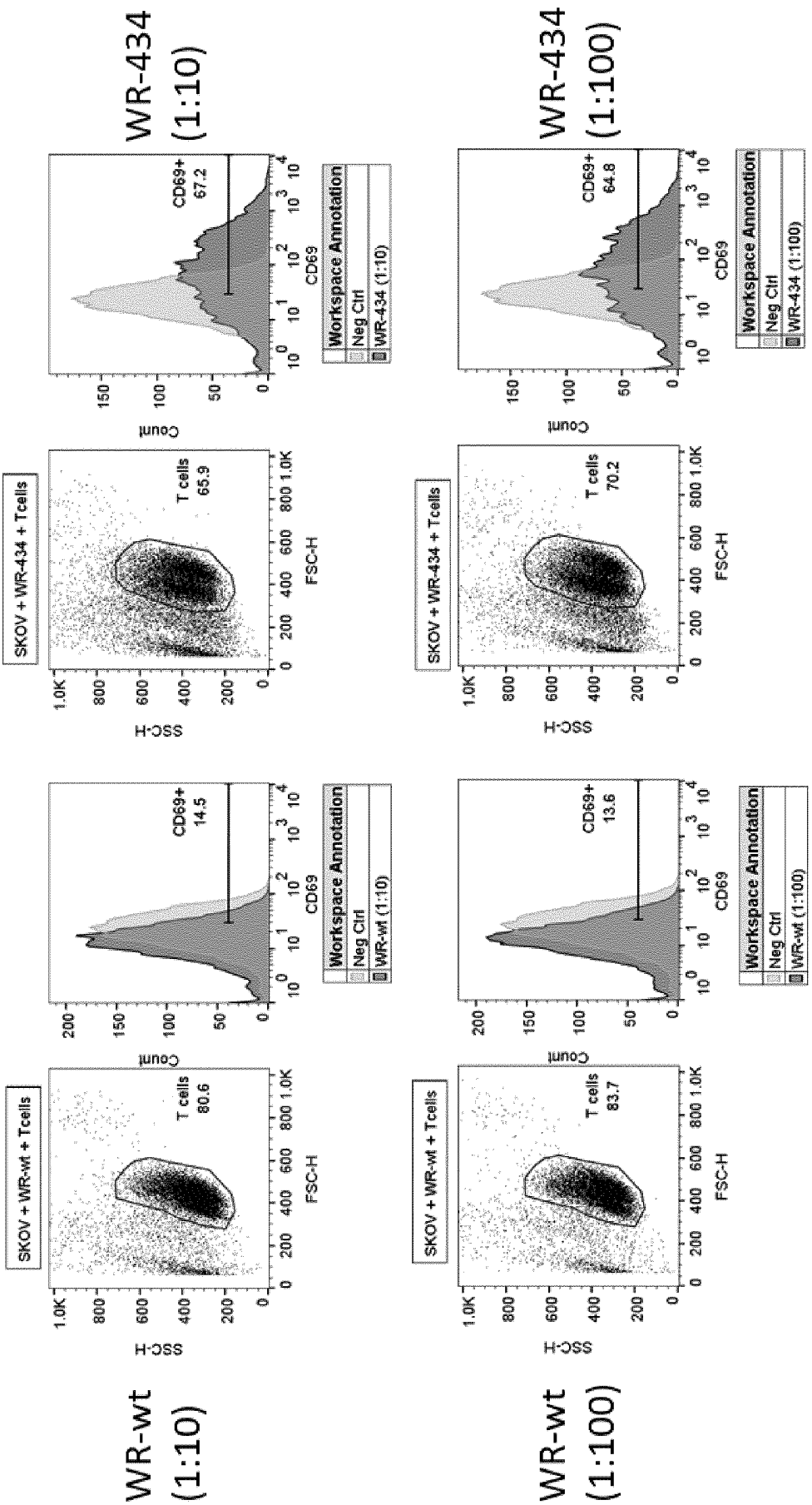


Figure 53B Upregulation of CD69 by T cells exposed to SKOV cells infected with wild type viruses (left side) or transgene-expressing viruses (right side)



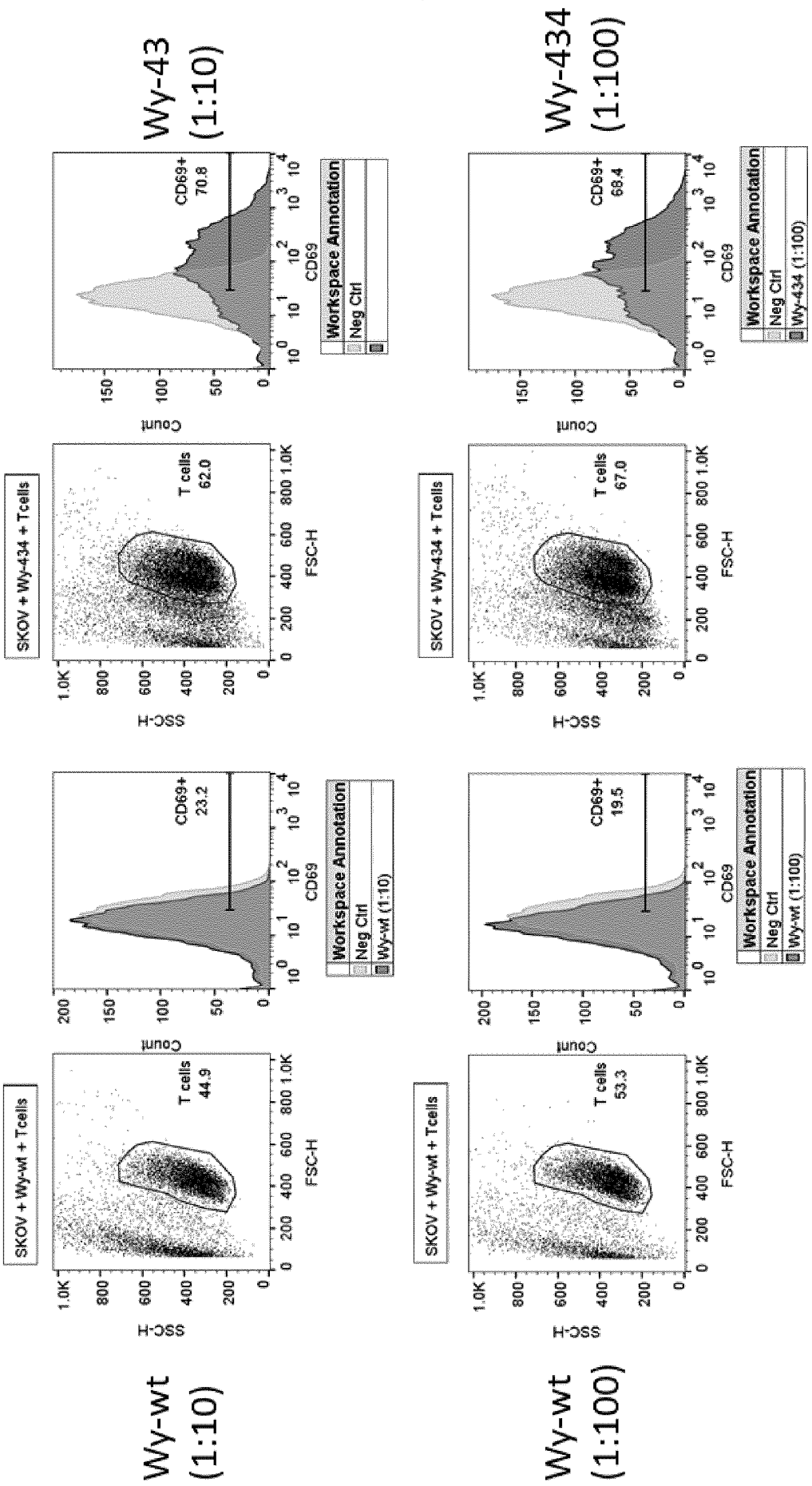


Figure 53B continued

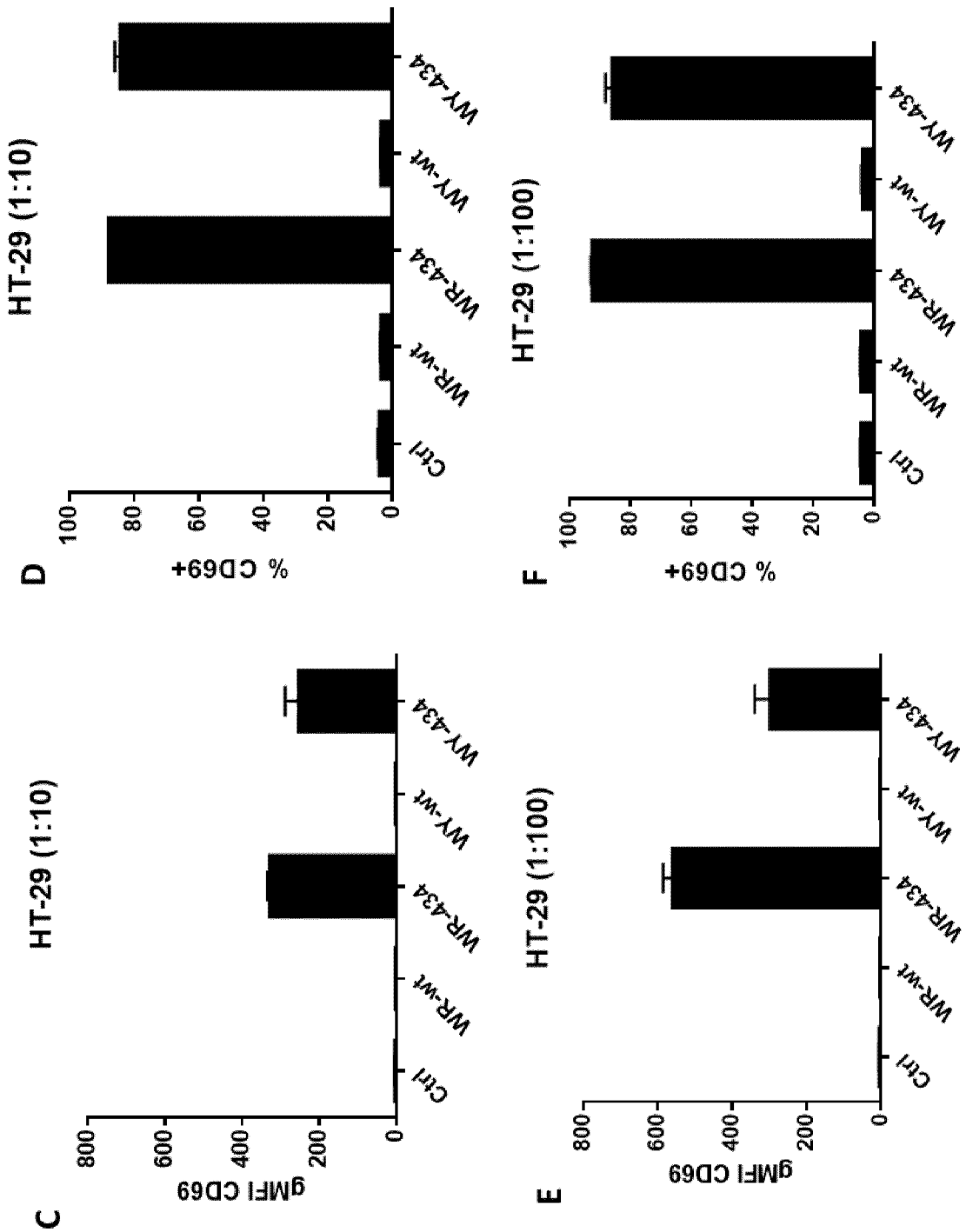

C
HT-29 (1:10)
gMFI CD69
0
200
400
600
800
Ctrl
WR-wt
WR-434
WV-wt
WV-434
D
HT-29 (1:10)
% CD69+
0
20
40
60
80
100
Ctrl
WR-wt
WR-434
WV-wt
WV-434
E
HT-29 (1:100)
gMFI CD69
0
200
400
600
800
Ctrl
WR-wt
WR-434
WV-wt
WV-434
F
HT-29 (1:100)
% CD69+
0
20
40
60
80
100
Ctrl
WR-wt
WR-434
WV-wt
WV-434

Figure 53C-F

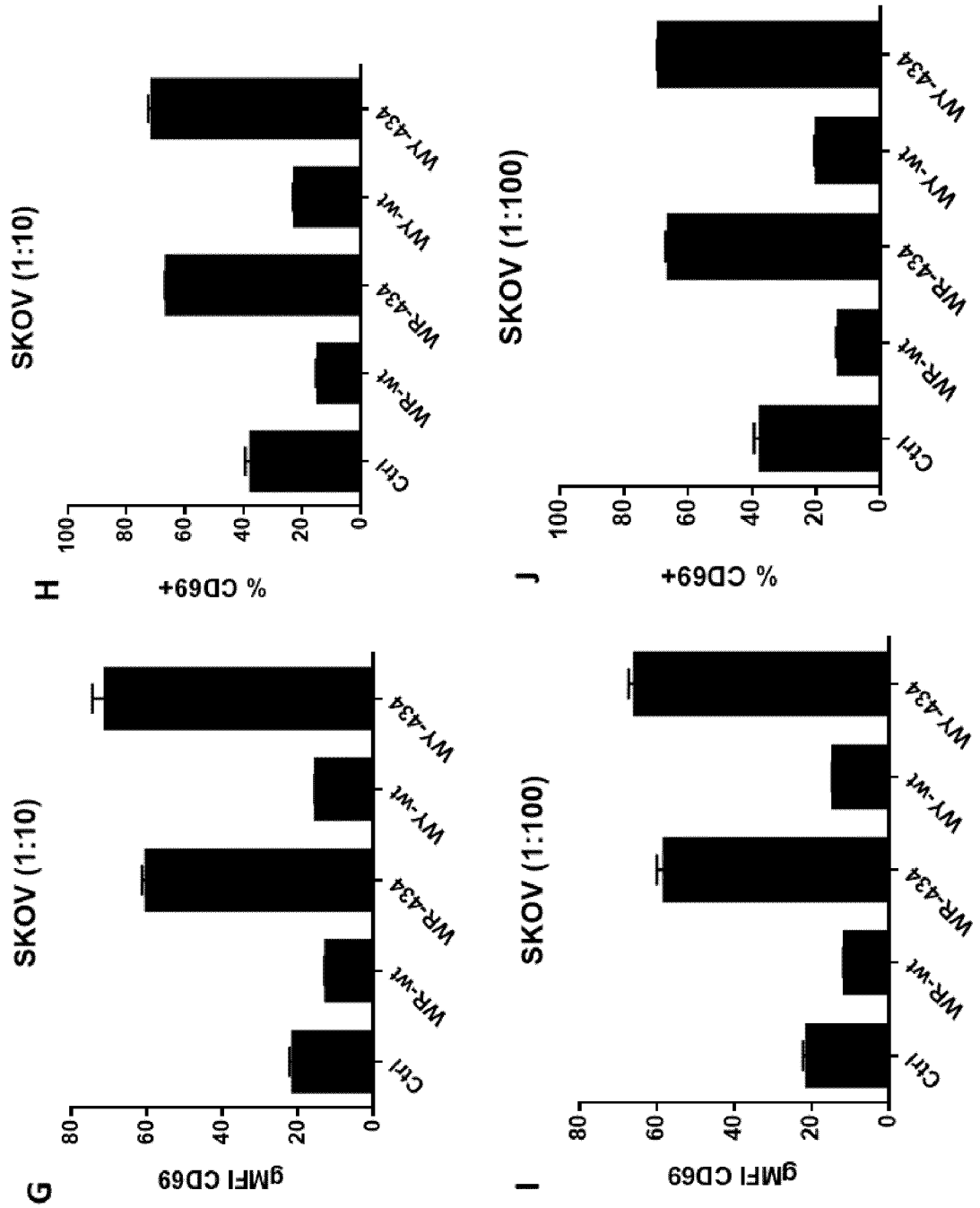

G
SKOV (1:10)
gMFI CD69
0
20
40
60
80
Ctrl
WR-wt
WR-434
WY-wt
WY-434
H
SKOV (1:10)
% CD69+
0
20
40
60
80
100
Ctrl
WR-wt
WR-434
WY-wt
WY-434
I
SKOV (1:100)
gMFI CD69
0
20
40
60
80
Ctrl
WR-wt
WR-434
WY-wt
WY-434
J
SKOV (1:100)
% CD69+
0
20
40
60
80
100
Ctrl
WR-wt
WR-434
WY-wt
WY-434

Figure 53G-J

Figure 53K

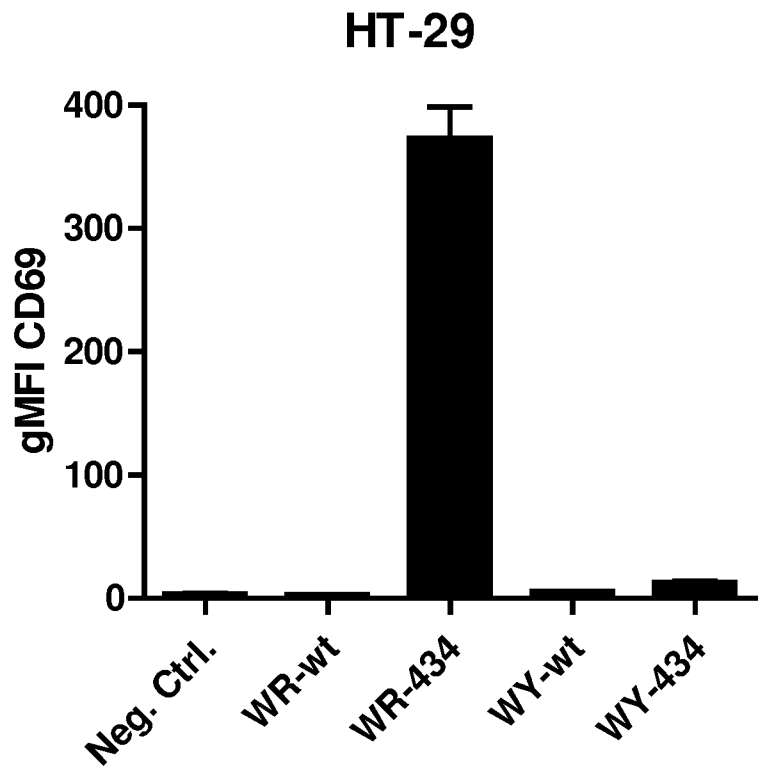
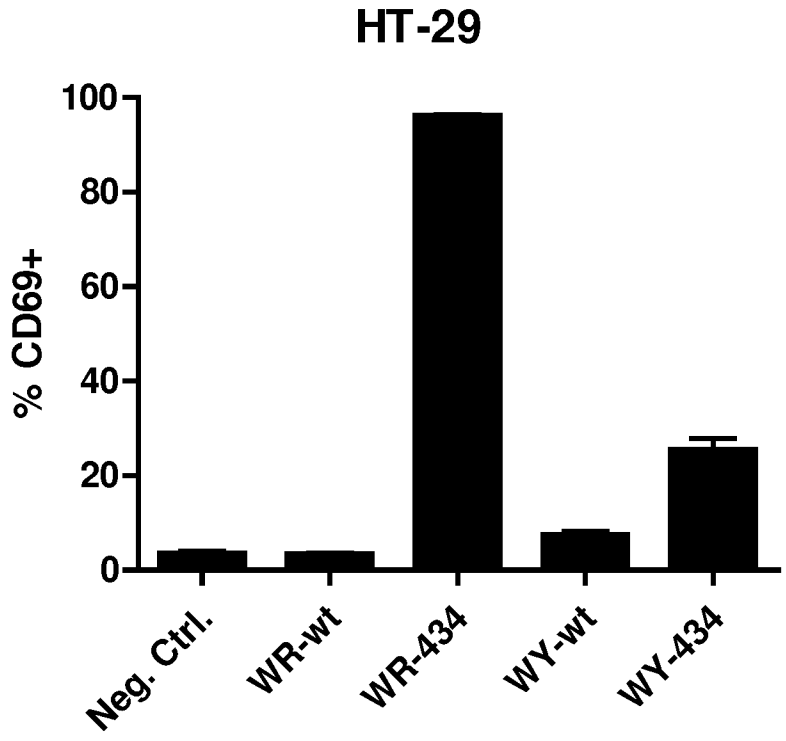


Figure 53L



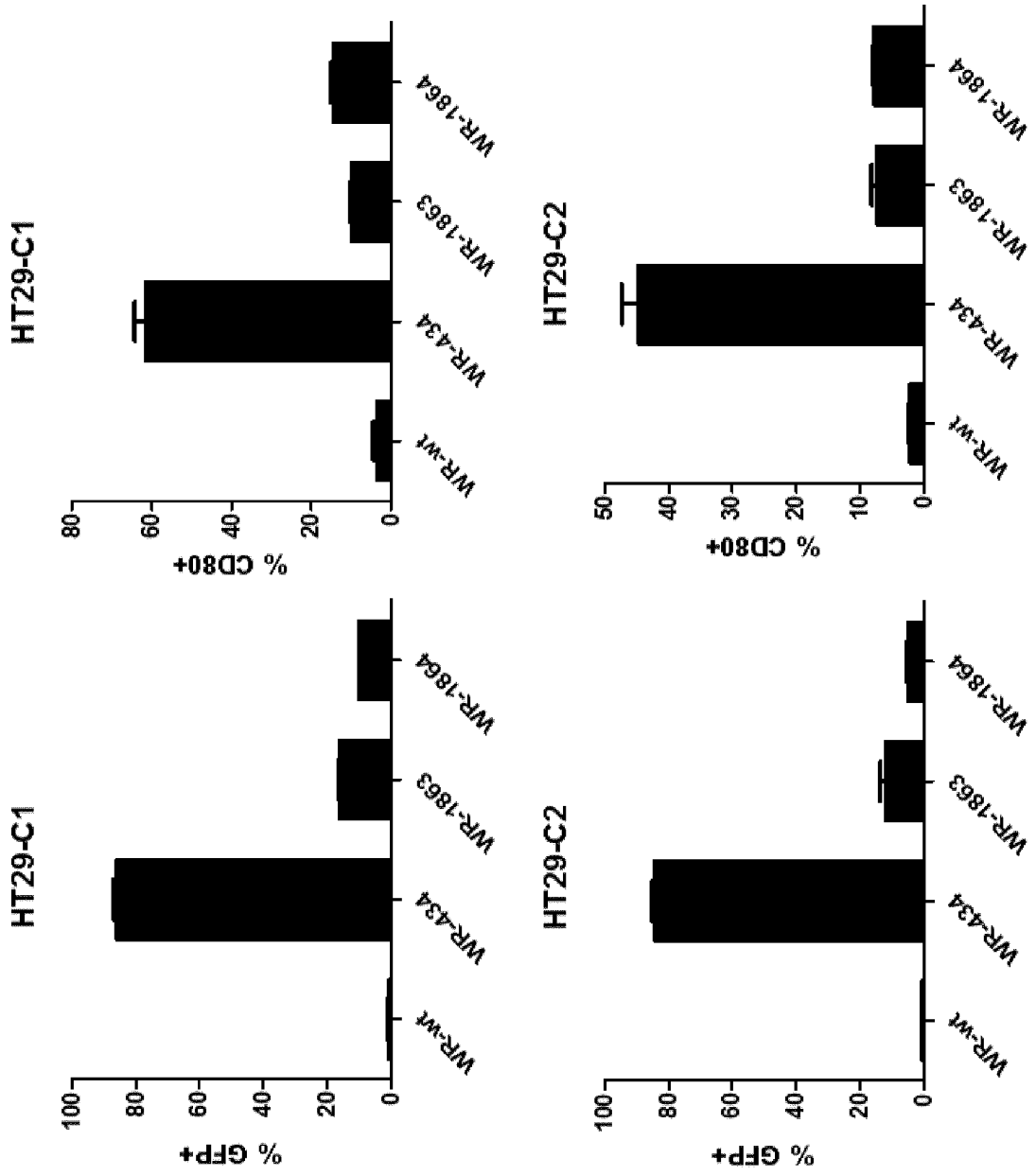

HT29-C1
% CD80+
0
20
40
60
80
WR-wt
WR-434
WR-1863
WR-1864
HT29-C2
% CD80+
0
10
20
30
40
50
WR-wt
WR-434
WR-1863
WR-1864
HT29-C1
% GFP+
0
20
40
60
80
100
WR-wt
WR-434
WR-1863
WR-1864
HT29-C2
% GFP+
0
20
40
60
80
100
WR-wt
WR-434
WR-1863
WR-1864

Figure 54A

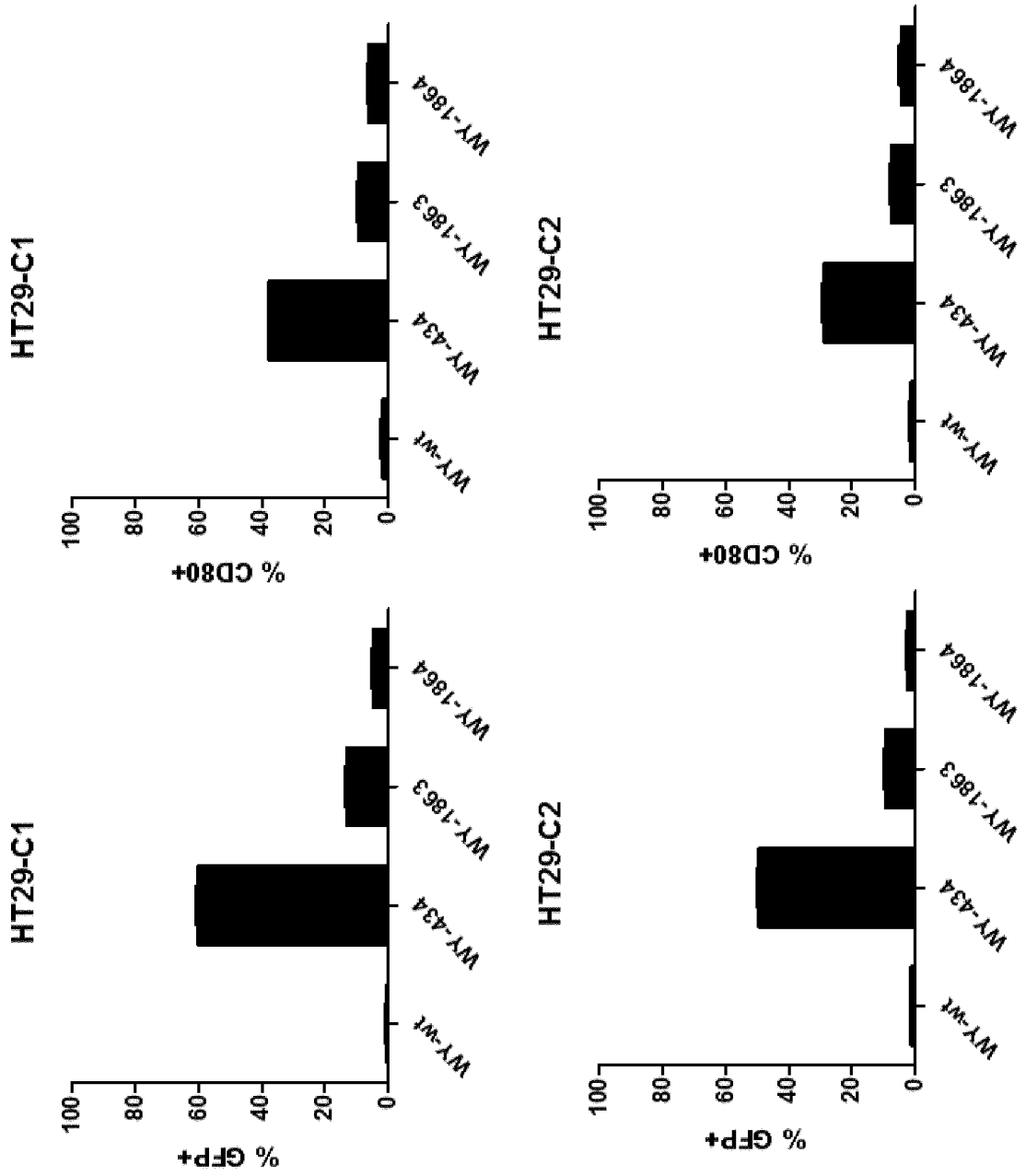


Figure 54A continued

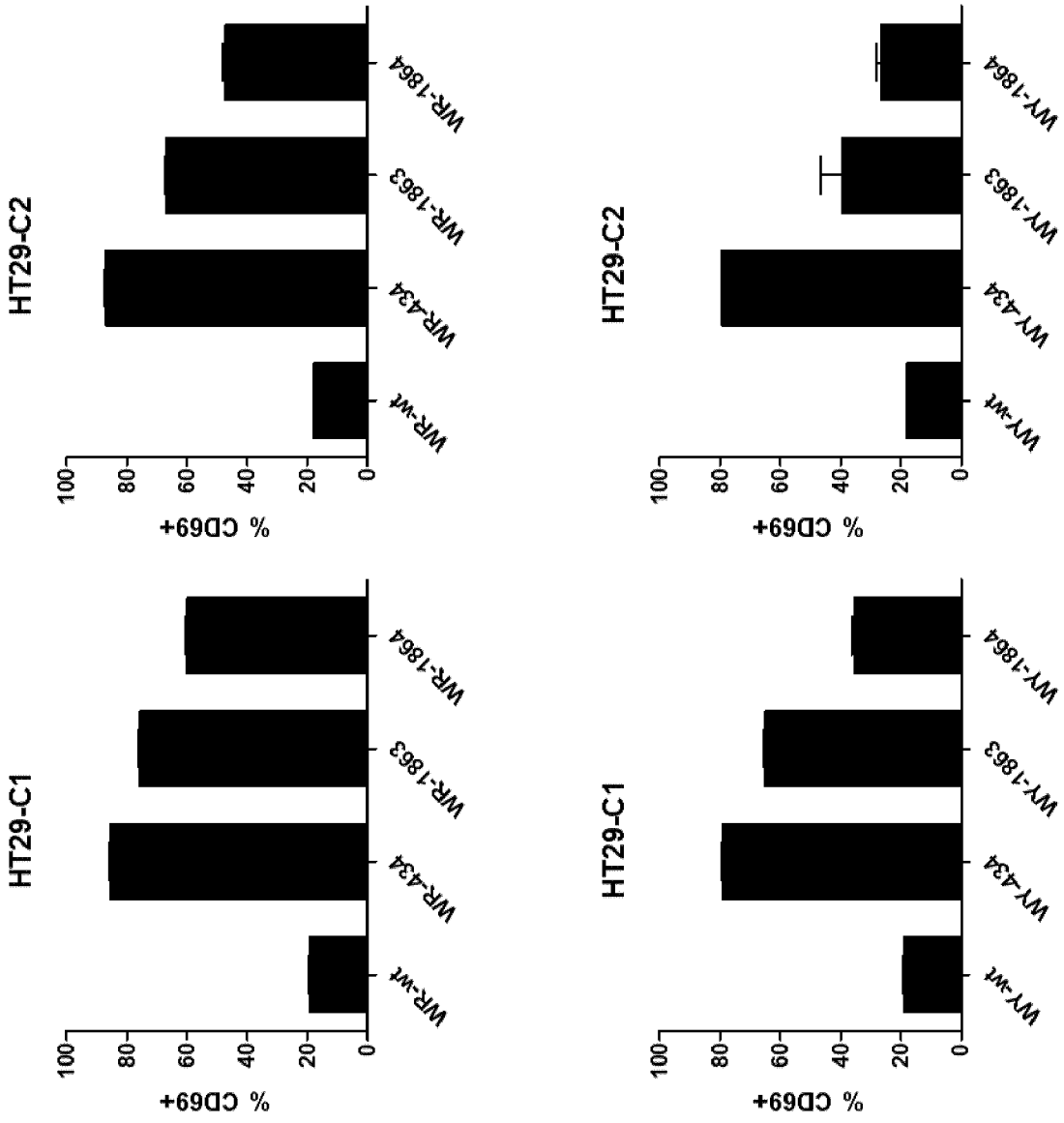

HT29-C2
% CD69+
0
20
40
60
80
100
WR-wt
WR-434
WR-1863
WR-1864
HT29-C1
% CD69+
0
20
40
60
80
100
WR-wt
WR-434
WR-1863
WR-1864
HT29-C2
% CD69+
0
20
40
60
80
100
WY-wt
WY-434
WY-1863
WY-1864
HT29-C1
% CD69+
0
20
40
60
80
100
WY-wt
WY-434
WY-1863
WY-1864

Figure 54B

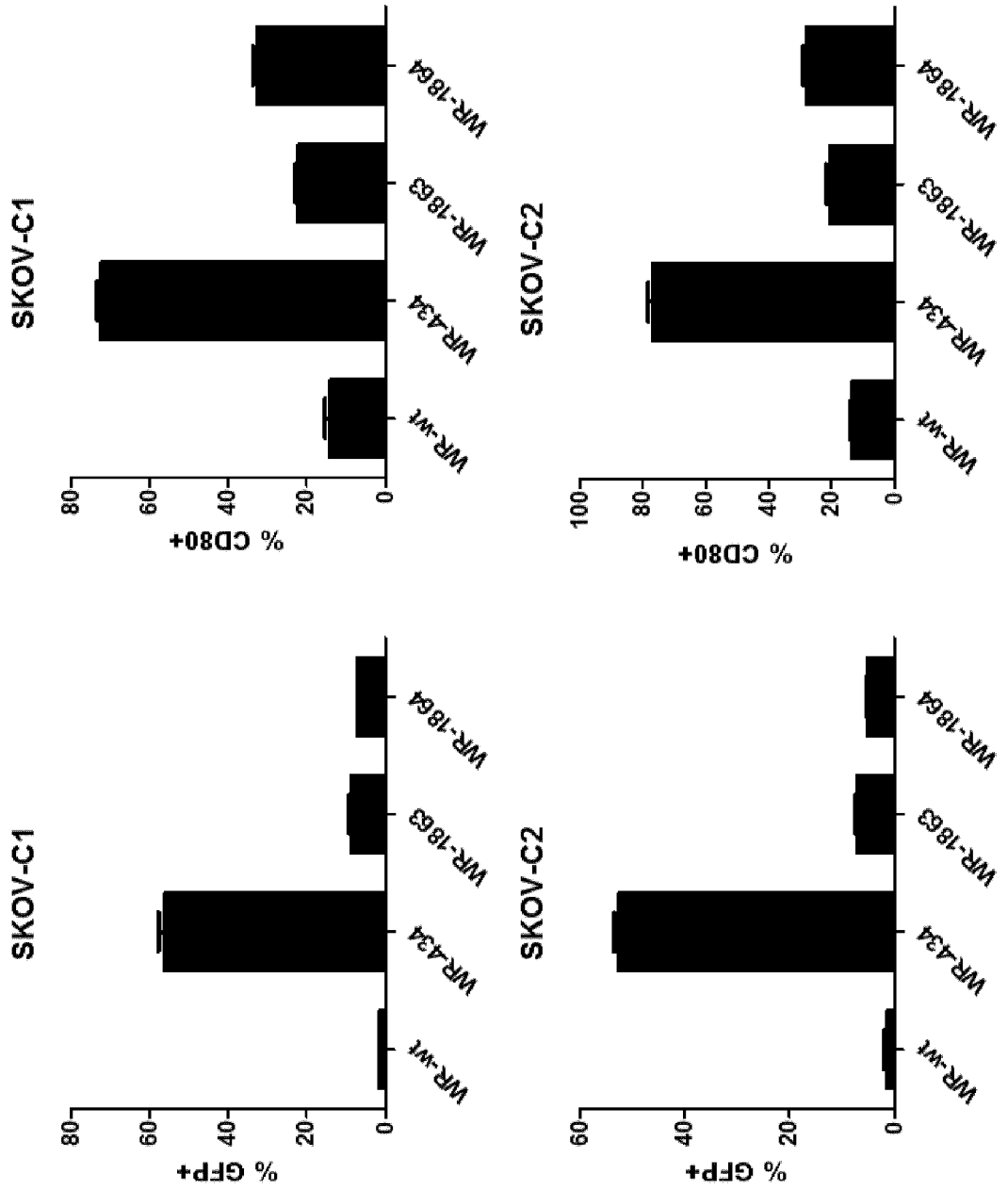

SKOV-C1
% CD80+
0
20
40
60
80
WR-wt
WR-434
WR-1863
WR-1864
SKOV-C2
% CD80+
0
20
40
60
80
100
WR-wt
WR-434
WR-1863
WR-1864
SKOV-C1
% GFP+
0
20
40
60
80
WR-wt
WR-434
WR-1863
WR-1864
SKOV-C2
% GFP+
0
20
40
60
WR-wt
WR-434
WR-1863
WR-1864

Figure 54C

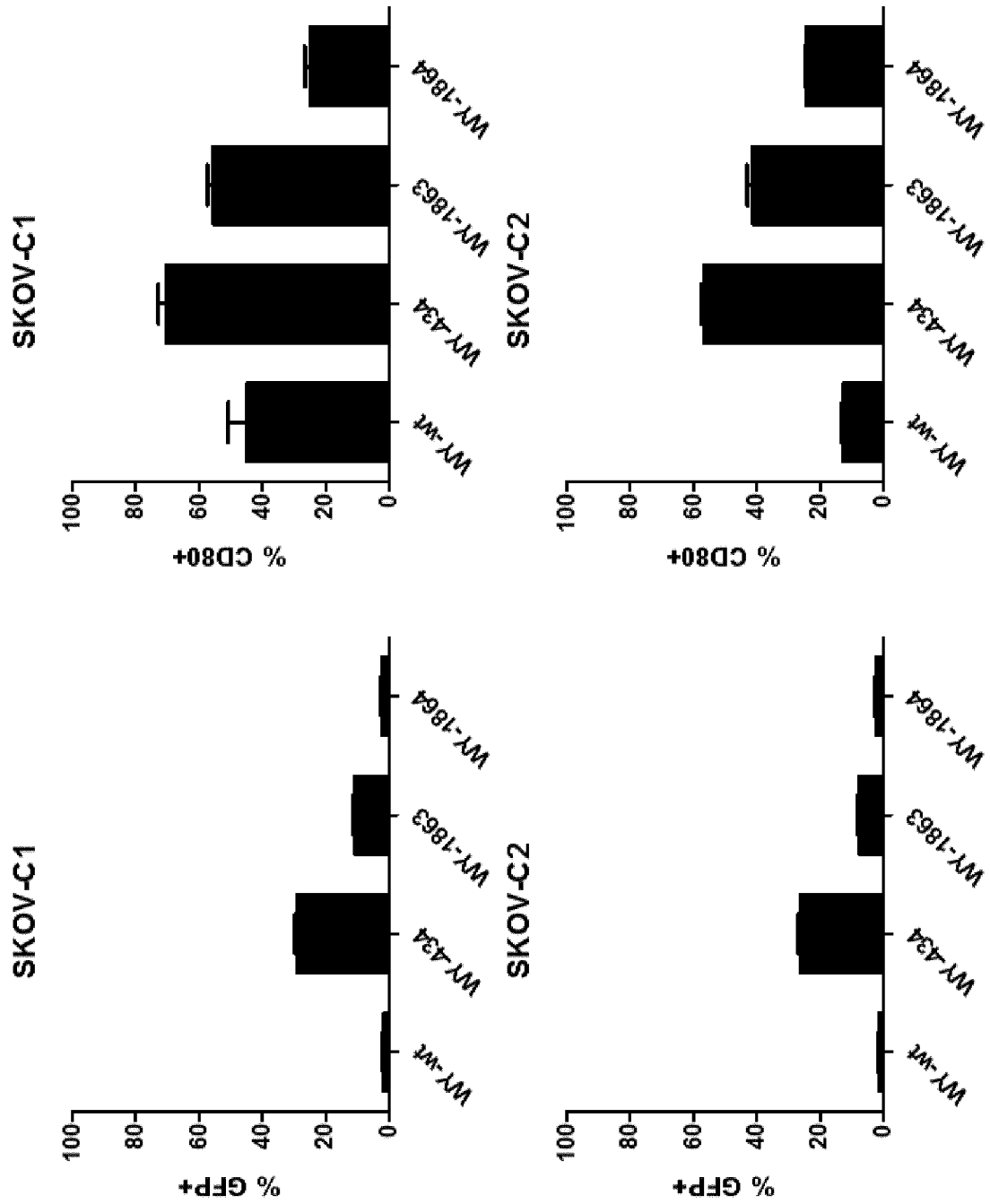


Figure 54C continued

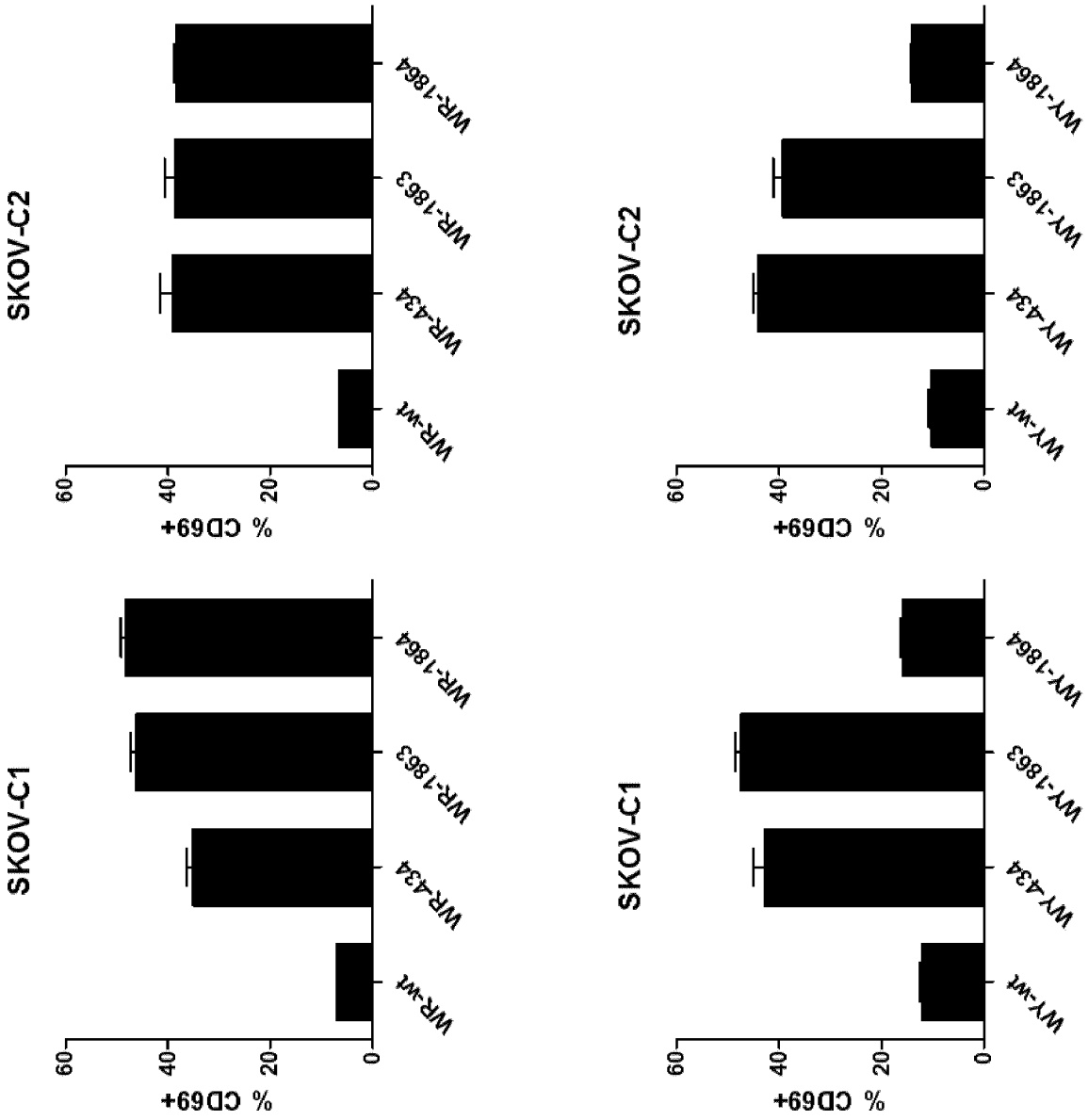

SKOV-C2
SKOV-C1
% CD69+
0
20
40
60
WR-wt
WR-434
WR-1863
WR-1864
WV-wt
WV-434
WV-1863
WV-1864

Figure 54D

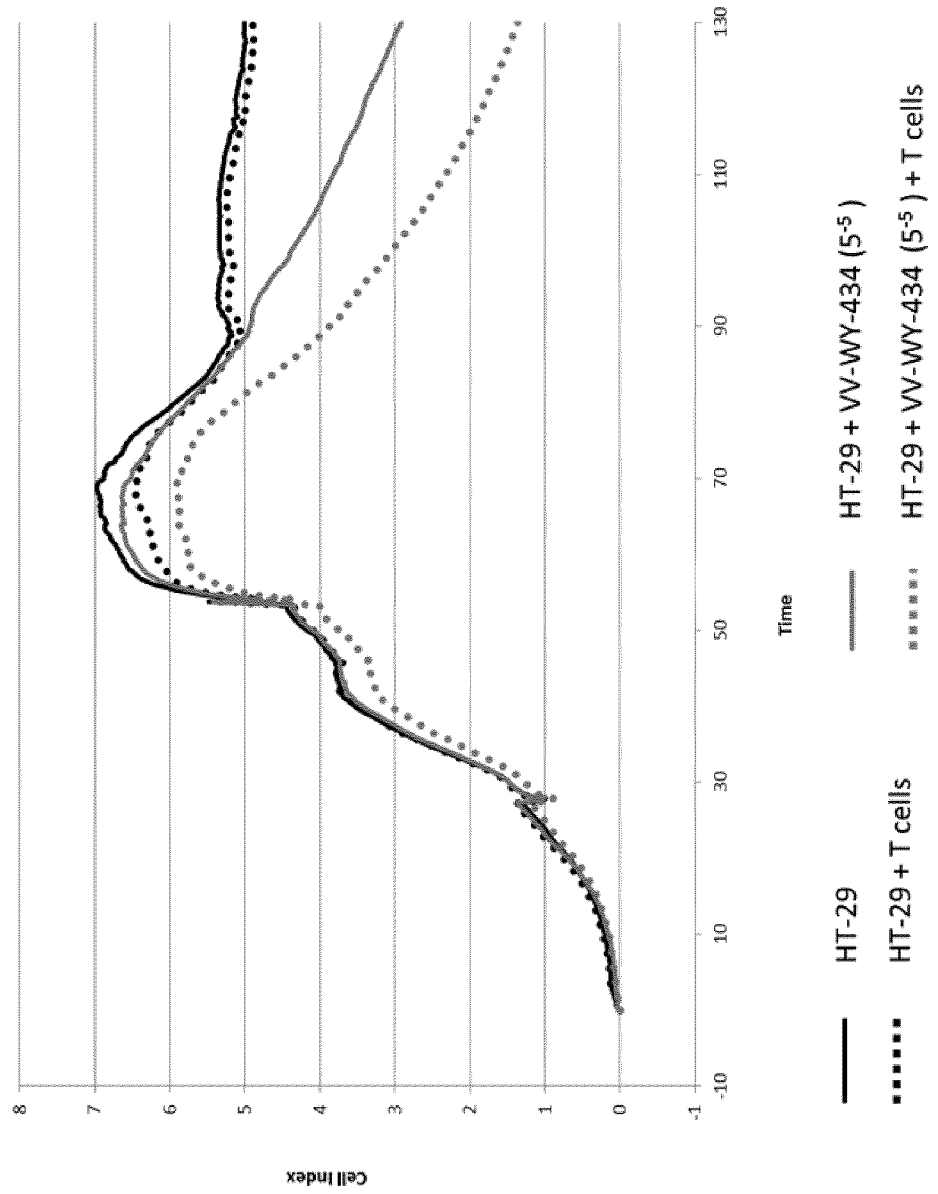

HT-29 + VV-WY-434 (C1)
Cell Index
8
7
6
5
4
3
2
1
0
-1
-10
10
30
50
70
90
110
130
Time
HT-29
HT-29 + T cells
HT-29 + VV-WY-434 (5-5)
HT-29 + VV-WY-434 (5-5) + T cells

Figure 55A

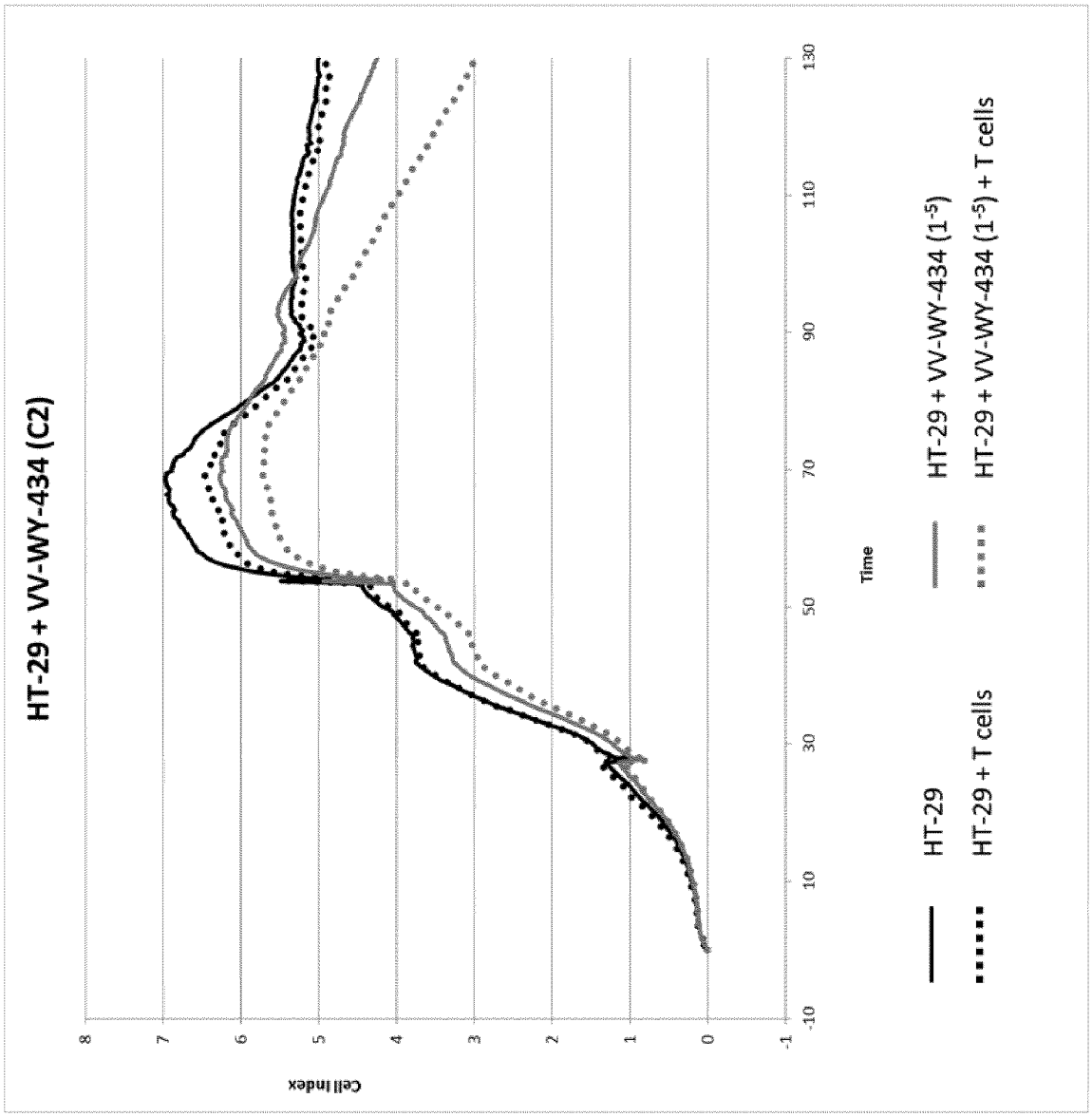

HT-29 + VV-WY-434 (C2)
Cell Index
Time
HT-29
HT-29 + T cells
HT-29 + VV-WY-434 (1^{-5})
HT-29 + VV-WY-434 (1^{-5}) + T cells

Figure 55B

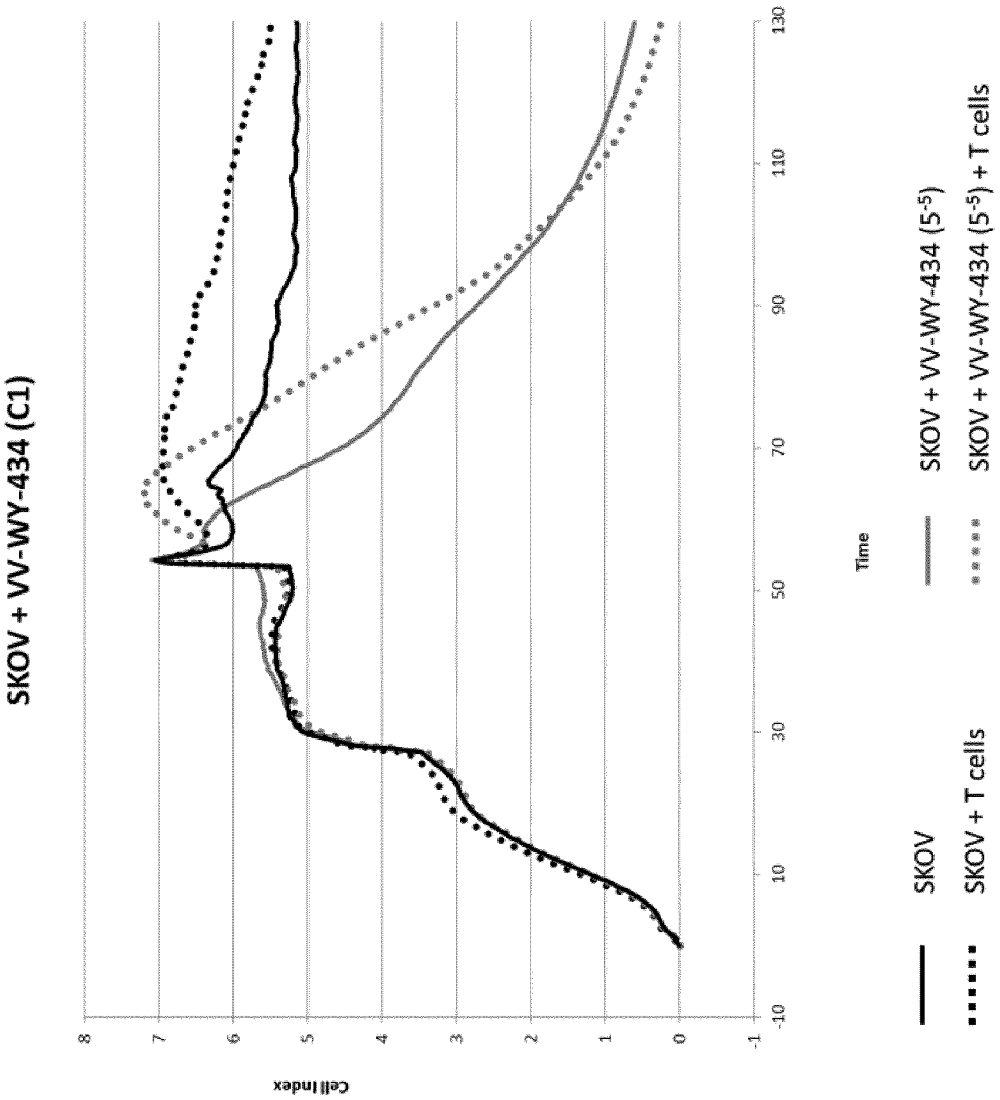

SKOV + VV-WY-434 (C1)
Cell Index
8
7
6
5
4
3
2
1
0
-1
-10
10
30
50
70
90
110
130
Time
SKOV
SKOV + T cells
SKOV + VV-WY-434 (5-5)
SKOV + VV-WY-434 (5-5) + T cells

Figure 55C

Figure 56A

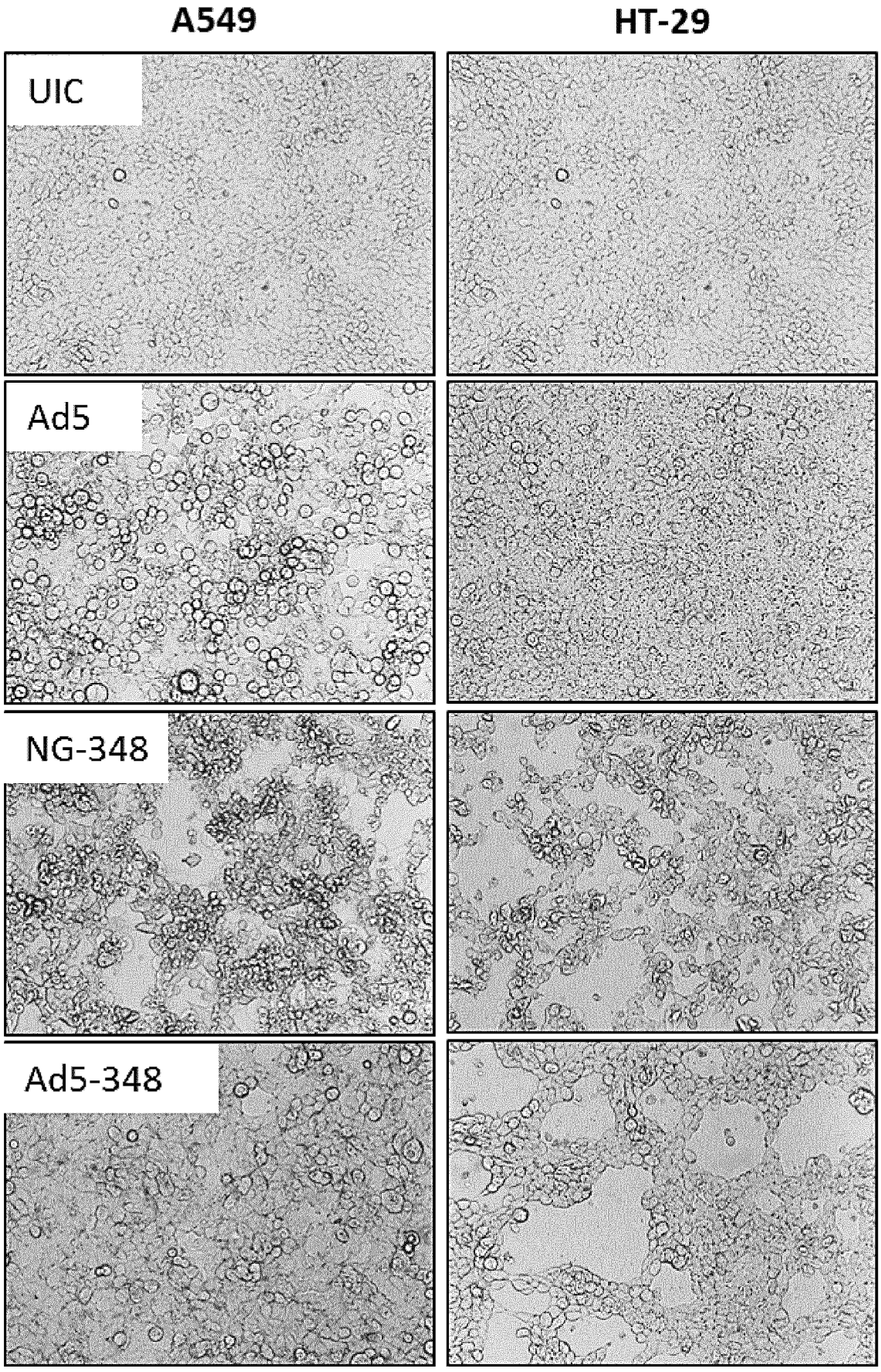


Figure 56B

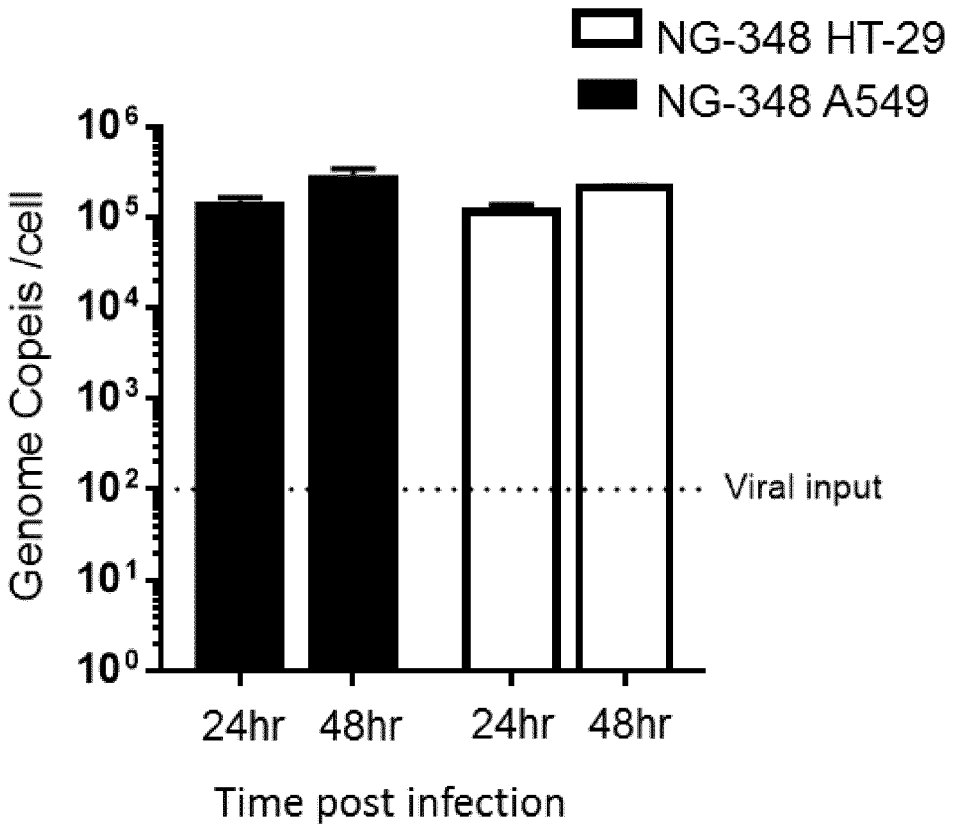
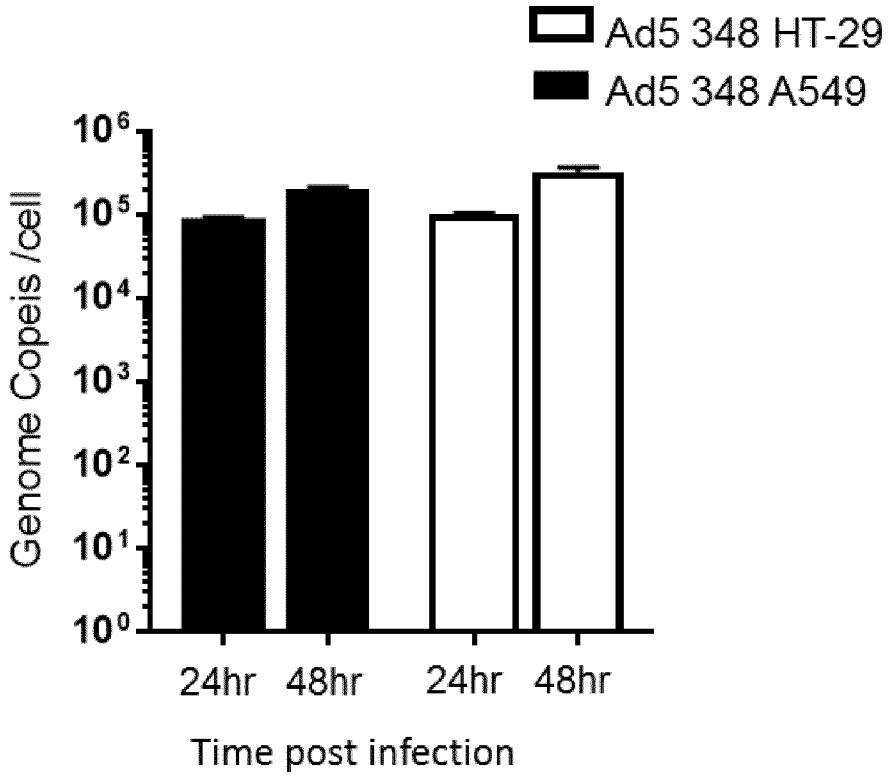


Figure 56C



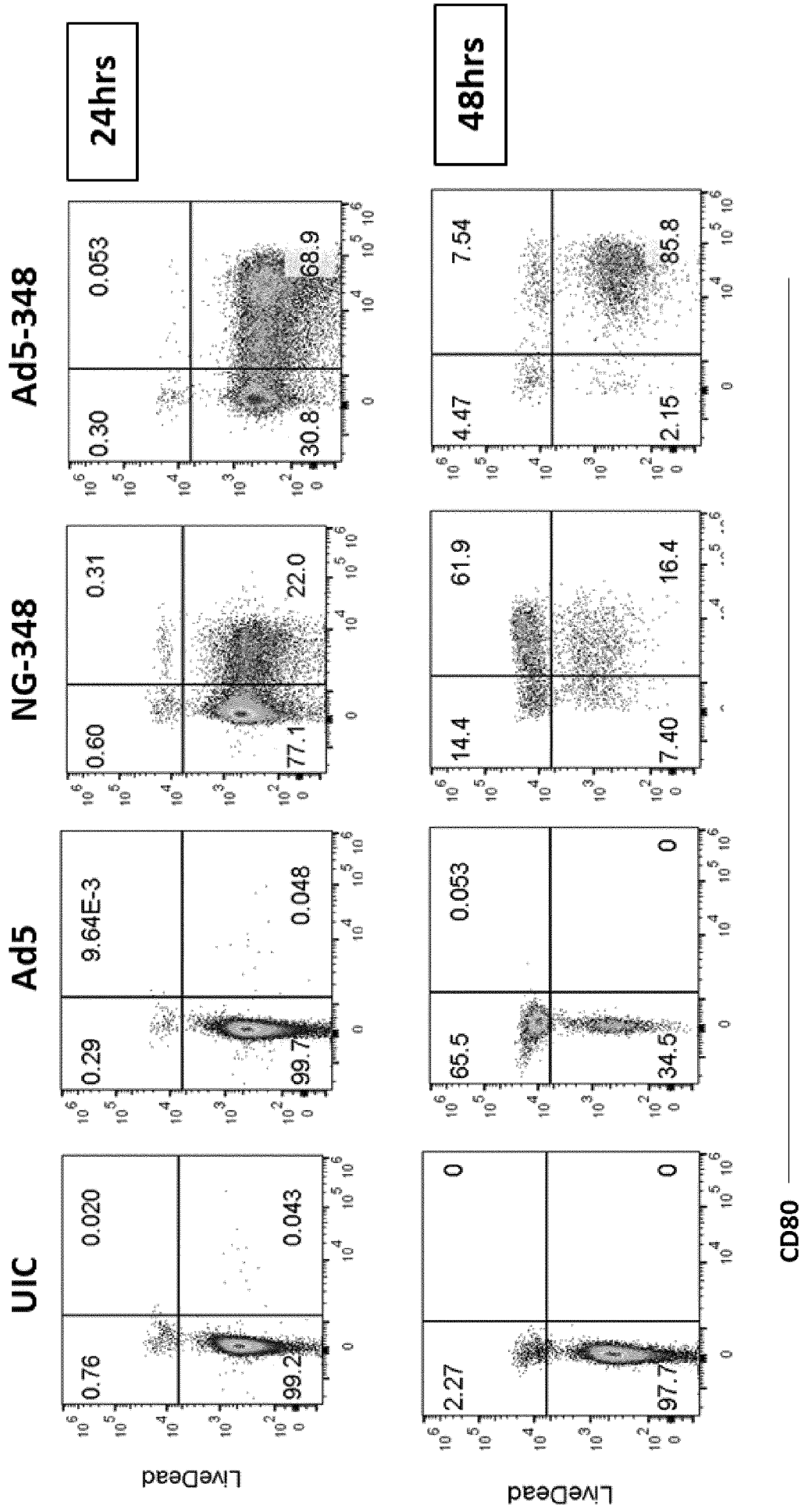

24hrs
48hrs
UIC
Ad5
NG-348
Ad5-348
LiveDead
CD80
0.76
0.020
99.2
0.043
0.29
9.64E-3
99.7
0.048
0.60
0.31
77.1
22.0
0.30
0.053
30.8
68.9
2.27
0
97.7
0
65.5
0.053
34.5
0
14.4
61.9
7.40
16.4
4.47
7.54
2.15
85.8

Figure 57A

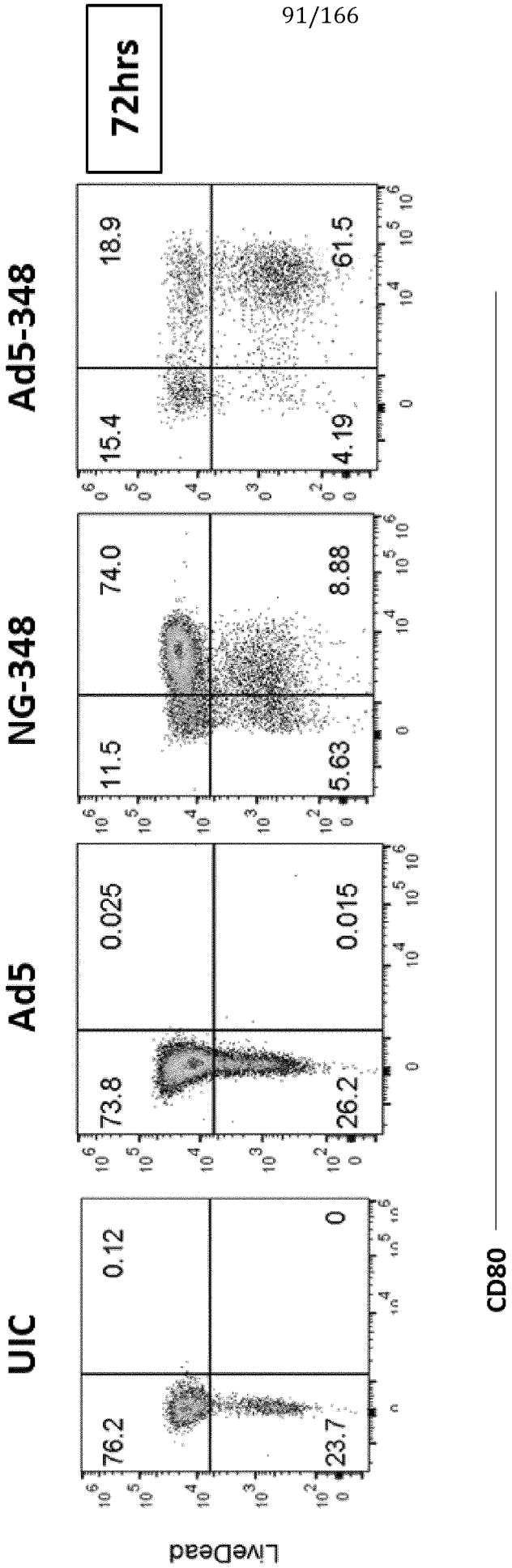


Figure 57A continued

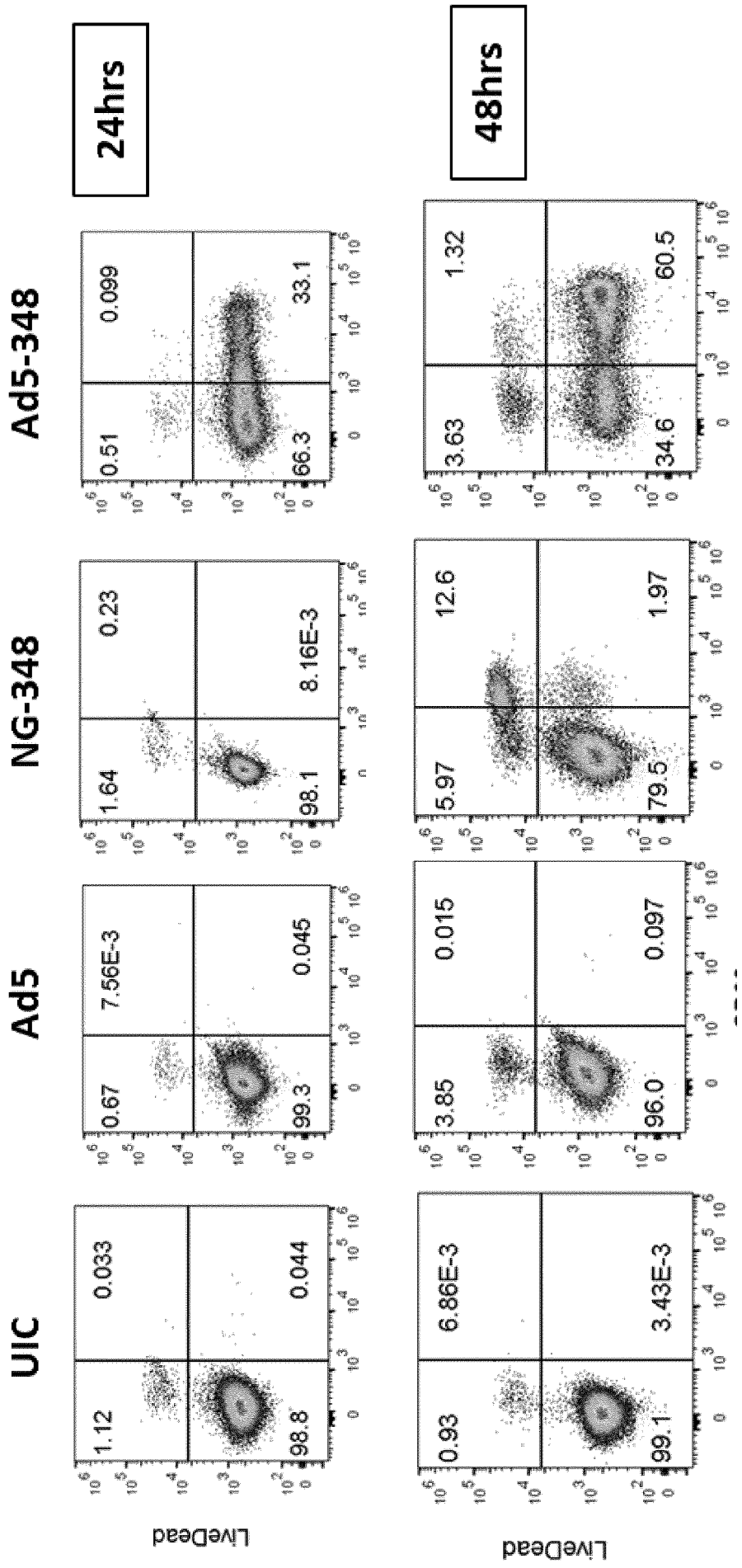

24hrs
48hrs
UIC
Ad5
NG-348
Ad5-348
LiveDead
CD80
1.12
0.033
98.8
0.044
0.67
7.56E-3
99.3
0.045
1.64
0.23
98.1
8.16E-3
0.51
0.099
66.3
33.1
0.93
6.86E-3
99.1
3.43E-3
3.85
0.015
96.0
0.097
5.97
12.6
79.5
1.97
3.63
1.32
34.6
60.5

Figure 57B

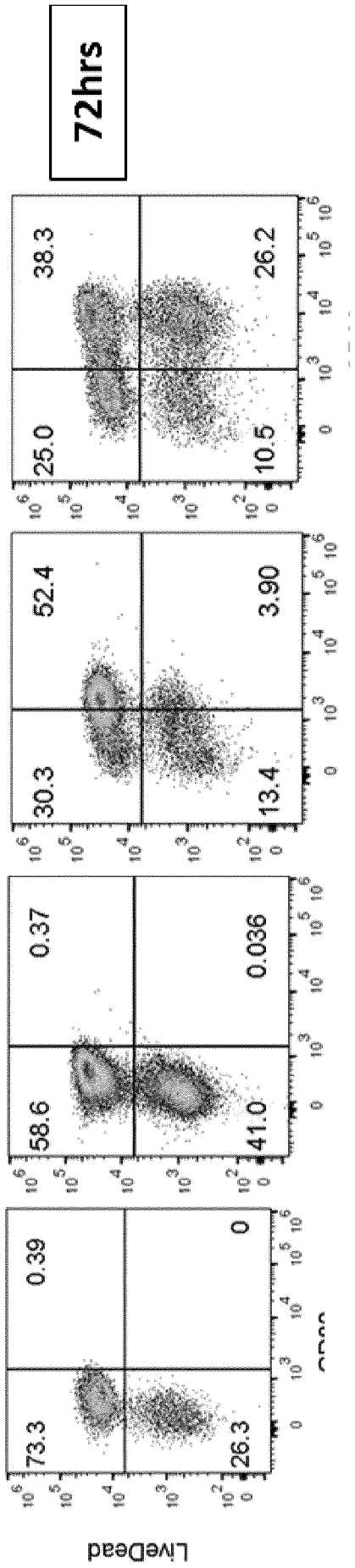


Figure 57B continued

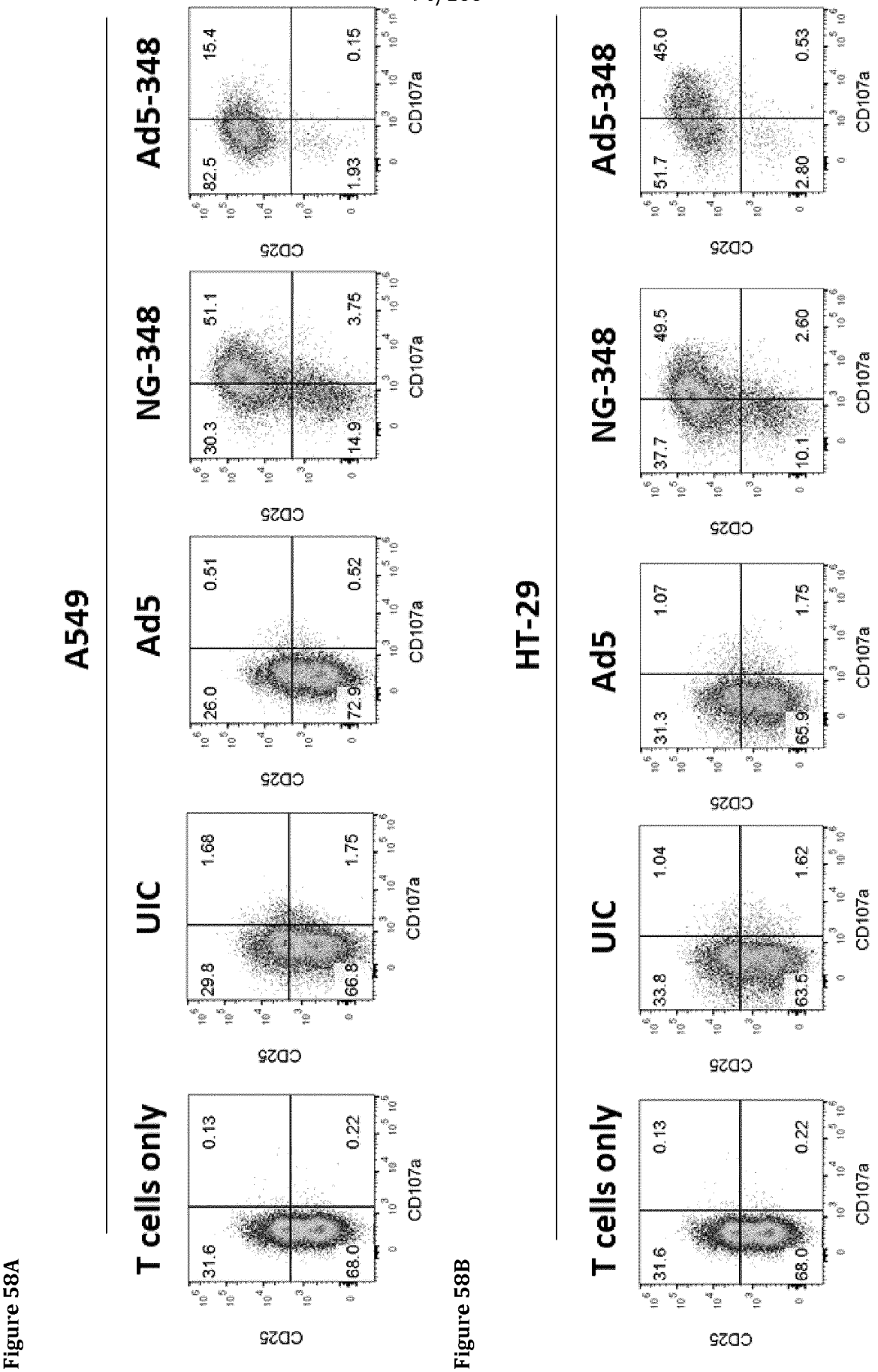


Figure 58A

Figure 58B

Figure 58C

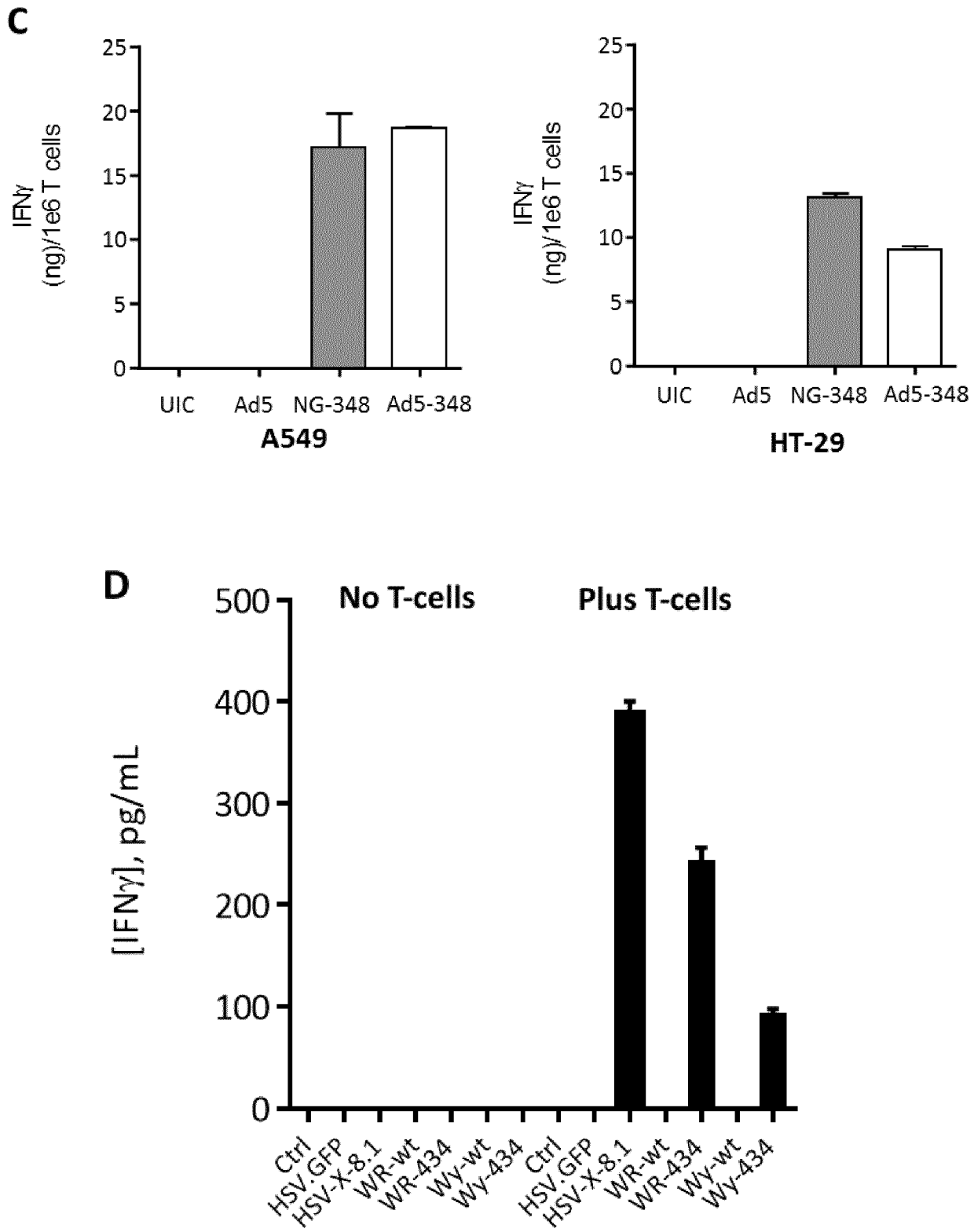

C
IFNγ (ng)/1e6 T cells
25
20
15
10
5
0
UIC
Ad5
NG-348
Ad5-348
A549
HT-29
D
No T-cells
Plus T-cells
[IFNγ], pg/mL
500
400
300
200
100
0
Ctrl
HSV.GFP
HSV-X-8.1
WR-wt
WR-434
Wy-wt
Wy-434

Figure 59

SEQ ID NO: 1 Muromonab-CD3 (OKT3) VH

QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKAT
LTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS

SEQ ID NO: 2 Muromonab-CD3 (OKT3) VL

DIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLASGVPAHFRGSGSGTS
YSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINR

SEQ ID NO: 3 Muromonab –CD3 (OKT3) single chain Fv

QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKAT
LTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSGGGGSGGGGSGGGGSDIVL
TQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLASGVPAHFRGSGSGTSYSLT
ISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINR

SEQ ID NO: 4 Membrane anchored form of the anti-human CD3 single chain Fv

MGWSCIILFLVATATGVHSQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGY
INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS
GGGGSGGGGSGGGGSDIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLA
SGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINRGSEQKLISEEDLNAVG
QDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

SEQ ID NO: 5 Membrane anchored form of anti-human CD3 single chain Fv with C-terminal V5 tag

MGWSCIILFLVATATGVHSQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGY
INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS
GGGGSGGGGSGGGGSDIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLA
SGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINRGSEQKLISEEDLNAVG
QDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPRGS**IPNPLLGLD** **Tag in bold**

SEQ ID NO: 6 Teplizumab VH sequence

QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKVKDRFT
ISRDNSKNTAFLQMDSLRPEDTGVYFCARYYDDHYCLDYWGQGTPVTVSS

Figure 60

SEQ ID NO: 7 Teplizumab VL sequence

DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTD
YTFTISSLQPEDIATYYCQQWSSNPFTFGQGTKLQITR

SEQ ID NO: 8 Teplizumab Heavy chain sequence

QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKVKDRFT
ISRDNSKNTAFLQMDSLRPEDTGVYFCARYYDDHYCLDYWGQGTPVTVSSASTKGPSVFPLAPSSKSTS
GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK
PSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 9 Teplizumab Light Chain Sequence

DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTD
YTFTISSLQPEDIATYYCQQWSSNPFTFGQGTKLQITRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF
YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC

SEQ ID NO: 15 PDGFR TM Domain

AVGQDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

SEQ ID NO: 18 PDGFR TM Domain with N-terminal c-myc tag

gsEQKLISEEDLnAVGQDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR

Figure 61

SEQ ID NO: 19 HuVH human VH leader sequence

MGWSCIILFLVATATGVHS

SEQ ID NO. 21 EnAd Genome

TCTATCTATATAATATACCTTATAGATGGAATGGTGCCAATATGTAAATGAGGTGATTTTAAAAAGTGT
GGATCGTGTGGTGATTGGCTGTGGGGTTAACGGCTAAAAGGGGCGGTGCGACCGTGGGAAAATGACGTT
TTGTGGGGGTGGAGTTTTTTTGCAAGTTGTCGCGGGAAATGTGACGCATAAAAAGGCTTTTTTCTCACG
GAACTACTTAGTTTTCCCACGGTATTTAACAGGAAATGAGGTAGTTTTGACCGGATGCAAGTGAAAATT
GTTGATTTTCGCGCGAAAACTGAATGAGGAAGTGTTTTTCTGAATAATGTGGTATTTATGGCAGGGTGG
AGTATTTGTTCAGGGCCAGGTAGACTTTGACCCATTACGTGGAGGTTTCGATTACCGTGTTTTTTACCT
GAATTTCCGCGTACCGTGTCAAAGTCTTCTGTTTTTACGTAGGTGTCAGCTGATCGCTAGGGTATTTAT
ACCTCAGGGTTTGTGTCAAGAGGCCACTCTTGAGTGCCAGCGAGAAGAGTTTTCTCCTCTGCGCCGGCA
GTTTAATAATAAAAAAATGAGAGATTTGCGATTTCTGCCTCAGGAAATAATCTCTGCTGAGACTGGAAA
TGAAATATTGGAGCTTGTGGTGCACGCCCTGATGGGAGACGATCCGGAGCCACCTGTGCAGCTTTTTGA
GCCTCCTACGCTTCAGGAACTGTATGATTTAGAGGTAGAGGGATCGGAGGATTCTAATGAGGAAGCTGT
AAATGGCTTTTTTACCGATTCTATGCTTTTAGCTGCTAATGAAGGGTTAGAATTAGATCCGCCTTTGGA
CACTTTTGATACTCCAGGGGTAATTGTGGAAAGCGGTACAGGTGTAAGAAAATTACCTGATTTGAGTTC
CGTGGACTGTGATTTGCACTGCTATGAAGACGGGTTTCCTCCGAGTGATGAGGAGGACCATGAAAAGGA
GCAGTCCATGCAGACTGCAGCGGGTGAGGGAGTGAAGGCTGCCAATGTTGGTTTTCAGTTGGATTGCCC
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TGTTAGAGAACGCTTCGGACGGAGTCTCCGGTTTTTGGAGATTCTGGTTCGCTAGTGAATTAGCTAGGG
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TCATGCCAGCGGTTCTGGAGGAGGAACAGCAAGAGGACAACCCGAGAGCCGGCCTGGACCCTCCAGTGG
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TGGATGTTTTATTTTGATTAAGGGAAATGCCAGCGTAAAGCATAACATGATTTGCGGTGCTTCCGATGA
GAGGCCTTATCAAATGCTCACTTGTGCTGGTGGGCATTGTAATATGCTGGCTACTGTGCATATTGTTTC
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GCGTAGAGGAATGTTTATGCCTTACCAGTGTAACATGAATCATGTGAAAGTGTTGTTGGAACCAGATGC
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CAAACTGAGTCTGCTGTCGGCACGGCAAAGTCTAAATAAAAAAATCCCAGAATCAATGAATAAATAAAC
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GTTTGGACGGCTCCTGGAATAGGGTATGAGACGATGGGCGTCCAGCGCTGCCAGGGTTCGGTCCTTCCA
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TACCCGTTTCTTGTAGGCGGGGTTGGGCAAAGCGAAAGTAACATCATTGAAGAGAATCTTGCCGGCCCT
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CCGCTGGTCTCCAGAGAGTTTCATGACCAGCATGAAGGGGATTAGCTGCTTGCCAAAGGACCCCATCCA
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CTTTTGGAGGGCGTGCGGGAGGTTCAGATAGTACTTGATCTCAACGGGTCCGTTGGTGGAGATGTCGAT
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AGGATTTCTTGCACGTCACCAGAGTTGTCCTGGTAGGCGATCTCCGCCATGAACTGCTCGATCTCTTCC
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CACTGTGTTGGTGAAAAAGCACAGCTAGATCAAAAGAAATGCGATTTTCAAGGTGCTCAACGGTGGCTT
CCAGCAAAGCCTCCACGCGCACATCCAAGAACAAAAGAATACCAAAAGAAGGAGCATTTTCTAACTCCT
CAATCATCATATTACATTCCTGCACCATTCCCAGATAATTTTCAGCTTTCCAGCCTTGAATTATTCGTG
TCAGTTCTTGTGGTAAATCCAATCCACACATTACAAACAGGTCCCGGAGGGCGCCCTCCACCACCATTC
TTAAACACACCCTCATAATGACAAAATATCTTGCTCCTGTGTCACCTGTAGCGAATTGAGAATGGCAAC
ATCAATTGACATGCCCTTGGCTCTAAGTTCTTCTTTAAGTTCTAGTTGTAAAAACTCTCTCATATTATC
ACCAAACTGCTTAGCCAGAAGCCCCCCGGGAACAAGAGCAGGGGACGCTACAGTGCAGTACAAGCGCAG
ACCTCCCCAATTGGCTCCAGCAAAAACAAGATTGGAATAAGCATATTGGGAACCGCCAGTAATATCATC
GAAGTTGCTGGAAATATAATCAGGCAGAGTTTCTTGTAAAAATTGAATAAAAGAAAAATTTGCCAAAAA
AACATTCAAAACCTCTGGGATGCAAATGCAATAGGTTACCGCGCTGCGCTCCAACATTGTTAGTTTTGA
ATTAGTCTGCAAAAATAAAAAAAAAAACAAGCGTCATATCATAGTAGCCTGACGAACAGATGGATAAAT
CAGTCTTTCCATCACAAGACAAGCCACAGGGTCTCCAGCTCGACCCTCGTAAAACCTGTCATCATGATT
AAACAACAGCACCGAAAGTTCCTCGCGGTGACCAGCATGAATAATTCTTGATGAAGCATACAATCCAGA
CATGTTAGCATCAGTTAACGAGAAAAAACAGCCAACATAGCCTTTGGGTATAATTATGCTTAATCGTAA
GTATAGCAAAGCCACCCCTCGCGGATACAAAGTAAAAGGCACAGGAGAATAAAAAATATAATTATTTCT
CTGCTGCTGTTCAGGCAACGTCGCCCCCGGTCCCTCTAAATACACATACAAAGCCTCATCAGCCATGGC

TTACCAGACAAAGTACAGCGGGCACACAAAGCACAAGCTCTAAAGTGACTCTCCAACCTCTCCACAATA
TATATATACACAAGCCCTAAACTGACGTAATGGGAGTAAAGTGTAAAAAATCCCGCCAAACCCAACACA
CACCCCGAAACTGCGTCACCAGGGAAAAGTACAGTTTCACTTCCGCAATCCCAACAGGCGTAACTTCCT
CTTTCTCACGGTACGTGATATCCCACTAACTTGCAACGTCATTTTCCCACGGTCGCACCGCCCCTTTTA
GCCGTTAACCCCACAGCCAATCACCACACGATCCACACTTTTTAAAATCACCTCATTTACATATTGGCA
CCATTCCATCTATAAGGTATATTATATAGATAGA

Figure 62

SEQ ID NO: 87 DNA CORRESPONDING TO E2B REGION OF THE ENAD GENOME (BP 10355-5068)

CTATGGCATCTCGATCCAGCAGACCTCCTCGTTTCGCGGGTTTGGACGGCTCCTGGAATAGGGTATGAG
ACGATGGGCGTCCAGCGCTGCCAGGGTTCGGTCCTTCCAGGGTCTCAGTGTTCGAGTCAGGGTTGTTTC
CGTCACAGTGAAGGGGTGTGCGCCTGCTTGGGCGCTTGCCAGGGTGCGCTTCAGACTCATCCTGCTGGT
CGAAAACTTCTGTCGCTTGGCGCCCTGTATGTCGGCCAAGTAGCAGTTTACCATGAGTTCGTAGTTGAG
CGCCTCGGCTGCGTGGCCTTTGGCGCGGAGCTTACCTTTGGAAGTTTTCTTGCATACCGGGCAGTATAG
GCATTTCAGCGCATACAACTTGGGCGCAAGGAAAACGGATTCTGGGGAGTATGCATCTGCGCCGCAGGA
GGCGCAAACAGTTTCACATTCCACCAGCCAGGTTAAATCCGGTTCATTGGGGTCAAAAACAAGTTTTCC
GCCATATTTTTTGATGCGTTTCTTACCTTTGGTCTCCATGAGTTCGTGTCCTCGTTGAGTGACAAACAG
GCTGTCCGTGTCCCCGTAGACTGATTTTACAGGCCTCTTCTCCAGTGGAGTGCCTCGGTCTTCTTCGTA
CAGGAACTCTGACCACTCTGATACAAAGGCGCGCGTCCAGGCCAGCACAAAGGAGGCTATGTGGGAGGG
GTAGCGATCGTTGTCAACCAGGGGGTCCACCTTTTCCAAAGTATGCAAACACATGTCACCCTCTTCAAC
ATCCAGGAATGTGATTGGCTTGTAGGTGTATTTCACGTGACCTGGGGTCCCCGCTGGGGGGGTATAAAA
GGGGGCGGTTCTTTGCTCTTCCTCACTGTCTTCCGGATCGCTGTCCAGGAACGTCAGCTGTTGGGGTAG
GTATTCCCTCTCGAAGGCGGGCATGACCTCTGCACTCAGGTTGTCAGTTTCTAAGAACGAGGAGGATTT
GATATTGACAGTGCCGGTTGAGATGCCTTTCATGAGGTTTTCGTCCATCTGGTCAGAAAACACAATTTT
TTTATTGTCAAGTTTGGTGGCAAATGATCCATACAGGGCGTTGGATAAAAGTTTGGCAATGGATCGCAT
GGTTTGGTTCTTTTCCTTGTCCGCGCGCTCTTTGGCGGCGATGTTGAGTTGGACATACTCGCGTGCCAG
GCACTTCCATTCGGGGAAGATAGTTGTTAATTCATCTGGCACGATTCTCACTTGCCACCCTCGATTATG
CAAGGTAATTAAATCCACACTGGTGGCCACCTCGCCTCGAAGGGGTTCATTGGTCCAACAGAGCCTACC
TCCTTTCCTAGAACAGAAAGGGGGAAGTGGGTCTAGCATAAGTTCATCGGGAGGGTCTGCATCCATGGT
AAAGATTCCCGGAAGTAAATCCTTATCAAAATAGCTGATGGGAGTGGGGTCATCTAAGGCCATTTGCCA
TTCTCGAGCTGCCAGTGCGCGCTCATATGGGTTAAGGGGACTGCCCCATGGCATGGGATGGGTGAGTGC
AGAGGCATACATGCCACAGATGTCATAGACGTAGATGGGATCCTCAAAGATGCCTATGTAGGTTGGATA
GCATCGCCCCCCTCTGATACTTGCTCGCACATAGTCATATAGTTCATGTGATGGCGCTAGCAGCCCCGG
ACCCAAGTTGGTGCGATTGGGTTTTTCTGTTCTGTAGACGATCTGGCGAAAGATGGCGTGAGAATTGGA
AGAGATGGTGGGTCTTTGAAAAATGTTGAAATGGGCATGAGGTAGACCTACAGAGTCTCTGACAAAGTG
GGCATAAGATTCTTGAAGCTTGGTTACCAGTTCGGCGGTGACAAGTACGTCTAGGGCGCAGTAGTCAAG
TGTTTCTTGAATGATGTCATAACCTGGTTGGTTTTTCTTTTCCCACAGTTCGCGGTTGAGAAGGTATTC
TTCGCGATCCTTCCAGTACTCTTCTAGCGGAAACCCGTCTTTGTCTGCACGGTAAGATCCTAGCATGTA
GAACTGATTAACTGCCTTGTAAGGGCAGCAGCCCTTCTCTACGGGTAGAGAGTATGCTTGAGCAGCTTT
TCGTAGCGAAGCGTGAGTAAGGGCAAAGGTGTCTCTGACCATGACTTTGAGGAATTGGTATTTGAAGTC
GATGTCGTCACAGGCTCCCTGTTCCCAGAGTTGGAAGTCTACCCGTTTCTTGTAGGCGGGGTTGGGCAA
AGCGAAAGTAACATCATTGAAGAGAATCTTGCCGGCCCTGGGCATGAAATTGCGAGTGATGCGAAAAGG
CTGTGGTACTTCCGCTCGGTTATTGATAACCTGGGCAGCTAGGACGATCTCGTCGAAACCGTTGATGTT
GTGTCCTACGATGTATAATTCTATGAAACGCGGCGTGCCTCTGACGTGAGGTAGCTTACTGAGCTCATC
AAAGGTTAGGTCTGTGGGGTCAGATAAGGCGTAGTGTTCGAGAGCCCATTCGTGCAGGTGAGGATTCGC
TTTAAGGAAGGAGGACCAGAGGTCCACTGCCAGTGCTGTTTGTAACTGGTCCCGGTACTGACGAAAATG
CCGTCCGACTGCCATTTTTTCTGGGGTGACGCAATAGAAGGTTTGGGGGTCCTGCCGCCAGCGATCCCA
CTTGAGTTTTATGGCGAGGTCATAGGCGATGTTGACGAGCCGCTGGTCTCCAGAGAGTTTCATGACCAG
CATGAAGGGGATTAGCTGCTTGCCAAAGGACCCCATCCAGGTGTAGGTTTCCACATCGTAGGTGAGAAA

GAGCCTTTCTGTGCGAGGATGAGAGCCAATCGGGAAGAACTGGATCTCCTGCCACCAGTTGGAGGAATG
GCTGTTGATGTGATGGAAGTAGAACTCCCTGCGACGCGCCGAGCATTCATGCTTGTGCTTGTACAGACG
GCCGCAGTAGTCGCAGCGTTGCACGGGTTGTATCTCGTGAATGAGTTGTACCTGGCTTCCCTTGACGAG
AAATTTCAGTGGGAAGCCGAGGCCTGGCGATTGTATCTCGTGCTTTACTATGTTGTCTGCATCGGCCTG
TTCATCTTCTGTCTCGATGGTGGTCATGCTGACGAGCCCTCGCGGGAGGCAAGTCCAGACCTCGGCGCG
GCAGGGGCGGAGCTCGAGGACGAGAGCGCGCAGGCTGGAGCTGTCCAGGGTCCTGAGACGCTGCGGACT
CAGGTTAGTAGGCAGTGTCAGGAGATTAACTTGCATGATCTTTTGGAGGGCGTGCGGGAGGTTCAGATA
GTACTTGATCTCAACGGGTCCGTTGGTGGAGATGTCGATGGCTTGCAGGGTTCCGTGTCCCTTGGGCGC
TACCACCGTGCCCTTGTTTTTCATTTTGGACGGCGGTGGCTCTGTTGCTTCTTGCATGTTTAGAAGCGG
TGTCGAGGGCGCGCACCGGGCGGCAGGGGCGGCTCGGGACCCGGCGGCATGGCTGGCAGTGGTACGTCG
GCGCCGCGCGCGGGTAGGTTCTGGTACTGCGCCCTGAGAAGACTCGCATGCGCGACGACGCGGCGGTTG
ACATCCTGGATCTGACGCCTCTGGGTGAAAGCTACCGGCCCCGTGAGCTTGAACCTGAAAGAGAGTTCA
ACAGAATCAATCTCGGTATCGTTGACGGCGGCTTGCCTAAGGATTTCTTGCACGTCACCAGAGTTGTCC
TGGTAGGCGATCTCCGCCATGAACTGCTCGATCTCTTCCTCTTGAAGATCTCCGCGGCCCGCTCTCTCG
ACGGTGGCCGCGAGGTCGTTGGAGATGCGCCCAATGAGTTGAGAGAATGCATTCATGCCCGCCTCGTTC
CAGACGCGGCTGTAGACCACGGCCCCCACGGGATCTCTCGCGCGCATGACCACCTGGGCGAGGTTGAGC
TCCACGTGGCGGGTGAAGACCGCATAGTTGCATAGGCGCTGGAAAAGGTAGTTGAGTGTGGTGGCGATG
TGCTCGGTGACGAAGAAATACATGATCCATCGTCTCAGCGGCATCTCGCTGACATCGCCCAGAGCTTCC
AAGCGCTCCATGGCCTCGTAGAAGTCCACGGCAAAATTAAAAAACTGGGAGTTTCGCGCGGACACGGTC
AACTCCTCTTCCAGAAGACGGATAAGTTCGGCGATGGTGGTGCGCACCTCGCGCTCGAAAGCCCCTGGG
ATTTCTTCCTCAATCTCTTCTTCTTCCACTAACATCTCTTCCTCTTCAGGTGGGGCTGCAGGAGGAGGG
GGAACGCGGCGACGCCGGCGGCGCACGGGCAGACGGTCGATGAATCTTTCAATGACCTCTCCGCGGCGG
CGGCGCATGGTTTCAGTGACGGCGCGGCCGTTCTCGCGCGGTCGCAGAGTAAAAACACCGCCGCGCATC
TCCTTAAAGTGGTGACTGGGAGGTTCTCCGTTTGGGAGGGAGAGGGCGCTGATTATACATTTTATTAAT
TGGCCCGTAGGGACTGCACGCAGAGATCTGATCGTGTCAAGATCCACGGGATCTGAAAACCTTTCGACG
AAAGCGTCTAACCAGTCACAGTCACAAGGTAGGCTGAGTACGGCTTCTTGTGGGCGGGGGTGGTTATGT
GTTCGGTCTGGGTCTTCTGTTTCTTCTTCATCTCGGGAAGGTGAGACGATGCTGCTGGTGATGAAATTA
AAGTAGGCAGTTCTAAGACGGCGGATGGTGGCGAGGAGCACCAGGTCTTTGGGTCCGGCTTGCTGGATA
CGCAGGCGATTGGCCATTCCCCAAGCATTATCCTGACATCTAGCAAGATCTTTGTAGTAGTCTTGCATG
AGCCGTTCTACGGGCACTTCTTCCTCACCCGTTCTGCCATGCATACGTGTGAGTCCAAATCCGCGCATT
GGTTGTACCAGTGCCAAGTCAGCTACGACTCTTTCGGCGAGGATGGCTTGCTGTACTTGGGTAAGGGTG
GCTTGAAAGTCATCAAAATCCACAAAGCGGTGGTAAGCTCCTGTATTAATGGTGTAAGCACAGTTGGCC
ATGACTGACCAGTTAACTGTCTGGTGACCAGGGCGCACGAGCTCGGTGTATTTAAGGCGCGAATAGGCG
CGGGTGTCAAAGATGTAATCGTTGCAGGTGCGCACCAGATACTGGTACCCTATAAGAAAATGCGGCGGT
GGTTGGCGGTAGAGAGGCCATCGTTCTGTAGCTGGAGCGCCAGGGGCGAGGTCTTCCAACATAAGGCGG
TGATAGCCGTAGATGTACCTGGACATCCAGGTGATTCCTGCGGCGGTAGTAGAAGCCCGAGGAAACTCG
CGTACGCGGTTCCAAATGTTGCGTAGCGGCATGAAGTAGTTCAT

SEQ ID NO: 88 A NON-CODING SEQUENCE SUITABLE FOR INCLUSION INTO BX

AAAATGATTAATAAAAAATCACTTACTTGAAATCAGCAATAAGGTCTCTGTTGAAATTTTCTCCCAGCA
GCACCTCACTTCCCTCTTCCCAACTCTGGTATTCTAAACCCCGTTCAGCGGCATACTTTCTCCATACTT
TAAAGGGGATGTCAAATTTTAGCTCCTCTCCTGTACCCACAATCTTCATGTCTTTCTTCCCAG

SEQ ID NO: 89 A NON-CODING SEQUENCE SUITABLE FOR INCLUSION INTO BY

CAAATAAAGTTTAACTTGTTTATTTGAAAATCAA

SEQ ID NO. 90 SPLICE ACCEPTOR SEQUENCE

TTTCTCTCTT CAGG

SEQ ID NO. 91 SPLICE ACCEPTOR SEQUENCE

TGCTAATCTT CCTTTCTCTC TTCAGG

Figure 63

SEQ ID NO: 93 Internal Ribosome Entry Sequence (IRES)

CGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATT
GCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCT
TTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTC
TTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCT
CTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAG
TTGGATAGTTGTGGAAAGAGTCAAATGGCTCCCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCA
GAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTTCATGTGTTTAGTCGAG
GTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATAATA

SEQ ID NO: 94 High efficiency self-cleavable P2A peptide sequence

GSGATNFSLLKQAGDVEENPGP

SEQ ID NO: 95 High efficiency self-cleavable F2A peptide sequence

GSGEGRGSLLTCGDVEENPGP

SEQ ID NO: 96 High efficiency self-cleavable E2A peptide sequence

GSGQCTNYALLKLAGDVESNPGP

SEQ ID NO: 97 High efficiency self-cleavable T2A peptide sequence

GSGVKQTLNFDLLKLAGDVESNPGP

SEQ ID NO: 98 Human CD80 amino acid sequence

MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCSGVIHVTKEVKEVATLSCGHNVSVEELAQTRIYWQKE
KKMVLTMMSGDMNIWPEYKNRTIFDITNNLSIVILALRPSDEGTYECVVLKYEKDAFKREHLAEVTLSV
KADFPTPSISDFEIPTSNIRRIICSTSGGFPEPHLSWLENGEELNAINTTVSQDPETELYAVSSKLDFN
MTTNHSFMCLIKYGHLRVNQTFNWNTTKQEHFPDNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRR
NERLRRESVRPV

SEQ ID NO. 99 poly adenylation sequence (SV40 late polyA sequence)

CAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTA
TTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACA
ACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTT

Figure 64

SEQ ID NO. 100 NG-348 virus genome sequence comprising the EnAd genome with a transgene cassette that encodes a membrane-anchored chimeric form of the single chain Fv anti-human CD3e and the T lymphocyte activation antigen, CD80 inserted in the region B_γ. The transgene cassette contains a 5' SSA, membrane-anchored anti-$CD3_\varepsilon$ cDNA sequence, P2A peptide, human CD80 cDNA sequence and a 3' poly(A) sequence

TCTATCTATATAATATACCTTATAGATGGAATGGTGCCAATATGTAAATGAGGTGATTTTAAAAAGTGT
GGATCGTGTGGTGATTGGCTGTGGGGTTAACGGCTAAAAGGGGCGGTGCGACCGTGGGAAAATGACGTT
TTGTGGGGGTGGAGTTTTTTTGCAAGTTGTCGCGGGAAATGTGACGCATAAAAAGGCTTTTTTCTCACG
GAACTACTTAGTTTTCCCACGGTATTTAACAGGAAATGAGGTAGTTTTGACCGGATGCAAGTGAAAATT
GTTGATTTTCGCGCGAAAACTGAATGAGGAAGTGTTTTTCTGAATAATGTGGTATTTATGGCAGGGTGG
AGTATTTGTTCAGGGCCAGGTAGACTTTGACCCATTACGTGGAGGTTTCGATTACCGTGTTTTTTACCT
GAATTTCCGCGTACCGTGTCAAAGTCTTCTGTTTTTACGTAGGTGTCAGCTGATCGCTAGGGTATTTAT
ACCTCAGGGTTTGTGTCAAGAGGCCACTCTTGAGTGCCAGCGAGAAGAGTTTTCTCCTCTGCGCCGGCA
GTTTAATAATAAAAAAATGAGAGATTTGCGATTTCTGCCTCAGGAAATAATCTCTGCTGAGACTGGAAA
TGAAATATTGGAGCTTGTGGTGCACGCCCTGATGGGAGACGATCCGGAGCCACCTGTGCAGCTTTTTGA
GCCTCCTACGCTTCAGGAACTGTATGATTTAGAGGTAGAGGGATCGGAGGATTCTAATGAGGAAGCTGT
AAATGGCTTTTTTACCGATTCTATGCTTTTAGCTGCTAATGAAGGGTTAGAATTAGATCCGCCTTTGGA
CACTTTTGATACTCCAGGGGTAATTGTGGAAAGCGGTACAGGTGTAAGAAAATTACCTGATTTGAGTTC

CGTGGACTGTGATTTGCACTGCTATGAAGACGGGTTTCCTCCGAGTGATGAGGAGGACCATGAAAAGGA
GCAGTCCATGCAGACTGCAGCGGGTGAGGGAGTGAAGGCTGCCAATGTTGGTTTTCAGTTGGATTGCCC
GGAGCTTCCTGGACATGGCTGTAAGTCTTGTGAATTTCACAGGAAAAATACTGGAGTAAAGGAACTGTT
ATGTTCGCTTTGTTATATGAGAACGCACTGCCACTTTATTTACAGTAAGTGTGTTTAAGTTAAAATTTA
AAGGAATATGCTGTTTTTCACATGTATATTGAGTGTGAGTTTTGTGCTTCTTATTATAGGTCCTGTGTC
TGATGCTGATGAATCACCATCTCCTGATTCTACTACCTCACCTCCTGAGATTCAAGCACCTGTTCCTGT
GGACGTGCGCAAGCCCATTCCTGTGAAGCTTAAGCCTGGGAAACGTCCAGCAGTGGAAAAACTTGAGGA
CTTGTTACAGGGTGGGGACGGACCTTTGGACTTGAGTACACGGAAACGTCCAAGACAATAAGTGTTCCA
TATCCGTGTTTACTTAAGGTGACGTCAATATTTGTGTGACAGTGCAATGTAATAAAAATATGTTAACTG
TTCACTGGTTTTTATTGCTTTTTGGGCGGGGACTCAGGTATATAAGTAGAAGCAGACCTGTGTGGTTAG
CTCATAGGAGCTGGCTTTCATCCATGGAGGTTTGGGCCATTTTGGAAGACCTTAGGAAGACTAGGCAAC
TGTTAGAGAACGCTTCGGACGGAGTCTCCGGTTTTTGGAGATTCTGGTTCGCTAGTGAATTAGCTAGGG
TAGTTTTTAGGATAAAACAGGACTATAAACAAGAATTTGAAAAGTTGTTGGTAGATTGCCCAGGACTTT
TTGAAGCTCTTAATTTGGGCCATCAGGTTCACTTTAAAGAAAAAGTTTTATCAGTTTTAGACTTTTCAA
CCCCAGGTAGAACTGCTGCTGCTGTGGCTTTTCTTACTTTTATATTAGATAAATGGATCCCGCAGACTC
ATTTCAGCAGGGGATACGTTTTGGATTTCATAGCCACAGCATTGTGGAGAACATGGAAGGTTCGCAAGA
TGAGGACAATCTTAGGTTACTGGCCAGTGCAGCCTTTGGGTGTAGCGGGAATCCTGAGGCATCCACCGG
TCATGCCAGCGGTTCTGGAGGAGGAACAGCAAGAGGACAACCCGAGAGCCGGCCTGGACCCTCCAGTGG
AGGAGGCGGAGTAGCTGACTTGTCTCCTGAACTGCAACGGGTGCTTACTGGATCTACGTCCACTGGACG
GGATAGGGGCGTTAAGAGGGAGAGGGCATCTAGTGGTACTGATGCTAGATCTGAGTTGGCTTTAAGTTT
AATGAGTCGCAGACGTCCTGAAACCATTTGGTGGCATGAGGTTCAGAAAGAGGGAAGGGATGAAGTTTC
TGTATTGCAGGAGAAATATTCACTGGAACAGGTGAAAACATGTTGGTTGGAGCCTGAGGATGATTGGGA
GGTGGCCATTAAAAATTATGCCAAGATAGCTTTGAGGCCTGATAAACAGTATAAGATTACTAGACGGAT
TAATATCCGGAATGCTTGTTACATATCTGGAAATGGGGCTGAGGTGGTAATAGATACTCAAGACAAGGC
AGTTATTAGATGCTGCATGATGGATATGTGGCCTGGGGTAGTCGGTATGGAAGCAGTAACTTTTGTAAA
TGTTAAGTTTAGGGGAGATGGTTATAATGGAATAGTGTTTATGGCCAATACCAAACTTATATTGCATGG
TTGTAGCTTTTTTGGTTTCAACAATACCTGTGTAGATGCCTGGGGACAGGTTAGTGTACGGGGATGTAG
TTTCTATGCGTGTTGGATTGCCACAGCTGGCAGAACCAAGAGTCAATTGTCTCTGAAGAAATGCATATT
TCAAAGATGTAACCTGGGCATTCTGAATGAAGGCGAAGCAAGGGTCCGCCACTGCGCTTCTACAGATAC
TGGATGTTTTATTTTGATTAAGGGAAATGCCAGCGTAAAGCATAACATGATTTGCGGTGCTTCCGATGA
GAGGCCTTATCAAATGCTCACTTGTGCTGGTGGGCATTGTAATATGCTGGCTACTGTGCATATTGTTTC
CCATCAACGCAAAAAATGGCCTGTTTTTGATCACAATGTGATGACGAAGTGTACCATGCATGCAGGTGG
GCGTAGAGGAATGTTTATGCCTTACCAGTGTAACATGAATCATGTGAAAGTGTTGTTGGAACCAGATGC
CTTTTCCAGAATGAGCCTAACAGGAATTTTTGACATGAACATGCAAATCTGGAAGATCCTGAGGTATGA
TGATACGAGATCGAGGGTACGCGCATGCGAATGCGGAGGCAAGCATGCCAGGTTCCAGCCGGTGTGTGT
AGATGTGACTGAAGATCTCAGACCGGATCATTTGGTTATTGCCCGCACTGGAGCAGAGTTCGGATCCAG
TGGAGAAGAAACTGACTAAGGTGAGTATTGGGAAAACTTTGGGGTGGGATTTTCAGATGGACAGATTGA
GTAAAAATTTGTTTTTTCTGTCTTGCAGCTGTCATGAGTGGAAACGCTTCTTTTAAGGGGGGAGTCTTC
AGCCCTTATCTGACAGGGCGTCTCCCATCCTGGGCAGGAGTTCGTCAGAATGTTATGGGATCTACTGTG
GATGGAAGACCCGTCCAACCCGCCAATTCTTCAACGCTGACCTATGCTACTTTAAGTTCTTCACCTTTG
GACGCAGCTGCAGCTGCCGCCGCCGCTTCTGTTGCCGCTAACACTGTGCTTGGAATGGGTTACTATGGA
AGCATCATGGCTAATTCCACTTCCTCTAATAACCCTTCTACCCTGACTCAGGACAAGTTACTTGTCCTT
TTGGCCCAGCTGGAGGCTTTGACCCAACGTCTGGGTGAACTTTCTCAGCAGGTGGTCGAGTTGCGAGTA
CAAACTGAGTCTGCTGTCGGCACGGCAAAGTCTAAATAAAAAAATCCCAGAATCAATGAATAAATAAAC
AAGCTTGTTGTTGATTTAAAATCAAGTGTTTTTATTTCATTTTTCGCGCACGGTATGCCCTAGACCACC
GATCTCTATCATTGAGAACTCGGTGGATTTTTTCCAGGATCCTATAGAGGTGGGATTGAATGTTTAGAT
ACATGGGCATTAGGCCGTCTTTGGGGTGGAGATAGCTCCATTGAAGGGATTCATGCTCCGGGGTAGTGT
TGTAAATCACCCAGTCATAACAAGGTCGCAGTGCATGGTGTTGCACAATATCTTTTAGAAGTAGGCTGA
TTGCCACAGATAAGCCCTTGGTGTAGGTGTTTACAAACCGGTTGAGCTGGGATGGGTGCATTCGGGGTG
AAATTATGTGCATTTTGGATTGGATTTTTAAGTTGGCAATATTGCCGCCAAGATCCCGTCTTGGGTTCA
TGTTATGAAGGACCACCAAGACGGTGTATCCGGTACATTTAGGAAATTTATCGTGCAGCTTGGATGGAA
AAGCGTGGAAAAATTTGGAGACACCCTTGTGTCCTCCAAGATTTTCCATGCACTCATCCATGATAATAG

CAATGGGGCCGTGGGCAGCGGCGCGGGCAAACACGTTCCGTGGGTCTGACACATCATAGTTATGTTCCT
GAGTTAAATCATCATAAGCCATTTTAATGAATTTGGGGCGGAGAGTACCAGATTGGGGTATGAATGTTC
CTTCGGGCCCCGGAGCATAGTTCCCCTCACAGATTTGCATTTCCCAAGCTTTCAGTTCCGAGGGTGGAA
TCATGTCCACCTGGGGGGCTATGAAAAACACCGTTTCTGGGGCGGGGGTGATTAATTGTGATGATAGCA
AATTTCTGAGCAATTGAGATTTGCCACATCCGGTGGGGCCATAAATGATTCCGATTACGGGTTGCAGGT
GGTAGTTTAGGGAACGGCAACTGCCGTCTTCTCGAAGCAAGGGGGCCACCTCGTTCATCATTTCCCTTA
CATGCATATTTTCCCGCACCAAATCCATTAGGAGGCGCTCTCCTCCTAGTGATAGAAGTTCTTGTAGTG
AGGAAAAGTTTTTCAGCGGTTTCAGACCGTCAGCCATGGGCATTTTGGAGAGAGTTTGCTGCAAAAGTT
CTAGTCTGTTCCACAGTTCAGTGATGTGTTCTATGGCATCTCGATCCAGCAGACCTCCTCGTTTCGCGG
GTTTGGACGGCTCCTGGAATAGGGTATGAGACGATGGGCGTCCAGCGCTGCCAGGGTTCGGTCCTTCCA
GGGTCTCAGTGTTCGAGTCAGGGTTGTTTCCGTCACAGTGAAGGGGTGTGCGCCTGCTTGGGCGCTTGC
CAGGGTGCGCTTCAGACTCATCCTGCTGGTCGAAAACTTCTGTCGCTTGGCGCCCTGTATGTCGGCCAA
GTAGCAGTTTACCATGAGTTCGTAGTTGAGCGCCTCGGCTGCGTGGCCTTTGGCGCGGAGCTTACCTTT
GGAAGTTTTCTTGCATACCGGGCAGTATAGGCATTTCAGCGCATACAACTTGGGCGCAAGGAAAACGGA
TTCTGGGGAGTATGCATCTGCGCCGCAGGAGGCGCAAACAGTTTCACATTCCACCAGCCAGGTTAAATC
CGGTTCATTGGGGTCAAAAACAAGTTTTCCGCCATATTTTTTGATGCGTTTCTTACCTTTGGTCTCCAT
GAGTTCGTGTCCTCGTTGAGTGACAAACAGGCTGTCCGTGTCCCCGTAGACTGATTTTACAGGCCTCTT
CTCCAGTGGAGTGCCTCGGTCTTCTTCGTACAGGAACTCTGACCACTCTGATACAAAGGCGCGCGTCCA
GGCCAGCACAAAGGAGGCTATGTGGGAGGGGTAGCGATCGTTGTCAACCAGGGGGTCCACCTTTTCCAA
AGTATGCAAACACATGTCACCCTCTTCAACATCCAGGAATGTGATTGGCTTGTAGGTGTATTTCACGTG
ACCTGGGGTCCCCGCTGGGGGGGTATAAAAGGGGGCGGTTCTTTGCTCTTCCTCACTGTCTTCCGGATC
GCTGTCCAGGAACGTCAGCTGTTGGGGTAGGTATTCCCTCTCGAAGGCGGGCATGACCTCTGCACTCAG
GTTGTCAGTTTCTAAGAACGAGGAGGATTTGATATTGACAGTGCCGGTTGAGATGCCTTTCATGAGGTT
TTCGTCCATCTGGTCAGAAAACACAATTTTTTTATTGTCAAGTTTGGTGGCAAATGATCCATACAGGGC
GTTGGATAAAAGTTTGGCAATGGATCGCATGGTTTGGTTCTTTTCCTTGTCCGCGCGCTCTTTGGCGGC
GATGTTGAGTTGGACATACTCGCGTGCCAGGCACTTCCATTCGGGGAAGATAGTTGTTAATTCATCTGG
CACGATTCTCACTTGCCACCCTCGATTATGCAAGGTAATTAAATCCACACTGGTGGCCACCTCGCCTCG
AAGGGGTTCATTGGTCCAACAGAGCCTACCTCCTTTCCTAGAACAGAAAGGGGGAAGTGGGTCTAGCAT
AAGTTCATCGGGAGGGTCTGCATCCATGGTAAAGATTCCCGGAAGTAAATCCTTATCAAAATAGCTGAT
GGGAGTGGGGTCATCTAAGGCCATTTGCCATTCTCGAGCTGCCAGTGCGCGCTCATATGGGTTAAGGGG
ACTGCCCCATGGCATGGGATGGGTGAGTGCAGAGGCATACATGCCACAGATGTCATAGACGTAGATGGG
ATCCTCAAAGATGCCTATGTAGGTTGGATAGCATCGCCCCCCTCTGATACTTGCTCGCACATAGTCATA
TAGTTCATGTGATGGCGCTAGCAGCCCCGGACCCAAGTTGGTGCGATTGGGTTTTTCTGTTCTGTAGAC
GATCTGGCGAAAGATGGCGTGAGAATTGGAAGAGATGGTGGGTCTTTGAAAAATGTTGAAATGGGCATG
AGGTAGACCTACAGAGTCTCTGACAAAGTGGGCATAAGATTCTTGAAGCTTGGTTACCAGTTCGGCGGT
GACAAGTACGTCTAGGGCGCAGTAGTCAAGTGTTTCTTGAATGATGTCATAACCTGGTTGGTTTTTCTT
TTCCCACAGTTCGCGGTTGAGAAGGTATTCTTCGCGATCCTTCCAGTACTCTTCTAGCGGAAACCCGTC
TTTGTCTGCACGGTAAGATCCTAGCATGTAGAACTGATTAACTGCCTTGTAAGGGCAGCAGCCCTTCTC
TACGGGTAGAGAGTATGCTTGAGCAGCTTTTCGTAGCGAAGCGTGAGTAAGGGCAAAGGTGTCTCTGAC
CATGACTTTGAGGAATTGGTATTTGAAGTCGATGTCGTCACAGGCTCCCTGTTCCCAGAGTTGGAAGTC
TACCCGTTTCTTGTAGGCGGGGTTGGGCAAAGCGAAAGTAACATCATTGAAGAGAATCTTGCCGGCCCT
GGGCATGAAATTGCGAGTGATGCGAAAAGGCTGTGGTACTTCCGCTCGGTTATTGATAACCTGGGCAGC
TAGGACGATCTCGTCGAAACCGTTGATGTTGTGTCCTACGATGTATAATTCTATGAAACGCGGCGTGCC
TCTGACGTGAGGTAGCTTACTGAGCTCATCAAAGGTTAGGTCTGTGGGGTCAGATAAGGCGTAGTGTTC
GAGAGCCCATTCGTGCAGGTGAGGATTCGCTTTAAGGAAGGAGGACCAGAGGTCCACTGCCAGTGCTGT
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TAAGGTATATTATATAGATAGA

SEQ ID NO: 101 V5 TAG

IPNPLLGLD

Figure 65

SEQUENCE ID NO. 102 NG-348A virus genome sequence comprising the EnAd genome with a transgene cassette that encodes a membrane-anchored chimeric form of the single chain Fv anti-human CD3e with C-terminal V5 tag and the T lymphocyte activation antigen, CD80 inserted in the region B_γ. The transgene cassette contains a 5' SSA, membrane-anchored anti-CD3Ɛ cDNA sequence, V5 tag, P2A peptide, human CD80 cDNA sequence and a 3' poly(A) sequence

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CACCTCATTTACATATTGGCACCATTCCATCTATAAGGTATATTATATAGATAGA

Figure 66

SEQ ID NO. 103 NG-420 virus genome sequence comprising the EnAd genome with a transgene cassette that encodes a membrane-anchored chimeric form of the single chain Fv anti-human CD3e inserted in the region B_{γ}. The transgene cassette contains a 5' SSA, membrane-anchored anti-CD3Ɛ cDNA sequence and a 3' poly(A) sequence

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Figure 67

SEQ ID NO. 104 NG-420A virus genome sequence comprising the EnAd genome with a transgene cassette that encodes a membrane-anchored chimeric form of the single chain Fv anti-human CD3e and a C-terminal V5 tag, inserted in the region B_γ. The transgene cassette contains a 5' SSA, membrane-anchored anti-CD3Ɛ cDNA sequence, V5 tag sequence and a 3' poly(A) sequence.

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Figure 68

SEQ ID NO: 105 NG-444-R in pUC57-Kan plasmid

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TCTAATACCTGGAATGCTGTTTTCCCAGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGG
ATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTA
ACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAAT
CGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCC
ATGTTGGAATTTAATCGCGGCCTAGAGCAAGACGTTTCCCGTTGAATATGGCTCATACTCTTCCTTTTT
CAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAA
AATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATT
ATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC

Figure 69

SEQ ID NO: 106: 434-MVA-GFP+transgene

GCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTT
TCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCA
GGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGG
AAACAGCTATGACCATGATTACGCCAAGCTTGCATGCATCTGGAAACGGGCATCTCCATTTAAGACTAG
ATGCCACGGGGTTTAAAATACTAATCATGACATTTTGTAGAGCGTAATTACTTAGTAAATCCGCCGTAC
TAGGTTCATTTCCTCCTCGTTTGGATCTCACATCAGAAATTAAAATAATCTTAGAAGGATGCAGTTGTT
TTTTGATGGATCGTAGATATTCCTCATCAACGAACCGAGTCACTAGAGTCACATCACGCAATCCATTTA
AAATAGGATCATGATGGCGGCCGTCAATTAGCATCCATTTGATGATCACTCCTAAATTATAGAAATGAT
CTCTCAAATAACGTATATGTGTACCGGGAGCAGATCCTATATACACTACGGTGGCACCATCTAATATAC
CGTGTCGCTGTAACTTACTAAGAAAAAATAATTCTCCTAGTAATAGTTTTAACTGTCCTTGATACGGTA
GTTTTTTTGCGACCTCATTTGCACTTTCTGGTTCGTAATCTAACTCATTATCAATTTCCTCAAAATACA
TAAACGGTTTATCTAACGACACAACATCCATTTTTAAGTATTATATTAAAATTTAATCAATGTTTATTT
TTAGTTTTTTAGATAAAAAATATAATATTATGAGTCGATGTAACACTTTCTACACACCGATTGATACAT
ATCATTACCTCCTATTATCTCTATCTCGGTTTCCTCACCCAATCGTTTAGAAAAGGAAGCCTCCTTAAA
GCATTTCATACACACAGCAGTTAGTTTTACCACCATTTCAGATAATGGAATAAGATTCAAAATATTATT
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GATCCTACGTACTCGAGCCCGGGATAGCTGACGACTACCACATTTGTAGAGGTTTTACTTGCTTTAAAA
AACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATT
GCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTG
CATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGTGAGCTGAAGGTACGCTGT
ATCTCAGTCAGTCAAGCTAGCTTATACAGGGCGTACACTTTCCCTTCTCAATCTCTCATTCCTCCTTCT
CTCTCTGCATCTTGGGGCAAAGCAGTAGGTCAGGCAGCATATCACAAAAATTCCATTTACTGAGATTAA
GGTAATGGCCCAGGATGGGAGCAGGTTATCAGGAAAATGCTCTTGCTTGGTTGTATTCCAGTTGAAGGT
CTGATTCACTCTTAAATGTCCATACTTGATGAGACACATGAAGCTGTGGTTGGTTGTCATATTGAAATC
CAGTTTGCTGCTAACAGCATAGAGCTCAGTTTCAGGATCTTGGGAAACTGTTGTGTTGATGGCATTTAA
TTCTTCTCCATTTTCCAACCAGGAGAGGTGAGGCTCTGGAAAACCTCCAGAGGTTGAGCAAATTATCCT
TCTAATATTAGAAGTTGGAATTTCAAAGTCAGATATACTAGGTGTAGGGAAGTCAGCTTTGACTGATAA
CGTCACTTCAGCCAGGTGTTCCCGCTTGAAAGCGTCTTTTTCATACTTCAGAACAACACACTCGTATGT
GCCCTCGTCAGATGGGCGCAGAGCCAGGATCACAATGGAGAGGTTATTAGTGATATCAAAGATGGTCCG
GTTCTTGTACTCGGGCCATATATTCATGTCCCCAGACATCATAGTCAGCACCATTTTCTTCTCCTTTTG
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CTCCTCCACGTCTCCAGCCTGCTTCAGCAGGCTGAAGTTAGTAGCTCCGCTTCCACGTGGCTTCTTCTG
CCAAAGCATGATGAGGATGATAAGGGAGATGATGGTGAGCACCACCAGGGCCAGGATGGCTGAGATCAC
CACCACCTTAAAGGGCAAGGAGTGTGGCACCACGATGACCTCCTGCGTGTCCTGGCCCACAGCATTCAG
ATCCTCTTCTGAGATGAGTTTTTGTTCGGATCCCCGGTTTATTTCCAACTTTGTCCCCGAGCCGAACGT
GAATGGGTTACTACTCCACTGCTGGCAGTAATAAGTGGCAGCATCTTCAGCCTCCATGCCGCTGATTGT
GAGAGAGTAAGAGGTCCCAGACCCACTGCCCCTGAAGTGAGCAGGGACTCCAGAAGCCAGTTTGGATGT
GTCATAAATCCATCTTTTGGGGGAGGTGCCTGACTTCTGCTGGTACCAGTTCATGTAACTTACACTTGA
GCTGGCACTGCAGGTCATGGTGACCTTCTCCCCTGGAGATGCAGACATGATTGCTGGAGACTGAGTGAG
CACGATATCAGATCCGCCGCCACCAGATCCACCACCGCCCGAGCCACCGCCACCTGAGGAGACTGTGAG
AGTGGTGCCTTGGCCCCAGTAGTCAAGGCAGTAATGATCATCATAATATCTTGCACAGTAATAGACTGC
AGAGTCCTCAGATGTCAGGCTGCTCAGTTGCATGTAGGCTGTGCTGGAGGATTTGTCTGTAGTCAATGT
GGCCTTGTCCTTGAACTTCTGATTGTAATTAGTATAACCACGGCTAGGATTAATGTATCCAATCCATTC
CAGACCCTGTCCAGGCCTCTGTTTTACCCAGTGCATCGTGTACCTAGTAAAGGTGTAGCCAGAAGCCTT
GCAGGACATCTTCACTGAGGCCCCAGGTCTTGCCAGTTCAGCCCCAGACTGCTGCAGCTGCACCTGGGA
GTGGACACCTGTAGCTGTTGCTACCAAGAAGAGGATGATACAGCTCCATCCCATGGTGGGCTCGAGGGT
ACCGGATCTAGATGGGGATCCGTCACTGTTCTTTATGATTCTACTTCCTTACCGTGCAATAAATTAGAA
TATATTTTCTACTTTTACGAGAAATTAATTATTGTATTTATTATTTATGGGTGAAAAACTTACTATAAA

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TCGAGAACATTACCCACATGATAAGAGATTGTATCAGTTTCGTAGTCTTGAGTATTGGTATTACTATAT
AGTATATAGATGTCGACCTGCAGGTCGACGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGCAGCC
AAGCTGGAATTCATCCACTTTGGATAAGAAATCTGCATGATAAATATATTGATATCCTACCACCTATTA
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GGAATCTGATTCTAATATAGCGATTGAAGAGGATCCACCGGTCGCCACCATGGTGAGCAAGGGCGAGGA
GCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGT
GTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAA
GCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCC
CGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCAT
CTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAA
CCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAA
CTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGAT
CCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGA
CGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGA
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GTACAAGTAAAGCGGCCGCGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCAACAATGTCTGGAAAG
AACTGTCCTTCATCGATACCTATCACGGAGAAATCTGTAATTGATTCCAAGACATCACATAGTTTAGTT
GCTTCCAATGCTTCAAAATTATTCTTATCATGCGTCCATAGTCCCGTTCCGTATCTATTATCGTTAGAA
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TTATTGTTCACTTTATTCGACTTTAATATATCCATCACGTTAGAAAATGCGATATCGCGACGAGGATCT
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ACAAAGTCGAACAAATATCCTTTATTAAGTTTGACCCTTCCATCTGTAACAATAGGGACCTTGTTAAAC
AGTTTTTTAAAATCTTGAGAGTCTGTGAATTTTGTCAATTGTCTGTATTCCTCTGAAAGAGATTCATAA
CAATGACCCACGGCTTCTAATTTATTTTTTGATTGGATCAATAATAATAACAGAAAGTCTAGATATTGA
GTGATTTGCAATATATCAGATAATGAAGATTCATCATCTTGACTAGCCAAATACTTAAAAAATGAATCA
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TGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCAC
ATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCA
GCCTGAATGGCGAATGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCA
TATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACAC
CCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCG
GGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATAC
GCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAA
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AACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCC
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AGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTAT
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CTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCA
TACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTG
GCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGAC
CACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGT
CTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGG
GGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATT
GGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAA

GGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACT
GAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCT
GCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTT
TTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAG
GCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTG
CTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGC
GGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGAT
ACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAA
GCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTC
CTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTAT
GGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCT
TTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCC
GCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGA

Figure 70

SEQ ID NO 107: 1863-MVA-GFP+transgene

GCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTT
TCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCA
GGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGG
AAACAGCTATGACCATGATTACGCCAAGCTTGCATGCATCTGGAAACGGGCATCTCCATTTAAGACTAG
ACGCCACGGGGTTTAAAATACTAATCATGACATTTTGTAGAGCGTAATTACTTAGTAAATCCGCCGTAC
TAGGTTCATTTCCTCCTCGTTTGGATCTCACATCAGAAATTAAAATAATCTTAGAAGGATGCAGTTGTT
TTTTGATGGATCGTAGATATTCCTCATCAACGAACCGAGTCACTAGAGTCACATCACGCAATCCATTTA
AAATAGGATCATGATGGCGGCCGTCAATTAGCATCCATTTGATGATCACTCCTAAATTATAGAAATGAT
CTCTCAAATAACGTATATGTGTACCGGGAGCAGATCCTATATACACTACGGTGGCACCATCTAATATAC
CGTGTCGCTGTAACTTACTAAGAAAAAATAATTCTCCTAGTAATAGTTTTAACTGTCCTTGATACGGCA
GTTTTTTTGCGACCTCATTTGCACTTTCTGGTTCGTAATCTAACTCATTATCAATTTCCTCAAAATACA
TAAACGGTTTATCTAACGACACAACATCCATTTTTAAGTATTATATTAAAATTTAATCAATGTTTATTT
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ATCATTACCTCCTATTATTTCTATCTCGGTTTCCTCACCCAATCGTTTAGAAAAGGAAGCCTCCTTAAA
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GATCCTACGTACTCGAGCCCGGGATAGCTGACGACTACCACATTTGTAGAGGTTTTACTTGCTTTAAAA
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GCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTG
CATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGTGAGCTGAAGGTACGCTGT
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CTCTCTGCATCTTGGGGCAAAGCAGTAGGTCAGGCAGCATATCACAAAAATTCCATTTACTGAGATTAA
GGTAATGGCCCAGGATGGGAGCAGGTTATCAGGAAAATGCTCTTGCTTGGTTGTATTCCAGTTGAAGGT
CTGATTCACTCTTAAATGTCCATACTTGATGAGACACATGAAGCTGTGGTTGGTTGTCATATTGAAATC
CAGTTTGCTGCTAACAGCATAGAGCTCAGTTTCAGGATCTTGGGAAACTGTTGTGTTGATGGCATTTAA
TTCTTCTCCATTTTCCAACCAGGAGAGGTGAGGCTCTGGAAAACCTCCAGAGGTTGAGCAAATTATCCT
TCTAATATTAGAAGTTGGAATTTCAAAGTCAGATATACTAGGTGTAGGGAAGTCAGCTTTGACTGATAA
CGTCACTTCAGCCAGGTGTTCCCGCTTGAAAGCGTCTTTTTCATACTTCAGAACAACACACTCGTATGT
GCCCTCGTCAGATGGGCGCAGAGCCAGGATCACAATGGAGAGGTTATTAGTGATATCAAAGATGGTCCG
GTTCTTGTACTCGGGCCATATATTCATGTCCCCAGACATCATAGTCAGCACCATTTTCTTCTCCTTTTG
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CTCCTCCACGTCTCCAGCCTGCTTCAGCAGGCTGAAGTTAGTAGCTCCGCTTCCACGTGGCTTCTTCTG
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CACCACCTTAAAGGGCAAGGAGTGTGGCACCACGATGACCTCCTGCGTGTCCTGGCCCACAGCATTCAG
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GAATGGGTTACTACTCCACTGCTGGCAGTAATAAGTGGCAGCATCTTCAGCCTCCATGCCGCTGATTGT
GAGAGAGTAAGAGGTCCCAGACCCACTGCCCCTGAAGTGAGCAGGGACTCCAGAAGCCAGTTTGGATGT

GTCATAAATCCATCTTTTGGGGGAGGTGCCTGACTTCTGCTGGTACCAGTTCATGTAACTTACACTTGA
GCTGGCACTGCAGGTCATGGTGACCTTCTCCCCTGGAGATGCAGACATGATTGCTGGAGACTGAGTGAG
CACGATATCAGATCCGCCGCCACCAGATCCACCACCGCCCGAGCCACCGCCACCTGAGGAGACTGTGAG
AGTGGTGCCTTGGCCCCAGTAGTCAAGGCAGTAATGATCATCATAATATCTTGCACAGTAATAGACTGC
AGAGTCCTCAGATGTCAGGCTGCTCAGTTGCATGTAGGCTGTGCTGGAGGATTTGTCTGTAGTCAATGT
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CAGACCCTGTCCAGGCCTCTGTTTTACCCAGTGCATCGTGTACCTAGTAAAGGTGTAGCCAGAAGCCTT
GCAGGACATCTTCACTGAGGCCCCAGGTCTTGCCAGTTCAGCCCCAGACTGCTGCAGCTGCACCTGGGA
GTGGACACCTGTAGCTGTTGCTACCAAGAAGAGGATGATACAGCTCCATCCCATGGTGGGCTCGAGGCG
GCCGCGGTACCGGATCCTACGATACAAACTTAACGGATATCGCGATAATGAAATTATTTATGATTATTT
CTCGCTTTCAATTTAACACAACCCTCAAGAACCTTTGTATTTATTTTCAATTTTTCTGCAGGTCGACGA
AGTTCCTATACTTTCTAGAGAATAGGAACTTCGCAGCCAAGCTGGAATTCATCCACTTTGGATAAGAAA
TCTGCATGATAAATATATTGATATCCTACCACCTATTAAAGTACCATTATCTAATAGCAATAAGATAGA
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GAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTAC
GGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACC
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GACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAG
CTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTAC
CTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTC
GTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGCGAAGTTCCTATA
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TTTGATAACGTCTAACTCGTCTAATTAATTCTGTACTTTTACCTGAAAACATGGGGCCGATTATCAACT
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CCATCACGTTAGAAAATGCGATATTGCGACGAGGATCTATGTATCTAACAGGATCTATTGCGGTGGTAG
CTAGAGAGGATTCTTTTTTGAATCGCATCAAACTAATCACAAAGTCGAACAAATATCCTTTATTAAGTT
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TCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAA
AAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTC
CTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGG
GTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAA
TGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAAC
TCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTA
CGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACT
TACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAA
CTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGC
CTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAAC

AATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCT
GGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAG
ATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATA
GACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATA
TACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATC
TCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAG
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CGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGC
AGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGC
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AGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCA
CGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGA
GGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGC
GTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTAC
GGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATA
ACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAG
TGAGCGAGGAAGCGGAAGA

Figure 71

SEQ ID NO 108: 1864-MVA-GFP+transgene

GCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTT
TCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCA
GGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGG
AAACAGCTATGACCATGATTACGCCAAGCTTGCATGCATCTGGAAACGGGCATCTCCATTTAAGACTAG
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SEQ ID NO. 109 CMV promoter

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Figure 72

SEQ ID NO. 110 Ad5-348 Genome

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<151> 2015-12-17

<150> GB1607463.5
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Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
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Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
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Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser
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Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu
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Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe
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Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
        115                 120                 125

Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Ala Ile
    130                 135                 140

Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser
145                 150                 155                 160

Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser
                165                 170                 175

Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro
            180                 185                 190

Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
        195                 200                 205

Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
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Arg
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<210> 4
<211> 322
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<220>
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<400> 4

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Val His Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
            20                  25                  30

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
        35                  40                  45

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

Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn
65                  70                  75                  80

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
                85                  90                  95

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

Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser
145                 150                 155                 160

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
                165                 170                 175

Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser
            180                 185                 190

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser
        195                 200                 205

Gly Val Pro Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser
    210                 215                 220

Leu Thr Ile Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
225                 230                 235                 240

Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu
                245                 250                 255
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Glu Ile Asn Arg Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
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Asn Ala Val Gly Gln Asp Thr Gln Glu Val Ile Val Val Pro His Ser
        275                 280                 285

Leu Pro Phe Lys Val Val Val Ile Ser Ala Ile Leu Ala Leu Val Val
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Leu Thr Ile Ile Ser Leu Ile Ile Leu Ile Met Leu Trp Gln Lys Lys
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Pro Arg
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<211> 333
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<213> Artificial Sequence

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<400> 5

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Val His Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
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Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
        35                  40                  45

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

Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn
65                  70                  75                  80

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
                85                  90                  95

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

Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
        115                 120                 125
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Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Ser Gly
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Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser
145                 150                 155                 160

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
                165                 170                 175

Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser
            180                 185                 190

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser
        195                 200                 205

Gly Val Pro Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser
    210                 215                 220

Leu Thr Ile Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
225                 230                 235                 240

Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu
                245                 250                 255

Glu Ile Asn Arg Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
            260                 265                 270

Asn Ala Val Gly Gln Asp Thr Gln Glu Val Ile Val Val Pro His Ser
        275                 280                 285

Leu Pro Phe Lys Val Val Val Ile Ser Ala Ile Leu Ala Leu Val Val
    290                 295                 300

Leu Thr Ile Ile Ser Leu Ile Ile Leu Ile Met Leu Trp Gln Lys Lys
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Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Ala Phe
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Pro Val Thr Val Ser Ser
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<210>  7
<211>  107
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Teplizumab VL sequence

<400>  7

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95
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```
Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
            100                 105


<210>  8
<211>  449
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Teplizumab Heavy chain sequence

<400>  8

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30


Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
        35                  40                  45


Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Val
    50                  55                  60


Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Ala Phe
65                  70                  75                  80


Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95


Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110


Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125


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


Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160


Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175


Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190
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```
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

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

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445
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Lys

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<212> PRT
<213> Artificial Sequence

<220>
<223> Teplizumab Light Chain Sequence

<400> 9

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
        35                  40                  45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg Thr Val Ala Ala Pro
            100                 105                 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
        115                 120                 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
    130                 135                 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145                 150                 155                 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
                165                 170                 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
            180                 185                 190
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Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
        195                 200                 205


Asn Arg Gly Glu Cys
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<213>  Artificial Sequence

<220>
<223>  PDGFR Receptor A

<400>  10

Ala Val Leu Val Leu Leu Val Ile Val Ile Ile Ser Leu Ile Val Leu
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Val Val Ile Trp
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<213>  Artificial Sequence

<220>
<223>  PDGFR Receptor B

<400>  11

Val Val Ile Ser Ala Ile Leu Ala Leu Val Val Leu Thr Ile Ile Ser
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Leu Ile Ile Leu Ile
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<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Insulin-Like growth factor 1

<400>  12

Ile Ile Ile Gly Pro Pro Leu Ile Phe Val Phe Leu Phe Ser Val Val
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Ile Gly Ser Ile Tyr Leu Phe Leu
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<210> 13
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<212> PRT
<213> Artificial Sequence

<220>
<223> IL6-R

<400> 13

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Ala Phe Gly Thr Leu Leu Cys Ile Ala Ile Val Leu
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<210> 14
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<212> PRT
<213> Artificial Sequence

<220>
<223> CD28

<400> 14

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
1 5 10 15

Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val
20 25

<210> 15
<211> 49
<212> PRT
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<220>
<223> PDGFR TM Domain

<400> 15

Ala Val Gly Gln Asp Thr Gln Glu Val Ile Val Val Pro His Ser Leu
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Pro Phe Lys Val Val Val Ile Ser Ala Ile Leu Ala Leu Val Val Leu
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Thr Ile Ile Ser Leu Ile Ile Leu Ile Met Leu Trp Gln Lys Lys Pro
35 40 45

Arg

<210> 16

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<213> Artificial Sequence

<220>
<223> c-myc tag

<400> 16

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
1 5 10

<210> 17
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<212> PRT
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<220>
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<400> 17

Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn
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<210> 18
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<220>
<223> PDGFR TM Domain with N-terminal c-myc tag

<400> 18

Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ala Val Gly
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Gln Asp Thr Gln Glu Val Ile Val Val Pro His Ser Leu Pro Phe Lys
20 25 30

Val Val Val Ile Ser Ala Ile Leu Ala Leu Val Val Leu Thr Ile Ile
35 40 45

Ser Leu Ile Ile Leu Ile Met Leu Trp Gln Lys Lys Pro Arg
50 55 60

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<212> PRT
<213> Artificial Sequence

<220>
<223> HuVH human VH leader sequence

<400> 19

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Val His Ser

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<220>
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<400> 20

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Xaa Gly Gly Gly Ser Xaa Gly Gly Gly Ser Gly Ala Ser Ala Ser
20 25 30

<210> 43
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<220>
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<400> 43

Ala Ala Ala Gly Ser Gly Xaa Ser Gly Ala Ser Ala Ser
1 5 10

<210> 44
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
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<400> 44

Pro Gly Gly Asn Arg Gly Thr Thr Thr Thr Arg Arg Pro Ala Thr Thr
1 5 10 15

Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser His Tyr
20 25

<210> 45
<211> 11
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<400> 45

Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr
1 5 10

<210> 46
<211> 6
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<220>
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<400> 46

Ala Thr Thr Thr Gly Ser
1 5

<210> 47
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<220>
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<400> 47

Glu Pro Ser Gly Pro Ile Ser Thr Ile Asn Ser Pro Pro Ser Lys Glu
1 5 10 15

Ser His Lys Ser Pro
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<210> 48
<211> 15
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<400> 48

Gly Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
1 5 10 15

<210> 49
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<400> 49

Gly Gly Gly Gly Ile Ala Pro Ser Met Val Gly Gly Gly Gly Ser
1 5 10 15

<210> 50
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<400> 50

Gly Gly Gly Gly Lys Val Glu Gly Ala Gly Gly Gly Gly Gly Ser
1 5 10 15

<210> 51
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<400> 51

Gly Gly Gly Gly Ser Met Lys Ser His Asp Gly Gly Gly Gly Ser
1 5 10 15

<210> 52
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<400> 52

Gly Gly Gly Gly Asn Leu Ile Thr Ile Val Gly Gly Gly Gly Ser
1 5 10 15

<210> 53
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
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<400> 53

Gly Gly Gly Gly Val Val Pro Ser Leu Pro Gly Gly Gly Gly Ser
1 5 10 15

<210> 54
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
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<400> 54

Gly Gly Glu Lys Ser Ile Pro Gly Gly Gly Gly Ser
1   5   10

<210> 55
<211> 18
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<400> 55

Arg Pro Leu Ser Tyr Arg Pro Pro Phe Pro Phe Gly Phe Pro Ser Val
1   5   10   15

Arg Pro

<210> 56
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<212> PRT
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<220>
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<400> 56

Tyr Pro Arg Ser Ile Tyr Ile Arg Arg Arg His Pro Ser Pro Ser Leu
1   5   10   15

Thr Thr

<210> 57
<211> 18
<212> PRT
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<220>
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<400> 57

Thr Pro Ser His Leu Ser His Ile Leu Pro Ser Phe Gly Leu Pro Thr
1   5   10   15

Phe Asn

<210> 58
<211> 18
<212> PRT
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<220>
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<400> 58

Arg Pro Val Ser Pro Phe Thr Phe Pro Arg Leu Ser Asn Ser Trp Leu
1 5 10 15

Pro Ala

<210> 59
<211> 18
<212> PRT
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<220>
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<400> 59

Ser Pro Ala Ala His Phe Pro Arg Ser Ile Pro Arg Pro Gly Pro Ile
1 5 10 15

Arg Thr

<210> 60
<211> 18
<212> PRT
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<220>
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<400> 60

Ala Pro Gly Pro Ser Ala Pro Ser His Arg Ser Leu Pro Ser Arg Ala
1 5 10 15

Phe Gly

<210> 61
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<212> PRT
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<220>
<223> Flexible linker sequence

<400> 61

Pro Arg Asn Ser Ile His Phe Leu His Pro Leu Leu Val Ala Pro Leu
1 5 10 15

Gly Ala

<210> 62
<211> 18
<212> PRT
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<220>
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<400> 62

Met Pro Ser Leu Ser Gly Val Leu Gln Val Arg Tyr Leu Ser Pro Pro
1 5 10 15

Asp Leu

<210> 63
<211> 18
<212> PRT
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<220>
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<400> 63

Ser Pro Gln Tyr Pro Ser Pro Leu Thr Leu Thr Leu Pro Pro His Pro
1 5 10 15

Ser Leu

<210> 64
<211> 18
<212> PRT
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<220>
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<400> 64

Asn Pro Ser Leu Asn Pro Pro Ser Tyr Leu His Arg Ala Pro Ser Arg
1 5 10 15

Ile Ser

<210> 65
<211> 17
<212> PRT
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<220>
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<400> 65

Leu Pro Trp Arg Thr Ser Leu Leu Pro Ser Leu Pro Leu Arg Arg Arg
1 5 10 15

Pro

<210> 66
<211> 18
<212> PRT
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<220>
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<400> 66

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

Pro Pro

<210> 67
<211> 18
<212> PRT
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<220>
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<400> 67

Val Pro Pro Ala Pro Val Val Ser Leu Arg Ser Ala His Ala Arg Pro
1 5 10 15

Pro Tyr

<210> 68
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Flexible linker sequence

<400> 68

Leu Arg Pro Thr Pro Pro Arg Val Arg Ser Tyr Thr Cys Cys Pro Thr
1 5 10 15

Pro

<210> 69
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
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<400> 69

Pro Asn Val Ala His Val Leu Pro Leu Leu Thr Val Pro Trp Asp Asn
1 5 10 15

Leu Arg

<210> 70
<211> 18
<212> PRT
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<220>
<223> Flexible linker sequence

<400> 70

Cys Asn Pro Leu Leu Pro Leu Cys Ala Arg Ser Pro Ala Val Arg Thr
1 5 10 15

Phe Pro

<210> 71
<211> 11
<212> PRT
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<220>
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<400> 71

Gly Ala Pro Ala Pro Ala Ala Pro Ala Pro Ala
1 5 10

<210> 72
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
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<400> 72

Pro Pro Pro Pro
1

<210> 73
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<400> 73

Asp Leu Cys Leu Arg Asp Trp Gly Cys Leu Trp
1   5   10

<210> 74
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<400> 74

Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp
1   5   10

<210> 75
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<400> 75

Met Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp Gly Asp
1   5   10   15

<210> 76
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<400> 76

Gln Arg Leu Met Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp

1 5 10 15

Glu Asp Asp Glu
20

<210> 77
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<400> 77

Gln Gly Leu Ile Gly Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp
1 5 10 15

Gly Arg Ser Val
20

<210> 78
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<400> 78

Gln Gly Leu Ile Gly Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp
1 5 10 15

Gly Arg Ser Val Lys
20

<210> 79
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<400> 79

Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp Glu Asp Asp
1 5 10 15

<210> 80
<211> 18
<212> PRT
<213> Artificial Sequence

<220>

<223> Linker sequence

<400> 80

Arg Leu Met Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp Glu
1 5 10 15

Asp Asp

<210> 81
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<400> 81

Met Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp Glu Asp Asp
1 5 10 15

<210> 82
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<400> 82

Met Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp Glu Asp
1 5 10 15

<210> 83
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<400> 83

Arg Leu Met Glu Asp Ile Cys Leu Ala Arg Trp Gly Cys Leu Trp Glu
1 5 10 15

Asp Asp

<210> 84
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<400> 84

Glu Val Arg Ser Phe Cys Thr Arg Trp Pro Ala Glu Lys Ser Cys Lys
1 5 10 15

Pro Leu Arg Gly
20

<210> 85
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<400> 85

Arg Ala Pro Glu Ser Phe Val Cys Tyr Trp Glu Thr Ile Cys Phe Glu
1 5 10 15

Arg Ser Glu Gln
20

<210> 86
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker sequence

<400> 86

Glu Met Cys Tyr Phe Pro Gly Ile Cys Trp Met
1 5 10

<210> 87
<211> 5288
<212> DNA
<213> Artificial Sequence

<220>
<223> DNA sequence corresponding to E2B region of the ENAD GENOME (BP 10355-5068)

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tcgagtcagg gttgtttccg tcacagtgaa ggggtgtgcg cctgcttggg cgcttgccag 180

ggtgcgcttc agactcatcc tgctggtcga aaacttctgt cgcttggcgc cctgtatgtc 240

ggccaagtag cagtttacca tgagttcgta gttgagcgcc tcggctgcgt ggcctttggc 300

gcggagctta cctttggaag ttttcttgca taccgggcag tataggcatt tcagcgcata 360

caacttgggc gcaaggaaaa cggattctgg ggagtatgca tctgcgccgc aggaggcgca 420

aacagtttca cattccacca gccaggttaa atccggttca ttggggtcaa aaacaagttt 480

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agtgacaaac aggctgtccg tgtccccgta gactgatttt acaggcctct tctccagtgg 600

agtgcctcgg tcttcttcgt acaggaactc tgaccactct gatacaaagg cgcgcgtcca 660

ggccagcaca aaggaggcta tgtgggaggg gtagcgatcg ttgtcaacca gggggtccac 720

cttttccaaa gtatgcaaac acatgtcacc ctcttcaaca tccaggaatg tgattggctt 780

gtaggtgtat ttcacgtgac ctggggtccc cgctgggggg gtataaaagg gggcggttct 840

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agaaaacaca atttttttat tgtcaagttt ggtggcaaat gatccataca gggcgttgga 1080

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aagtgtttct tgaatgatgt cataacctgg ttggtttttc ttttcccaca gttcgcggtt 1920

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<210> 88
<211> 201
<212> DNA
<213> Artificial Sequence

<220>
<223> A non-coding sequence suitable for inclusion into BX

<400> 88
aaaatgatta ataaaaaatc acttacttga aatcagcaat aaggtctctg ttgaaatttt 60

ctcccagcag cacctcactt ccctcttccc aactctggta ttctaaaccc cgttcagcgg 120

catactttct ccatacttta aaggggatgt caaattttag ctcctctcct gtacccacaa 180

tcttcatgtc tttcttccca g 201

<210> 89
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> A non-coding sequence suitable for inclusion into BY

<400> 89
caaataaagt ttaacttgtt tatttgaaaa tcaa 34

<210> 90
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Splice acceptor sequence

<400> 90
tttctctctt cagg 14

<210> 91
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Splice acceptor sequence

<400> 91
tgctaatctt cctttctctc ttcagg 26

<210> 92
<211> 13
<212> RNA
<213> Artificial Sequence

<220>
<223> sequence comprising a start codon

<400> 92
gccgccrcca ugg 13

<210> 93
<211> 550
<212> DNA
<213> Artificial Sequence

<220>
<223> Internal Ribosome Entry Sequence (IRES)

<400> 93
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accatattgc cgtcttttgg caatgtgagg gcccggaaac ctggccctgt cttcttgacg 120

agcattccta ggggtctttc ccctctcgcc aaaggaatgc aaggtctgtt gaatgtcgtg 180

aaggaagcag ttcctctgga agcttcttga agacaaacaa cgtctgtagc gacccttttgc 240

aggcagcgga acccccccacc tggcgacagg tgcctctgcg gccaaaagcc acgtgtataa 300

gatacacctg caaaggcggc acaaccccag tgccacgttg tgagttggat agttgtggaa 360

agagtcaaat ggctcccctc aagcgtattc aacaaggggc tgaaggatgc ccagaaggta 420

ccccattgta tgggatctga tctggggcct cggtgcacat gcttttcatg tgtttagtcg 480

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gatgataata 550

<210> 94
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> High efficiency self-cleavable P2A peptide sequence

<400> 94

Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val
1 5 10 15

Glu Glu Asn Pro Gly Pro
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<210> 95
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> High efficiency self-cleavable F2A peptide sequence

<400> 95

Gly Ser Gly Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu
1 5 10 15

Glu Asn Pro Gly Pro
20

<210> 96
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> High efficiency self-cleavable E2A peptide sequence

<400> 96

Gly Ser Gly Gln Cys Thr Asn Tyr Ala Leu Leu Lys Leu Ala Gly Asp
1 5 10 15

Val Glu Ser Asn Pro Gly Pro
            20

<210> 97
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> High efficiency self-cleavable T2A peptide sequence

<400> 97

Gly Ser Gly Val Lys Gln Thr Leu Asn Phe Asp Leu Leu Lys Leu Ala
1   5   10   15

Gly Asp Val Glu Ser Asn Pro Gly Pro
            20   25

<210> 98
<211> 288
<212> PRT
<213> Artificial Sequence

<220>
<223> Human CD80 amino acid sequence

<400> 98

Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro Ser Lys Cys Pro Tyr
1   5   10   15

Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly Leu Ser His Phe Cys
            20   25   30

Ser Gly Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu
        35   40   45

Ser Cys Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile
    50   55   60

Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp
65   70   75   80

Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr
                85   90   95

Asn Asn Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly
            100   105   110

Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg

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Glu His Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr
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Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile
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Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu
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Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp
180 185 190

Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met
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Thr Thr Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg
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Val Asn Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro
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Asp Asn Leu Leu Pro Ser Trp Ala Ile Thr Leu Ile Ser Val Asn Gly
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Glu Arg Arg Arg Asn Glu Arg Leu Arg Arg Glu Ser Val Arg Pro Val
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<211> 192
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<213> Artificial Sequence

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<223> poly adenylation sequence (SV40 late polyA sequence)

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ataaacaagt taacaacaac aattgcattc attttatgtt tcaggttcag ggggaggtgt 180

gggaggtttt tt 192

<210> 100
<211> 34522

<212> DNA
<213> Artificial Sequence

<220>
<223> NG-348 virus genome sequence comprising the EnAd genome with a transgene cassette that encodes a membrane-anchored chimeric form of the single chain Fv anti-human CD3e and the T lymphocyte activation antigen, CD80 inserted in the region BY.

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<212> PRT
<213> Artificial Sequence

<220>
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<400> 111

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Lys Ser Ser Cys Ser Val Gly Cys Asp Leu Pro Gln Thr His Ser Leu
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Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser
        35                  40                  45

Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu
    50                  55                  60

Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His
65                  70                  75                  80

Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser
                85                  90                  95

Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr
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Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val
        115                 120                 125

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

Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro
145                 150                 155                 160

Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu
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<223> Human soluble Flt3 ligand amino acid sequence

<400> 112

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Gln His Ser Pro Ile Ser Ser Asp Phe Ala Val Lys Ile Arg Glu Leu
35 40 45

Ser Asp Tyr Leu Leu Gln Asp Tyr Pro Val Thr Val Ala Ser Asn Leu
50 55 60

Gln Asp Glu Glu Leu Cys Gly Gly Leu Trp Arg Leu Val Leu Ala Gln
65 70 75 80

Arg Trp Met Glu Arg Leu Lys Thr Val Ala Gly Ser Lys Met Gln Gly
85 90 95

Leu Leu Glu Arg Val Asn Thr Glu Ile His Phe Val Thr Lys Cys Ala
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Phe Gln Pro Pro Pro Ser Cys Leu Arg Phe Val Gln Thr Asn Ile Ser
115 120 125

Arg Leu Leu Gln Glu Thr Ser Glu Gln Leu Val Ala Leu Lys Pro Trp
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Ile Thr Arg Gln Asn Phe Ser Arg Cys Leu Glu Leu Gln Cys Gln Pro
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Asp Ser Ser Thr Leu Pro Pro Pro Trp Ser Pro Arg Pro Leu Glu Ala
165 170 175

Thr Ala Pro

<210> 113
<211> 93
<212> PRT
<213> Artificial Sequence

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<223> Human Macrophage Inflammatory protein 1a amino acid sequence
(LD78b isoform)

<400> 113

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Leu Cys Asn Gln Val Leu Ser Ala Pro Leu Ala Ala Asp Thr Pro Thr
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Ala Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile
        35                  40                  45

Ala Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Ser Val Ile
    50                  55                  60

Phe Leu Thr Lys Arg Gly Arg Gln Val Cys Ala Asp Pro Ser Glu Glu
65                  70                  75                  80

Trp Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala
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<210> 114
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<213> Artificial Sequence

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<223> Membrane anchored form of the anti-human CD3 single chain Fv

<400> 114

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            20                  25                  30

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
        35                  40                  45

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

Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn
65                  70                  75                  80

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
                85                  90                  95

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

Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
        115                 120                 125
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Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
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Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser
145                 150                 155                 160

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
                165                 170                 175

Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser
            180                 185                 190

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser
        195                 200                 205

Gly Val Pro Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser
    210                 215                 220

Leu Thr Ile Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
225                 230                 235                 240

Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu
                245                 250                 255

Glu Ile Asn Arg Gly Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
            260                 265                 270

Asn Ala Val Gly Gln Asp Thr Gln Glu Val Ile Val Val Pro His Ser
        275                 280                 285

Leu Pro Phe Lys Val Val Val Ile Ser Ala Ile Leu Ala Leu Val Val
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Pro Arg
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<210> 115
<211> 33493
<212> DNA
<213> Artificial Sequence

<220>
<223> NG-330 virus genome sequence comprising the EnAd genome with a transgene cassette that encodes the T lymphocyte activation antigen, CD80, inserted in the region By. The transgene cassette

contains a 5' SSA, human CD80 cDNA sequence and a 3' poly(A)

<400> 115

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