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(54) Title: NOVEL PD-1 IMMUNE MODULATING AGENTS

(57) Abrégé/Abstract:
The present application provides constructs comprising an anti-PD-1 antigen-binding protein or a fragment thereof, as well as nucleic acids or CAR T cells expressing such antigen-binding protein or fragment. Also provided are methods of regulating T cells or treating patients using such constructs or cells.

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**(54) Title**: NOVEL PD-1 IMMUNE MODULATING AGENTS

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# NOVEL PD-1 IMMUNE MODULATING AGENTS

## Cross Reference to Related Applications

**[001]** This application claims priority to U.S. provisional application no. 62/183,297 filed June 23, 2015 and U.S. provisional application no. 62/266,398 filed December 11, 2015; the contents of each are hereby incorporated by reference into the present disclosure.

## Sequence Listing

**[002]** This application contains a Sequence Listing, created on June 16, 2016; the file, in ASCII format, is designated 3314070AWO_SequenceListing_ST25.txt and is 104 kilobytes in size. The file is hereby incorporated by reference in its entirety into the present application.

## Technical Field

**[003]** The present disclosure relates generally to antigen-binding proteins involved in immune function. More particularly, the present disclosure relates to recombinant antibodies, chimeric antigen receptors and fragments thereof with specificity for PD-1.

## Background of the Disclosure

**[004]** The goal of cancer immunotherapy is to treat malignant disease by modulating cancer specific immune responses. A prime target is the programmed cell death (PD-1) receptor, which is expressed on the surface of activated T cells and leads to an intracellular inhibitory signal when bound to one of its ligands, PD-L1 and PD-L2.

**[005]** PD-1 has been shown to play a role in cancer. In humans, expression of PD-1 and/or PD-L1 has been found in a number of primary tumor biopsies assessed by immunohistochemistry. Such tissues include cancers of the lung, liver, ovary, cervix, skin, colon, glioma, bladder, breast, kidney, esophagus, stomach, oral squamous cell, urothelial cell, and pancreas as well as tumors of the head and neck. Furthermore, PD-ligand expression on tumor cells has been correlated to poor prognosis of cancer patients across multiple tumor types.

**[006]** There is an ongoing need for new therapeutics, including antibodies and other antigen-binding proteins that target PD-1 and function either as agonists of PD-1 or antagonists thereof.

## Summary of the Disclosure

**[007]** The present disclosure describes antigen-binding proteins such as antibodies and chimeric antigen receptors that are able to specifically bind a protein receptor associated with programmed cell death, PD-1, on T cells, thereby modulating immune response by the T cells. By inhibiting the binding of PD-1 to its ligand, PD-L1, blockade of the PD-1 signaling pathway inhibits apoptosis of the T cells.

**[008]** In one aspect, therefore, the disclosure relates to recombinant antigen-binding proteins, antibodies and chimeric antigen receptors or antigen-binding portions thereof that bind specifically to PD-1 and prevent binding of its ligand.

**[009]** In one aspect, therefore, the disclosure relates to a recombinant antigen-binding protein or antigen-binding fragment thereof comprising one of:

(A) an antigen binding region having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 21, SEQ ID NO: 32, SEQ ID NO: 43, SEQ ID NO: 53, SEQ ID NO: 61, SEQ ID NO: 72, SEQ ID NO: 83, SEQ ID NO: 94, SEQ ID NO: 103, SEQ ID NO: 114, SEQ ID NO: 125, SEQ ID NO: 133, SEQ ID NO: 142; a fragment thereof, and a homologous sequence thereof;

(B) an antigen binding region comprising a variable light chain (VL) and variable heavy chain (VH), respectively, with amino acid sequences selected from SEQ ID NOS: 6 and 8; SEQ ID NOS: 17 and 19; SEQ ID NOS: 28 and 30; SEQ ID NOS: 39 and 41; SEQ ID NOS: 49 and 51; SEQ ID NOS: 57 and 59; SEQ ID NOS: 68 and 70; SEQ ID NOS: 79 and 81; SEQ ID NOS: 90 and 92; SEQ ID NOS: 99 and 101; SEQ ID NOS: 110 and 112; SEQ ID NOS: 121 and 123; SEQ ID NOS: 129 and 131; SEQ ID NOS: 138 and 140; fragments thereof, and homologous sequences thereof; or

(C) an antigen binding region comprising:

(i) a light chain (LC) comprising light chain complementarity determining regions (LCCDR) LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence QSISSY (SEQ ID NO: 1), AAS and QQSYSTPLT (SEQ ID NO: 2) and a heavy chain (HC) comprising heavy chain complementarity determining regions (HCCDR) HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTSSSYW (SEQ ID NO: 4), IKQDGSEK (SEQ ID NO. 5) and ARGGWSYDM (SEQ ID NO: 6); fragments thereof or homologous sequences thereof;

(ii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGAGYA (SEQ ID NO: 12), TNN and QSYDSSLSGVI (SEQ ID NO: 13) and a heavy chain (HC) comprising

HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTLTELS (SEQ ID NO: 14), FDPEDGET (SEQ ID NO. 15) and ARAYYGFDQ (SEQ ID NO: 16); fragments thereof or homologous sequences thereof;

(iii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGNNA (SEQ ID NO: 23), YND and AAWDDSVNGYV (SEQ ID NO: 24) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTRFG (SEQ ID NO: 25), ISVNNGNT (SEQ ID NO. 26) and ARYMYGRRDS (SEQ ID NO: 27); fragments thereof or homologous sequences thereof;

(iv) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QVWDNHSDVV (SEQ ID NO: 35) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences RNKFSSYA (SEQ ID NO: 36), ISGSGGTT (SEQ ID NO. 37) and ARWYSSYYDV (SEQ ID NO: 38); fragments thereof or homologous sequences thereof;

(v) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QVWDSSSDYV (SEQ ID NO: 45) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARNYISMFDS (SEQ ID NO: 48); fragments thereof or homologous sequences thereof;

(vi) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QVWDSSSDHV (SEQ ID NO: 55) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARGYSSYYDA (SEQ ID NO: 56); fragments thereof or homologous sequences thereof;

(vii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence RSNIGENT (SEQ ID NO: 63), SNN and AAWDDRLNGYV (SEQ ID NO: 64) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTNYG (SEQ ID NO: 65), IGAQKGDT (SEQ ID NO. 66) and ARSQGVPFDS (SEQ ID NO: 67); fragments thereof or homologous sequences thereof;

(viii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence RSNIGSNT (SEQ ID NO: 74), NNN and ATWDDSLNEYV (SEQ ID NO: 75) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTRYG (SEQ ID NO: 76), ISGYNGNT (SEQ ID NO. 77) and ARHGYGYHGD (SEQ ID NO: 78); fragments thereof or homologous sequences thereof;

(ix) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGAGYV (SEQ ID NO: 85), HNN and QSYDSSLSGWV (SEQ ID NO: 86) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFKDYY (SEQ ID NO: 87), ISTSGNSV (SEQ ID NO. 88) and

ARSPGHSDYDS (SEQ ID NO: 89); fragments thereof or homologous sequences thereof;

(x) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGDKS (SEQ ID NO: 96), YDS and QVWASGTDHPYVI (SEQ ID NO: 97) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARMYGSYTDM (SEQ ID NO: 98); and a fragment or homologous sequence thereof;

(xi) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGYNY (SEQ ID NO: 105), RNN and TSWDDSLSGYV (SEQ ID NO: 106) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GNAFTNFY (SEQ ID NO: 107), INPSGTDLT (SEQ ID NO. 108) and ARQYAYGYSGFDM (SEQ ID NO: 109); fragments thereof or homologous sequences thereof;

(xii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence QSVSNW (SEQ ID NO: 116), AAS and QQSYSTPIT (SEQ ID NO: 117) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTSYY (SEQ ID NO: 118), INPNTGGS (SEQ ID NO. 119) and ARGDVTYDE (SEQ ID NO: 120); fragments thereof or homologous sequences thereof;

(xiii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDD and QVWDINDHYV (SEQ ID NO: 127) and a heavy chain (HC) comprising

HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARSQASFMDI (SEQ ID NO: 128); fragments thereof or homologous sequences thereof; or

(xiv) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), DDS and QVWDSSSDQGV (SEQ ID NO: 135) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), IGTGGGT (SEQ ID NO. 136) and ARGTGYDGDQ (SEQ ID NO: 137) and fragments thereof or homologous sequences thereof.

**[0010]** In some embodiments, the recombinant antigen-binding protein or antigen-binding fragment thereof comprises a fragment of at least one of the recited SEQ ID NOS that is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the entire length of the at least one recited SEQ ID NO. In some embodiments, the recombinant antigen-binding protein or antigen-binding fragment thereof comprises a sequence homologous to at least one of the recited SEQ ID NOS that has at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% identity to the at least one recited SEQ ID NO.

**[0011]** In a related aspect, the disclosure relates to recombinant antigen-binding proteins or antigen-binding fragments thereof, wherein the recombinant antigen-binding protein is an antibody, chimeric antigen receptor (CAR), fusion protein or conjugate thereof. In an embodiment, the recombinant antigen-binding protein or antigen-binding fragment thereof is

conjugated to a therapeutic agent, for example, a drug, toxin or cytotoxic moiety, radioisotope, protein or peptide.

**[0012]** An antibody of the disclosure is a full-length antibody, an intact antibody, fragments and homologous sequences thereof, including but not limited to, an Fab fragment, an $F(ab')_2$ fragment or a single chain variable fragment (scFv).

**[0013]** In the recombinant antigen-binding protein, the antigen-binding region specifically binds to an epitope of human PD-1 and blocks binding of PD-1 to its ligand(s).

**[0014]** In a related aspect, the disclosure relates to nucleic acids encoding an antigen-binding protein of the disclosure as well as vectors and cells comprising such nucleic acids or antigen-binding proteins.

**[0015]** In yet another aspect, the disclosure relates to a method of increasing a T cell response in a subject comprising administering a therapeutically effective amount of an antigen-binding protein or an antigen binding fragment thereof. The administration of a therapeutically effective amount of the antigen-binding protein or antigen binding fragment thereof inhibits, reduces, modulates or abolishes signal transduction mediated by PD-1.

**[0016]** In yet another related aspect, the disclosure relates to a method for treatment of a subject having a PD1-positive disease comprising administering to the subject a therapeutically effective amount of an antigen-binding protein or antigen binding fragment thereof. In an embodiment, a pharmaceutical composition comprising the antigen-binding protein or antigen binding fragment thereof is administered.

**[0017]** In another aspect of the invention, the disclosure relates to a vector comprising a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

**[0018]** In yet another aspect, the disclosure relates to a cell comprising the vector described herein. In a related aspect, the disclosure relates to a cell comprising a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor. In another related aspect, the disclosure relates to a cell comprising a recombinant anti-PD-1 antigen-binding protein and a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

**[0019]** In some embodiments of the vectors or the cells described herein, the chimeric antigen receptor does not specifically bind to PD-1.

**[0020]** In another aspect of the invention, the disclosure provides a method of increasing a T cell response in a subject comprising administering to the subject a therapeutically effective amount of a recombinant anti-PD-1 antigen-binding protein, a vector, a cell, or a pharmaceutical composition described herein, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 antagonist.

**[0021]** In yet another aspect of the invention, the disclosure provides a method of decreasing a T cell response in a subject comprising administering to the subject a therapeutically effective amount of a recombinant anti-PD-1

antigen-binding protein, a vector, a cell, or a pharmaceutical composition described herein, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 agonist.

**[0022]** In another aspect of the invention, the disclosure provides a method for treatment of a subject having a PD1-positive disease, comprising administering to the subject a therapeutically effective amount of a recombinant anti-PD-1 antigen-binding protein, a vector, a cell, or a pharmaceutical composition described herein.

**[0023]** In a related aspect, the disclosure provides a method for treatment of a subject having a PD1-positive disease, comprising transducing at least one T cell of the subject with a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

**[0024]** In some embodiments of the methods described herein, the chimeric antigen receptor does not specifically bind to PD-1. In some embodiments, the PD1-positive disease is a cancer. In some embodiments, the PD1-positive disease is an autoimmune disease.

## Brief Description of the Drawings

**[0025]** **Figure 1** shows relative binding affinity of scFv-Fc clones to PD-1 monomer. The ability of scFv-Fc clones to bind PD-1 was determined by detecting level of binding to PD-1 monomer. Binding affinity of the scFv-Fc clones was ranked where clone 31 bound weakly and clones 26 and 27 bound the most (31 < 23 < 40 < 18 < 16 = 27 < 26.)

**[0026]** **Figures 2A** and **B** show disruption of the interaction of PD-1 with its ligand, PD-L1. **A**. is a schematic of the competitive binding assay used to measure the ability of the scFv-Fc to disrupt the PD-1/PD-L1 interaction. (1) biotinylated PD-1-Fc was mixed with serially diluted ET901 ScFv-Fc (negative control) or anti-PD1 ScFv-Fcs; and (2) then added into a PD-L1-Fc coated plate. In step (3) PD-1-Fc binding towards coated PD-L1 was visualized via HRP-conjugated streptavidin. **B**. In order to compare the competitive binding assay results, points where the concentration of the scFv was 1.7-5.25 ug/ml are circled. Clones 40 and 23 had the weakest ability to disrupt the interaction and clone 26 had the strongest (26 > 27 = 16 > 18 > 31 > 23 = 40).

**[0027]** **Figure 3** is a schematic of the construct used. A secreteable scFv was designed to include a murine kappa leader sequence to allow exportation of the scFv from the cell. A serine glycine linker ($G_4S$) was used to link the variable heavy chain and variable light chain. A HIS/HA tag was included to allow detection of the scFv. The schematic of SFG-1928z/scFv retroviral construct depicting the 5' and 3' long terminal repeats (LTR), splice donor (SD), splice acceptor (SA), packaging element (ψ), CD8 leader sequence (CD8), variable heavy (VH) and variable light (VL) chains of the single chain variable fragment (scFv), transmembrane domain (TM), human CD28 signaling domain (hCD28), human zeta chain signaling domain (hζ chain) and position of the anti-PD-1 scFv.

**[0028]** **Figure 4** Expansion of human peripheral blood T cells modified to express the 1928z CAR alone or with a PD-1 scFv. Transduced T cells were cultured in 50 IU/ml recombinant human IL-2 and expansion was monitored. T cells modified to express the CAR and secrete anti-PD-1 scFv clone 23 or 27 had expansion that was at least equivalent to T cells

expressing the CAR alone. Cells modified to express the 1928z CAR and secrete anti-PD-1 scFv clones 16, 18, 26, 31 or 40 did not expand *in vitro*. Data shown is representative of two independent experiments.

**[0029]** **Figure 5** INF-γ secretion (pg/ml) from tumor targeted T cells that secrete an anti-PD-1 scFv. T cells expressing the CAR or CAR and secreting an anti-PD-1 scFv were cocultured with CD19+ Raji tumor cells. Supernatants were analysed with Luminex technology to detect the levels of cytokines secreted from the T cells. T cells modified to express the 1928z CAR and secrete anti-PD-1scFv clones 23 and 27 had increased secretion of IFN-γ compared to cells modified to express the CAR alone. Cells modified to express the 1928z CAR and secrete anti-PD-1 scFv clones 16, 18, 2, 31 or 40 secreted less IFN-γ compared to cells modified with the CAR alone.

**[0030]** **Figure 6** shows the results of transduction of human T cells with 1928z CAR and Eureka anti-PD-1 scFvs. (Upper panel) Following transduction, human T cells were assessed by flow cytometry to detect CAR expression using antibody specific for the 1928z CAR (19E3). Transduction efficiency was greater than 55% for all contructs tested and reproducible. (Lower panel) Constructs containing Eureka anti-PD-1 scFv clones 26, 27 and 40 had significantly lower transductions efficiency, *$p<0.05$. Transduction efficiency from 4 independent experiments, data shown is average +/- SEM.

**[0031]** **Figure 7** shows the effect of the presence of anti-PD-1 scFv in T cells modified to express the 1928z CAR and Eureka anti-PD-1 scFv. Human T cells transduced to express the CAR and the Eureka anti-PD-1 scFvs were incubated with golgi inhibitors for 4 hours prior to preparation of western blot lysates. Western blot analysis was used to detect anti-PD-1 scFv by probing

the membrane with anti-HA antibody. Membranes were probed with anti-GAPDH antibody as loading control.

**[0032]** **Figure 8** shows the expansion of human T cells modified to express the 1928z CAR and anti-PD-1 scFv in the presence/absence of PD-L1/L2. Human T cells modified to express the CAR and secrete anti-PD-1 scFv clones 23, 26, 27,and 40 were placed on artificial antigen presenting cells (aAPCs, murine 3T3 fibroblasts) expressing human PD-1L1/L2 or not. After 24 hours incubation with aAPCs, CD3/CD28 activating beads were added to the cells to stimulate proliferation (1:2 bead:T cell ratio). After 3 days, T cells were enumerated using trypan blue exclusion. T cells modified to express the 1928zCAR and anti-PD-1 scFv clone 26 and 23 had decreased proliferation on aAPCs expressing PD-L1/L2 to aAPCs not expressing inhibitory ligands. In contrast, T cells modified to express the 1928zCAR and anti-PD-1 scFv clone 27 had increased expansion on aAPCs expressing PD-L1/L2. Data shown is representative of one experiment.

**[0033]** **Figure 9** shows the amino acid sequences for the complementarity determining regions (CDR) for light and heavy variable chains of some antigen-binding proteins of the disclosure.

**[0034]** **Figure 10** shows that monoclonal antibodies generated from the anti-PD-1 specific scFvs bind to PD-1 on human T cells. Human monoclonal antibodies from the PD-1 specific scFvs were generated and incubated with human T cells modified to overexpress human PD-1 (1µg/ml). Flow cytometry was used to detect bound antibody using a goat anti-human Ig FITC conjugated antibody. Clones 23, 26 and 27 monoclonal antibodies bound P-1 human T cells at 51%, 78% and 67% respectively. Control antibody (clone

901) did not bind human PD-1 on T cells. Data shown is representative of one experiment.

**[0035] Figure 11** shows that T cells modified to express a first generation CAR incubated on aAPCs expressing PD-L1/L2 expand when anti-PD-1 clone 27 monoclonal antibody is present. Human T cells modified to express the first generation CD19-specific CAR (19z1) were incubated with anti-PD-1 monoclonal antibodies for 24 hours then placed on aAPCs expressing PD-L1/L2 or not. After 24 hours stimulation with aAPCs, the cells were then stimulated with CD3/CD28 beads. After three days, the cells were enumerated. 19z1 T cells incubated with no monoclonal antibody (stippled bar), control antibody, 901 (checkered bar) and clones 23 (horizontal striped bar) and 26 (vertical striped bar) monoclonal antibody expanded much less on aAPCs expressing PD-1 ligands (corresponding open bars) compared to aAPCs with no inhibitory ligand. However, 19z1 T cells incubated with anti-PD-1 clone 27 monoclonal antibody (diagonally striped bar) expanded on PD-L1/L2 aAPCs to a greater extent. Data shown is representative of one experiment.

**[0036] Figures 12A-12D** show the generation of CAR T cells further modified to secrete PD-1 blocking scFv, E27. **A**. Bicistronic retroviral constructs were generated encoding a CD19-specific CAR (termed 1928z) or an ovarian tumor antigen specific CAR (termed 4H1128z) and the PD-1 blocking scFv, E27. The E27 was preceded by a signal peptide, mouse IgK, to allow secretion of the scFv. A HA/His tag was also included to detect the scFv once secreted from the T cells. **B**. Human peripheral blood T cells were transduced with the retroviral constructs encoding the CAR, 1928z, or the CAR and the E27 PD-1 blocking scFv, 1928z-E27. Following transduction, flow cytometry was used to detect expression of the CAR, using an antibody that specifically binds the

CD19-targeted CAR, termed 19E3. **C**. Western blot analysis of supernatant from transduced human T cells was utilized to detect the PD-1 blocking scFv with an anti-HA antibody. We also investigated scFv secretion from T cells modified to express the CAR and a control scFv, B6, which was detected using an anti-c-myc tag antibody. **D**. A standard $^{51}Cr$ release assay against two $CD19^{+}$ tumor targets was performed to ensure that secretion of an scFv did not interrupt the ability of the CAR to redirect T cells cytolytic capacity. CAR T cells expressing either the CAR alone (1928z or 4H1128z control CAR), the CAR and the E27 scFv (1928z-E27 or 4H1128z-E27), or the CAR and a control scFv (1928z-B6H12.2 or 4H1128z-B6H12.2) were incubated with $^{51}Cr$ labeled tumor cells (Raji or Nalm6) for 4 hrs. T cells expressing the CD19 specific CAR were able to lyse the tumor targets at equivalent levels, and the ovarian-targeted CAR T cells were unable to lyse Raji or Nalm6. Therefore, we conclude that secretion of the scFv did not interrupt the ability of the CAR to redirect T cell lytic capacity.

**[0037] Figures 13A-13D** show that T cells modified to express the CAR and secrete a PD-1 blocking scFv resist inhibition from PD-L1-PD-1 interactions, *in vitro*. **A**. T cells expressing the CAR alone (1928z), or the CAR and the PD-1 blocking scFv (1928z-E27) were cultured on 3T3 cells empty cells or 3T3 cells modified to express human PD-L1. Following 24 hours on the 3T3 feeder cells, cells were stimulated with CD3/CD28 beads added to the cultures at a 1:3 bead: T cell ratio. Expansion of T cells was determined with trypan blue enumeration and fresh beads were added twice to the cultures (indicated by the arrows). 1928z T cells expanded on 3T3 empty feeder cells, however did not expand on the 3T3-PD-L1 feeder cells. In contrast, 1928z-E27 T cells expanded on both the 3T3 Empty and 3T3-PD-L1 feeder cells, indicating a resistance to PD-L1-PD-1 mediated suppression. **B**. T cells incubated on 3T3 empty or 3T3-PD-L1 cells as shown in **Figure 13A** were analyzed by flow

cytometry to detect expression on inhibitory receptors, PD-1, 2B4 and LAG3. 1928z cells expressed increased levels of PD-1 than 1928z-E27 cells (not shown). When gated on PD-1$^+$ cells, analysis of 2B4 and LAG3 revealed that 1928z cells had a higher proportion of PD-1$^+$, 2B4$^+$ and LAG3$^+$ cells compared to 1928z-E27 cells. **C**. Transduced T cells were cultured with Raji-PDL1 or Nalm6-PDL1 tumor cells at varying effector to target (E:T) ratios (1:1, 1:3, 1:5) for 72 hours. Flow cytometry following staining with anti-CD3 and anti-CD19 antibodies and enumeration beads were used to monitor lysis of tumor targets and expansion of T cells over time. 1928z-E27 cells (upper curves) continued to expand to greater levels compared to 1928z T cells (lower curves) when cultured with PDL1$^+$ tumor cells. **D**. Transduced T cells were stimulated with Nalm6-PDL1 tumor cells as shown in Figure 3C were re-stimulated with Nalm6-PDL1 tumor cells at the 1:5 T E:T ratio. After 48 hours co-culture flow cytometry was used to determine lysis of tumor targets. 1928z-E27 retained ability to lyse PD-L1 tumor targets upon re-stimulation compared to 1928z cells.

**[0038] Figure 14** shows *in vivo* anti-tumor efficacy of T cells modified to express the CAR and secrete the PD-1 blocking scFv. A. SCID-beige mice were inoculated with Raji-PD-L1 tumor cells via intravenous infusion on Day 0. On Day 1, mice were infused intravenously with $10^6$ CAR+ T cells and survival was monitored clinically. Mice were euthanized upon development of hind limb paralysis.

**[0039] Figures 15A-15D** relate to the selection of PD-1 blocking scFv, E27. **A** PD-1 blocking mAb candidates E27, E26 and E23 were used in a competitive binding assay to detect interruption of PD-1 binding to PD-L1 at varying concentrations, compared to a control mAb, targeted to a hapten not present in humans. E23, E26 and E27 mAbs all prevented PD-1 binding to PD-L1. **B**

Schematic of design of PD-1 blocking scFv designed from the E23, E26 and E27 mAbs used in **A**, where the signal peptide was linked to the variable heavy sequence and a serine glycine linker and the the variable light sequence. This HIS/HA tag was included for detection of the scFv. **C** Western blot on SN from 293Glv9 packaging cells transduced to express the secretable scFvs with the 1928z CAR, stained with anti-HA antibody. The E27 scFv was detected at the highest levels and therefore was used in the remainder of the publication. **D** Western blot on SN from PBMCs + 4H1128z and PBMCs +4H1128z stained with anti-HA mAb.

**[0040] Figures 16A-16E** show that T cells can be co-modified to express CAR and secrete PD-1 blocking scFv, E27. **A** Representative flow cytometry plot demonstrating equivalent CAR expression following transduction with the 1928z CAR alone (1928z) or the 1928z CAR and the E27 PD-1 blocking scFv (1928z-E27), following stainting with 19E3 mAb that specifically binds the 1928z CAR. **B** Western blot on SN from 1928z and 1928z-E27 T cells stained with anti-HA mAb, showing only a ~30 kDa protein in the 1928z-E27 cells, demonstrating that the E27 scFv is secreted from the 1928z-E27 transduced T cells and not those transduced with the CAR alone. **C** Representative flow cytometry demonstrating lower levels of PD-1 expression on 1928z-E27 T cells compared to 1928z T cells following transduction. **D** Expression of PD-1 was statistically significantly lower on 1928z-E27 T cells compared to 1928z T cells, data shown is mean +/- SEM from 4 independent experiments. **E** 4 hr $^{51}Cr$ release assay demonstrating that lysis of Raji tumor cells was unaffected by secretion of the E27 scFv. 1928z and 1928z-E27 T cells lysed Raji tumor cells equivalently. Control 4H1128z-E27 T cells mediated no increase in lysis of Raji cells compared to 4H1128z T cells. Data shown is representative of two independent experiments.

**[0041] Figures 17A-17G** show that expression of CAR and E27 protects proliferative and lytic capacity of T cells in the context of $CD19^+$ $PD\text{-}L1^+$ tumor cells. **A** Raji tumor cells were retrovirally modified to express human PD-L1 (Raji-PDL1) and were stained with mAb specific for PD-L1. Parental Raji tumor (Raji) express no PD-L1 and Raji-PDL1 tumor cells expressed high levels of PD-L1. **B** Representative flow cytometry plots showing1928z-E27 T cells lyse more Raji-PDL1 tumor cells compared to 1928z T cells as determined with flow cytometry following 72 hrs co-culture. **C** 1928z-E27 T cells lyse statistically significantly more Raji-PDL1 tumor cells compared to 1928z T cells, data shown the mean +/- SEM from 4 independent experiments. **D** 1928z-E27 T cells expand to greater numbers following co-culture with Raji-PDL1 tumor cells as determined by flow cytometry and enumeration beads, data shown is the average total number of T cells +/- SEM from 4 independent experiments. **E** Representative flow cytometry plot showing increased PD-1 expression on 1928z T cells compared to 1928z-E27 T cells following 7 days co-culture with Raji-PDL1 tumor cells. **F** 1928z T cells express significantly more PD-1 compared to 1928z-E27 T cells, with regard to percentage positive cells and mean fluorescence intensity (MFI) of PD-1 staining. Data show in the mean +/- SEM from 4 independent experiments. **G** Representative flow cytometry plots showing increased percentage of 2B4+PD-1+ 1928z T cells compared to 1928z-E27 cells following coculture with Raji-PDL1 for 7 days. 1928z-E27 T cells also express less BTLA and TIM3 on the 2B4+PD-1+ population. Data shown is representative of 3 independent experiments.

**[0042] Figures 18A-18C** show that E27 protects proliferative capacity of CD3/CD28 stimulated T cells in the context of PD-L1. **A** NIH3T3 cells were retrovirally modified to express human PD-L1 (3T3-PDL1) and were stained with mAb specific for PD-L1. Parental NIH3T3 (3T3-EMPTY) express no PD-

L1 and 3T3-PDL1 tumor cells expressed high levels of PD-L1. **B** 1928z and 1928z-E27 T cells were cultured with 3T3-EMPTY or 3T3-PDL1 cells and stimulated with CD3/CD28 beads. Cells were enumerated and re-plated on new 3T3 cells on days 3, 6, 9 and 12. 1928z T cells had reduced expansion when cultured with 3T3-PDL1 cells compared to 3T3-EMPTY cells. 1928z-E27 cells had equivalent expansion when cultured on 3T3-EMPTY or 3T3-PDL1 cells. Data shown is the mean fold expansion +/- SEM from 4 independent experiments. **C** Representative flow cytometry plots showing increased expression of 2B4, PD-1, BTLA and TIM3 on 1928z T cells cultured with 3T3-PDL1 compared to1928z T cells cultured on 3T3-EMPTY cells. 1928z-E27 cells had equivalent expression of 2B4, PD-1, BTLA-4 and TIM3 when cultured with 3T3-EMPTY and 3T3-PDL1. Data shown is representative of 3 independent experiments.

**[0043] Figure 19** shows that CAR T cells secreting E27 scFv have increased anti-tumor function in vivo. SCID-Beige mice were inoculated with Raji-PDL1 tumor cells intravenously, and the following day were infused intravenously with CAR T cells. Mice treated with 1928z-E27 T cells had enhanced survival compared to mice treated with 1928z T cells. Mice treated with 1928z T cells survived longer than untreated mice, and mice treated with CAR T cells targeted to an irrelevant antigen, 4H1128z and 4H1128z-E27 T cells. Data shown is from 2 independent experiments.

**[0044] Figure 20** shows the results of a PD1/PDL1 blocking ELISA using the anti-PD-1 antibodies, ET130-23, ET130-26 and ET130-27. ET901 (negative control) showed no binding, while ET130-23, ET130-26 and ET130-27 showed a blocking effect to PD1/PDL1 binding over a range of concentrations between 0.031 and 10 µg/ml.

**[0045] Figure 21** shows the results of a PD1/PDL2 blocking ELISA using the anti-PD-1 antibodies, ET130-23, ET130-26 and ET130-27. ET901 (negative control) showed no binding, while ET130-23, ET130-26 and ET130-27 showed a blocking effect to PD1/PDL1 binding over a range of concentrations between 0.031 and 10 µg/ml.

## Detailed Description of the Disclosure

**[0046]** All publications, patents and other references cited herein are incorporated by reference in their entirety into the present disclosure.

**[0047]** In practicing the present disclosure, many conventional techniques in molecular biology, microbiology, cell biology, biochemistry, and immunology are used, which are within the skill of the art. These techniques are described in greater detail in, for example, Molecular Cloning: a Laboratory Manual 3rd edition, J.F. Sambrook and D.W. Russell, ed. Cold Spring Harbor Laboratory Press 2001; Recombinant Antibodies for Immunotherapy, Melvyn Little, ed. Cambridge University Press 2009; "Oligonucleotide Synthesis" (M. J. Gait, ed., 1984); "Animal Cell Culture" (R. I. Freshney, ed., 1987); "Methods in Enzymology" (Academic Press, Inc.); "Current Protocols in Molecular Biology" (F. M. Ausubel et al., eds., 1987, and periodic updates); "PCR: The Polymerase Chain Reaction", (Mullis et al., ed., 1994); "A Practical Guide to Molecular Cloning" (Perbal Bernard V., 1988); "Phage Display: A Laboratory Manual" (Barbas et al., 2001). The contents of these references and other references containing standard protocols, widely known to and relied upon by those of skill in the art, including manufacturers' instructions are hereby incorporated by reference as part of the present disclosure.

**[0048]** In the description that follows, certain conventions will be followed as regards the usage of terminology. Generally, terms used herein are intended to be interpreted consistently with the meaning of those terms as they are known to those of skill in the art.

**[0049]** An “antigen-binding protein” is a protein or polypeptide that comprises an antigen-binding region or antigen-binding portion, that is, has a strong affinity to another molecule to which it binds. Antigen-binding proteins encompass antibodies, chimeric antigen receptors and fusion proteins.

**[0050]** "Antibody" and "antibodies" as those terms are known in the art refer to antigen binding proteins of the immune system. The term "antibody" as referred to herein includes whole, full length antibodies and any fragment thereof in which the "antigen-binding portion" or "antigen-binding region" is retained, or single chains thereof. A naturally occurring "antibody" is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant (CH) region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant CL region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies

may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system.

**[0051]** The term "antigen-binding portion" or "antigen-binding region" of an antibody, as used herein, refers to that region or portion of the antibody that confers antigen specificity; fragments of antigen-binding proteins, for example, antibodies therefore, includes one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., an HLA-peptide complex). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of antigen-binding fragments encompassed within the term "antibody fragments" of an antibody include a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a F(ab)2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a Fab fragment (Ward et al., 1989 Nature 341:544-546), which consists of a VH domain; and an isolated complementarity determining region (CDR).

**[0052]** Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules. These are known as single chain Fv (scFv); see e.g., Bird et al., 1988 Science 242:423-426; and Huston et al., 1988 Proc. Natl. Acad. Sci. 85:5879-5883. Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to

those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

**[0053]** A "recombinant antibody" or “recombinant antigen-binding protein” is one which has an antigen binding portion that has been identified and selected based on binding characteristics for inclusion in a recombinantly generated antigen-binding protein, for example an antibody.

**[0054]** The term “homologous sequence thereof” refers to amino acid and nucleotide sequences that are between 60 and 99.9% identical to the sequences shown in Tables 1-14. In some embodiments, a homologous sequence has at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% identity. In an embodiment, a homologous sequence has 95-99.9% identity; in another embodiment the homologous sequence has 98-99.9%.

**[0055]** In one embodiment, single chain variable fragments (scFv) that specifically bind to human PD-1 were selected and tested. scFvs were isolated from a phage display library that is a proprietary fully human antibody scFv phage library (Eureka Therapeutics, Emeryville CA). The library is composed of human antibody repertoires from more than 100 Caucasian and Asian healthy donors, and from donors with autoimmune disease, such as systemic lupus erythematosus, scleroderma, etc.

**[0056]** The antigen used for antibody phage panning was a recombinant fusion protein, PD-1 extracellular domain fused to human IgG1 Fc (PD-1 ECD-Fc domain. DNA sequences encoding PD-1 ECD and hIgG1 Fc were synthesized by Genewiz, Inc. (South Plainfield, NJ). The DNA sequences were then subcloned into Eureka’s proprietary mammalian expression vector, which was then transfected into HEK293 cells for fusion protein expression.

PD-1 ECD-Fc fusion protein was purified by standard FPLC method from HEK293 cell culture medium after the cells died off.

**[0057]** A human scFv antibody phage display library is used for the selection of mAb clones. In brief, biotinylated antigens (PD-1 ECD-Fc fusion protein) can be first mixed with the human scFv phage library, then the antigen-scFv antibody complexes can be pulled down by streptavidin-conjugated Dynabeads M-280 through a magnetic rack. Bound clones can then be eluted and used to infect *E.Coli* XL1 -Blue. The scFv phage clones expressed in the bacteria can be purified (Yasmina NA, et al. *Probing the binding mechanism and affinity of tanezumab, a recombinant humanized anti-NGF monoclonal antibody, using a repertoire of biosensors*. Protein Science 2008; 17(8): 1326-1335; Roberts WK, et al. *Vaccination with CD20 peptides induces a biologically active, specific immune response in mice*. Blood 2002: 99 (10): 3748-3755). Panning can be performed for 3-4 cycles to enrich scFv phage clones that bind to PD-1 specifically. Positive clones can be determined by standard ELISA method against biotinylated single chain PD-1. Positive clones can be further tested for their binding to PD-1 on live cell surfaces by flow cytometry, using a PD-1$^+$ cell line, for example a 3T3 cell line.

**[0058]** Some clones encompassed by the disclosure are referred to herein as clones 14, 16, 18, 19, 23, 26, 27, 31, 36, 37, 40, 42, 46, and 47. Variable light (VL) and variable heavy (VH) chain amino acid sequences and the nucleotide sequences that code for these embodiments are shown in Tables 1-14 below. In some embodiments, the VL and VH sequences were linked with a serine glycine linker to form an scFv. In some embodiments, a HA/His tag can be included to allow for detection of the scFv.

**[0059]** In some embodiments, the disclosure includes anti-bodies that have the scFv sequence fused to one or more constant domains of the heavy chain to form an antibody with an Fc region of a human immunoglobulin to yield a bivalent protein, increasing the overall avididty and stability of the antibody. In addition, the Fc portion allows the the direct conjugation of other molecules, including but not limited to fluorescent dyes, cytotoxins, radioisotopes etc. to the antibody for example, for use in antigen quantitation studies, to immobilize the antibody for affinity measurements, for targeted delivery of a therapeutic agent, to test for Fc-mediated cytotoxicity using immune effector cells and many other applications.

**[0060]** In some embodiments, the anti-PD-1 antigen-binding proteins may comprise one or more framework region amino acid substitutions designed to improve protein stability, antibody binding, expression levels or to introduce a site for conjugation of therapeutic agents. These scFv are then used to produce recombinant human monoclonal Igs in accordance with methods known to those of skill in the art.

**[0061]** In some embodiments, the antigen-binding protein is a chimeric antigen receptor (CAR). Chimeric antigen receptor therapy (CAR-T therapy) is a new form of targeted immunotherapy. It merges the exquisite targeting specificity of monoclonal antibodies with the potent cytotoxicity and long-term persistence provided by cytotoxic T cells. This technology enables T cells to acquire long-term novel antigenic specificity independent of the endogenous TCR. Clinical trials have shown clinically significant antitumor activity of CAR-T therapy in neuroblastoma (Louis C.U. *et al.*, *Blood* 118(23):6050-6056), B-ALL (Maude S.L. *et al.*, *N. Engl. J. Med.* 371(16):1507-1517, 2014), CLL (Brentjens R.J. *et al.*, *Blood* 118(18):4817-4828, 2011), and B cell lymphoma (Kochenderfer J.N. *et al.*, Blood. 116(20):4099-4102, 2010). In one study,

a 90% complete remission rate in 30 patients with B-ALL treated with CD19-CAR T therapy was reported (Maude S.L. *et al.*, *supra*).

**[0062]** In some embodiments, the chimeric antigen receptor comprises an extracellular domain comprising the antibody moiety, a transmembrane domain, and an intracellular signaling domain. In some embodiments, the intracellular signaling domain comprises a CD3ζ intracellular signaling sequence and a co-stimulatory signaling sequence. In some embodiments, the co-stimulatory signaling sequence is a CD28 intracellular signaling sequence.

**[0063]** Other aspects of the disclosure include without limitation, the use of antigen-binding proteins and nucleic acids that encode them for treatment of PD1 associated disease, for diagnostic and prognostic applications as well as use as research tools for the detection of PD1 in cells and tissues. Pharmaceutical compositions comprising the disclosed antigen-binding proteins and nucleic acids are encompassed by the disclosure. Vectors comprising the nucleic acids of the disclosure for antibody-based treatment by vectored immunotherapy are also contemplated by the present disclosure. Vectors include expression vectors which enable the expression and secretion of antibodies, as well as vectors which are directed to cell surface expression of the antigen binding proteins, such as chimeric antigen receptors (CAR).

**[0064]** Cells comprising the nucleic acids, for example cells that have been transfected with the vectors of the disclosure are also encompassed by the disclosure.

**[0065]** For use in diagnostic and research applications, kits are also provided that contain a PD1 antibody or nucleic acids of the disclosure, assay reagents, buffers, and the like.

Table 1: PD1-16

| Antigen | PD1 ECD-hIgG1 Fc fusion | | |
|---|---|---|---|
| CDRs: | 1 | 2 | 3 |
| VL | QSISSY (SEQ ID NO: 1) | AAS | QQSYSTPLT (SEQ ID NO: 2) |
| VH | GFTSSSYW (SEQ ID NO: 3) | IKQDGSEK (SEQ ID NO: 4) | ARGGWSYDM (SEQ ID NO: 5) |
| | | | |
| Full VL | DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIKRSR (SEQ ID NO: 6) | | |
| DNA | gacatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgccgggcaag tcagagcattagcagctatttaaattggtatcagcagaaaccagggaaagcccctaagctcctgatctatgctgcatc cagtttgcaaagtggggtcccatcaaggttcagtggcagtggatctgggacagatttcactctcaccatcagcagtct gcaacctgaagattttgcaacttactactgtcaacagagttacagtaccccgctcactttcggcggagggaccaagg tggagatcaaacgt (SEQ ID NO: 7) | | |
| | | | |
| Full VH | EVQLVESGGGLVQPGGSLRLSCAASGFTSSSYWMSWVRQAPGRGLEWVANIKQ DGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGGWSYDMW GQGTLVTVSS (SEQ ID NO: 8) | | |
| DNA | gaggtgcagctggtggagtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcagcctctgg attcacctctagtagctattggatgagctgggtccgccaggctccagggagagggctggagtgggtggccaacata aagcaagatggaagtgagaagtactatgtggactctgtgaagggccgattcaccatctccagagacaacgccaa gaactcactgtatctgcaaatgaacagcctgagagccgaggacactgccgtgtattactgtgcgcgcggtggttggt cttacgatatgtggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 9 ) | | |
| | | | |
| scFv PD1-16 | DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIKRSRG GGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTSSSYWMSWVR QAPGRGLEWVANIKQDGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTA VYYCARGGWSYDMWGQGTLVTVSS HHHHHHGAYPYDVPDYAS* (SEQ ID NO: 10) | | |
| DNA (5’ -3’) | gacatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgccgggcaag tcagagcattagcagctatttaaattggtatcagcagaaaccagggaaagcccctaagctcctgatctatgctgcatc cagtttgcaaagtggggtcccatcaaggttcagtggcagtggatctgggacagatttcactctcaccatcagcagtct gcaacctgaagattttgcaacttactactgtcaacagagttacagtaccccgctcactttcggcggagggaccaagg tggagatcaaacgtggtggtggtggtagcggcggcggcggctctggtggtggtggatccgaggtgcagctggtgga gtctgggggaggcttggtccagcctgggggtccctgagactctcctgtgcagcctctggattcacctctagtagctatt ggatgagctgggtccgccaggctccagggagagggctggagtgggtggccaacataaagcaagatggaagtga gaagtactatgtggactctgtgaagggccgattcaccatctccagagacaacgccaagaactcactgtatctgcaa atgaacagcctgagagccgaggacactgccgtgtattactgtgcgcgcggtggttggtcttacgatatgtggggtca aggtactctggtgaccgtctcctca (SEQ ID NO: 11) | | |

Table 2: PD1-18

| Antigen | PD1 ECD-hIgG1 Fc fusion | | |
|---|---|---|---|
| CDRs: | 1 | 2 | 3 |
| VL | SSNIGAGYA (SEQ ID NO: 12) | TNN | QSYDSSLSGVI SEQ ID NO: 13) |
| VH | GYTLTELS (SEQ ID NO: 14) | FDPEDGET (SEQ ID NO: 15) | ARAYYGFDQ (SEQ ID NO: 16) |
| | | | |
| Full VL | QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYAVNWYQLLPGTAPKLLISTNNNR PSGVPDRFSGSQFGASASLAITGLQAEDEADYYCQSYDSSLSGVIFGGGTKLTVLG (SEQ ID NO: 17) | | |
| DNA | cagtctgtgttgacgcagccgccctcagtgtctggggccccagggcagagggtcaccatctcctgcactgggagcag ctccaacatcggggcaggttatgctgtaaattggtaccagcttcttccaggaacagcccccaaactcctcatctctacta acaacaatcggccctcaggggtccctgaccgattctctggctcccagtttggcgcctctgcctccctggccatcactgg actccaggctgaggatgaggctgattattactgccagtcctatgacagtagtctgagtggtgtgatattcggcggaggg accaagctgaccgtcctaggt (SEQ ID NO: 18) | | |
| | | | |
| Full VH | EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDP EDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCARAYYGFDQWG QGTLVTVSS (SEQ ID NO: 19) | | |
| DNA | gaagtgcagctggtgcagtctggggctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggtttccgg atacaccctcactgaattatccatgcactgggtgcgacaggctcctggaaaagggcttgagtggatgggaggttttgat cctgaagatggtgaaacaatctacgcacagaagttccagggcagagtcaccatgaccgaggacacatctacagac acagcctacatggagctgagcagcctgaggtctgaggacactgccgtgtattactgtgcgcgcgcttactacggtttcg atcagtggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 20) | | |
| | | | |
| scFv PD-1-18 | DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIKR GGGGSGGGGSGGGGS EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDP EDGETIYAQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCARAYYGFDQWG QGTLVTVSSHHHHHHGAYPYDVPDYAS* (SEQ ID NO: 21) | | |
| DNA (5’ -3’) | cagtctgtgttgacgcagccgccctcagtgtctggggccccagggcagagggtcaccatctcctgcactgggagcag ctccaacatcggggcaggttatgctgtaaattggtaccagcttcttccaggaacagcccccaaactcctcatctctacta acaacaatcggccctcaggggtccctgaccgattctctggctcccagtttggcgcctctgcctccctggccatcactgg actccaggctgaggatgaggctgattattactgccagtcctatgacagtagtctgagtggtgtgatattcggcggaggg accaagctgaccgtcctaggtggtggtggtggtagcggcggcggcggctctggtggtggtggatcc gaagtgcagctggtgcagtctggggctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggtttccgg atacaccctcactgaattatccatgcactgggtgcgacaggctcctggaaaagggcttgagtggatgggaggttttgat cctgaagatggtgaaacaatctacgcacagaagttccagggcagagtcaccatgaccgaggacacatctacagac acagcctacatggagctgagcagcctgaggtctgaggacactgccgtgtattactgtgcgcgcgcttactacggtttcg atcagtggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 22) | | |

Table 3: PD1-23

| Antigen | PD1 ECD-hIgG1 Fc fusion | | |
|---|---|---|---|
| CDRs: | 1 | 2 | 3 |
| VL | SSNIGNNA (SEQ ID NO: 23) | YND | AAWDDSVNGYV (SEQ ID NO: 24) |
| VH | GYTFTRFG (SEQ ID NO: 25) | ISVNNGNT (SEQ ID NO: 26) | ARYMYGRRDS (SEQ ID NO: 27) |
| | | | |
| Full VL | QAVLTQPPSMSEAPRQRVTISCSGSSSNIGNNAVNWYQQLPGKAPKLLIYYNDLLSS GVSDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSVNGYVFGTGTKVTVLG (SEQ ID NO: 28) | | |
| DNA | caggctgtgctgactcagccaccctcgatgtctgaagccccaggcagagggtcaccatctcctgttctggaagcagc tccaacatcggaaataatgctgtaaactggtaccagcagctcccaggaaaggctcccaaactcctcatctattataatg atctgctgtcctcaggggtctctgaccgattctctggctccaagtctggcacctcagcctccctggccatcagtgggctcc agtctgaggatgaggctgattattactgtgcagcatgggatgacagtgtgaatggttatgtcttcggaactgggaccaag gtcaccgtcctaggt (SEQ ID NO: 29) | | |
| | | | |
| Full VH | EVQLVQSGAEVKKPGDSVKVSCKASGYTFTRFGFSWVRQAPGQGLEWMGWISVN NGNTKYAQKYQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARYMYGRRDSWG QGTLVTVSS (SEQ ID NO: 30) | | |
| DNA | Gaggtccagctggtgcagtctggagctgaggtgaagaagcctggggactcagtgaaggtctcctgcaaggcttctgg ttacacctttaccagatttggtttcagctgggtgcgacaggcccctggacaagggcttgagtggatgggatggatcagc gttaataatggtaacacaaagtatgcacagaagtaccagggcagagtcaccatgaccacagacacatccacgagc acagcctacatggagctgaggagcctgaggtctgacgacactgccgtgtattactgtgcgcgctacatgtacggtcgtc gtgattcttggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 31) | | |
| | | | |
| scFv PD-1-23 | QAVLTQPPSMSEAPRQRVTISCSGSSSNIGNNAVNWYQQLPGKAPKLLIYYNDLLSS GVSDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSVNGYVFGTGTKVTVLG GSRGGGGSGGGGSGGGGS EVQLVQSGAEVKKPGDSVKVSCKASGYTFTRFGFSWVRQAPGQGLEWMGWISVN NGNTKYAQKYQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARYMYGRRDSWG QGTLVTVSS GQHHHHHHGAYPYDVPDYAS* (SEQ ID NO: 32) | | |
| DNA (5’ -3’) | caggctgtgctgactcagccaccctcgatgtctgaagccccaggcagagggtcaccatctcctgttctggaagcagc tccaacatcggaaataatgctgtaaactggtaccagcagctcccaggaaaggctcccaaactcctcatctattataatg atctgctgtcctcaggggtctctgaccgattctctggctccaagtctggcacctcagcctccctggccatcagtgggctcc agtctgaggatgaggctgattattactgtgcagcatgggatgacagtgtgaatggttatgtcttcggaactgggaccaag gtcaccgtcctaggtggttctagaggtggtggtggtagcggcggcggcggctctggtggtggtggatccgaggtccag ctggtgcagtctggagctgaggtgaagaagcctggggactcagtgaaggtctcctgcaaggcttctggttacacctttac cagatttggtttcagctgggtgcgacaggcccctggacaagggcttgagtggatgggatggatcagcgttaataatggt aacacaaagtatgcacagaagtaccagggcagagtcaccatgaccacagacacatccacgagcacagcctacat ggagctgaggagcctgaggtctgacgacactgccgtgtattactgtgcgcgctacatgtacggtcgtcgtgattcttggg gtcaaggtactctggtgaccgtctcctcagccggccagcaccatcaccatcaccatggcgcatacccgtacgacgttc cggactacgcttcttag (SEQ ID NO: 33) | | |

Table 4: PD1-26

| Antigen | PD1 ECD-hIgG1 Fc fusion | | |
|---|---|---|---|
| CDRs: | 1 | 2 | 3 |
| VL | NIGSKS (SEQ ID NO: 34) | YDS | QVWDNHSDVV (SEQ ID NO: 35) |
| VH | RNKFSSYA (SEQ ID NO: 36) | ISGSGGTT (SEQ ID NO: 37) | ARWYSSYYDV (SEQ ID NO: 38) |
| | | | |
| Full VL | QSVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSG IPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDNHSDVVFGGGTKLTVLG (SEQ ID NO: 39) | | |
| DNA | Cagtctgtgctgactcagccaccctcagtgtcagtggccccaggaaagacggccaggattacctgtgggggaaaca acattggaagtaaaagtgtgcactggtaccagcagaagccaggccaggcccctgtgctggtcatctattatgatagcg accggccctcagggatccctgagcgattctctggctccaactctgggaacacggccaccctgaccatcagcagggtc gaagccggggatgaggccgactattactgtcaggtctgggataatcatagtgatgtggtattcggcggagggaccaag ctgaccgtcctaggt (SEQ ID NO: 40) | | |
| | | | |
| Full VH | QVQLVESGGGLVQPGGSLRLSCAASGYTRNKFSSYAMSWVRQAPGKGLEWVSGI SGSGGTTYYADSVKGRFTISRDNSKNTQYLQLDSLRAEDTAVYYCARWYSSYYDV WGQGTLVTVSS (SEQ ID NO: 41) | | |
| DNA | Caggtgcagctggtggagtctgggggaggcttggtacagcctgggggtccctgagactctcctgtgcagcctctgga tacacccgtaacaaatttagcagctatgccatgagctgggtccgccaggctccagggaagggcctggaatgggtctc aggtattagtggtagtggtggtactacatactatgcagactccgtgaagggccggttcaccatctccagagacaattcca agaacacgcagtatctgcaattggacagcctgagagccgaggacacggccgtatattactgtgcgcgctggtactctt cttactacgatgttttggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 42) | | |
| | | | |
| scFv PD1-26 | QSVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSG IPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDNHSDVVFGGGTKLTVLGGGG GSGGGGSGGGGS QVQLVESGGGLVQPGGSLRLSCAASGYTRNKFSSYAMSWVRQAPGKGLEWVSGI SGSGGTTYYADSVKGRFTISRDNSKNTQYLQLDSLRAEDTAVYYCARWYSSYYDV WGQGTLVTVSS HHHHHHGAYPYDVPDYAS* (SEQ ID NO: 43) | | |
| DNA (5' -3') | cagtctgtgctgactcagccaccctcagtgtcagtggccccaggaaagacggccaggattacctgtgggggaaaca acattggaagtaaaagtgtgcactggtaccagcagaagccaggccaggcccctgtgctggtcatctattatgatagcg accggccctcagggatccctgagcgattctctggctccaactctgggaacacggccaccctgaccatcagcagggtc gaagccggggatgaggccgactattactgtcaggtctgggataatcatagtgatgtggtattcggcggagggaccaag ctgaccgtcctaggtggtggtggtggtagcggcggcggcggctctggtggtggtggatcccaggtgcagctggtggag tctgggggaggcttggtacagcctgggggtccctgagactctcctgtgcagcctctggatacacccgtaacaaattta gcagctatgccatgagctgggtccgccaggctccagggaagggcctggaatgggtctcaggtattagtggtagtggtg gtactacatactatgcagactccgtgaagggccggttcaccatctccagagacaattccaagaacacgcagtatctgc aattggacagcctgagagccgaggacacggccgtatattactgtgcgcgctggtactcttcttactacgatgtttggggtc aaggtactctggtgaccgtctcctcacaccatcaccatcaccatggcgcatacccgtacgacgttccggactacgcttct tag (SEQ ID NO: 44) | | |

Table 5: PD-1-27

| Antigen | PD1 ECD-hIgG1 Fc fusion | | |
|---|---|---|---|
| CDRs: | 1 | 2 | 3 |
| VL | NIGSKS (SEQ ID NO: 34) | YDS | QVWDSSSDYV (SEQ ID NO: 45) |
| VH | GFTFSSYA (SEQ ID NO: 46) | ISGSGGST (SEQ ID NO: 47) | ARNYISMFDS (SEQ ID NO: 48) |
| | | | |
| Full VL | QSVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQRPGQAPVLVIYYDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVFGIGTKVTVLG (SEQ ID NO: 49) | | |
| DNA | cagtctgtgctgactcagccaccctcagtgtcagtggccccaggaaagacggccaggattacctgtgggggaaaca acattggaagtaaaagtgtgcactggtaccagcagaggccaggccaggcccctgtgctggtcatctattatgatagc gaccggccctcagggatccctgagcgattctctggctccaactctgggaacacggccaccctgaccatcagcaggg tcgaagccggggatgaggccgactattactgtcaggtgtgggatagtagtagtgattatgtcttcggaattgggaccaa ggtcaccgtcctaggt (SEQ ID NO: 50) | | |
| | | | |
| Full VH | EVQLVESGGGLIQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSG GSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARNYISMFDSWGQG TLVTVSS (SEQ ID NO: 51) | | |
| DNA | gaggtgcagctggtggagtctggaggaggcttgatccagcctgggggtccctgagactctcctgtgcagcctctgg attcacctttagcagctatgccatgagctgggtccgccaggctccagggaaggggctggagtgggtctcagctattag tggtagtggtggtagcacatactacgcagactccgtgaagggccggttcaccatctccagagacaattccaagaaca cgctgtatctgcaaatgaacagcctgagagccgaggacacggccgtatattactgtgcgcgcaactacatctctatgtt cgattcttggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 52) | | |
| | | | |
| scFv PD1- 27 | QSVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQRPGQAPVLVIYYDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVFGIGTKVTVLG GGGGSGGGGSGGGGS EVQLVESGGGLIQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSG GSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARNYISMFDSWGQG TLVTVSSHHHHHHGAYPYDVPDYAS*(SEQ ID NO: 53) | | |
| DNA (5’ -3’) | cagtctgtgctgactcagccaccctcagtgtcagtggccccaggaaagacggccaggattacctgtgggggaaaca acattggaagtaaaagtgtgcactggtaccagcagaggccaggccaggcccctgtgctggtcatctattatgatagc gaccggccctcagggatccctgagcgattctctggctccaactctgggaacacggccaccctgaccatcagcaggg tcgaagccggggatgaggccgactattactgtcaggtgtgggatagtagtagtgattatgtcttcggaattgggaccaa ggtcaccgtcctaggtggtggtggtggtagcggcggcggcggctctggtggtggtggatccgaggtgcagctggtgg agtctggaggaggcttgatccagcctgggggtccctgagactctcctgtgcagcctctggattcacctttagcagctat gccatgagctgggtccgccaggctccagggaaggggctggagtgggtctcagctattagtggtagtggtggtagcac atactacgcagactccgtgaagggccggttcaccatctccagagacaattccaagaacacgctgtatctgcaaatga acagcctgagagccgaggacacggccgtatattactgtgcgcgcaactacatctctatgttcgattcttggggtcaagg tactctggtgaccgtctcctcacaccatcaccatcaccatggcgcatacccgtacgacgttccggactacgcttcttag (SEQ ID NO: 54) | | |

Table 6: PD-1-31

| Antigen | PD1 ECD hIgG1 Fc fusion | | |
|---|---|---|---|
| CDRs: | 1 | 2 | 3 |
| VL | NIGSKS (SEQ ID NO: 34) | YDS | QVWDSSSDHV (SEQ ID NO: 55) |
| VH | GFTFSSYA (SEQ ID NO: 46) | ISGSGGST (SEQ ID NO: 47) | ARGYSSYYDA (SEQ ID NO: 56) |
| | | | |
| Full VL | QAVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHVFGTGTKVTVLG (SEQ ID NO: 57) | | |
| DNA | caggctgtgctgactcagccaccctcagtgtcagtggccccaggaaagacggccaggattacctgtgggggaaac aacattggaagtaaaagtgtgcactggtaccagcagaagccaggccaggcccctgtgctggtcatctattatgatag cgaccggccctcagggatccctgagcgattctctggctccaactctgggaacacggccaccctgaccatcagcagg gtcgaagccggggatgaggccgactattactgtcaggtgtgggatagtagtagtgatcatgtcttcggaactgggacc aaggtcaccgtcctaggt (SEQ ID NO: 58) | | |
| | | | |
| Full VH | QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGS GGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGYSSYYDAWG QGTLVTVSS (SEQ ID NO: 59) | | |
| DNA | caggtgcagctggtggagtctgggggaggcttggtacagcctgggggtccctgagactctcctgtgcagcctctgg attcacctttagcagctatgccatgagctgggtccgccaggctccagggaaggggctggagtgggtctcagctattag tggtagtggtggtagcacatactacgcagactccgtgaagggccggttcaccatctccagagacaattccaagaaca cgctgtatctgcaaatgaacagcctgagagccgaggacacggccgtatattactgtgcgcgcggttactcttcttacta cgatgcttggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 60) | | |
| | | | |
| scFv PD-1- 31 | QAVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHVFGTGTKVTVLGGG GGSGGGGSGGGGS QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGS GGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGYSSYYDAWG QGTLVTVSS HHHHHHGAYPYDVPDYAS* (SEQ ID NO: 61) | | |
| DNA (5’ -3’) | caggctgtgctgactcagccaccctcagtgtcagtggccccaggaaagacggccaggattacctgtgggggaaac aacattggaagtaaaagtgtgcactggtaccagcagaagccaggccaggcccctgtgctggtcatctattatgatag cgaccggccctcagggatccctgagcgattctctggctccaactctgggaacacggccaccctgaccatcagcagg gtcgaagccggggatgaggccgactattactgtcaggtgtgggatagtagtagtgatcatgtcttcggaactgggacc aaggtcaccgtcctaggtggtggtggtagcggcggcggcggctctggtggtggtggatcccaggtgcagctggt ggagtctgggggaggcttggtacagcctgggggtccctgagactctcctgtgcagcctctggattcacctttagcagc tatgccatgagctgggtccgccaggctccagggaaggggctggagtgggtctcagctattagtggtagtggtggtagc acatactacgcagactccgtgaagggccggttcaccatctccagagacaattccaagaacacgctgtatctgcaaat gaacagcctgagagccgaggacacggccgtatattactgtgcgcgcggttactcttcttactacgatgcttggggtcaa ggtactctggtgaccgtctcctcacaccatcaccatcaccatggcgcatacccgtacgacgttccggactacgcttctta g (SEQ ID NO: 62) | | |

Table 7: PD-1-40

| Antigen | PD1 ECD-hIgG1 Fc fusion | | |
|---|---|---|---|
| CDRs: | 1 | 2 | 3 |
| VL | RSNIGENT (SEQ ID NO: 63) | SNN | AAWDDRLNGYV (SEQ ID NO: 64) |
| VH | GYTFTNYG (SEQ ID NO: 65) | IGAQKGDT (SEQ ID NO: 66) | ARSQGVPFDS (SEQ ID NO: 67) |
| | | | |
| Full VL | QSVLTQPPSASGTPGQRVTISCSGSRSNIGENTVNWYQQLPGTAPKLLIYSNNQRP SGVPDRFSGSKSGTSASLAISGLHSDDEADYFCAAWDDRLNGYVFGTGTKVTVLG (SEQ ID NO: 68) | | |
| DNA | Cagtctgtgttgactcagccaccctcagcgtctgggacccccgggcagagagtcaccatctcttgttctggaagcaggt ccaacatcggagaaaatactgtcaactggtaccagcagctcccaggaacggcccccaaactcctcatctacagtaat aatcagcggccctcaggggtccctgaccgattctctggctccaagtctggcacctcagcctccctggccatcagtgggc ttcactctgacgatgaggctgactattttgtgcagcatgggatgaccgcctcaatggttatgtcttcggaactgggaccaa ggtcaccgtcctaggt (SEQ ID NO: 69) | | |
| | | | |
| Full VH | QVQLVQSGPEVKKPGASVKVSCKASGYTFTNYGFTWVRQAPGQGLEWMGWIGAQ KGDTEYAQKFQGRVTMTTDTSTSTVYLELRSLRSDDTAVYYCARSQGVPFDSWGQ GTLVTVSS (SEQ ID NO: 70) | | |
| DNA | Caggtgcagctggtgcaatctggacctgaggtgaagaagcctggggcctcggtgaaggtctcctgcaaggcttctggt tacacctttaccaactatggtttcacctgggtgcgacaggcccctggacaaggtcttgagtggatgggatggatcggcg ctcaaaagggtgacacagagtatgcacaaaaattccagggcagagtcaccatgacgacagacacatccacgagc acagtctacttggagttgaggagcctgaggtctgacgacacggccgtgtattactgtgcgcgctctcagggtgttccgttc gattcttggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 71) | | |
| | | | |
| scFv PD-1-40 | QSVLTQPPSASGTPGQRVTISCSGSRSNIGENTVNWYQQLPGTAPKLLIYSNNQRP SGVPDRFSGSKSGTSASLAISGLHSDDEADYFCAAWDDRLNGYVFGTGTKVTVLG GGGGSGGGGSGGGGS QVQLVQSGPEVKKPGASVKVSCKASGYTFTNYGFTWVRQAPGQGLEWMGWIGAQ KGDTEYAQKFQGRVTMTTDTSTSTVYLELRSLRSDDTAVYYCARSQGVPFDSWGQ GTLVTVSSHHHHHHGAYPYDVPDYAS* (SEQ ID NO: 72) | | |
| DNA (5' -3') | cagtctgtgttgactcagccaccctcagcgtctgggacccccgggcagagagtcaccatctcttgttctggaagcaggt ccaacatcggagaaaatactgtcaactggtaccagcagctcccaggaacggcccccaaactcctcatctacagtaat aatcagcggccctcaggggtccctgaccgattctctggctccaagtctggcacctcagcctccctggccatcagtgggc ttcactctgacgatgaggctgactattttgtgcagcatgggatgaccgcctcaatggttatgtcttcggaactgggaccaa ggtcaccgtcctaggtggtggtggtggtagcggcggcggcggctctggtggtggtggatcccaggtgcagctggtgca atctggacctgaggtgaagaagcctggggcctcggtgaaggtctcctgcaaggcttctggttacacctttaccaactatg gtttcacctgggtgcgacaggcccctggacaaggtcttgagtggatgggatggatcggcgctcaaaagggtgacaca gagtatgcacaaaaattccagggcagagtcaccatgacgacagacacatccacgagcacagtctacttggagttga ggagcctgaggtctgacgacacggccgtgtattactgtgcgcgctctcagggtgttccgttcgattcttggggtcaaggta ctctggtgaccgtctcctcacaccatcaccatcaccatggcgcatacccgtacgacgttccggactacgcttcttag (SEQ ID NO: 73) | | |

Table 8: PD-1-36

| Antigen | PD1 ECD hIgG1 Fc fusion | | |
|---|---|---|---|
| CDRs: | 1 | 2 | 3 |
| VL | RSNIGSNT (SEQ ID NO: 74) | NNN | ATWDDSLNEYV (SEQ ID NO: 75) |
| VH | GYTFTRYG (SEQ ID NO: 76) | I SGYNGNT (SEQ ID NO: 77) | ARHGYGYHGD (SEQ ID NO: 78) |
| | | | |
| Full VL | QSVLTQPPSASATPGQRGTISCSGGRSNIGSNTVNWYQQLPGTAPKLLIYNNNLRP SGVPDRFSGSKSGTSASLAIRGLQSEDEADYYCATWDDSLNEYVFGTGTKVTVLG (SEQ ID NO: 79) | | |
| DNA | Cagtctgtgttgactcagccaccctcagcgtctgcgacccccgggcagaggggcaccatttcgtgttctggaggcagg tccaacatcggaagtaacactgttaactggtaccagcagctcccaggaacggcccccaaactcctcatctataataat aatctgcggccctcaggggtccctgaccgattctctggctccaagtctggcacctcagcctccctggccatcaggggc tccagtctgaggatgaggctgattattactgtgcaacatgggatgacagcctgaatgaatatgtcttcggaactgggacc aaggtcaccgtcctaggt (SEQ ID NO: 80) | | |
| | | | |
| Full VH | QVQLVQSGAEVKKPGASVKVSCKASGYTFTRYGISWVRQAPGQGLEWMGWISGY NGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARHGYGYHGDWG QGTLVTVSS (SEQ ID NO: 81) | | |
| DNA | Caggtgcagctggtgcaatctggagctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggcttctggt tacacctttaccagatatggtatcagctgggtgcgacaggcccctggacaagggcttgagtggatgggatggatcagc ggttacaacggtaacacaaactatgcacagaagctccagggcagagtcaccatgaccacagacacatccacgagc acagcctacatggagctgaggagcctgaggtctgacgacacggccgtgtattactgtgcgcgccatggttacggttac catggtgattggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 82) | | |
| | | | |
| scFv PD-1-36 | QSVLTQPPSASATPGQRGTISCSGGRSNIGSNTVNWYQQLPGTAPKLLIYNNNLRP SGVPDRFSGSKSGTSASLAIRGLQSEDEADYYCATWDDSLNEYVFGTGTKVTVLG GGGGSGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGYTFTRYGISWVRQAPGQGLEWMGWISGY NGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARHGYGYHGDWG QGTLVTVSS HHHHHHGAYPYDVPDYAS* (SEQ ID NO: 83) | | |
| DNA (5' -3') | cagtctgtgttgactcagccaccctcagcgtctgcgacccccgggcagaggggcaccatttcgtgttctggaggcaggt ccaacatcggaagtaacactgttaactggtaccagcagctcccaggaacggcccccaaactcctcatctataataata atctgcggccctcaggggtccctgaccgattctctggctccaagtctggcacctcagcctccctggccatcaggggct ccagtctgaggatgaggctgattattactgtgcaacatgggatgacagcctgaatgaatatgtcttcggaactgggacc aaggtcaccgtcctaggtggtggtggtggtagcggcggcggcggctctggtggtggtggatcc caggtgcagctggtgcaatctggagctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggcttctggt tacacctttaccagatatggtatcagctgggtgcgacaggcccctggacaagggcttgagtggatgggatggatcagc ggttacaacggtaacacaaactatgcacagaagctccagggcagagtcaccatgaccacagacacatccacgagc acagcctacatggagctgaggagcctgaggtctgacgacacggccgtgtattactgtgcgcgccatggttacggttac catggtgattggggtcaaggtactctggtgaccgtctcctcacaccatcaccatcaccatggcgcatacccgtacgacg ttccggactacgcttcttag (SEQ ID NO: 84) | | |

Table 9: PD-1-37

| Antigen | PD1 ECD hIgG1 Fc fusion | | |
|---|---|---|---|
| CDRs: | 1 | 2 | 3 |
| VL | SSNIGAGYV (SEQ ID NO: 85) | HNN | QSYDSSLSGWV (SEQ ID NO: 86) |
| VH | GFTFKDYY (SEQ ID NO: 87) | ISTSGNSV (SEQ ID NO: 88) | ARSPGHSDYDS (SEQ ID NO: 89) |
| | | | |
| Full VL | QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYVVQWYQQLPGTAPKLLIYHNNDR PSGVPYRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSGWVFGGGTKLTVLG (SEQ ID NO: 90) | | |
| DNA | Cagtctgtgctgacgcagccgccctcagtgtctggggccccagggcagagggtcaccatctcctgtactgggagcag ctccaacatcggggcaggttatgttgtacagtggtatcagcagcttccaggaacagcccccaaactcctcatctatcata acaacgatcggccctcaggggtcccttaccgattctctggctccaagtctggcacctcagcctccctggccatcactgg gctccaggctgaggatgaggctgattattactgccagtcctatgacagcagcctgagtggttgggtgttcggcggaggg accaagctgaccgtcctaggt (SEQ ID NO: 91) | | |
| | | | |
| Full VH | EVQLVESGGGLVKPGGSLRLSCAASGFTFKDYYMNWIRQAPGKGLEWISHISTSGN SVDYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARSPGHSDYDSWGQG TLVTVSS (SEQ ID NO: 92) | | |
| DNA | gaggtgcagctggtggagtctgggggaggcctagtcaagcctggagggtccctgagactctcctgtgcagcctctgga ttcacctttaaagactactacatgaactggatccgccaggctccagggaagggcctggagtggatttcacacattagta ccagcggtaatagtgtagattatgcagactctgtcaagggccggttcaccatctccagggacaacgccaagaattcac tgtacctgcaaatgaacagcctgagagccgaggacacggccgtatattactgtgcgcgctctccgggtcattctgacta cgattcttggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 93) | | |
| | | | |
| scFv PD-1-37 | QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYVVQWYQQLPGTAPKLLIYHNNDR PSGVPYRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSGWVFGGGTKLTVLG GGGGSGGGGSGGGGS EVQLVESGGGLVKPGGSLRLSCAASGFTFKDYYMNWIRQAPGKGLEWISHISTSGN SVDYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARSPGHSDYDSWGQG TLVTVSS HHHHHHGAYPYDVPDYAS*(SEQ ID NO: 94) | | |
| DNA (5’ -3’) | cagtctgtgctgacgcagccgccctcagtgtctggggccccagggcagagggtcaccatctcctgtactgggagcag ctccaacatcggggcaggttatgttgtacagtggtatcagcagcttccaggaacagcccccaaactcctcatctatcata acaacgatcggccctcaggggtcccttaccgattctctggctccaagtctggcacctcagcctccctggccatcactgg gctccaggctgaggatgaggctgattattactgccagtcctatgacagcagcctgagtggttgggtgttcggcggaggg accaagctgaccgtcctaggtggtggtggtggtagcggcggcggcggctctggtggtggtggatcc gaggtgcagctggtggagtctgggggaggcctagtcaagcctggagggtccctgagactctcctgtgcagcctctgga ttcacctttaaagactactacatgaactggatccgccaggctccagggaagggcctggagtggatttcacacattagta ccagcggtaatagtgtagattatgcagactctgtcaagggccggttcaccatctccagggacaacgccaagaattcac tgtacctgcaaatgaacagcctgagagccgaggacacggccgtatattactgtgcgcgctctccgggtcattctgacta cgattcttggggtcaaggtactctggtgaccgtctcctca caccatcaccatcaccatggcgcatacccgtacgacgttccggactacgcttcttag (SEQ ID NO: 95) |

Table 10: PD-1-19

| Antigen | PD1 ECD hIgG1 Fc fusion | | |
|---|---|---|---|
| CDRs: | 1 | 2 | 3 |
| VL | NIGDKS (SEQ ID NO: 96) | YDS | QVWASGTDHPYVI (SEQ ID NO: 97) |
| VH | GFTFSSYA (SEQ ID NO: 46) | ISGSGGST (SEQ ID NO: 47) | ARMYGSYTDM (SEQ ID NO: 98) |
| | | | |
| Full VL | SYVLTQPPSVSVAPGKTARITCGGNNIGDKSVHWYQQKPGQAPVLVIYYDSDRPSGI PERFSGSNSGNTATLTISRVEAGDEADYYCQVWASGTDHPYVIFGGGTKVTVLG (SEQ ID NO: 99) | | |
| DNA | Tcctatgtgctgactcagccaccctcagtgtcagtggccccaggaaagacggccaggattacctgtgggggaaaca acattggagataaaagtgtgcactggtaccagcagaagccaggccaggcccctgtgctggtcatctattatgatagcg accggccctcagggatccctgagcgattctctggctccaactctgggaacacggccaccctgaccatcagcagggtc gaagccggggacgaggccgactattactgtcaggtgtgggctagtggtactgatcatccctatgtgatattcggcggag gaccaaggtcaccgtcctaggt (SEQ ID NO: 100) | | |
| | | | |
| Full VH | EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSG GSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARMYGSYTDMWGQG TLVTVSS (SEQ ID NO: 101) | | |
| DNA | Gaggtgcagctggtggagtctgggggaggcttggtacagcctgggggtccctgagactctcctgtgcagcctctgga ttcacctttagcagctatgccatgagctgggtccgccaggctccagggaaggggctggagtgggtctcagctattagtg gtagtggtggtagcacatactacgcagactccgtgaagggccggttcaccatctccagagacaattccaagaacacg ctgtatctgcaaatgaacagcctgagagccgaggacacggccgtatattactgtgcgcgcatgtacggttcttacactg atatgtggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 102) | | |
| | | | |
| scFv PD-1-19 | SYVLTQPPSVSVAPGKTARITCGGNNIGDKSVHWYQQKPGQAPVLVIYYDSDRPSGI PERFSGSNSGNTATLTISRVEAGDEADYYCQVWASGTDHPYVIFGGGTKVTVLGGG GGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQA PGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC ARMYGSYTDMWGQGTLVTVSS HHHHHHGAYPYDVPDYAS*(SEQ ID NO: 103) | | |
| DNA (5’ -3’) | tcctatgtgctgactcagccaccctcagtgtcagtggccccaggaaagacggccaggattacctgtgggggaaacaa cattggagataaaagtgtgcactggtaccagcagaagccaggccaggcccctgtgctggtcatctattatgatagcga ccggccctcagggatccctgagcgattctctggctccaactctgggaacacggccaccctgaccatcagcagggtcg aagccggggacgaggccgactattactgtcaggtgtgggctagtggtactgatcatccctatgtgatattcggcggagg gaccaaggtcaccgtcctaggtggtggtggtggtagcggcggcggcggctctggtggtggtggatcc gaggtgcagctggtggagtctgggggaggcttggtacagcctgggggtccctgagactctcctgtgcagcctctggat tcacctttagcagctatgccatgagctgggtccgccaggctccagggaaggggctggagtgggtctcagctattagtgg tagtggtggtagcacatactacgcagactccgtgaagggccggttcaccatctccagagacaattccaagaacacgc tgtatctgcaaatgaacagcctgagagccgaggacacggccgtatattactgtgcgcgcatgtacggttcttacactgat atgtggggtcaaggtactctggtgaccgtctcctcacaccatcaccatcaccatggcgcatacccgtacgacgttccgg actacgcttcttag (SEQ ID NO: 104) | | |

Table 11: PD-1-14

| Antigen | PD1 ECD hIgG1 Fc fusion | | |
|---|---|---|---|
| CDRs: | 1 | 2 | 3 |
| VL | SSNIGYNY (SEQ ID NO: 105) | RNN | TSWDDSLSGYV (SEQ ID NO: 106) |
| VH | GNAFTNFY (SEQ ID NO: 107) | INPSGTDLT (SEQ ID NO: 108) | ARQYAYGYSGFDM (SEQ ID NO: 109) |
| | | | |
| Full VL | QSVLTQPPSASGTPGQRVTISCSGSSSNIGYNYVYWYQQLPGTAPKLLISRNNQRP SGVPDRFSGSKSGTSASLAISGLRSEDEADYYCTSWDDSLSGYVFGPGTKVTVLG (SEQ ID NO: 110) | | |
| DNA | cagtctgtgctgactcagccaccctcagcgtctgggacccccgggcagagggtcaccatctcttgttctggaagcagct ccaacatcggataataattatgtatactggtaccagcagctcccaggaacggcccccaaactcctcatctctagaaataa tcagcggccctcaggggtccctgaccgattctctggctccaagtctggcacctcagcctccctggccatcagtgggctc cggtccgaggatgaggctgactattactgtacatcgtgggatgacagcctgagtggttatgtcttcggacctgggacca aggtcaccgtcctaggt (SEQ ID NO: 111) | | |
| | | | |
| Full VH | EVQLVQSGAEVKKPGASVKVSCKASGNAFTNFYIHWVRQAPGQGLEWMGLINPSG TDLTRYAQKFQGRVTMTRDTPTSTVYMELSSLRSDDTAVYYCARQYAYGYSGFDM WGQGTLVTVSS (SEQ ID NO: 112) | | |
| DNA | Gaagtgcagctggtgcagtctggggctgaggtgaagaagcctggggcctcagtgaaggtttcctgcaaggcatctgg aaacgccttcaccaacttctatatacactgggtgcgacaggcccctggacaagggcttgagtggatgggattaatcaa ccctagtggtactgacctcacaaggtacgcacagaagttccagggcagagtcaccatgaccagggacacgcccac gagcacagtctacatggagctgagcagcctgaggtctgacgacacggctgtgtattactgtgcgcgccagtacgctta cggttactctggtttcgatatgtggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 113) | | |
| | | | |
| scFv PD-1-14 | QSVLTQPPSASGTPGQRVTISCSGSSSNIGYNYVYWYQQLPGTAPKLLISRNNQRP SGVPDRFSGSKSGTSASLAISGLRSEDEADYYCTSWDDSLSGYVFGPGTKVTVLGG GGGSGGGGSGGGGS EVQLVQSGAEVKKPGASVKVSCKASGNAFTNFYIHWVRQAPGQGLEWMGLINPSG TDLTRYAQKFQGRVTMTRDTPTSTVYMELSSLRSDDTAVYYCARQYAYGYSGFDM WGQGTLVTVSS HHHHHHGAYPYDVPDYAS*(SEQ ID NO: 114) | | |
| DNA (5' -3') | cagtctgtgctgactcagccaccctcagcgtctgggacccccgggcagagggtcaccatctcttgttctggaagcagct ccaacatcggataataattatgtatactggtaccagcagctcccaggaacggcccccaaactcctcatctctagaaataa tcagcggccctcaggggtccctgaccgattctctggctccaagtctggcacctcagcctccctggccatcagtgggctc cggtccgaggatgaggctgactattactgtacatcgtgggatgacagcctgagtggttatgtcttcggacctgggacca aggtcaccgtcctaggtggtggtggtggtagcggcggcggcggctctggtggtggtggatccgaagtgcagctggtgc agtctggggctgaggtgaagaagcctggggcctcagtgaaggtttcctgcaaggcatctggaaacgccttcaccaact tctatatacactgggtgcgacaggcccctggacaagggcttgagtggatgggattaatcaaccctagtggtactgacct cacaaggtacgcacagaagttccagggcagagtcaccatgaccagggacacgcccacgagcacagtctacatgg agctgagcagcctgaggtctgacgacacggctgtgtattactgtgcgcgccagtacgcttacggttactctggtttcgata tgtggggtcaaggtactctggtgaccgtctcctca caccatcaccatcaccatggcgcatacccgtacgacgttccggactacgcttcttag (SEQ ID NO: 115) | | |

Table 12: PD-1-47

| Antigen | PD1 ECD hIgG1 Fc fusion | | |
|---|---|---|---|
| CDRs: | 1 | 2 | 3 |
| VL | QSVSNW (SEQ ID NO: 116) | AAS | QQSYSTPIT (SEQ ID NO: 117) |
| VH | GYTFTSYY (SEQ ID NO: 118) | INPNTGGS (SEQ ID NO: 119) | ARGDVTYDE (SEQ ID NO: 120) |
| | | | |
| Full VL | DIQMTQSPSSVSASVGDRVTITCRASQSVSNWLAWYQLKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPITFGGGTKVEIKR (SEQ ID NO: 121) | | |
| DNA | Gacatccagatgacccagtctccatcttccgtgtctgcatctgtaggagacagagtcaccatcacttgtcgggcgagtc agagtgttagcaactggttagcctggtatcaactgaaaccagggaaagcccctaagctcctgatctatgctgcatccag tttgcaaagtggggtcccatcaaggttcagtggcagtggatctgggacagatttcactctcaccatcagcagtctgcaac ctgaagattttgcaacttactactgtcaacagagttacagtaccccgatcaccttcggcggagggaccaaggtggagat caaacgt (SEQ ID NO: 122) | | |
| | | | |
| Full VH | QVQLVQSGAEVKKPGTSVKVSCKASGYTFTSYYIHWVRQAPGQGLEWMGWINPNT GGSNFAQKFQGRVTMTRDTSISTAYMELNRLRSDDTAVYYCARGDVTYDEWGQGT LVTVSS (SEQ ID NO: 123) | | |
| DNA | Caggtccagctggtacagtctggggctgaggtgaagaagcctgggacctcagtgaaggtctcctgcaaggcttctgg atacaccttcacctcctactatatacactgggtgcgacaggcccctggacaagggcttgagtggatgggatggatcaa ccctaacactggtggctcaaactttgcacagaagtttcagggcagggtcaccatgaccagggacacgtccatcagca cagcctacatggagctgaacaggctgaggtctgacgacacggccgtgtattactgtgcgcgcggtgacgttacttacg atgaatggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 124) | | |
| | | | |
| scFv PD-1-47 | DIQMTQSPSSVSASVGDRVTITCRASQSVSNWLAWYQLKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPITFGGGTKVEIKR GGGGSGGGGSGGGGS QVQLVQSGAEVKKPGTSVKVSCKASGYTFTSYYIHWVRQAPGQGLEWMGWINPNT GGSNFAQKFQGRVTMTRDTSISTAYMELNRLRSDDTAVYYCARGDVTYDEWGQGT LVTVSS HHHHHHGAYPYDVPDYAS*(SEQ ID NO: 125) | | |
| DNA (5' -3') | gacatccagatgacccagtctccatcttccgtgtctgcatctgtaggagacagagtcaccatcacttgtcgggcgagtca gagtgttagcaactggttagcctggtatcaactgaaaccagggaaagcccctaagctcctgatctatgctgcatccagtt tgcaaagtggggtcccatcaaggttcagtggcagtggatctgggacagatttcactctcaccatcagcagtctgcaacc tgaagattttgcaacttactactgtcaacagagttacagtaccccgatcaccttcggcggagggaccaaggtggagatc aaacgtggtggtggtggtagcggcggcggcggctctggtggtggtggatcc caggtccagctggtacagtctggggctgaggtgaagaagcctgggacctcagtgaaggtctcctgcaaggcttctgga tacaccttcacctcctactatatacactgggtgcgacaggcccctggacaagggcttgagtggatgggatggatcaac cctaacactggtggctcaaactttgcacagaagtttcagggcagggtcaccatgaccagggacacgtccatcagcac agcctacatggagctgaacaggctgaggtctgacgacacggccgtgtattactgtgcgcgcggtgacgttacttacgat gaatggggtcaaggtactctggtgaccgtctcctcacaccatcaccatcaccatggcgcatacccgtacgacgttccg gactacgcttcttag (SEQ ID NO: 126) | | |

Table 13: PD-1-46

| Antigen | PD1 ECD hIgG1 Fc fusion | | |
|---|---|---|---|
| CDRs: | 1 | 2 | 3 |
| VL | NIGSKS (SEQ ID NO: 34) | YDD | QVWDINDHYV (SEQ ID NO: 127) |
| VH | GFTFSSYA (SEQ ID NO: 46) | ISGSGGST (SEQ ID NO: 47) | ARSQASFMDI (SEQ ID NO: 128) |
| | | | |
| Full VL | SYELTQPPSVSVAPGKTASITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDDMRPSGIPERFSGSSSGNTATLTISPVEAGDEADYYCQVWDINDHYVFASGTKVTVLG (SEQ ID NO: 129) | | |
| DNA | Tcctatgagctgactcagccaccctcagtgtcagtggccccaggaaagacggccagcattacctgtggggaaacaacattggaagtaaaagtgtgcactggtaccagcagaagccaggccaggcccctgtgctggtcatctattatgatgacatgcggccctcaggtatccctgagcgattctctggctccagctctgggaacacggccaccctgaccatcagcccggtcgaagccggggatgaggccgactattactgtcaggtgtgggatattaatgatcattatgtcttcgcatcggggaccaaggtcaccgtcctaggt (SEQ ID NO: 130) | | |
| | | | |
| Full VH | EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSQASFMDIWGQGTLVTVSS (SEQ ID NO: 131) | | |
| DNA | Gaggtgcagctggtggagtctgggggaggcttggtacagcctgggggtccctgagactctcctgtgcagcctctggattcacctttagcagctatgccatgagctgggtccgccaggctccagggaaggggctggagtgggtctcagctattagtggtagtggtggtagcacatactacgcagactccgtgaagggccggttcaccatctccagagacaattccaagaacacgctgtatctgcaaatgaacagcctgagagccgaggacacggccgtatattactgtgcgcgctctcaggcttctttcatggatatctggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 132) | | |
| | | | |
| scFv PD-1-46 | SYELTQPPSVSVAPGKTASITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDDMRPSGIPERFSGSSSGNTATLTISPVEAGDEADYYCQVWDINDHYVFASGTKVTVLGGGGGSGGGGSGGGGS EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSQASFMDIWGQGTLVTVSS HHHHHHGAYPYDVPDYAS* (SEQ ID NO: 133) | | |
| DNA (5' -3') | tcctatgagctgactcagccaccctcagtgtcagtggccccaggaaagacggccagcattacctgtggggaaacaacattggaagtaaaagtgtgcactggtaccagcagaagccaggccaggcccctgtgctggtcatctattatgatgacatgcggccctcaggtatccctgagcgattctctggctccagctctgggaacacggccaccctgaccatcagcccggtcgaagccggggatgaggccgactattactgtcaggtgtgggatattaatgatcattatgtcttcgcatcggggaccaaggtcaccgtcctaggtggtggtggtggtagcggcggcggcggctctggtggtggtggatccgaggtgcagctggtggagtctgggggaggcttggtacagcctgggggtccctgagactctcctgtgcagcctctggattcacctttagcagctatgccatgagctgggtccgccaggctccagggaaggggctggagtgggtctcagctattagtggtagtggtggtagcacatactacgcagactccgtgaagggccggttcaccatctccagagacaattccaagaacacgctgtatctgcaaatgaacagcctgagagccgaggacacggccgtatattactgtgcgcgctctcaggcttctttcatggatatctggggtcaaggtactctggtgaccgtctcctca caccatcaccatcaccatggcgcatacccgtacgacgttccggactacgcttcttag (SEQ ID NO: 134) | | |

Table 14: PD-1-42

| Antigen | PD1 ECD hIgG1 Fc fusion | | |
|---|---|---|---|
| CDRs: | 1 | 2 | 3 |
| VL | NIGSKS (SEQ ID NO: 34) | DDS | QVWDSSSDQGV (SEQ ID NO: 135) |
| VH | GFTFSSYA (SEQ ID NO: 46) | IGTGGGT (SEQ ID NO: 136) | ARGTGYDGDQ (SEQ ID NO: 137) |
| | | | |
| Full VL | LPVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDQGVFGTGTKVTVLG (SEQ ID NO: 138) | | |
| DNA | Ctgcctgtgctgactcagccaccctcggtgtcagtggccccaggacagacggccaggatcacctgtggggaaaca acattggaagtaaaagtgtgcactggtaccagcagaagccaggccaggcccctgtgctggtcgtctatgatgatagcg accggccctcagggatccctgagcgattctctggctccaattctgggaacacggccaccctgaccatcagcagggtcg aagccggggatgaggccgactattactgtcaggtgtgggatagtagtagtgatcagggcgtcttcggaactgggacca aggtcaccgtcctaggt (SEQ ID NO: 139) | | |
| | | | |
| Full VH | EVQLVQSGGGLVQPRGSLRLSCAGSGFTFSSYAMHWVRQAPGKGLEWVSAIGTG GGTYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAMYYCARGTGYDGDQWGQ GTLVTVSS (SEQ ID NO: 140) | | |
| DNA | Gaagtgcagctggtgcagtctgggggaggcttggtacagcctaggggtccctgagactctcctgtgcaggctctgga ttcaccttcagtagctatgctatgcactgggttcgccaggctccaggaaaaggtctggagtgggtatcagctattggtact ggtggtggcacatactatgcagactccgtgaagggccgattcaccatctccagggacaatgccaagaactccttgtat cttcaaatgaacagcctgagagccgaggacaccgccatgtattactgtgcgcgcggtactggttacgacggtgatcag tggggtcaaggtactctggtgaccgtctcctca (SEQ ID NO: 141) | | |
| | | | |
| scFv PD-1-42 | LPVLTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDQGVFGTGTKVTVLG GGGGSGGGGSGGGGS EVQLVQSGGGLVQPRGSLRLSCAGSGFTFSSYAMHWVRQAPGKGLEWVSAIGTG GGTYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAMYYCARGTGYDGDQWGQ GTLVTVSS HHHHHHGAYPYDVPDYAS* (SEQ ID NO: 142) | | |
| DNA (5’ -3’) | ctgcctgtgctgactcagccaccctcggtgtcagtggccccaggacagacggccaggatcacctgtggggaaaca acattggaagtaaaagtgtgcactggtaccagcagaagccaggccaggcccctgtgctggtcgtctatgatgatagcg accggccctcagggatccctgagcgattctctggctccaattctgggaacacggccaccctgaccatcagcagggtcg aagccggggatgaggccgactattactgtcaggtgtgggatagtagtagtgatcagggcgtcttcggaactgggacca aggtcaccgtcctaggtggtggtggtggtagcggcggcggcggctctggtggtggtggatcc gaagtgcagctggtgcagtctgggggaggcttggtacagcctaggggtccctgagactctcctgtgcaggctctggat tcaccttcagtagctatgctatgcactgggttcgccaggctccaggaaaaggtctggagtgggtatcagctattggtactg gtggtggcacatactatgcagactccgtgaagggccgattcaccatctccagggacaatgccaagaactccttgtatct tcaaatgaacagcctgagagccgaggacaccgccatgtattactgtgcgcgcggtactggttacgacggtgatcagtg gggtcaaggtactctggtgaccgtctcctcacaccatcaccatcaccatggcgcatacccgtacgacgttccggactac gcttcttag (SEQ ID NO: 143) | | |

**[0066]** In an embodiment in which the antigen-binding protein is a full length antibody, the heavy and light chains of an antibody of the disclosure may be full-length (e.g., an antibody can include at least one, and preferably two, complete heavy chains, and at least one, and preferably two, complete light chains) or may include an antigen-binding portion (a Fab, $F(ab')_2$, Fv or a single chain Fv fragment ("scFv")). In other embodiments, the antibody heavy chain constant region is chosen from, e.g., IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE. In some embodiments, the immunoglobulin isotype is selected from IgG1, IgG2, IgG3, and IgG4, more particularly, IgG1 (e.g., human IgG1). The choice of antibody type will depend on the immune effector function that the antibody is designed to elicit.

**[0067]** In constructing a recombinant immunoglobulin, appropriate amino acid sequences for constant regions of various immunoglobulin isotypes and methods for the production of a wide array of antibodies are known to those of skill in the art.

**[0068]** Nucleic acids that encode the antigen binding proteins identified herein can be used to engineer recombinant immune effector cells. Methods and vectors to generate genetically modified T-cells, for example, are known in the art (See Brentjens et al., Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias in Blood 118(18):4817-4828, November 2011).

**[0069]** Other embodiments of the disclosure include cells and expression vectors comprising nucleic acids encoding the antigen-binding proteins or antigen-binding fragments thereof of the disclosure. The cells may be

recombinant immune effector cells, such as T-cells genetically modified to express a chimeric antigen receptor comprising an antigen binding region in accordance with the present disclosure. Cells which have been engineered to produce antibodies in accordance with the disclosure are also encompassed by the disclosure.

**[0070]** Further, the disclosure comprises a method of producing an antibody or antibody fragment of the disclosure comprising: (a) culturing the recombinant cell comprising a nucleic acid encoding an antibody or antibody fragment of the disclosure in culture medium under conditions wherein the nucleic acid sequence is expressed, thereby producing polypeptides comprising the light and heavy chain variable regions; and (b) recovering the polypeptides from the host cell or culture medium.

**[0071]** Some embodiments of the antigen-binding protein of the disclosure encompass antagonistic anti-PD1 antibodies as well as anti-PD1 antibodies that function as agonists of PD1. While some anti-PD1 antibodies are antagonists, that is, they block binding of PD1 by its ligand, others are agonists, antibodies that have the effect of enhancing the immunosuppressive signal of PD-1, making them useful in the treatment of autoimmunity, for example. Antibodies of the disclosure that exhibit antagonist activity include clones 23 and 27 while clones 16, 18, 26, 31 and 40 appear to function as agonists.

**[0072]** The disclosure also comprises the use of an anti-PD-1 antibody or antibody fragment of the disclosure for the preparation of a medicament to increase immune response as well as the use of an anti-PD-1 antibody or antibody fragment of the disclosure for the preparation of a medicament to treat cancer.

**[0073]** Pharmaceutical compositions comprising the antigen binding protein, antibodies, nucleic acids, vectors or cells comprising the nucleic acids or antigen binding proteins of disclosed herein along with a pharmaceutically acceptable carrier are also encompassed by the disclosure.

**[0074]** The disclosure also comprises the use of an anti-PD-1 antibody or antibody fragment of the disclosure as a vaccine adjuvant.

**[0075]** In another aspect, the disclosure relates to an immunoconjugate comprising a first component which is an antigen-binding protein, antibody or antigen-binding fragment thereof as disclosed herein. The immunoconjugate comprises a second component that is a cytotoxin, a detectable label, a radioisotope, a therapeutic agent, a binding protein or a molecule having a second amino acid sequence. Where the second component is a binding protein or second antibody, the binding protein or second antibody has binding specificity for a target that is different from the HLA-peptide complex for which the first is specific.

**[0076]** The disclosure also relates to methods for treatment of a subject having a PD-1 associated disease, comprising administering to the subject a therapeutically effective amount of an antigen binding protein, antibody or antigen binding fragment thereof, a chimeric antigen receptor (CAR), a nucleic acid encoding the antigen binding protein or a cell comprising the nucleic acids or proteins as disclosed herein.

**[0077]** Those skilled in the art will recognize that several embodiments are possible within the scope and spirit of this invention. The invention will now be described in greater detail by reference to the following non-limiting examples. The following examples further illustrate the invention but, of course, should not be construed as in any limiting its scope.

## Examples

### Example 1

**[0078]** To test the ability of the scFvs to inhibit PD-1 ligation, scFv-Fc domain (scFv-Fc) fusion proteins were generated, where the scFvs were linked to a murine Fc (mouse IgG1a) domain. The scFv-Fc clones were then analyzed for the ability to bind PD-1 by coating an ELISA plate with human PD-1 monomer. Binding of the scFvs to PD-1 was quantified using a HRP-conjugated anti-mouse IgG1 Fc secondary antibody. All seven antibodies showed binding activity with respect to the PD1 monomer in a dose dependent manner (**Figure 1**). ET901 ScFv-Fc (mouse IgG1 Fc) served as the negative control. Binding affinity of the scFv-Fc clones was ranked where clone 31 bound weakly and clones 26 and 27 bound the most strongly (31 < 23 < 40 < 18 < 16 = 27 < 26).

### Example 2

**[0079]** To test the ability of the scFv-Fc to inhibit PD-1 interacting with PD-L1, a competitive ligand-binding assay as shown schematically in **Figure 2A** was performed. In this assay, PD-L1-Fc was coated onto ELISA plates. Biotinylated-PD1-Fc was mixed with serially diluted ET901 ScFv-Fc (negative control) or anti-PD1 ScFv-Fcs and then added into the PD-L1-Fc coated plate. PD-1-Fc binding to PD-L1 coated on the plates was visualized via HRP-conjugated streptavidin (**Figure 2B**). When comparing similar concentrations of scFv-Fc (circled), the ability to disrupt the PD-1-PDL1 interaction was ranked where clones 40 and 23 had the weakest and clone 26 had the strongest ability to do so ( 26 > 27 = 16 > 18 > 31 > 23 = 40, **Figure 2B**).

## Example 3

**[0080]** These clones were then investigated for their ability to regulate specific T cell function. We have previously generated tumor-targeted T cells, wherein T cells are retrovirally transduced to express a tumor-specific chimeric antigen receptor (CAR). We have previously demonstrated that expression of a CAR has redirected T cell function to target a given antigen (Brentjens, Santos et al. 2007). In our lab, we target B cell malignancies using a CAR specific for CD19, termed 1928z (Brentjens, Santos et al. 2007). We have previously demonstrated that CAR modified T cells have demonstrated significant anti-tumor activity *in vitro* and *in vivo* and in clinical studies (Brentjens, Latouche et al. 2003; Brentjens, Davila et al. 2013; Davila, Riviere et al. 2014). To determine whether the 7 scFv clones are agonistic (stimulate PD-1), antagonistic (block PD-1) or have no significant effect on PD-1, we generated a secretable scFv by including the murine Kappa leader sequence proximal to the anti-PD-1 scFv gene (**Figure 3**). We then generated a bicistronic retroviral vector to induce expression of the 1928z CAR and secretion of an anti PD-1 scFv from transduced human peripheral blood T cells.

## Example 4

**[0081]** Given the ability of PD-1 stimulation to inhibit T cell proliferation and function, we subsequently sought to characterize the effect of the anti-PD-1 scFvs on T cell proliferation. To achieve this, human T cells were isolated from peripheral blood of healthy donors and modified through retroviral transduction to express the CAR and secrete a PD-1-specific scFv, using methodology that has been previously described (Brentjens, Santos et al. 2007). Following transduction, modified T cells were monitored for expansion *in vitro* (**Figure 4**). Expansion of T cells expressing the 1928z CAR and secreting PD-1 specific scFv clones 23 and 27 expanded as well as T cells

modified to express the 1928z CAR alone. Cells modified to express the CAR and secrete PD-1-specific scFv clones 16, 18, 26, 31 or 40 did not proliferate, suggesting that these PD-1-specific scFv clones were agonistic, potentially stimulating PD-1 and in turn, resulting in decreased T cell proliferation.

**Example 5**

**[0082]** In addition to expansion studies, we co-cultured T cells (expressing the CAR or CAR and secreting a PD-1-specific scFv) with the $CD19^+$ Burket's lymphoma tumor cell line, Raji, and determined the level of cytokine secretion from the T cells. As shown in **Figure 5**, T cells modified to express the 1928z CAR and secrete the PD-1-specific scFv clones 23 or 27 had increased secretion of IFN-γ compared to cells modified to express the CAR alone, suggesting that these scFv clones were antagonistic to PD-1 signaling. Cells modified to express the 1928z CAR and secrete the PD-1-specific scFv clones 16, 18, 2, 31 or 40 secreted less IFN-γ compared to cells modified with the CAR alone, suggesting that these scFvs were agonistic to PD-1 and inhibited T cell function.

**Example 6**

**[0083]** Human T cells modified to express the 1928 CAR and a PD-1 blocking scFv were analyzed by flow cytometry. Following verification of expression of the CAR (**Figure 6**) the presence of the scFvs was determined using western blot (using an antibody specific for an HA tag incorporated in the scFv design) on lysates prepared from human T cells treated with golgi inhibitors to allow detection in the cell lysates. Human T cells modified to express 1928z CAR and clone 23 had significantly less scFv protein compared to the other clones (**Figure 7**). These cells were then placed on 3T3 murine fibroblasts (artificial antigen presenting cells, a APCs) that do or do not express PD-1 ligands, PD-L1/L2. Following 24 hour culture with the aAPCs, CD3/D28 beads were added to the cultures to activate the human T cells.

Three days following culture of human T cells, aAPCs and beads the human T cells were enumerated to determine if the presence of PD-1 ligands inhibited the bead-driven expansion of human T cells, or if the anti-PD-1 scFvs prevented the PD-1 ligand mediated inhibition of T cell expansion. T cells modified to express the 1928z CAR and anti-PD-1 scFv clone 26 and 23 have decreased proliferation on aAPCs expressing PD-L1/L2 to aAPCs not expressing inhibitory ligands (**Figure 8**). In contrast, T cells modified to express the 1928z CAR and anti-PD-1 scFv clone 27 have increased expansion on aAPCs expressing PD-L1/L2.

**Example 7**

**[0084]** Using recombinant technology know in the art, recombinant human monoclonal antibodies were generated from the PD-1 specific scFvs, that is, fully human Ig molecules were made with the same variable heavy and light chains found in the corresponding scFvs (see Tables 1-14 above and **Figure 9**). The binding of these monoclonal antibodies to PD-1 was demonstrated using flow cytometry (see **Figure 10**).

**[0085]** Human T cells were modified to overexpress human PD-1, then incubated with 1µg/ml of antibody. Clone 27 mAb bound to the PD-1 T cells the most, followed by clone 26 and then clone 23 (**Figure 9**). The control monoclonal antibody, 901, did not bind to the PD-1 T cells. T cells incubated with these antibodies were then placed on artificial antigen presenting cells (aAPCs) with beads to determine the impact of PD-1 ligands on the expansion of the T cells and the ability of the monoclonal antibodies to prevent this interaction. 19z1 T cells incubated with anti-PD-1 clone 27 monoclonal antibody expanded on PD-L1/L2 aAPCs to a greater extent.

## Example 8

**[0086]** T cells modified to express a first generation CAR incubated on aAPCs expressing PD-L1/L2 expand when anti-PD-1 clone 27 monoclonal antibody is present. Human T cells modified to express the first generation CD19-specific CAR (19z1) were incubated with anti-PD-1 monoclonal antibodies for 24 hours then placed on aAPCs expressing PD-L1/L2 or not. After 24 hours stimulation with aAPCs, the cells were then stimulated with CD3/CD28 beads. After three days, the cells were enumerated. 19z1 T cells incubated with no monoclonal antibody, control antibody (901) and clones 23 and 26 monoclonal antibody expanded much less on aAPCs expressing PD-1 ligands (**Figure 11** open bars) compared to aAPCs with no inhibitory ligands (**Figure 11** closed bars). However, 19z1 T cells incubated with anti-PD-1 clone 27 monoclonal antibody expanded on PD-L1/L2 aAPCs to a greater extent. Data shown is representative of one experiment.

## Example 9

**[0087]** Generation of CAR T cells further modified to secrete PD-1 blocking scFv, E27. Bicistronic retroviral constructs were generated encoding a CD19-specific CAR (termed 1928z) or an ovarian tumor antigen specific CAR (termed 4H1128z) and the PD-1 blocking scFv, E27 (**Figure 12A**). The E27 was preceded by a signal peptide, mouse IgK, to allow secretion of the scFv. A HA/His tag was also included to detect the scFv once secreted from the T cells. Human peripheral blood T cells were transduced with the retroviral constructs encoding the CAR, 1928z, or the CAR and the E27 PD-1 blocking scFv, 1928z-E27. Following transduction, flow cytometry was used to detect expression of the CAR, using an antibody that specifically binds the CD19-targeted CAR, termed 19E3 (**Figure 12 B**). Western blot analysis of supernatant from transduced human T cells was utilized to detect the PD-1 blocking scFv with an anti-HA antibody (**Figure 12C**). We also investigated

scFv secretion from T cells modified to express the CAR and a control scFv, B6, which was detected using an anti-c-myc tag antibody. A standard $^{51}Cr$ release assay against two $CD19^{+}$ tumor targets was performed to ensure that secretion of an scFv did not interrupt the ability of the CAR to redirect T cells cytolytic capacity. CAR T cells expressing either the CAR alone (1928z or 4H1128z control CAR), the CAR and the E27 scFv (1928z-E27 or 4H1128z-E27), or the CAR and a control scFv (1928z-B6H12.2 or 4H1128z-B6H12.2) were incubated with $^{51}Cr$ labeled tumor cells (Raji or Nalm6) for 4 hrs. T cells expressing the CD19 specific CAR were able to lyse the tumor targets at equivalent levels, and the ovarian-targeted CAR T cells were unable to lyse Raji or Nalm6 (**Figure 12D**). Therefore, we conclude that secretion of the scFv did not interrupt the ability of the CAR to redirect T cell lytic capacity.

**Example 10**

**[0088]** T cells modified to express the CAR and secrete a PD-1 blocking scFv resist inhibition from PD-L1-PD-1 interactions, *in vitro*. T cells expressing the CAR alone (1928z), or the CAR and the PD-1 blocking scFv (1928z-E27) were cultured on 3T3 cells empty cells or 3T3 cells modified to express human PD-L1. Following 24 hours on the 3T3 feeder cells, cells were stimulated with CD3/CD28 beads added to the cultures at a 1:3 bead: T cell ratio. Expansion of T cells was determined with trypan blue enumeration and fresh beads were added twice to the cultures (indicated by the arrows). 1928z T cells expanded on 3T3 empty feeder cells, however did not expand on the 3T3-PD-L1 feeder cells. In contrast, 1928z-E27 T cells expanded on both the 3T3 Empty and 3T3-PD-L1 feeder cells, indicating a resistance to PD-L1-PD-1 mediated suppression (**Figure 13A**). T cells incubated on 3T3 empty or 3T3-PD-L1 cells as shown in **Figure 13A** were analyzed by flow cytometry to detect expression on inhibitory receptors, PD-1, 2B4 and LAG3. 1928z cells expressed increased levels of PD-1 than 1928z-E27 cells (not shown). When

gated on PD-1$^+$ cells, analysis of 2B4 and LAG3 revealed that 1928z cells had a higher proportion of PD-1$^+$, 2B4$^+$ and LAG3$^+$ cells compared to 1928z-E27 cells (**Figure 13B**). Transduced T cells were cultured with Raji-PDL1 or Nalm6-PDL1 tumor cells at varying effector to target (E:T) ratios (1:1, 1:3, 1:5) for 72 hours. Flow cytometry following staining with anti-CD3 and anti-CD19 antibodies and enumeration beads were used to monitor lysis of tumor targets and expansion of T cells over time. 1928z-E27 cells continued to expand to greater levels compared to 1928z T cells when cultured with PDL1$^+$ tumor cells (**Figure 13C**). Transduced T cells were stimulated with Nalm6-PDL1 tumor cells as shown in Figure 3C were re-stimulated with Nalm6-PDL1 tumor cells at the 1:5 T E:T ratio. After 48 hours co-culture flow cytometry was used to determine lysis of tumor targets. 1928z-E27 retained ability to lyse PD-L1 tumor targets upon re-stimulation compared to 1928z cells (**Figure 13D**).

**Example 11**

**[0089]** *In vivo* anti-tumor efficacy of T cells modified to express the CAR and secrete the PD-1 blocking scFv is shown in **Figure 14**. SCID-beige mice were inoculated with Raji-PD-L1 tumor cells via intravenous infusion on Day 0. On Day 1, mice were infused intravenously with $10^6$ CAR+ T cells and survival was monitored clinically. Mice were euthanized upon development of hind limb paralysis.

**Example 12**

**[0090]** PD-1 blocking mAb candidates E27, E26 and E23 were used in a competitive binding assay to detect interruption of PD-1 binding to PD-L1 at varying concentrations, compared to a control mAb, targeted to a hapten not present in humans. E23, E26 and E27 mAbs all prevented PD-1 binding to PD-L1 (**Figure 15A**). Western blot on SN from 293Glv9 packaging cells transduced to express the secretable scFvs with the 1928z CAR, stained with

anti-HA antibody. The E27 scFv was detected at the highest levels and therefore was used in the remainder of the study (**Figure 15C**).

**Example 13**

**[0091]** T cells can be co-modified to express CAR and secrete PD-1 blocking scFv, E27. **Figure 16A** is a representative flow cytometry plot demonstrating equivalent CAR expression following transduction with the 1928z CAR alone (1928z) or the 1928z CAR and the E27 PD-1 blocking scFv (1928z-E27), following staining with 19E3 mAb that specifically binds the 1928z CAR. **Figure 16B** shows a Western blot on SN from 1928z and 1928z-E27 T cells stained with anti-HA mAb, showing only a ~30 kDa protein in the 1928z-E27 cells, demonstrating that the E27 scFv is secreted from the 1928z-E27 transduced T cells and not those transduced with the CAR alone. **Figure 16C** is a representative flow cytometry demonstrating lower levels of PD-1 expression on 1928z-E27 T cells compared to 1928z T cells following transduction. Expression of PD-1 was statistically significantly lower on 1928z-E27 T cells compared to 1928z T cells, data shown is mean +/- SEM from 4 independent experiments (**Figure 16D**). A 4 hr $^{51}Cr$ release assay demonstrating that lysis of Raji tumor cells was unaffected by secretion of the E27 scFv. 1928z and 1928z-E27 T cells lysed Raji tumor cells equivalently. Control 4H1128z-E27 T cells mediated no increase in lysis of Raji cells compared to 4H1128z T cells (**Figure 16 E**). Data shown is representative of two independent experiments.

**Example 14**

**[0092]** Expression of CAR and E27 protects proliferative and lytic capacity of T cells in the context of $CD19^+$ $PD\text{-}L1^+$ tumor cells. Raji tumor cells were retrovirally modified to express human PD-L1 (Raji-PDL1) and were stained with mAb specific for PD-L1. Parental Raji tumor (Raji) express no PD-L1 and

Raji-PDL1 tumor cells expressed high levels of PD-L1 (**Figure 17A**). **Figure 17B** shows representative flow cytometry plots showing1928z-E27 T cells lyse more Raji-PDL1 tumor cells compared to 1928z T cells as determined with flow cytometry following 72 hrs co-culture. 1928z-E27 T cells lyse statistically significantly more Raji-PDL1 tumor cells compared to 1928z T cells, data shown the mean +/- SEM from 4 independent experiments (**Figure 17C**). 1928z-E27 T cells expand to greater numbers following co-culture with Raji-PDL1 tumor cells as determined by flow cytometry and enumeration beads, data shown is the average total number of T cells +/- SEM from 4 independent experiments (**Figure 17D**). **Figure 17E** shows a representative flow cytometry plot showing increased PD-1 expression on 1928z T cells compared to 1928z-E27 T cells following 7 days co-culture with Raji-PDL1 tumor cells. 1928z T cells express significantly more PD-1 compared to 1928z-E27 T cells, with regard to percentage positive cells and mean fluorescence intensity (MFI) of PD-1 staining. Data show in the mean +/- SEM from 4 independent experiments (**Figure 17F**). **Figure 17G** shows representative flow cytometry plots showing increased percentage of 2B4+PD-1+ 1928z T cells compared to 1928z-E27 cells following coculture with Raji-PDL1 for 7 days. 1928z-E27 T cells also express less BTLA and TIM3 on the 2B4+PD-1+ population. Data shown is representative of 3 independent experiments.

**Example 15**

**[0093]** E27 protects proliferative capacity of CD3/CD28 stimulated T cells in the context of PD-L1. NIH3T3 cells were retrovirally modified to express human PD-L1 (3T3-PDL1) and were stained with mAb specific for PD-L1. Parental NIH3T3 (3T3-EMPTY) express no PD-L1 and 3T3-PDL1 tumor cells expressed high levels of PD-L1 (**Figure 18A**). 1928z and 1928z-E27 T cells were cultured with 3T3-EMPTY or 3T3-PDL1 cells and stimulated with CD3/CD28 beads. Cells were enumerated and re-plated on new 3T3 cells on

days 3, 6, 9 and 12. 1928z T cells had reduced expansion when cultured with 3T3-PDL1 cells compared to 3T3-EMPTY cells. 1928z-E27 cells had equivalent expansion when cultured on 3T3-EMPTY or 3T3-PDL1 cells (**Figure 18B**). Data shown is the mean fold expansion +/- SEM from 4 independent experiments. **Figure 18C** are representative flow cytometry plots showing increased expression of 2B4, PD-1, BTLA and TIM3 on 1928z T cells cultured with 3T3-PDL1 compared to1928z T cells cultured on 3T3-EMPTY cells. 1928z-E27 cells had equivalent expression of 2B4, PD-1, BTLA-4 and TIM3 when cultured with 3T3-EMPTY and 3T3-PDL1. Data shown is representative of 3 independent experiments.

**Example 16**

**[0094]** CAR T cells secreting E27 scFv have increased anti-tumor function in vivo. SCID-Beige mice were inoculated with Raji-PDL1 tumor cells intravenously, and the following day were infused intravenously with CAR T cells. As shown in **Figure 19** mice treated with 1928z-E27 T cells had enhanced survival compared to mice treated with 1928z T cells. Mice treated with 1928z T cells survived longer than untreated mice, and mice treated with CAR T cells targeted to an irrelevant antigen, 4H1128z and 4H1128z-E27 T cells. Data shown is from 2 independent experiments.

**Example 17**

**[0095]** Anti-PD-1 antibodies ET130-23, ET130-26 and ET130-27 were tested by ELISA to check the blocking effect to PD1/PDL1 binding. As shown in **Figure 20**, ET901 (negative control) showed no binding, while ET130-23, ET130-26 and ET130-27 showed a blocking effect to PD1/PDL1 binding over a range of concentrations between 0.031 and 10 µg/ml.

**[0096]** Similarly, ET130-23, ET130-26 and ET130-27 were tested by ELISA to check the blocking effect to PD1/PDL2 binding. As shown in **Figure 21**, ET901 (negative control) showed no binding, while ET130-23, ET130-26 and ET130-27 showed a blocking effect to PD1/PDL2 binding over the same range of concentrations. ET130-26 showed the highest blocking effect against PD1/PDL2, while ET130-23 showed the lowest effect. The blocking pattern is parallel to PD1/PDL1 binding.

**Example 18**

**[0097]** The application of the anti-PD-1 scFvs or monoclonal antibodies is investigated for the ability of the scFvs or monoclonal antibodies to dampen the immune response and subvert autoimmune diseases. This can also be investigated using murine models of GVHD. Infusion of human T cells into irradiated NOD.SCID.IL-2Rγ$^{-/-}$ results in engraftment of human cells and severe GVHD, where the human T cells attack the murine tissues. T cells secreting an anti-PD-1 scFv (or T cells augmented with injection of monoclonal antibodies) are infused into a subject and the development of GVHD is assessed. When the anti-PD-1 scFv/mAb is agonistic, the GVHD response is inhibited due to suppression of the human T cells.

***Exemplary Embodiments***

1. A recombinant antigen-binding protein or antigen-binding fragment thereof comprising one of:

(A) an antigen binding region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 21, SEQ ID NO: 32, SEQ ID NO: 43, SEQ ID NO: 53, SEQ ID NO: 61, SEQ ID NO: 72, SEQ ID NO: 83, SEQ ID NO: 94, SEQ ID NO: 103, SEQ ID NO: 114, SEQ ID

NO: 125, SEQ ID NO: 133, SEQ ID NO: 142; a fragment thereof and a homologous sequence thereof;

(B) an antigen binding region comprising a variable light chain (VL) and variable heavy chain (VH), respectively, with amino acid sequences selected from SEQ ID NOS: 6 and 8; SEQ ID NOS: 17 and 19; SEQ ID NOS: 28 and 30; SEQ ID NOS: 39 and 41; SEQ ID NOS: 49 and 51; SEQ ID NOS: 57 and 59; SEQ ID NOS: 68 and 70; SEQ ID NOS: 79 and 81; SEQ ID NOS: 90 and 92; SEQ ID NOS: 99 and 101; SEQ ID NOS: 110 and 112; SEQ ID NOS: 121 and 123; SEQ ID NOS: 129 and 131; SEQ ID NOS: 138 and 140; fragments thereof and homologous sequences thereof;

(C) an antigen binding region comprising:

(i) a light chain (LC) comprising light chain complementarity determining regions (LCCDR) LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence QSISSY (SEQ ID NO: 1), AAS and QQSYSTPLT (SEQ ID NO: 2) and a heavy chain (HC) comprising heavy chain complementarity determining regions (HCCDR) HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTSSSYW (SEQ ID NO: 4), IKQDGSEK (SEQ ID NO. 5) and ARGGWSYDM (SEQ ID NO: 6); fragments thereof and homologous sequences thereof;

(ii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGAGYA (SEQ ID NO: 12), TNN and QSYDSSLSGVI (SEQ ID NO: 13) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTLTELS (SEQ ID NO: 14), FDPEDGET (SEQ ID NO. 15) and ARAYYGFDQ (SEQ ID NO: 16); fragments thereof and homologous sequences thereof;

(iii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGNNA (SEQ ID NO: 23), YND and AAWDDSVNGYV (SEQ ID NO: 24) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTRFG (SEQ ID NO: 25), ISVNNGNT (SEQ ID NO. 26) and ARYMYGRRDS (SEQ ID NO: 27); fragments thereof and homologous sequences thereof;

(iv) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QVWDNHSDVV (SEQ ID NO: 35) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences RNKFSSYA (SEQ ID NO: 36), ISGSGGTT (SEQ ID NO. 37) and ARWYSSYYDV (SEQ ID NO: 38); fragments thereof and homologous sequences thereof;

(v) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QVWDSSSDYV (SEQ ID NO: 45) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARNYISMFDS (SEQ ID NO: 48); fragments thereof and homologous sequences thereof;

(vi) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QVWDSSSDHV (SEQ ID NO: 55) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and

ARGYSSYYDA (SEQ ID NO: 56); fragments thereof and homologous sequences thereof;

(vii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence RSNIGENT (SEQ ID NO: 63), SNN and AAWDDRLNGYV (SEQ ID NO: 64) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTNYG (SEQ ID NO: 65), IGAQKGDT (SEQ ID NO. 66) and ARSQGVPFDS (SEQ ID NO: 67); fragments thereof and homologous sequences thereof;

(viii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence RSNIGSNT (SEQ ID NO: 74), NNN and ATWDDSLNEYV (SEQ ID NO: 75) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTRYG (SEQ ID NO: 76), ISGYNGNT (SEQ ID NO. 77) and ARHGYGYHGD (SEQ ID NO: 78); fragments thereof and homologous sequences thereof;

(ix) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGAGYV (SEQ ID NO: 85), HNN and QSYDSSLSGWV (SEQ ID NO: 86) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFKDYY (SEQ ID NO: 87), ISTSGNSV (SEQ ID NO. 88) and ARSPGHSDYDS (SEQ ID NO: 89); fragments thereof and homologous sequences thereof;

(x) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGDKS (SEQ ID NO: 96), YDS and QVWASGTDHPYVI (SEQ ID NO: 97) and a heavy chain (HC) comprising

HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARMYGSYTDM (SEQ ID NO: 98); fragments thereof and homologous sequences thereof;

(xi) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGYNY (SEQ ID NO: 105), RNN and TSWDDSLSGYV (SEQ ID NO: 106) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GNAFTNFY (SEQ ID NO: 107), INPSGTDLT (SEQ ID NO. 108) and ARQYAYGYSGFDM (SEQ ID NO: 109); fragments thereof and homologous sequences thereof;

(xii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence QSVSNW (SEQ ID NO: 116), AAS and QQSYSTPIT (SEQ ID NO: 117) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTSYY (SEQ ID NO: 118), INPNTGGS (SEQ ID NO. 119) and ARGDVTYDE (SEQ ID NO: 120); fragments thereof and homologous sequences thereof;

(xiii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDD and QVWDINDHYV (SEQ ID NO: 127) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARSQASFMDI (SEQ ID NO: 128); fragments thereof and homologous sequences thereof; or

(xiv) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), DDS and QVWDSSSDQGV (SEQ ID NO: 135) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), IGTGGGT (SEQ ID NO. 136) and ARGTGYDGDQ (SEQ ID NO: 137); fragments thereof and homologous sequences thereof.

2. The recombinant antigen-binding protein of embodiment 1, wherein said protein is an antibody.

3. The recombinant antigen-binding protein of embodiment 2, wherein the antibody is a human antibody.

4. The recombinant antigen-binding protein of embodiment 2, wherein said antibody or antigen-binding fragment thereof is intact Ig, Fab, $F(ab')_2$, Fv, or scFv.

5. The antigen-binding protein of embodiment 1, wherein said antigen-binding protein is a PD-1 agonist.

6. The antigen-binding protein of embodiment 1, wherein said antigen-binding protein is a PD-1 antagonist.

7. The antigen-binding protein of embodiment 1, wherein said antigen-binding protein is a chimeric antigen receptor (CAR).

8. A nucleic acid encoding an antigen-binding protein of any one of embodiments 1-7.

9. A vector comprising a nucleic acid of embodiment 8.

10. A cell comprising an antigen-binding protein of any one of embodiments 1-7, a nucleic acid of embodiment 8 or a vector of embodiment 9.

11. An antigen-binding protein of any one of embodiments 1-7 conjugated to a therapeutic agent.

12. The antigen-binding protein of embodiment 11, wherein said therapeutic agent is a drug, toxin, radioisotope, protein, or peptide.

13. A pharmaceutical composition comprising an antigen-binding protein of any one of embodiments 1-7, a nucleic acid of embodiment 8, a vector of embodiment 9, a cell of embodiment 10 or an antigen-binding protein of embodiment 11 or 12.

14. The pharmaceutical composition of embodiment 13 further comprising a pharmaceutically acceptable carrier.

15. A method of increasing a T cell response in a subject comprising administering to the subject a therapeutically effective amount of an antigen-binding protein or an antigen binding fragment thereof of any one of embodiments 1-7, a nucleic acid of embodiment 8, a vector of embodiment 9, a cell of embodiment 10, an antigen-binding protein of either of embodiments 11 or 12 or a pharmaceutical composition of either of embodiments 13 or 14.

16. The method of embodiment 15, wherein the antigen-binding protein or antigen binding fragment thereof inhibits, reduces, modulates or abolishes signal transduction mediated by PD-1.

17. A method for treatment of a subject having a PD1-positive disease, comprising administering to the subject a therapeutically effective amount of an antigen-binding protein or antigen binding fragment thereof of any one of embodiments 1-7, a nucleic acid of embodiment 8, a vector of embodiment 9, a cell of embodiment 10, an antigen-binding protein of either of embodiments 11 or 12 or a pharmaceutical composition of either of embodiments 13 or 14.

18. The method of embodiment 17, wherein said antigen-binding protein or antigen binding fragment thereof is a conjugate having a cytotoxic moiety linked thereto.

19. The method of embodiment 17 or 18, wherein the PD-1 positive disease is cancer.

20. Use of a recombinant anti-PD1 antigen-binding protein or antigen-binding fragment thereof of any one of embodiments 1-7, a nucleic acid of embodiment 8, a vector of embodiment 9, a cell of embodiment 10, an antigen-binding protein of either of embodiments 11 or 12 or a pharmaceutical composition of either of embodiments 13 or 14 for the treatment of PD1-positive disease by inhibiting PD1 binding to a PD1 ligand.

21. The use of embodiment 20, wherein the PD1-positive disease is a cancer.

22. Use of a recombinant anti-PD1 antigen-binding protein or antigen-binding fragment thereof of any one of embodiments 1-7, a nucleic acid of embodiment 8, a vector of embodiment 9, a cell of embodiment 10, an antigen-binding protein of either of embodiments 11 or 12 or a pharmaceutical composition of either of embodiments 13 or 14 for immunomodulation by inhibiting the PD-1 signaling pathway.

23. A vector comprising a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

24. A cell comprising the vector of embodiment 23.

25. A cell comprising a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

26. A cell comprising a recombinant anti-PD-1 antigen-binding protein and a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

27. The vector or the cell of any one of embodiments 23-26, wherein the chimeric antigen receptor does not specifically bind to PD-1.

28. The vector or the cell of any one of embodiments 23-27, wherein the recombinant anti-PD-1 antigen-binding protein is an antibody.

29. The vector or the cell of any one of embodiments 23-28, wherein the recombinant anti-PD-1 antigen-binding protein is a human antibody.

30. The vector or the cell of any one of embodiments 23-29, wherein the recombinant anti-PD-1 antigen-binding protein is an intact Ig, Fab, $F(ab')_2$, Fv, or scFv.

31. The vector or the cell of any one of embodiments 23-30, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 agonist.

32. The vector or the cell of any one of embodiments 23-30, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 antagonist.

33. The vector or the cell of any one of embodiments 23-32, wherein the recombinant anti-PD-1 antigen-binding protein is a secretable protein.

34. The vector or the cell of any one of embodiments 23-33, wherein the recombinant anti-PD-1 antigen-binding protein comprises an antigen binding region recited in embodiment 1.

35. The vector or the cell of any one of embodiments 23-34, wherein the chimeric antigen receptor specifically binds to CD-19.

36. The vector or the cell of any one of embodiments 23-35, wherein the chimeric antigen receptor can be inserted in a human T cell membrane.

37. The cell of any one of embodiments 24-36, wherein the cell is a T cell.

38. A pharmaceutical composition comprising a vector or a cell of any one of embodiments 23-37.

39. The pharmaceutical composition of embodiment 38 further comprising a pharmaceutically acceptable carrier.

40. A method of increasing a T cell response in a subject comprising administering to the subject a therapeutically effective amount of a vector or cell of any one of embodiments 23-37, or a pharmaceutical composition of embodiment 38 or 39, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 antagonist.

41. The method of embodiment 40, wherein the recombinant anti-PD-1 antigen-binding protein inhibits, reduces, modulates or abolishes signal transduction mediated by PD-1.

42. A method of decreasing a T cell response in a subject comprising administering to the subject a therapeutically effective amount of a vector or cell of any one of embodiments 23-37, or a pharmaceutical composition of embodiment 38 or 39, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 agonist.

43. A method for treatment of a subject having a PD1-positive disease, comprising administering to the subject a therapeutically effective amount of a vector or cell of any one of embodiments 23-37, or a pharmaceutical composition of embodiment 38 or 39.

44. A method for treatment of a subject having a PD1-positive disease, comprising transducing at least one T cell of the subject with a

nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

45. The method of embodiment 44, wherein the chimeric antigen receptor does not specifically bind to PD-1.

46. The method of any one of embodiments 42-45, wherein the PD1-positive disease is a cancer.

47. Use of a vector or cell of any one of embodiments 23-37, or a pharmaceutical composition of embodiment 38 or 39 for the treatment of PD1-positive disease by inhibiting PD1 binding to a PD1 ligand.

48. The use of embodiment 47, wherein the PD1-positive disease is a cancer.

49. Use of a vector or cell of any one of embodiments 23-37, or a pharmaceutical composition of embodiment 38 or 39 for immunomodulation by inhibiting the PD-1 signaling pathway.

50. Use of an antigen-binding protein of embodiment 5, a vector or cell of any one of embodiments 23-37, or a pharmaceutical composition of embodiment 38 or 39, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 agonist, for the treatment of an autoimmune disease.

51. The vector or the cell of any one of embodiments 23-37, wherein at least one of the anti-PD-1 antigen-binding protein and chimeric antigen receptor is conjugated to a therapeutic agent.

52. The vector or the cell of embodiment 51, wherein said therapeutic agent is a drug, toxin, radioisotope, protein, or peptide.

53. The method of any one of embodiments 17 and 42-45, wherein the antigen-binding protein or the recombinant anti-PD-1 antigen-binding protein is a PD-1 agonist, and wherein the PD1-positive disease is an autoimmune disease.

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Davila, M. L., **I.** Riviere, et al. (2014). "Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia." Sci Transl Med 6(224): 224ra225.

# CLAIMS

We claim:

1. A recombinant antigen-binding protein or antigen-binding fragment thereof comprising one of:

(A) an antigen binding region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 21, SEQ ID NO: 32, SEQ ID NO: 43, SEQ ID NO: 53, SEQ ID NO: 61, SEQ ID NO: 72, SEQ ID NO: 83, SEQ ID NO: 94, SEQ ID NO: 103, SEQ ID NO: 114, SEQ ID NO: 125, SEQ ID NO: 133, SEQ ID NO: 142; a fragment thereof and a homologous sequence thereof;

(B) an antigen binding region comprising a variable light chain (VL) and variable heavy chain (VH), respectively, with amino acid sequences selected from SEQ ID NOS: 6 and 8; SEQ ID NOS: 17 and 19; SEQ ID NOS: 28 and 30; SEQ ID NOS: 39 and 41; SEQ ID NOS: 49 and 51; SEQ ID NOS: 57 and 59; SEQ ID NOS: 68 and 70; SEQ ID NOS: 79 and 81; SEQ ID NOS: 90 and 92; SEQ ID NOS: 99 and 101; SEQ ID NOS: 110 and 112; SEQ ID NOS: 121 and 123; SEQ ID NOS: 129 and 131; SEQ ID NOS: 138 and 140; fragments thereof and homologous sequences thereof;

(C) an antigen binding region comprising:

(i) a light chain (LC) comprising light chain complementarity determining regions (LCCDR) LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence QSISSY (SEQ ID NO: 1), AAS and QQSYSTPLT (SEQ ID NO: 2) and a heavy chain (HC) comprising heavy chain complementarity determining regions (HCCDR) HCCDR1, HCCDR2 and HCCDR3 respectively,

having amino acid sequences GFTSSSYW (SEQ ID NO: 4), IKQDGSEK (SEQ ID NO. 5) and ARGGWSYDM (SEQ ID NO: 6); fragments thereof and homologous sequences thereof;

(ii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGAGYA (SEQ ID NO: 12), TNN and QSYDSSLSGVI (SEQ ID NO: 13) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTLTELS (SEQ ID NO: 14), FDPEDGET (SEQ ID NO. 15) and ARAYYGFDQ (SEQ ID NO: 16); fragments thereof and homologous sequences thereof;

(iii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGNNA (SEQ ID NO: 23), YND and AAWDDSVNGYV (SEQ ID NO: 24) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTRFG (SEQ ID NO: 25), ISVNNGNT (SEQ ID NO. 26) and ARYMYGRRDS (SEQ ID NO: 27); fragments thereof and homologous sequences thereof;

(iv) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QVWDNHSDVV (SEQ ID NO: 35) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences RNKFSSYA (SEQ ID NO: 36), ISGSGGTT (SEQ ID NO. 37) and ARWYSSYYDV (SEQ ID NO: 38); fragments thereof and homologous sequences thereof;

(v) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS

and QVWDSSSDYV (SEQ ID NO: 45) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARNYISMFDS (SEQ ID NO: 48); fragments thereof and homologous sequences thereof;

(vi) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QVWDSSSDHV (SEQ ID NO: 55) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARGYSSYYDA (SEQ ID NO: 56); fragments thereof and homologous sequences thereof;

(vii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence RSNIGENT (SEQ ID NO: 63), SNN and AAWDDRLNGYV (SEQ ID NO: 64) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTNYG (SEQ ID NO: 65), IGAQKGDT (SEQ ID NO. 66) and ARSQGVPFDS (SEQ ID NO: 67); fragments thereof and homologous sequences thereof;

(viii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence RSNIGSNT (SEQ ID NO: 74), NNN and ATWDDSLNEYV (SEQ ID NO: 75) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTRYG (SEQ ID NO: 76), ISGYNGNT (SEQ ID NO. 77) and ARHGYGYHGD (SEQ ID NO: 78); fragments thereof and homologous sequences thereof;

(ix) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGAGYV (SEQ ID NO: 85), HNN and QSYDSSLSGWV (SEQ ID NO: 86) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFKDYY (SEQ ID NO: 87), ISTSGNSV (SEQ ID NO. 88) and ARSPGHSDYDS (SEQ ID NO: 89); fragments thereof and homologous sequences thereof;

(x) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGDKS (SEQ ID NO: 96), YDS and QVWASGTDHPYVI (SEQ ID NO: 97) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARMYGSYTDM (SEQ ID NO: 98); fragments thereof and homologous sequences thereof;

(xi) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGYNY (SEQ ID NO: 105), RNN and TSWDDSLSGYV (SEQ ID NO: 106) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GNAFTNFY (SEQ ID NO: 107), INPSGTDLT (SEQ ID NO. 108) and ARQYAYGYSGFDM (SEQ ID NO: 109); fragments thereof and homologous sequences thereof;

(xii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence QSVSNW (SEQ ID NO: 116), AAS and QQSYSTPIT (SEQ ID NO: 117) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTSYY (SEQ ID NO: 118), INPNTGGS (SEQ ID NO. 119) and

ARGDVTYDE (SEQ ID NO: 120); fragments thereof and homologous sequences thereof;

(xiii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDD and QVWDINDHYV (SEQ ID NO: 127) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARSQASFMDI (SEQ ID NO: 128); fragments thereof and homologous sequences thereof; or

(xiv) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), DDS and QVWDSSSDQGV (SEQ ID NO: 135) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), IGTGGGT (SEQ ID NO. 136) and ARGTGYDGDQ (SEQ ID NO: 137); fragments thereof and homologous sequences thereof.

2. The recombinant antigen-binding protein of claim 1, wherein said protein is an antibody.

3. The recombinant antigen-binding protein of claim 2, wherein the antibody is a human antibody.

4. The recombinant antigen-binding protein of claim 2, wherein said antibody or antigen-binding fragment thereof is intact Ig, Fab, $F(ab')_2$, Fv, or scFv.

5. The antigen-binding protein of claim 1, wherein said antigen-binding protein is a PD-1 agonist.

6. The antigen-binding protein of claim 1, wherein said antigen-binding protein is a PD-1 antagonist.

7. The antigen-binding protein of claim 1, wherein said antigen-binding protein is a chimeric antigen receptor (CAR).

8. A nucleic acid encoding an antigen-binding protein of any one of claims 1-7.

9. A vector comprising a nucleic acid of claim 8.

10. A cell comprising an antigen-binding protein of any one of claims 1-7, a nucleic acid of claim 8 or a vector of claim 9.

11. A pharmaceutical composition comprising an antigen-binding protein of any one of claims 1-7, a nucleic acid of claim 8, a vector of claim 9, a cell of claim 10.

12. A method of increasing a T cell response in a subject comprising administering to the subject a therapeutically effective amount of an antigen-binding protein or an antigen binding fragment thereof of any one of claims 1-7, a nucleic acid of claim 8, a vector of claim 9, a cell of claim 10, or a pharmaceutical composition of claim 11.

13. The method of claim 12, wherein the antigen-binding protein or antigen binding fragment thereof inhibits, reduces, modulates or abolishes signal transduction mediated by PD-1.

14. A method for treatment of a subject having a PD1-positive disease, comprising administering to the subject a therapeutically effective amount of an antigen-binding protein or antigen binding fragment thereof of any one of claims 1-7, a nucleic acid of claim 8, a vector of claim 9, a cell of claim 10, or a pharmaceutical composition of claim 11.

15. Use of a recombinant anti-PD1 antigen-binding protein or antigen-binding fragment thereof of any one of claims 1-7, a nucleic acid of claim 8, a vector of claim 9, a cell of claim 10, or a pharmaceutical composition of claim 11 for the treatment of PD1-positive disease by inhibiting PD1 binding to a PD1 ligand.

16. Use of a recombinant anti-PD1 antigen-binding protein or antigen-binding fragment thereof of any one of claims 1-7, a nucleic acid of claim 8, a vector of claim 9, a cell of claim 10, or a pharmaceutical composition of claim 11 for immunomodulation by inhibiting the PD-1 signaling pathway.

17. A vector comprising a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

18. A cell comprising the vector of claim 17.

19. A cell comprising a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

20. A cell comprising a recombinant anti-PD-1 antigen-binding protein and a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

21. The vector or the cell of any one of claims 17-20, wherein the chimeric antigen receptor does not specifically bind to PD-1.

22. The vector or the cell of any one of claims 17-21, wherein the recombinant anti-PD-1 antigen-binding protein is an antibody.

23. The vector or the cell of any one of claims 17-22, wherein the recombinant anti-PD-1 antigen-binding protein is a human antibody.

24. The vector or the cell of any one of claims 17-24, wherein the recombinant anti-PD-1 antigen-binding protein is an intact Ig, Fab, $F(ab')_2$, Fv, or scFv.

25. The vector or the cell of any one of claims 17-24, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 agonist.

26. The vector or the cell of any one of claims 17-24, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 antagonist.

27. The vector or the cell of any one of claims 17-26, wherein the recombinant anti-PD-1 antigen-binding protein is a secretable protein.

28. The vector or the cell of any one of claims 17-27, wherein the recombinant anti-PD-1 antigen-binding protein comprises an antigen binding region recited in claim 1.

29. The vector or the cell of any one of claims 17-28, wherein the chimeric antigen receptor specifically binds to CD-19.

30. The vector or the cell of any one of claims 17-29, wherein the chimeric antigen receptor can be inserted in a human T cell membrane.

31. The cell of any one of claims 17-30, wherein the cell is a T cell.

32. A pharmaceutical composition comprising a vector or a cell of any one of claims 17-31.

33. A method of increasing a T cell response in a subject comprising administering to the subject a therapeutically effective amount of a vector or cell of any one of claims 17-31, or a pharmaceutical composition of claim 32, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 antagonist.

34. The method of claim 33, wherein the recombinant anti-PD-1 antigen-binding protein inhibits, reduces, modulates or abolishes signal transduction mediated by PD-1.

35. A method of decreasing a T cell response in a subject comprising administering to the subject a therapeutically effective amount of a vector or cell of any one of claims 17-31, or a pharmaceutical composition of claim 32, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 agonist.

36. A method for treatment of a subject having a PD1-positive disease, comprising administering to the subject a therapeutically effective amount of a vector or cell of any one of claims 17-31, or a pharmaceutical composition of claim 32.

37. A method for treatment of a subject having a PD1-positive disease, comprising transducing at least one T cell of the subject with a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

38. The method of claim 37, wherein the chimeric antigen receptor does not specifically bind to PD-1.

39. The method of any one of claims 33-38, wherein the PD-1-positive disease is a cancer.

40. Use of a vector or cell of any one of claims 17-31, or a pharmaceutical composition of claim 32 for the treatment of PD1-positive disease by inhibiting PD1 binding to a PD1 ligand.

41. Use of a vector or cell of any one of claims 17-31, or a pharmaceutical composition of claim 38 or 39 for immunomodulation by inhibiting the PD-1 signaling pathway.

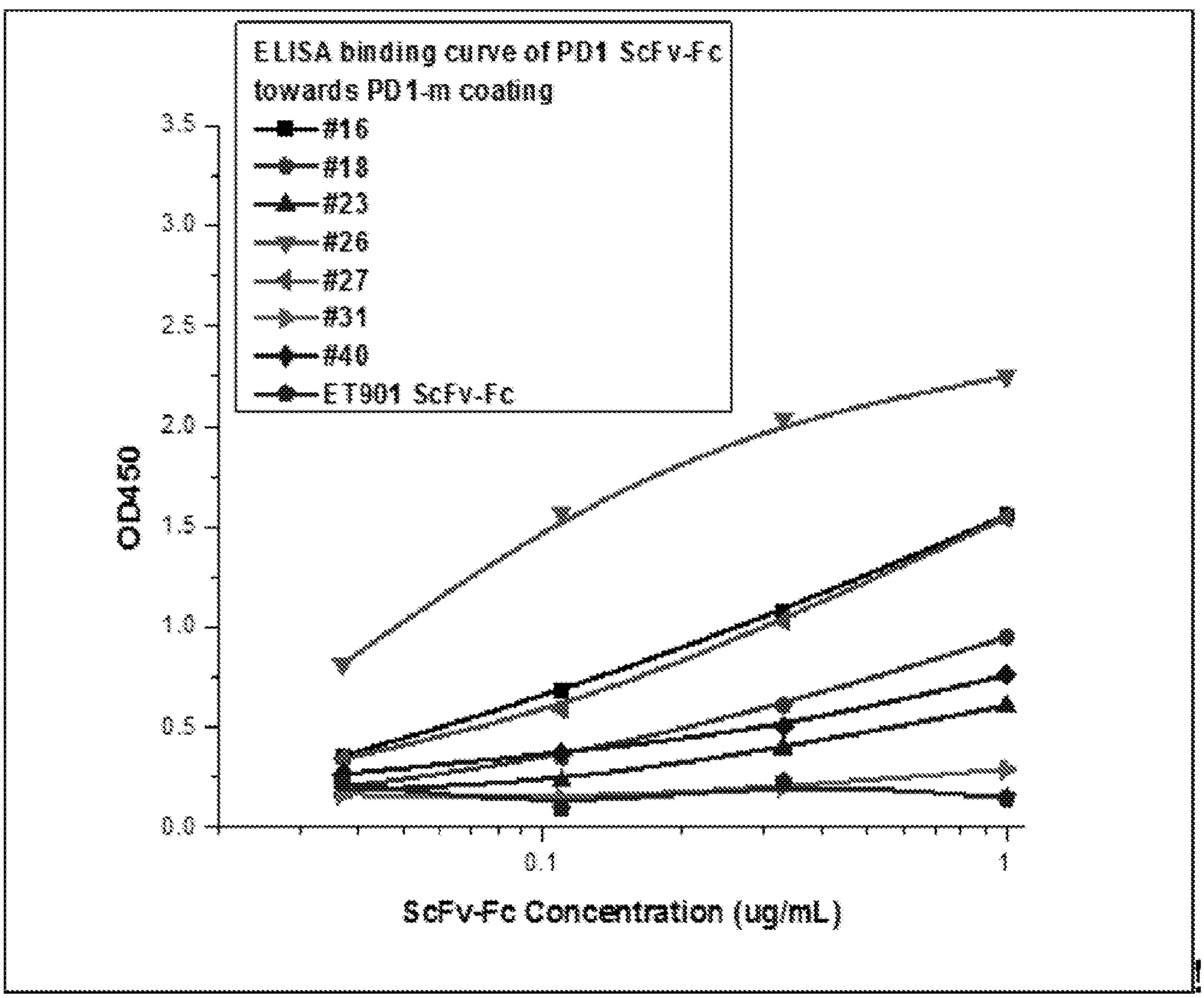
ELISA binding curve of PD1 ScFv-Fc towards PD1-m coating
#16
#18
#23
#26
#27
#31
#40
ET901 ScFv-Fc
OD450
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.1
1
ScFv-Fc Concentration (ug/mL)

**FIGURE 1**

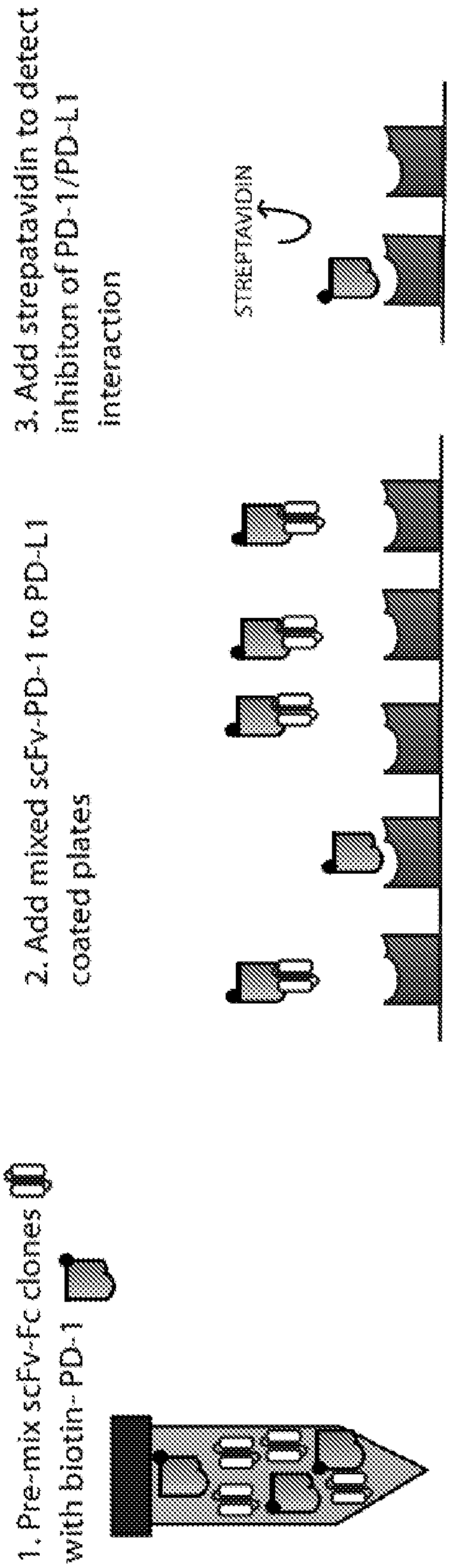
1. Pre-mix scFv-Fc clones with biotin- PD-1
2. Add mixed scFv-PD-1 to PD-L1 coated plates
3. Add strepatavidin to detect inhibiton of PD-1/PD-L1 interaction
STREPTAVIDIN

**FIGURE 2A**

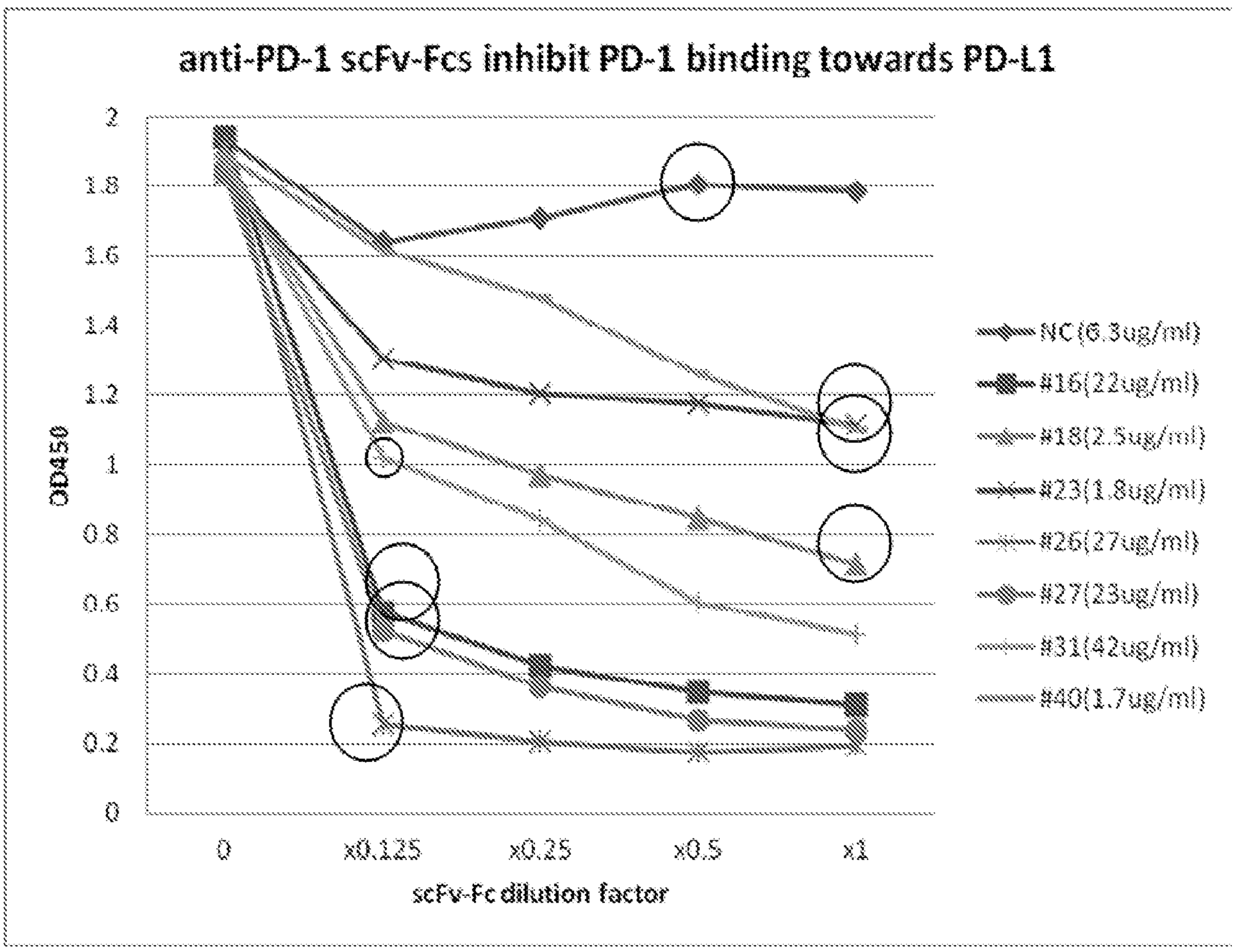
anti-PD-1 scFv-Fcs inhibit PD-1 binding towards PD-L1
OD450
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
x0.125
x0.25
x0.5
x1
scFv-Fc dilution factor
NC(6.3ug/ml)
#16(22ug/ml)
#18(2.5ug/ml)
#23(1.8ug/ml)
#26(27ug/ml)
#27(23ug/ml)
#31(42ug/ml)
#40(1.7ug/ml)

**FIGURE 2B**

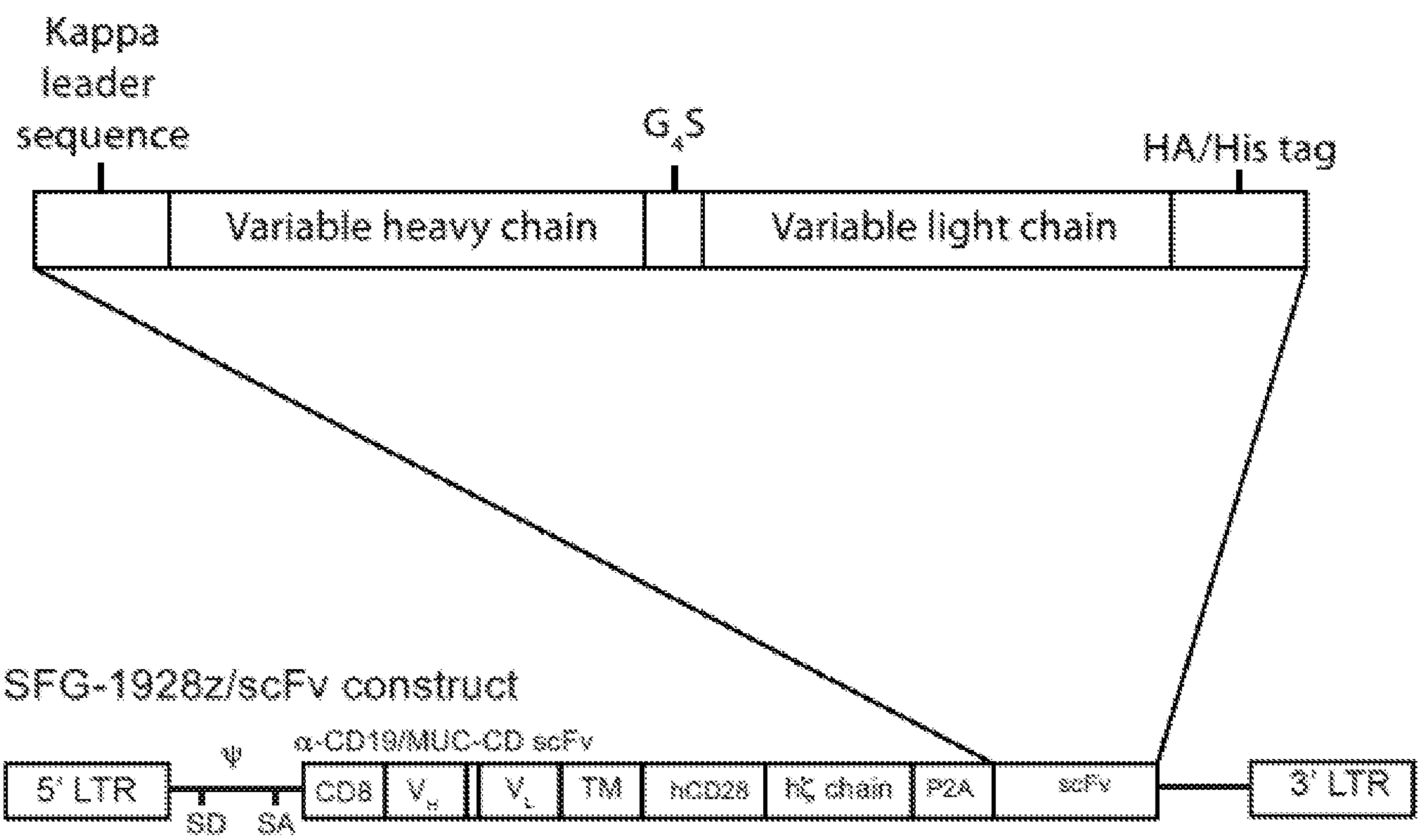
Kappa leader sequence
G4S
HA/His tag
Variable heavy chain
Variable light chain
SFG-1928z/scFv construct
α-CD19/MUC-CD scFv
Ψ
5' LTR
SD
SA
CD8
VH
VL
TM
hCD28
hζ chain
P2A
scFv
3' LTR

**FIGURE 3**

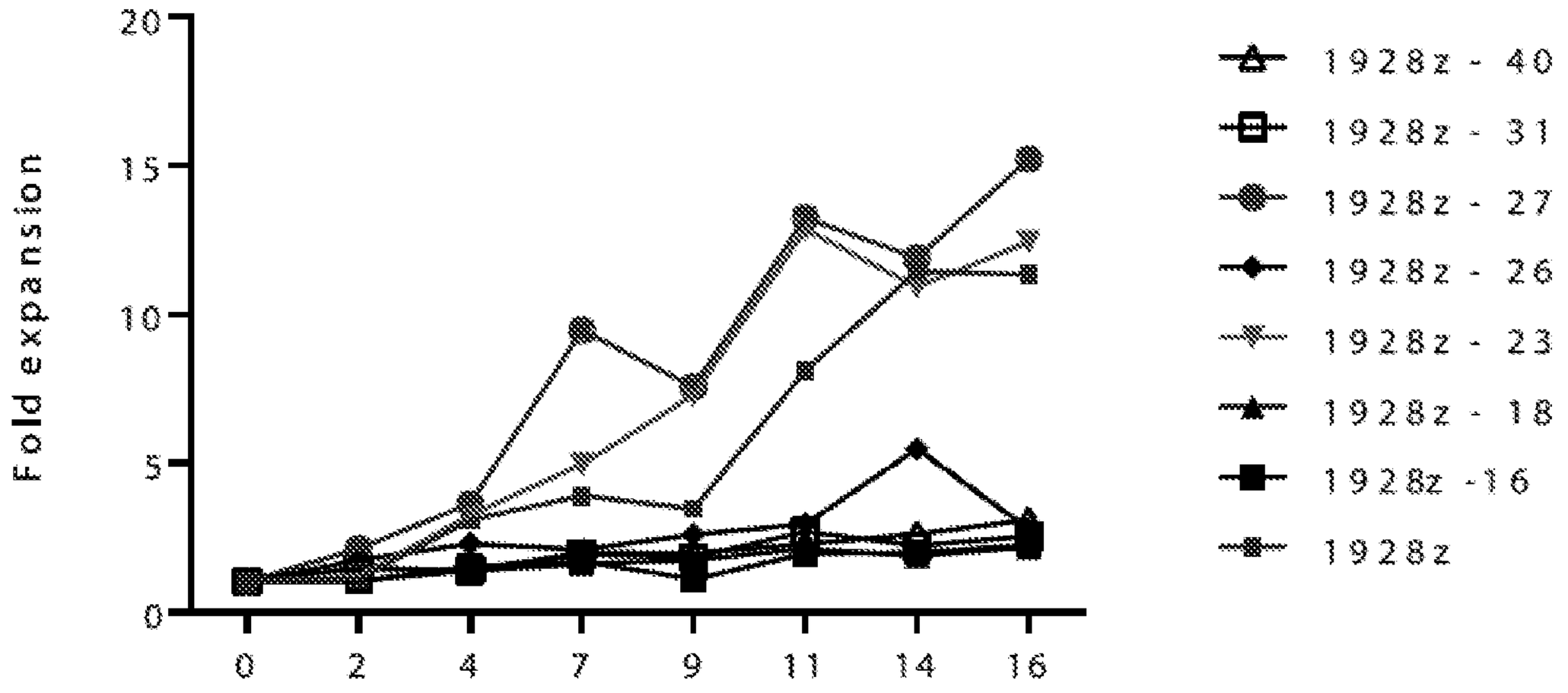
Fold expansion
20
15
10
5
0
0
2
4
7
9
11
14
16
Days post transduction
1928z - 40
1928z - 31
1928z - 27
1928z - 26
1928z - 23
1928z - 18
1928z -16
1928z

**FIGURE 4**

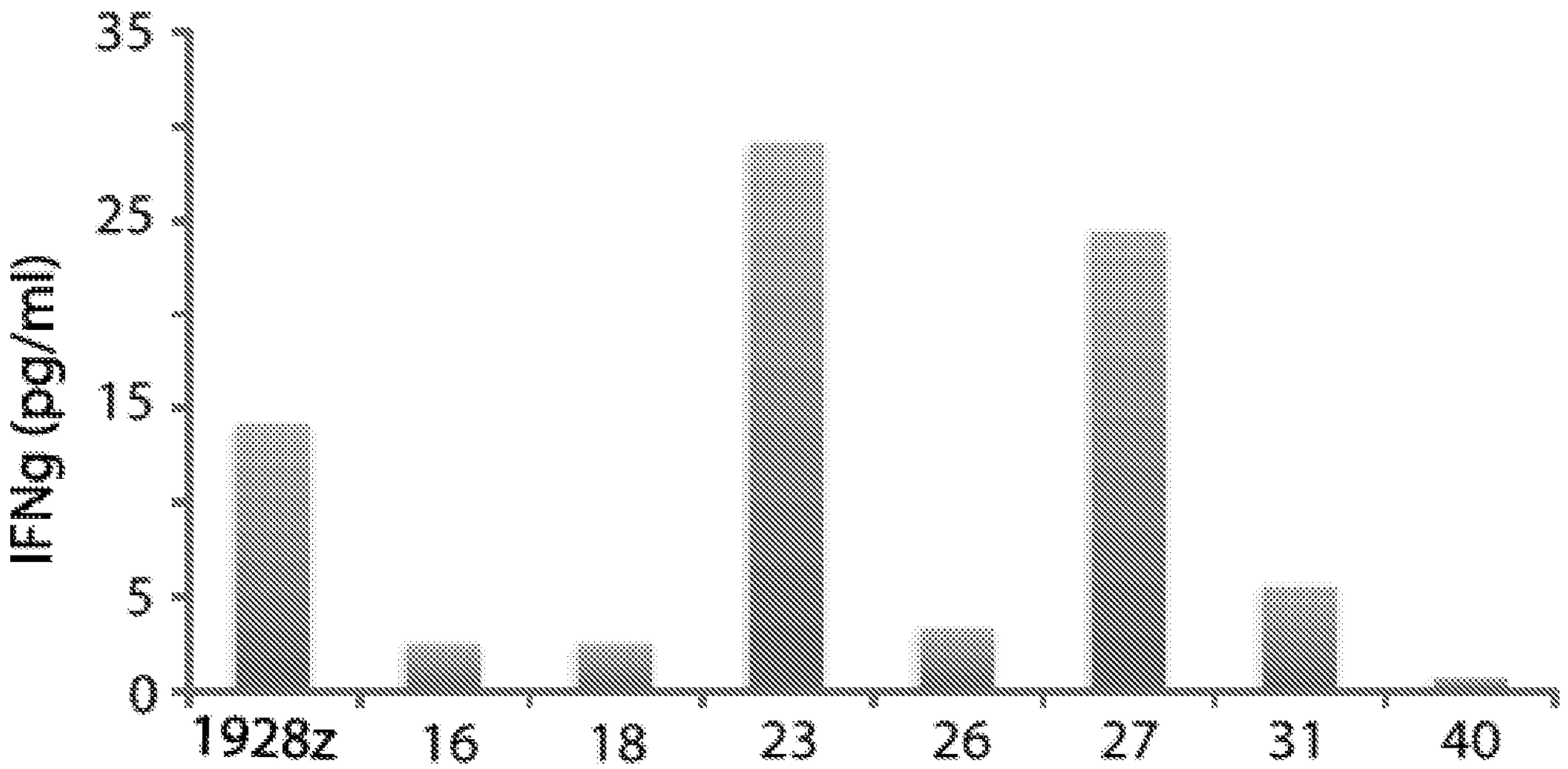


**FIGURE 5**

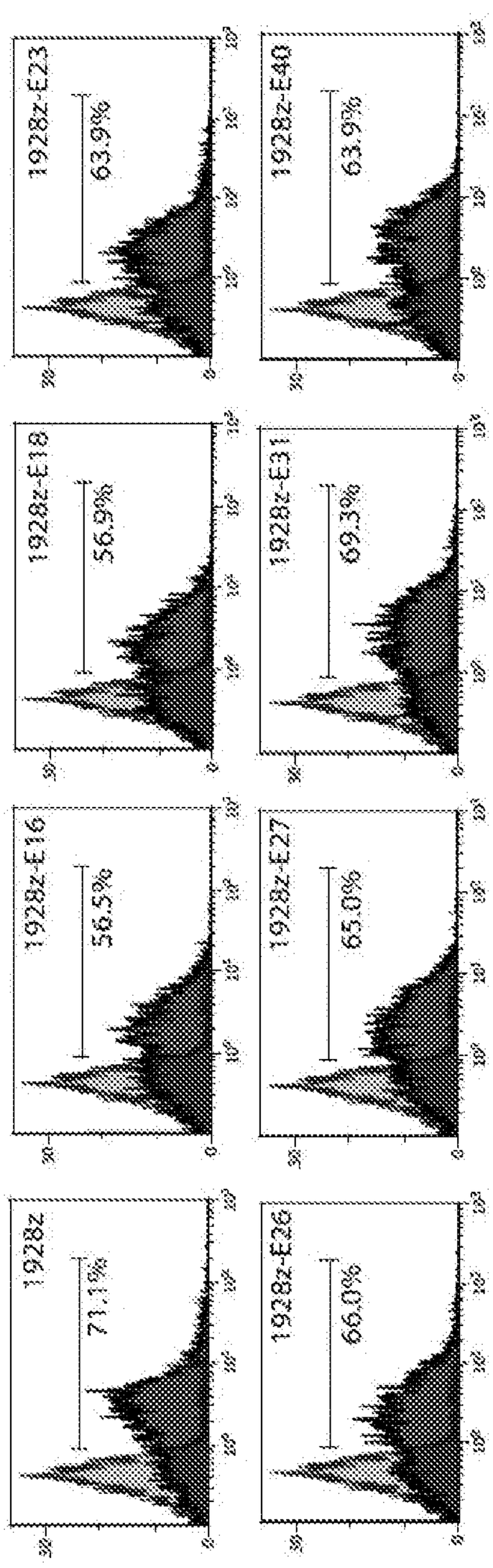
1928z-E23
63.9%
1928z-E40
63.9%
1928z-E18
56.9%
1928z-E31
69.3%
1928z-E16
56.5%
1928z-E27
65.0%
1928z-E26
71.1%
66.0%
Transduction Efficiency (%)
100
80
60
40
20
0
1928z
1928z-E16
1928z-E18
1928z-E26
1928z-E23
1928z-E27
1928z-E31
1928z-E40
*
*
*

**FIGURE 6**

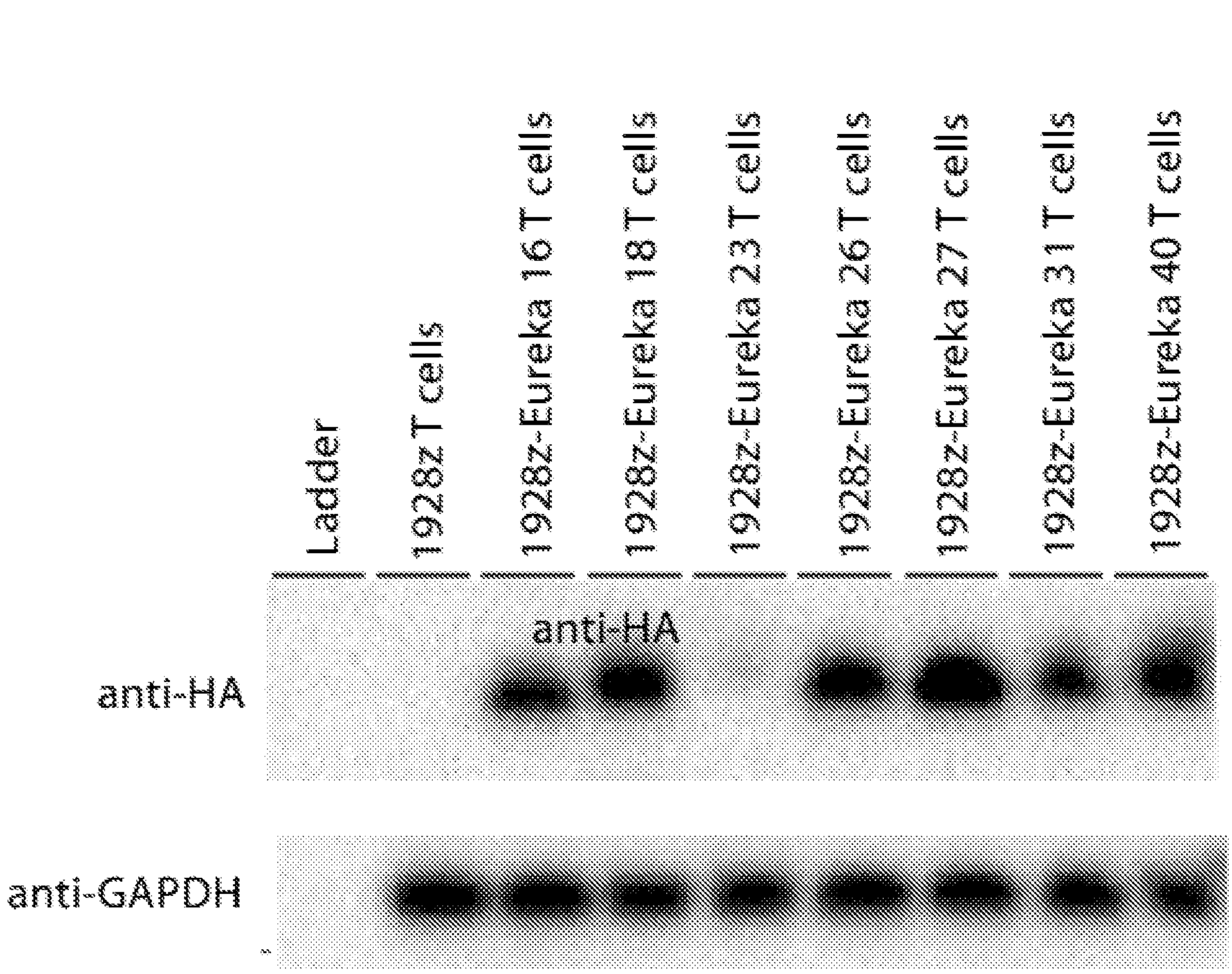
Ladder
1928z T cells
1928z-Eureka 16 T cells
1928z-Eureka 18 T cells
1928z-Eureka 23 T cells
1928z-Eureka 26 T cells
1928z-Eureka 27 T cells
1928z-Eureka 31 T cells
1928z-Eureka 40 T cells
anti-HA
anti-HA
anti-GAPDH

**FIGURE 7**

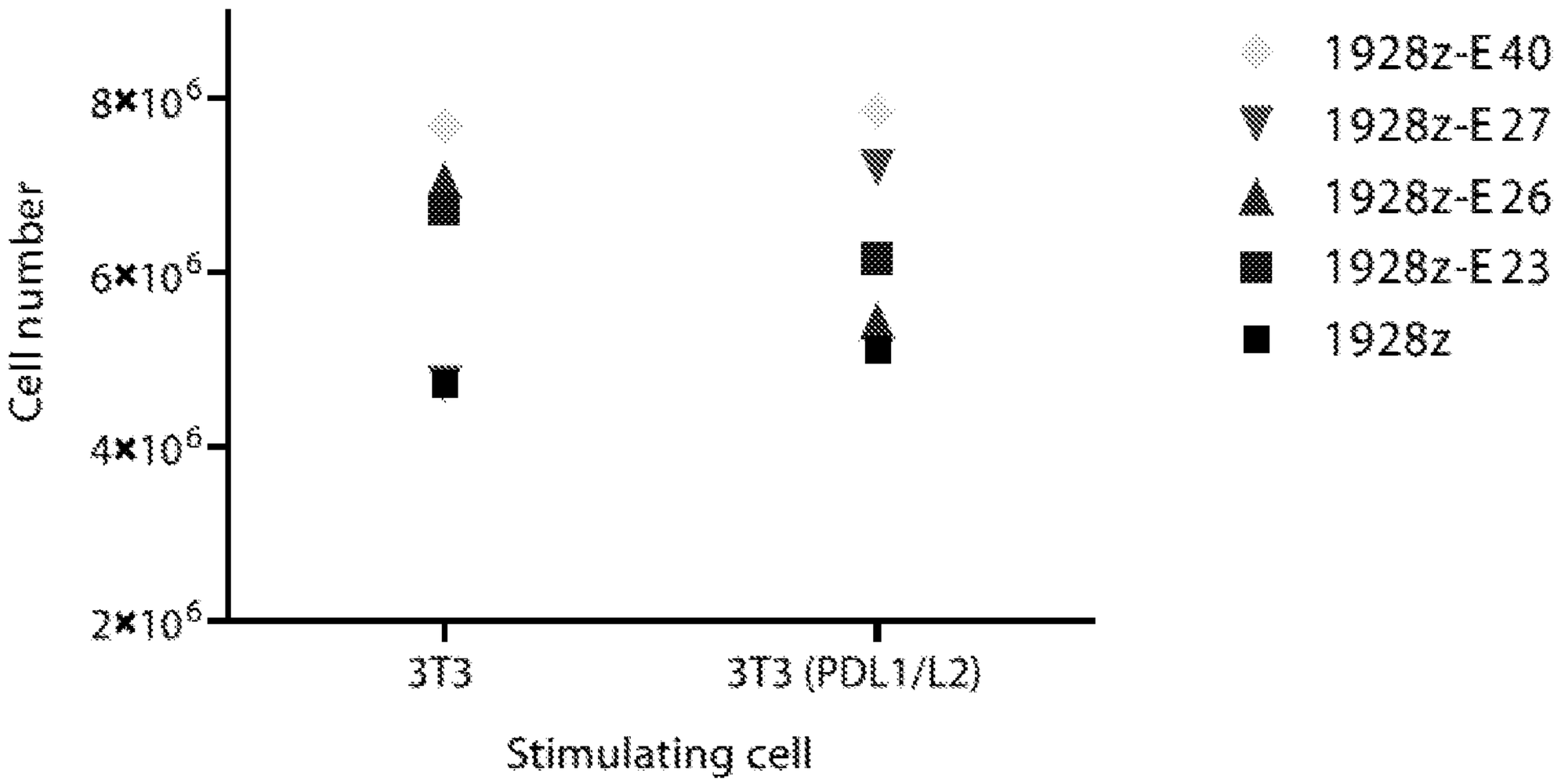
Cell number
8×10^6
6×10^6
4×10^6
2×10^6
3T3
3T3 (PDL1/L2)
Stimulating cell
1928z-E40
1928z-E27
1928z-E26
1928z-E23
1928z

**FIGURE 8**

| | LV | | | | | HV | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | CDR1 | | CDR2 | CDR3 | | CDR1 | | CDR2 | | CDR3 | |
| #16 | QSISSY | 1 | AAS | QQSYSTPLT | 2 | GFTSSSYW | 3 | IKQDGSEK | 4 | ARGGWSYDM | 5 |
| #18 | SSNIGAGYA | 12 | TNN | QSYDSSLSGVI | 13 | GYTLTELS | 14 | FDPEDGET | 15 | ARAYYGFDQ | 16 |
| #23 | SSNIGNNA | 23 | YND | AAWDDSVNGYV | 24 | GYTFTRFG | 25 | ISVNNGNT | 26 | ARYMYGRRDS | 27 |
| #26 | NIGSKS | 34 | YDS | QVWDNHSDVV | 35 | RNKFSSYA | 36 | ISGSGGTT | 37 | ARWYSSYYDV | 38 |
| #27 | NIGSKS | 34 | YDS | QVWDSSSDYV | 45 | GFTFSSYA | 46 | ISGSGGST | 47 | ARNYISMFDS | 48 |
| #31 | NIGSKS | 34 | YDS | QVWDSSSDHV | 55 | GFTFSSYA | 46 | ISGSGGST | 47 | ARGYSSYYDA | 56 |
| #40 | RSNIGENT | 63 | SNN | AAWDDRLNGYV | 64 | GYTFTNYG | 65 | IGAQRGDT | 66 | ARSQGVPFDS | 67 |
| #36 | RSNIGSNT | 74 | NNN | ATWDDSLNEYV | 75 | GYTFTRYG | 76 | ISGYNGNT | 77 | ARHGYGYHGD | 78 |
| #37 | SSNIGAGYV | 85 | HNN | QSYDSSLSGWV | 86 | GFTFKDYY | 87 | IGTSGNSV | 88 | ARSPGHSDYDS | 89 |
| #19 | NIGDKS | 96 | YDS | QVWASGTDHPYVI | 97 | GFTFSSYA | 46 | ISGSGGST | 47 | ARMYGSYTDM | 98 |
| #14 | SSNIGYNY | 105 | RNN | TSWDDSLSGYV | 106 | GNAFTNFY | 107 | INPSGTDLT | 108 | ARQYAYGYSGFDM | 109 |
| #47 | QSVSNW | 116 | AAS | QQSYSTPIT | 117 | GYTFTSYY | 118 | INPNTGGS | 119 | ARGDVTYDE | 120 |
| #46 | NIGSKS | 34 | YDD | QVWDINDHYV | 127 | GFTFSSYA | 46 | ISGSGGST | 47 | ARSQASFMDI | 128 |
| #42 | NIGSKS | 34 | DDS | QVWDSSSDQGV | 135 | GFTFSSYA | 46 | IGTGGGT | 136 | ARGTGYDGDQ | 137 |

FIGURE 9

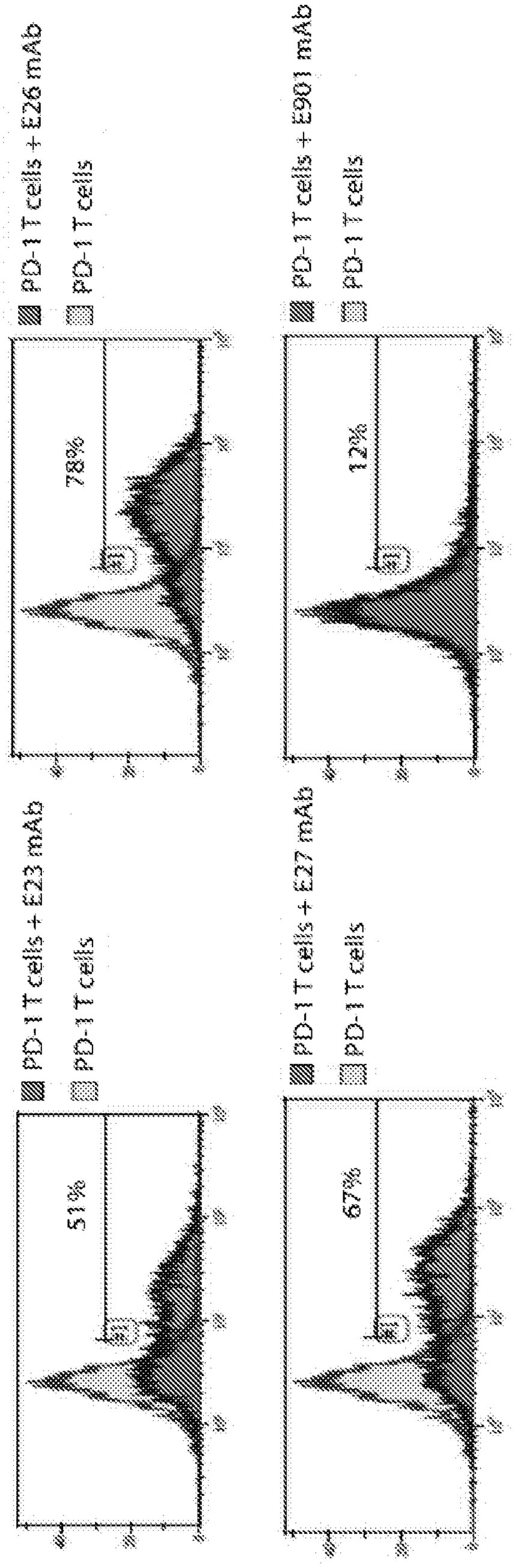
PD-1 T cells + E26 mAb
PD-1 T cells
78%
PD-1 T cells + E901 mAb
PD-1 T cells
12%
PD-1 T cells + E23 mAb
PD-1 T cells
51%
PD-1 T cells + E27 mAb
PD-1 T cells
67%

**FIGURE 10**

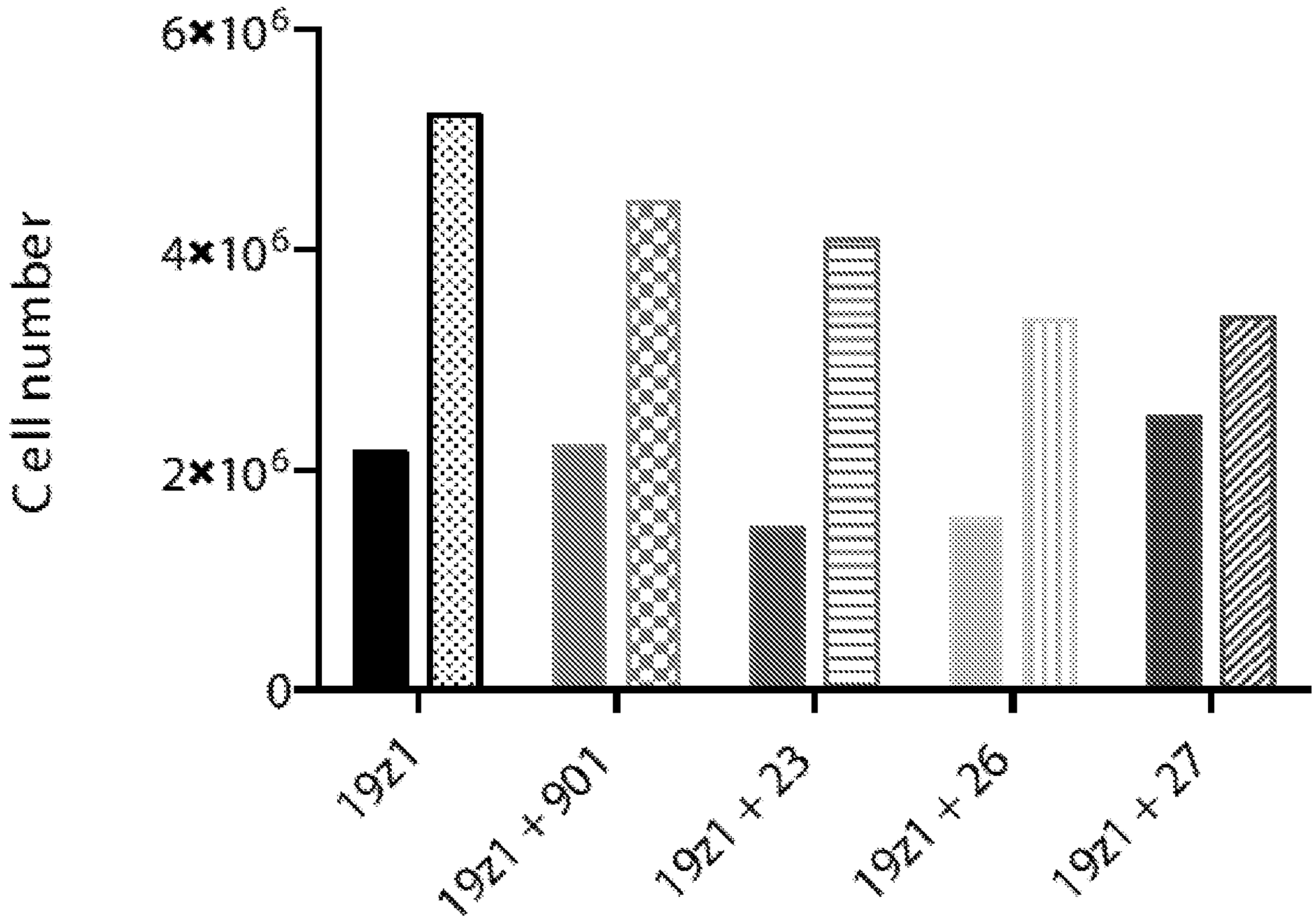
Cell number
6×10^6
4×10^6
2×10^6
0
1921
1921 + 901
1921 + 23
1921 + 26
1921 + 27

**FIGURE 11**

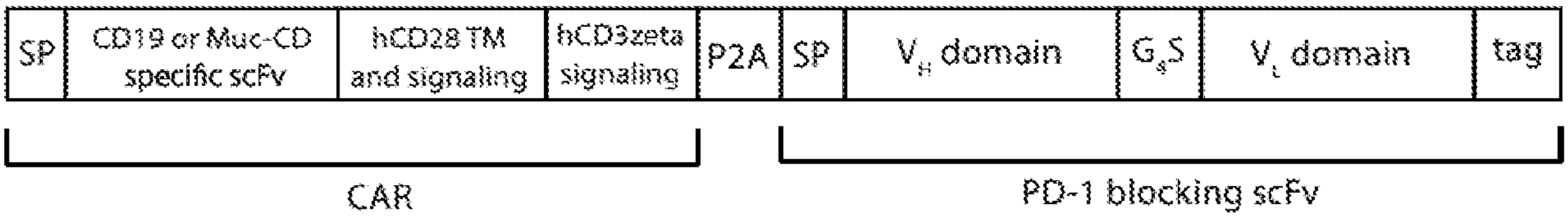


**FIGURE 12A**

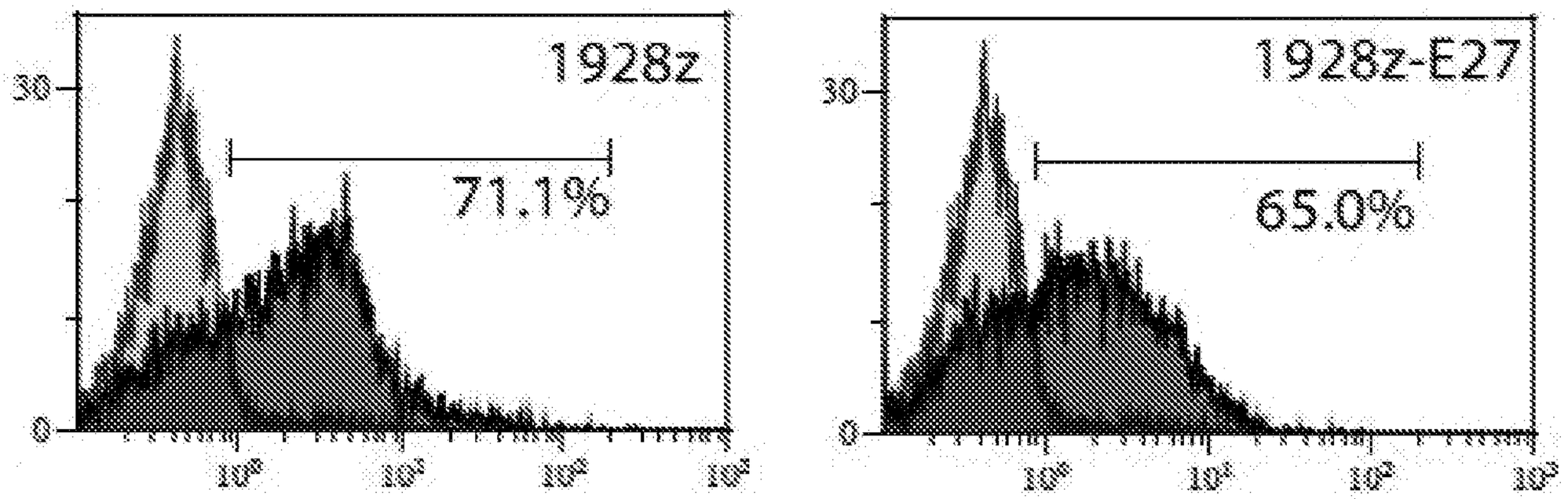


**FIGURE 12B**

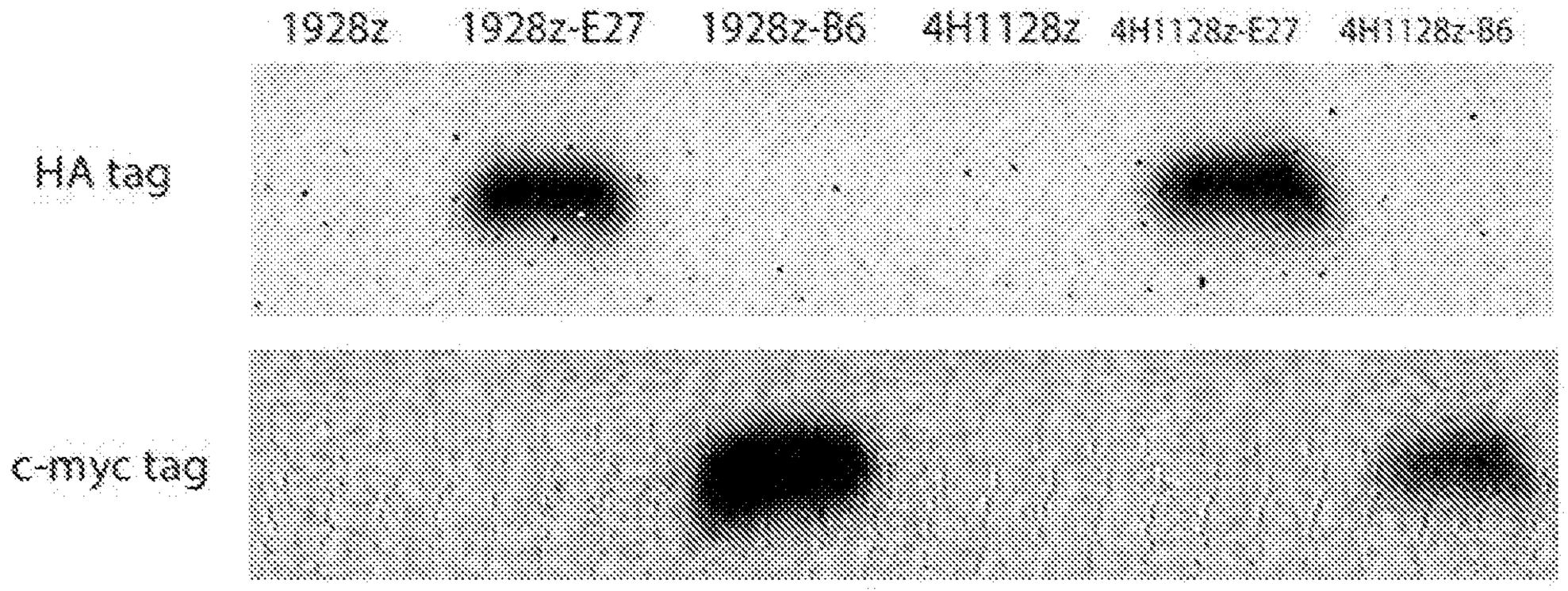


**FIGURE 12C**

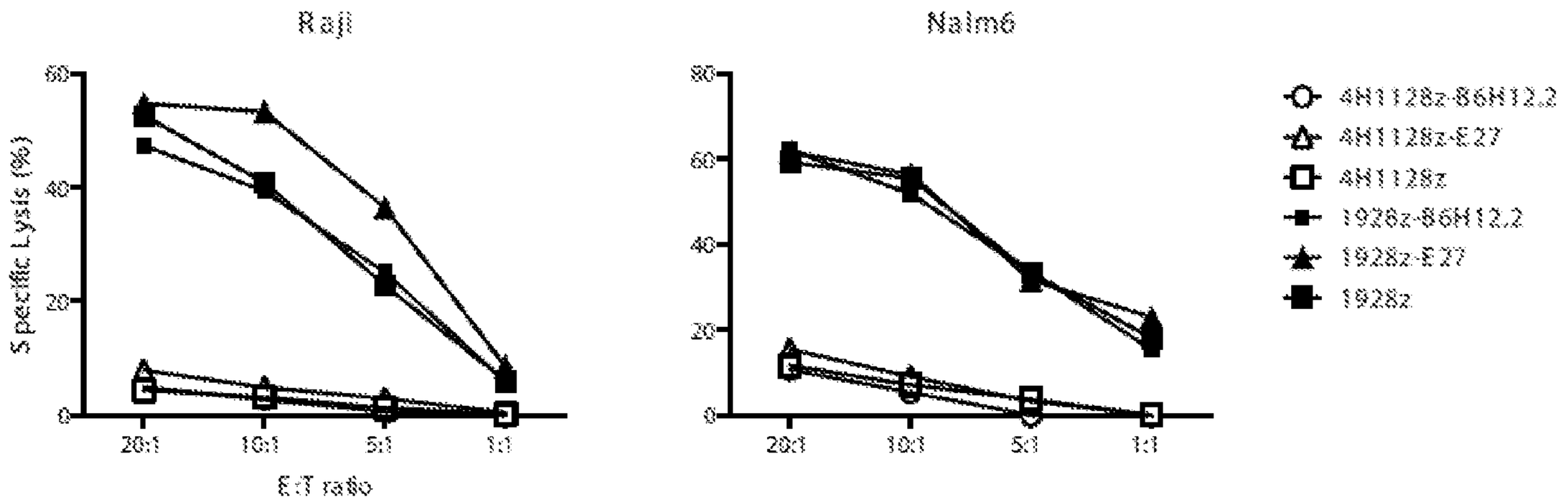


**FIGURE 12D**

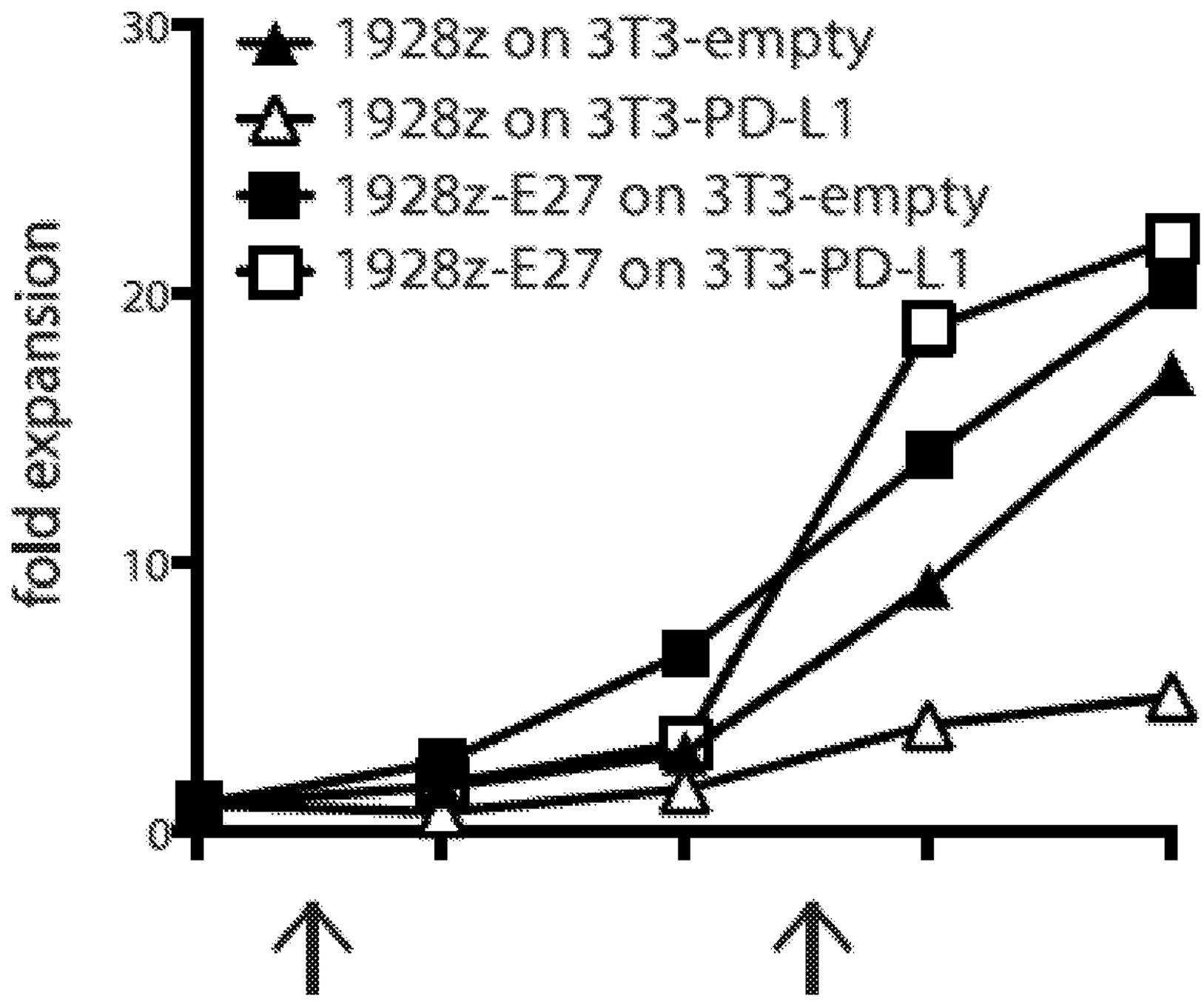
1928z on 3T3-empty
1928z on 3T3-PD-L1
1928z-E27 on 3T3-empty
1928z-E27 on 3T3-PD-L1
fold expansion
30
20
10
0

**FIGURE 13A**

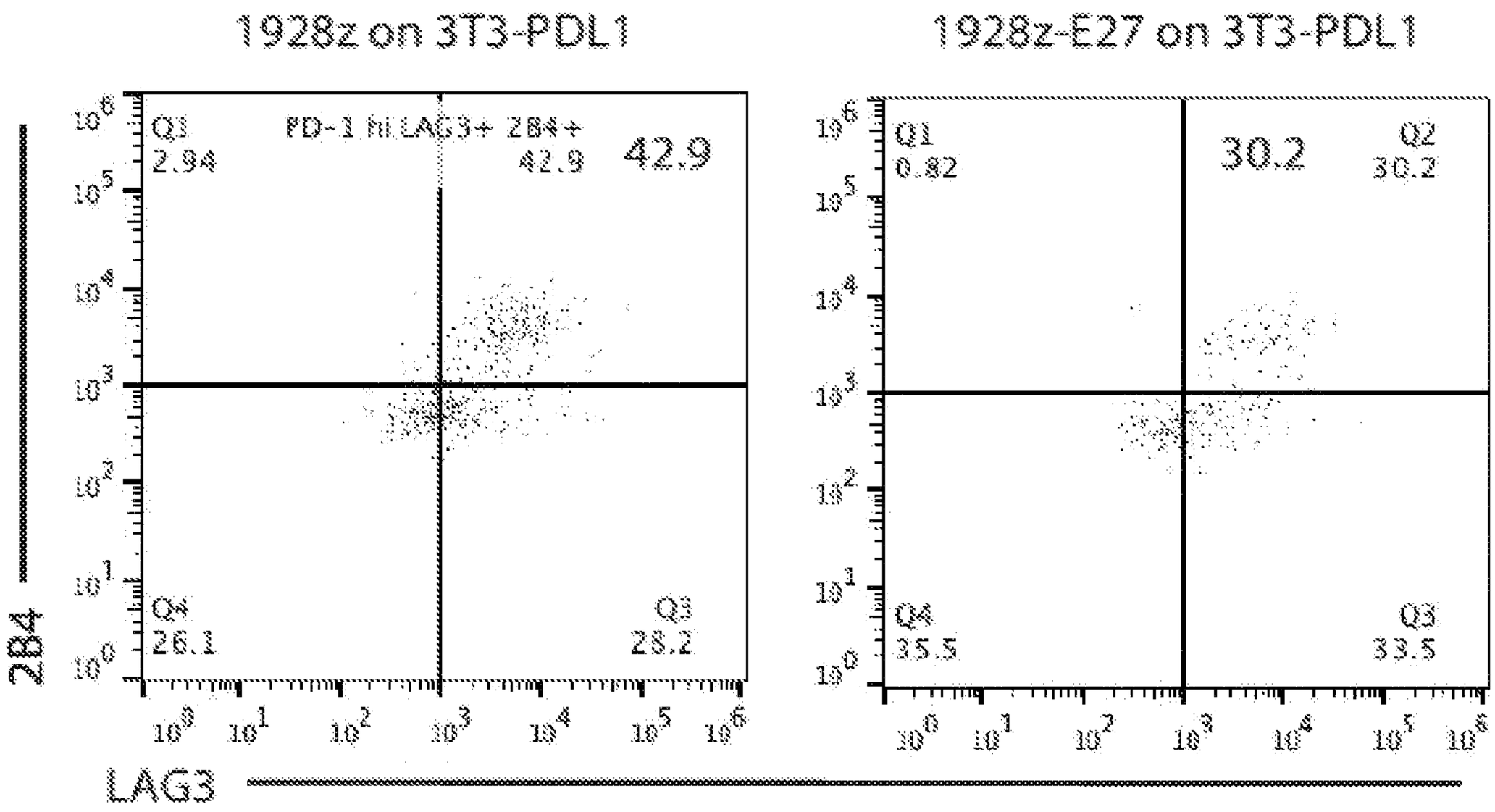
1928z on 3T3-PDL1
1928z-E27 on 3T3-PDL1
Q1
2.94
PD-1 hi LAG3+ 284+
42.9
42.9
Q1
0.82
30.2
Q2
30.2
Q4
26.1
Q3
28.2
Q4
35.5
Q3
33.5
284
LAG3

**FIGURE 13B**

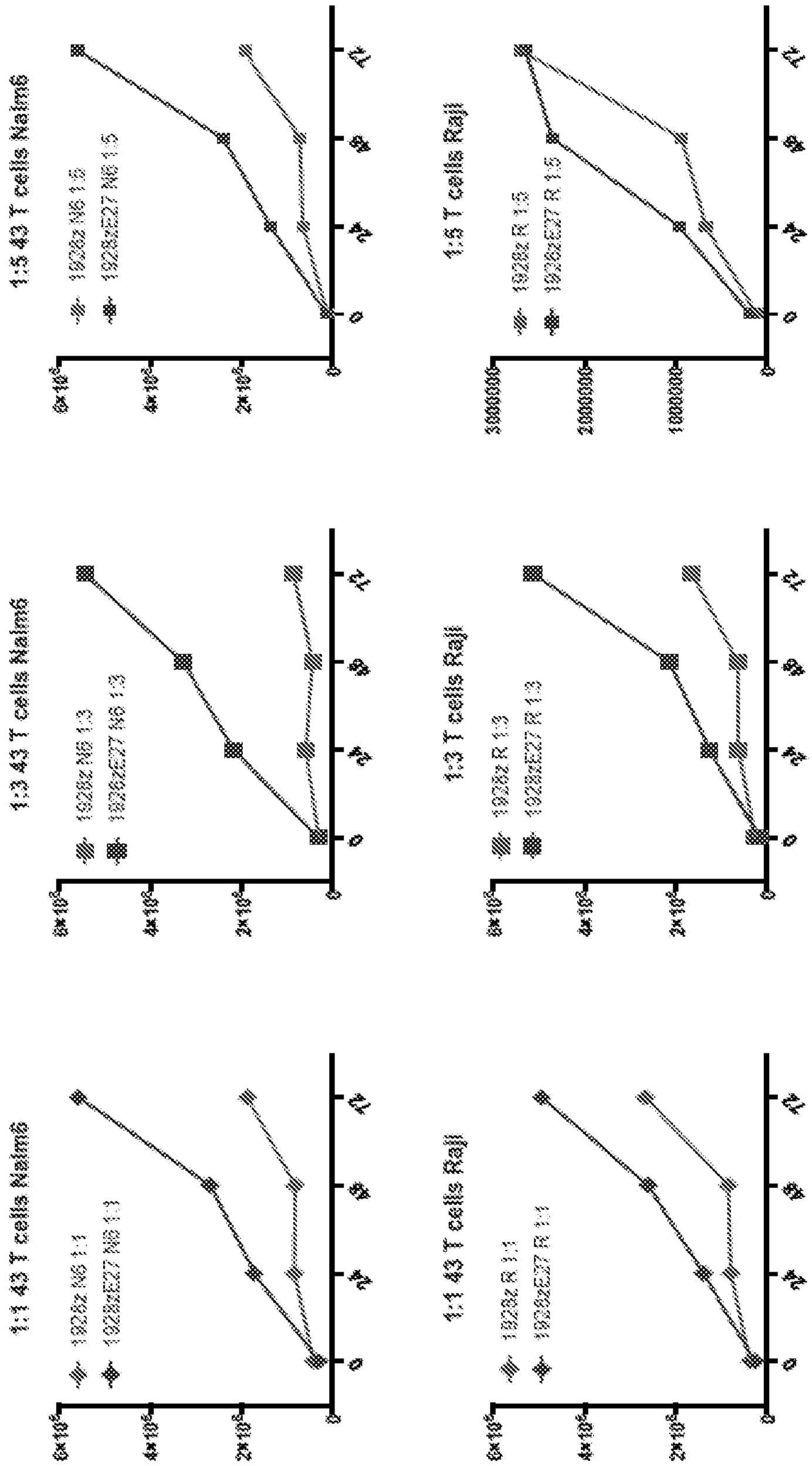
1:5 43 T cells Nalm6
1928z NB 1:5
1928zE27 NB 1:5
1:5 T cells Raji
1928z R 1:5
1928zE27 R 1:5
1:3 43 T cells Nalm6
1928z NB 1:3
1928zE27 NB 1:3
1:3 T cells Raji
1928z R 1:3
1928zE27 R 1:3
1:1 43 T cells Nalm6
1928z NB 1:1
1928zE27 NB 1:1
1:1 43 T cells Raji
1928z R 1:1
1928zE27 R 1:1
0
2x10⁵
4x10⁵
6x10⁵
1000000
2000000
3000000
4
8
12

FIGURE 13C

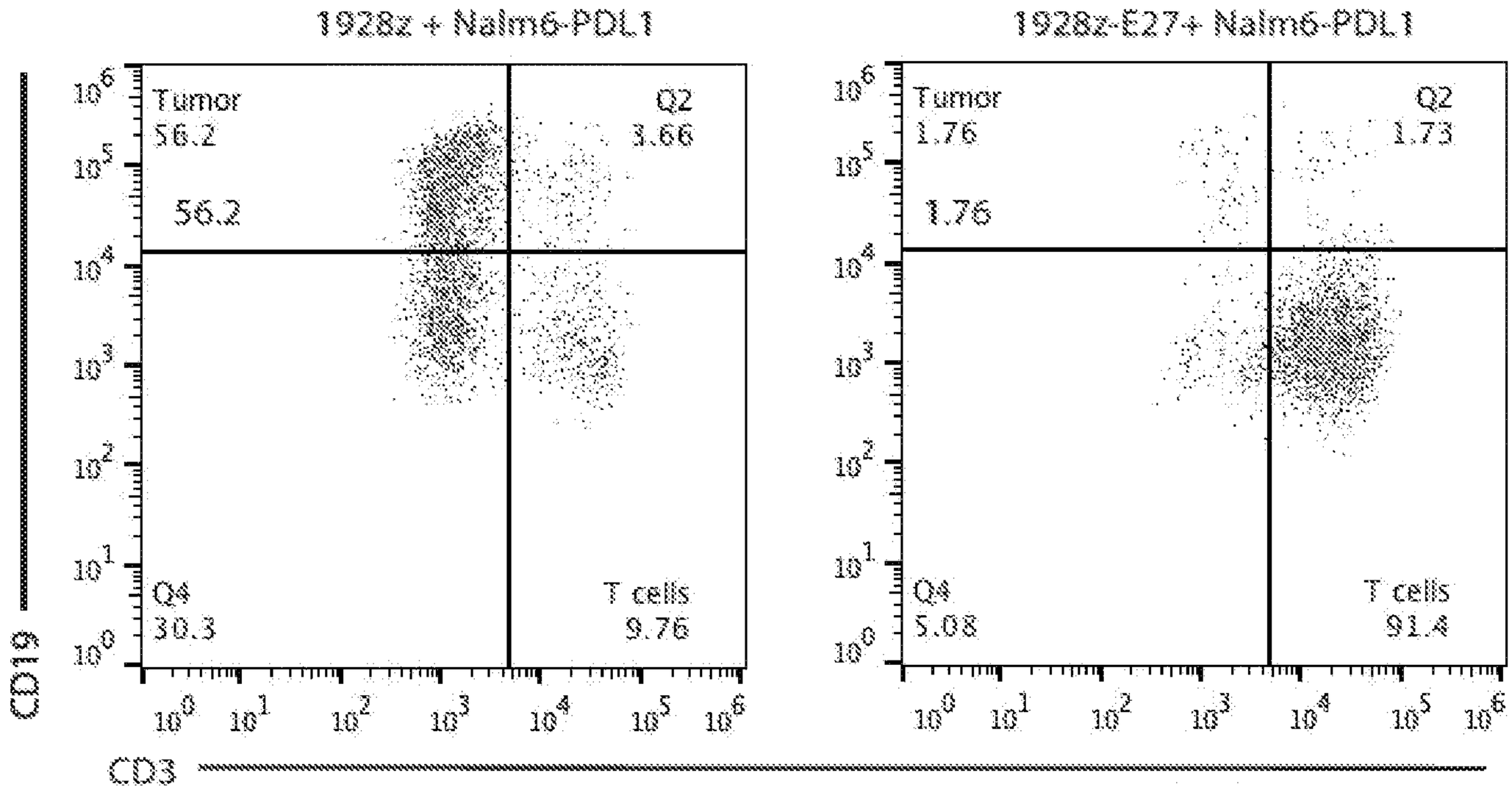
1928z + Nalm6-PDL1
1928z-E27+ Nalm6-PDL1
Tumor
56.2
56.2
Q2
3.66
Q4
30.3
T cells
9.76
Tumor
1.76
1.76
Q2
1.73
Q4
5.08
T cells
91.4
CD19
CD3

**FIGURE 13D**

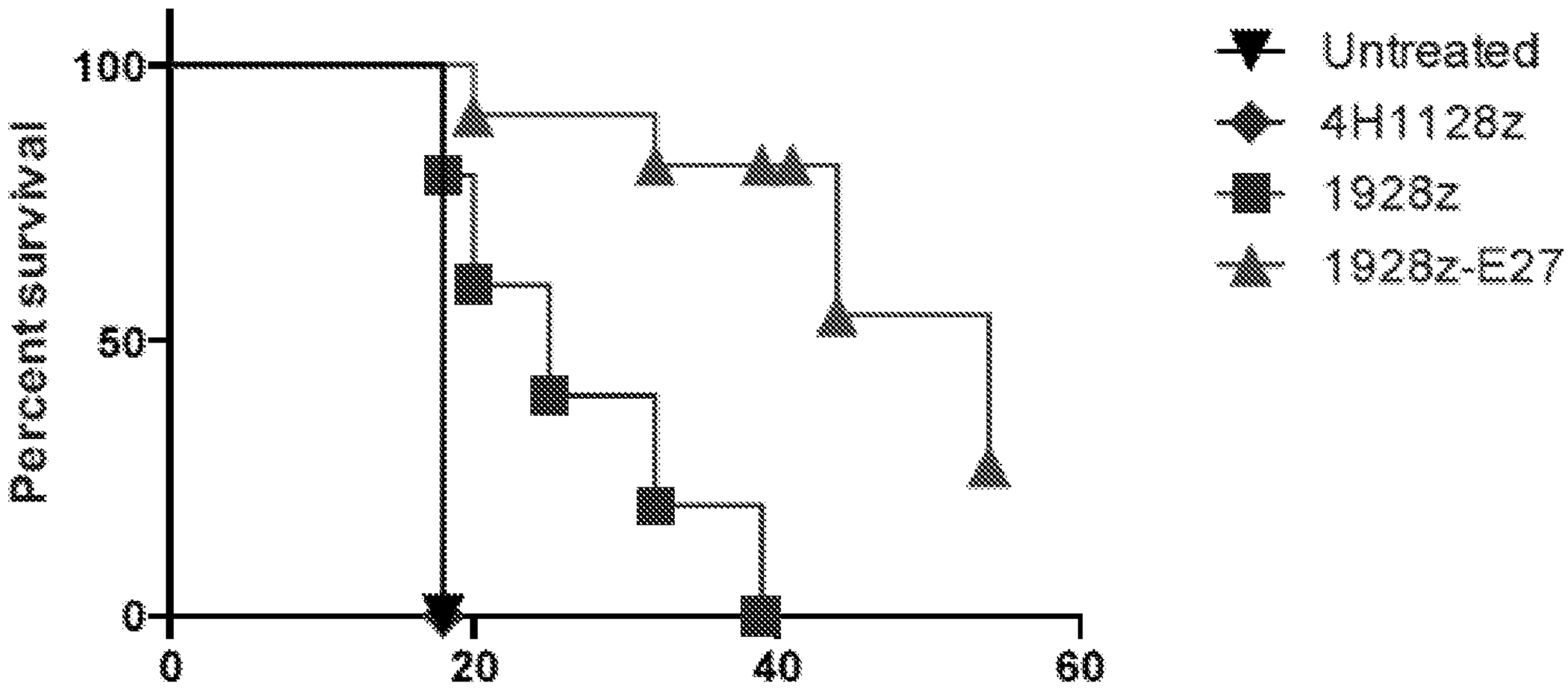
Percent survival
100
50
0
0
20
40
60
Days post tumor inoculation
Untreated
4H1128z
1928z
1928z-E27

**FIGURE 14**

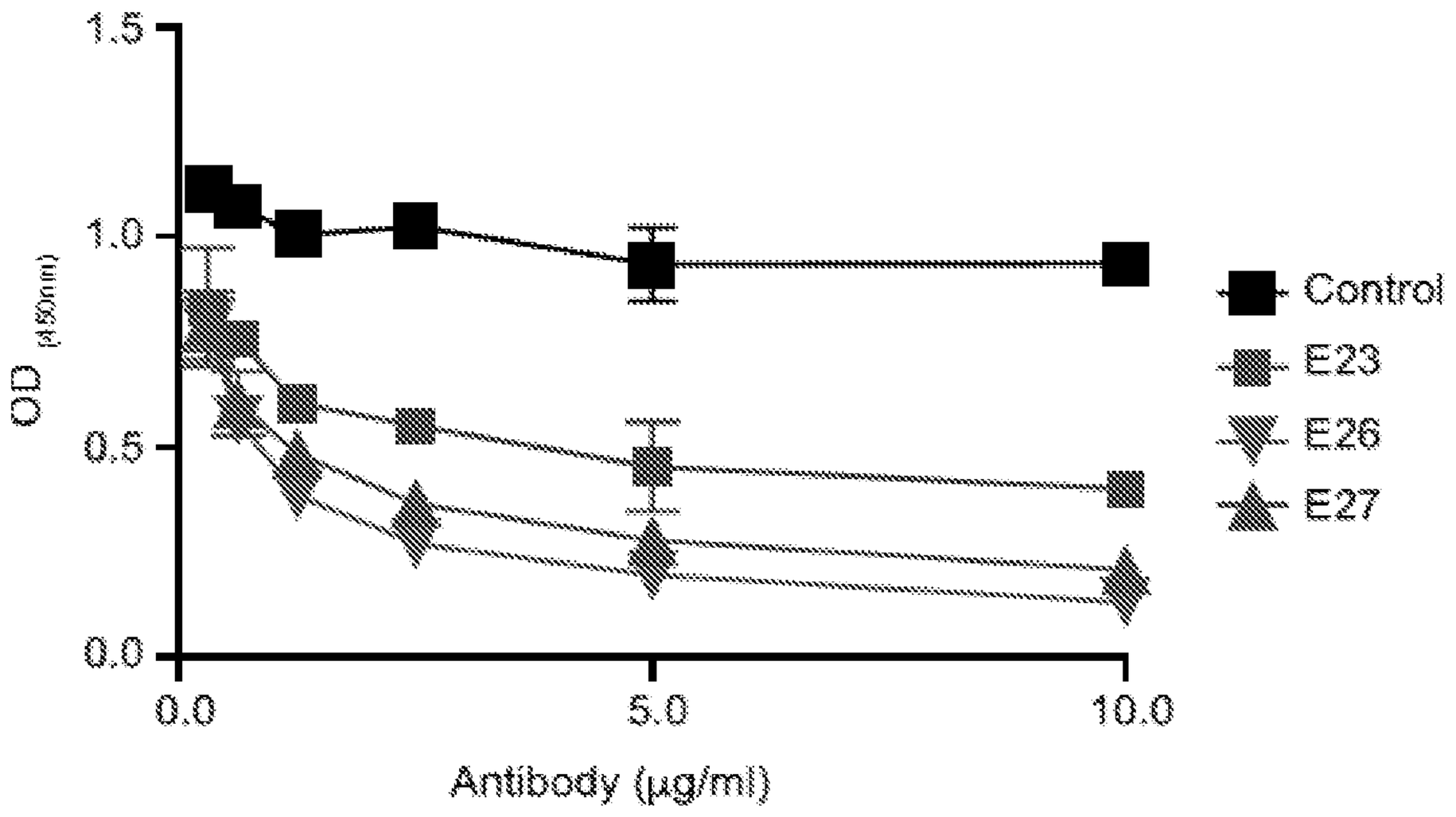


**FIGURE 15A**

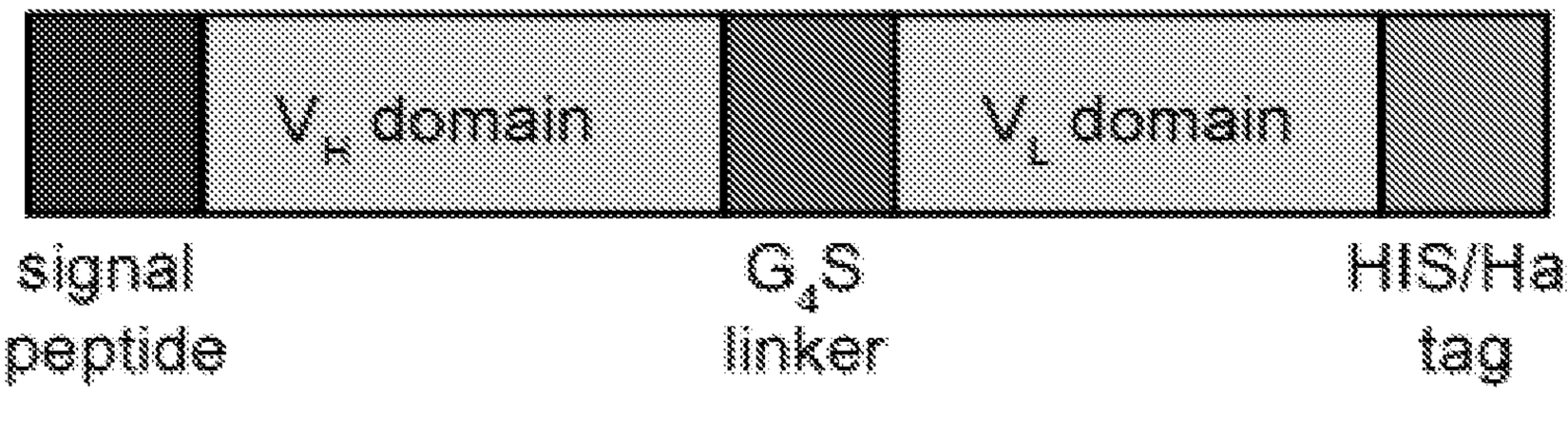


**FIGURE 15B**

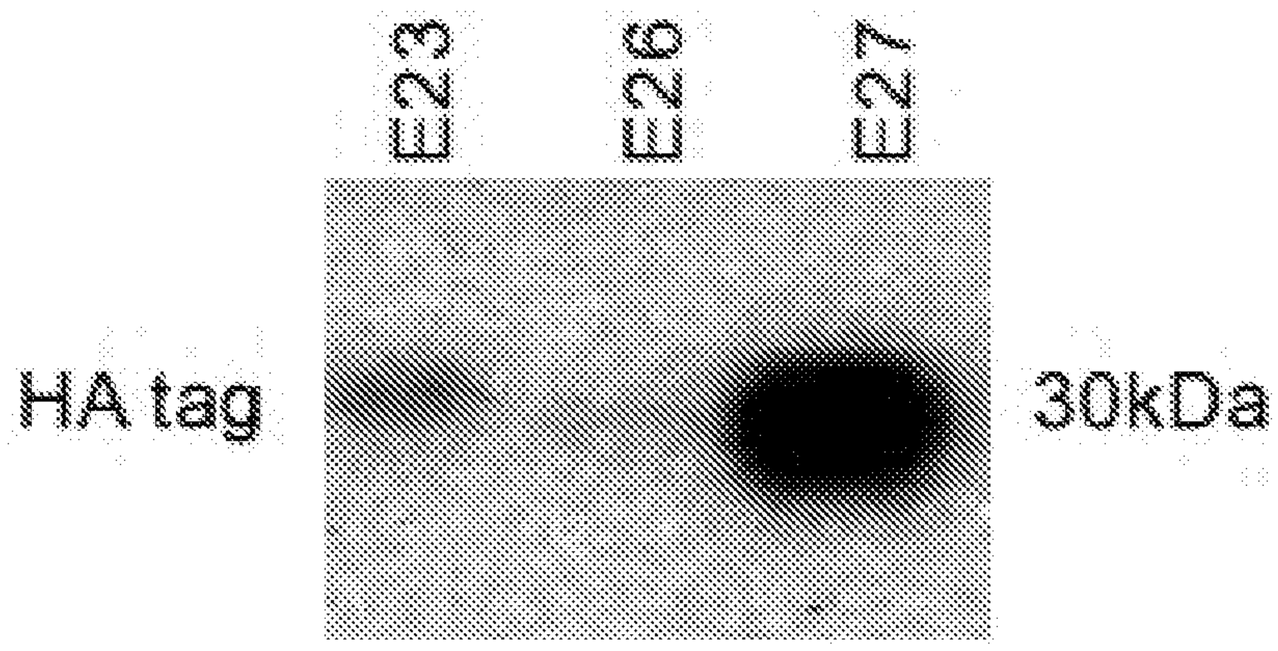


**FIGURE 15C**

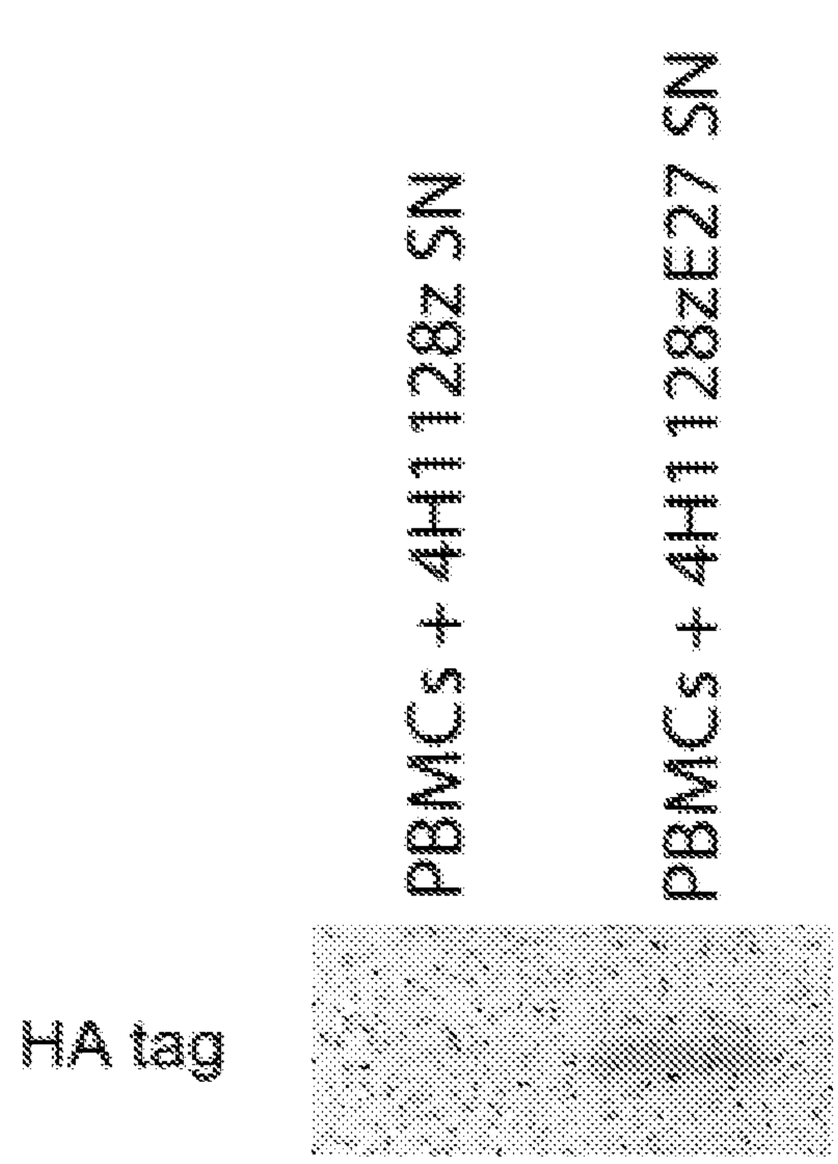


**FIGURE 15D**

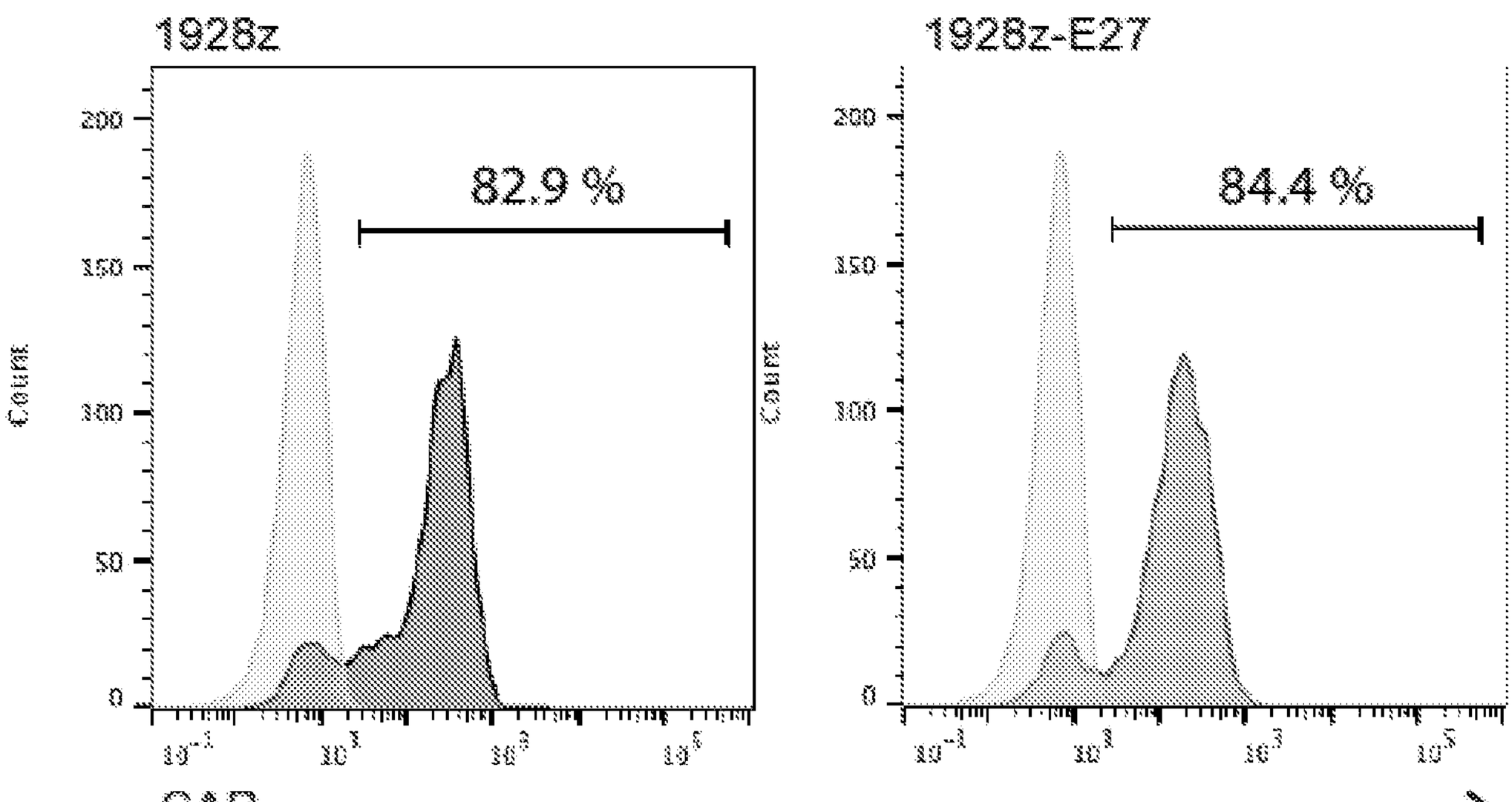


**FIGURE 16A**

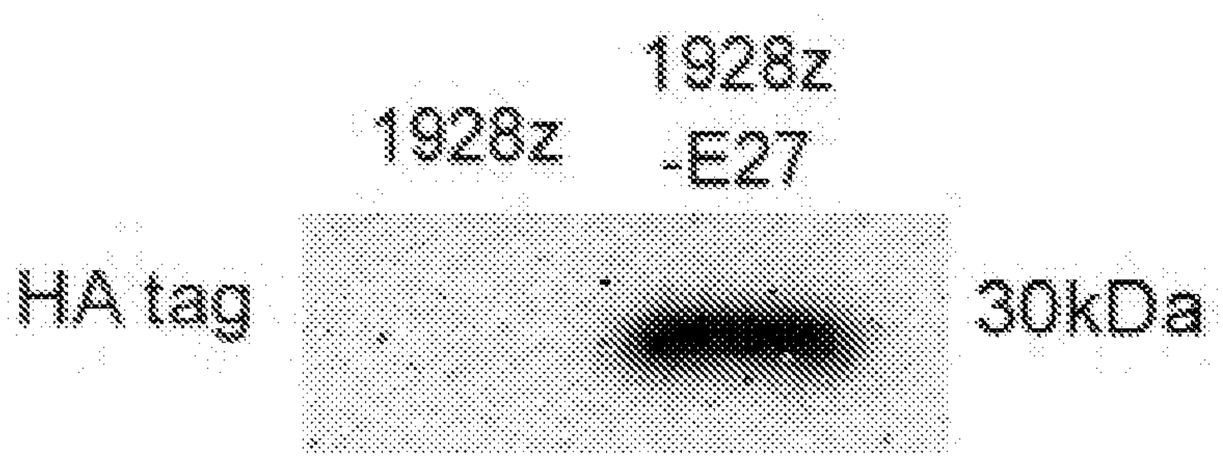


**FIGURE 16B**

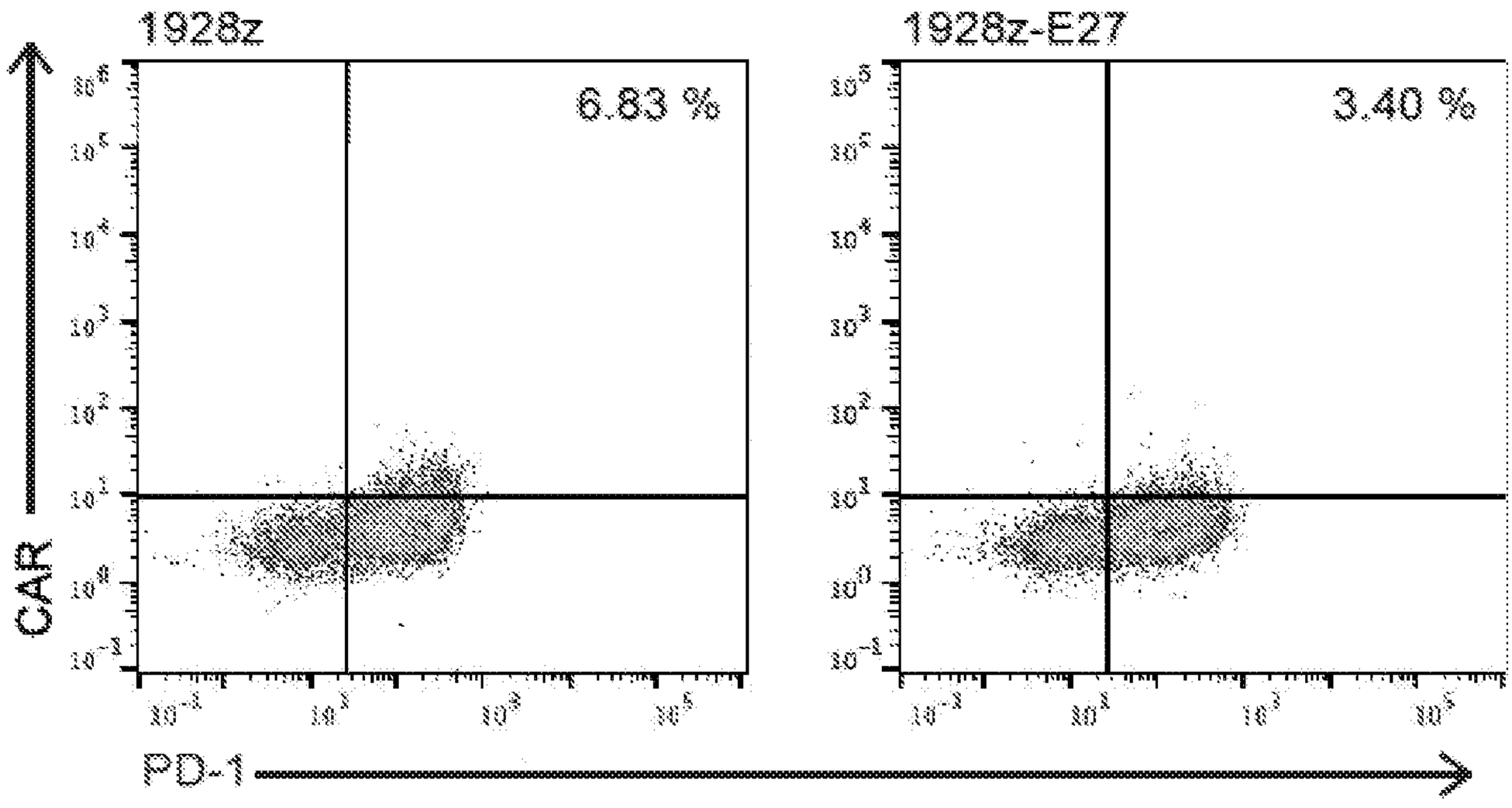


**FIGURE 16C**

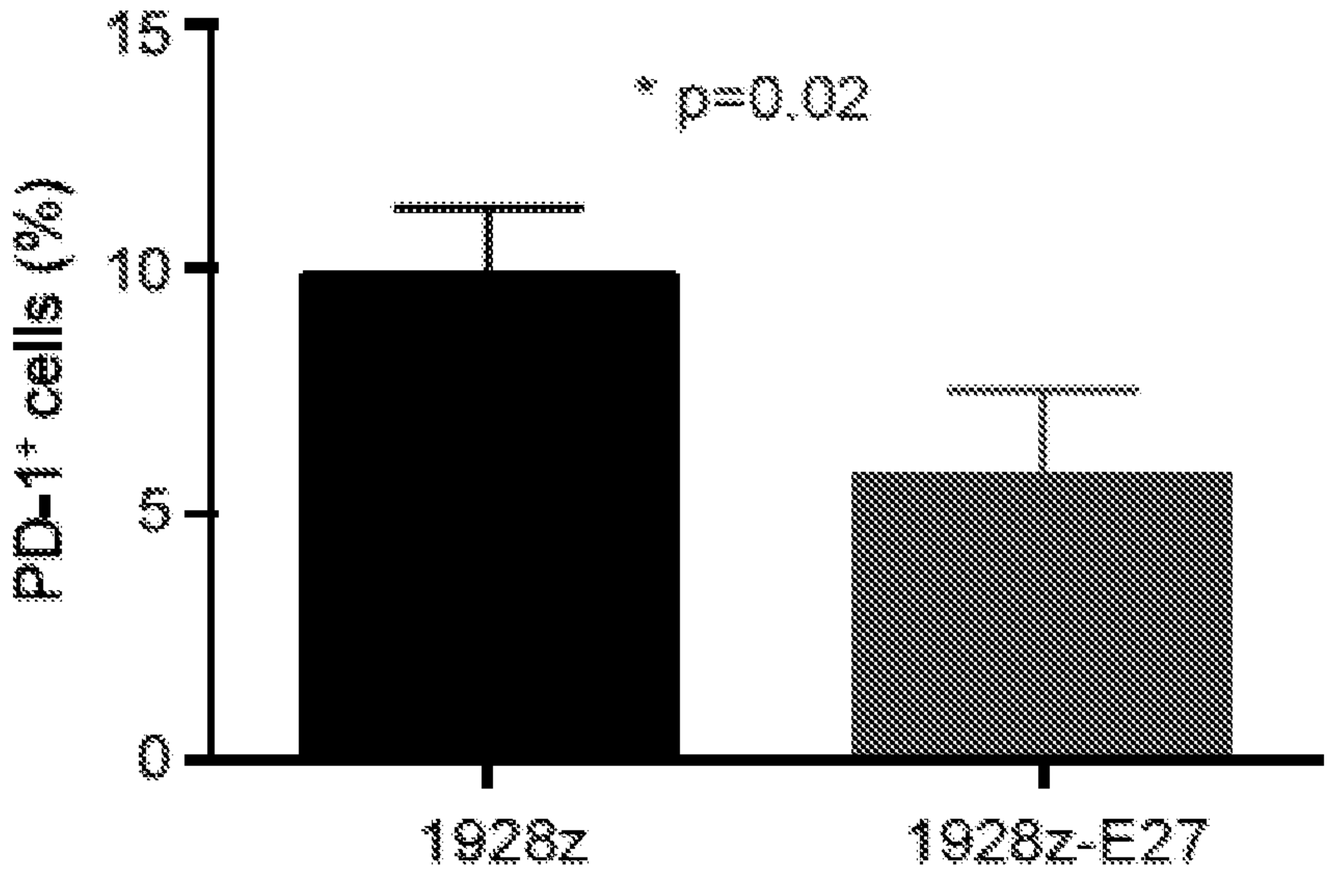


**FIGURE 16D**

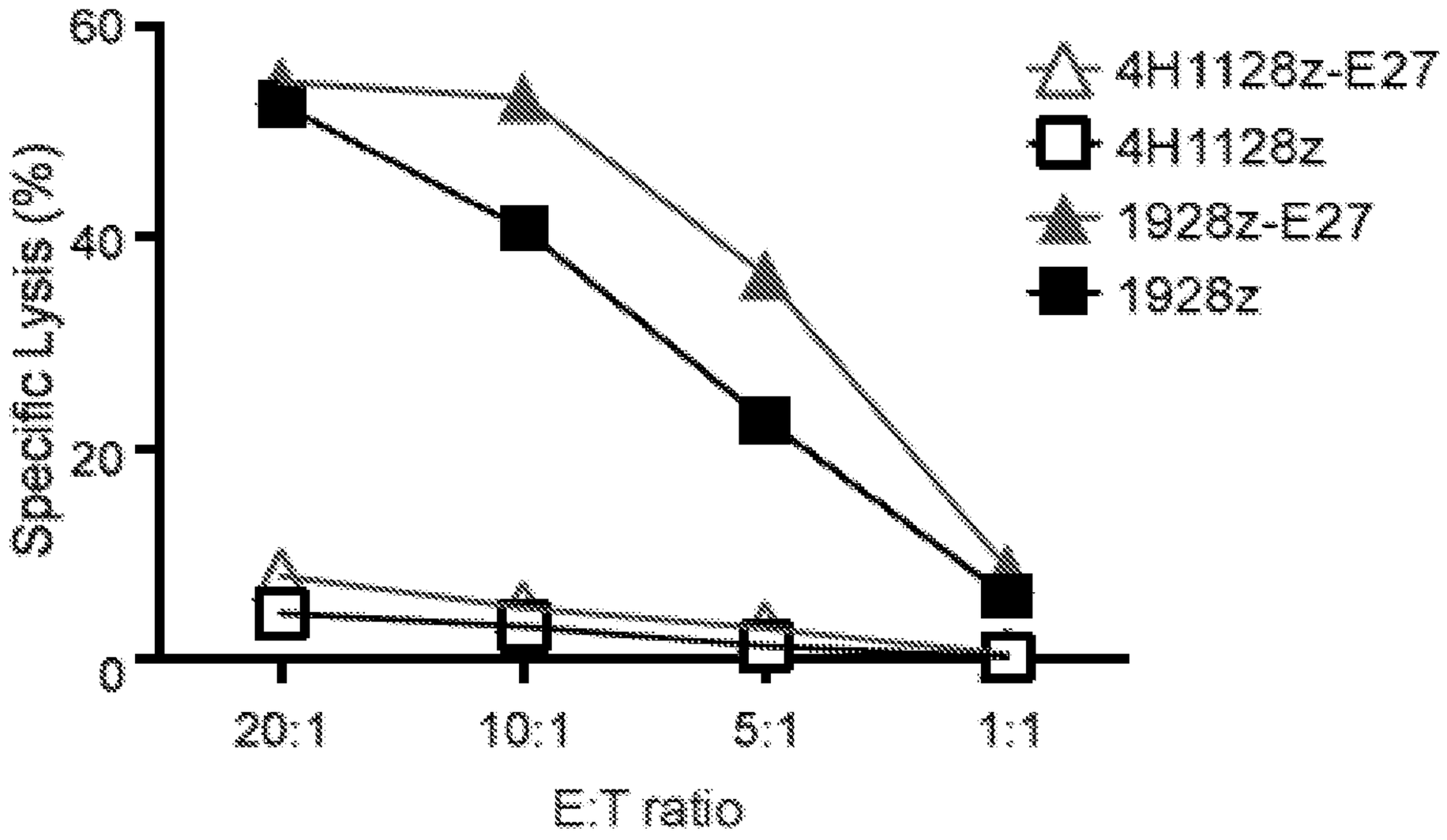
60
40
20
0
% Specific Lysis (%)
20:1
10:1
5:1
1:1
E:T ratio
4H1128z-E27
4H1128z
1928z-E27
1928z

**FIGURE 16E**

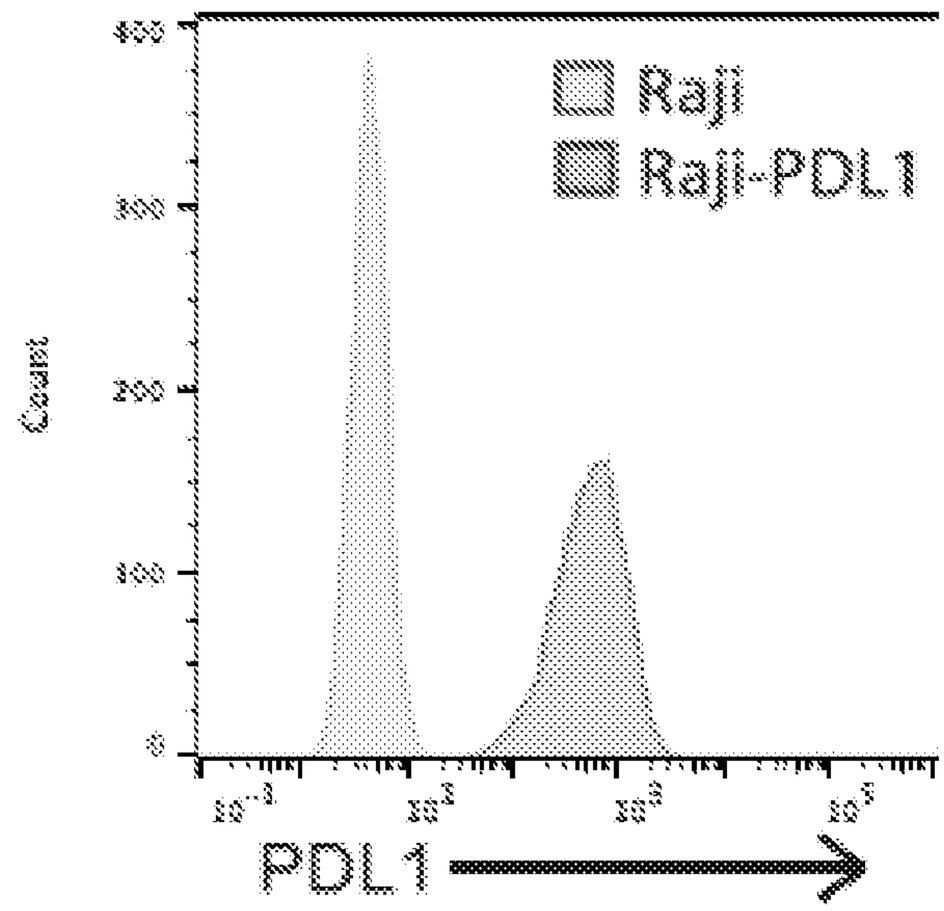


FIGURE 17A




FIGURE 17B

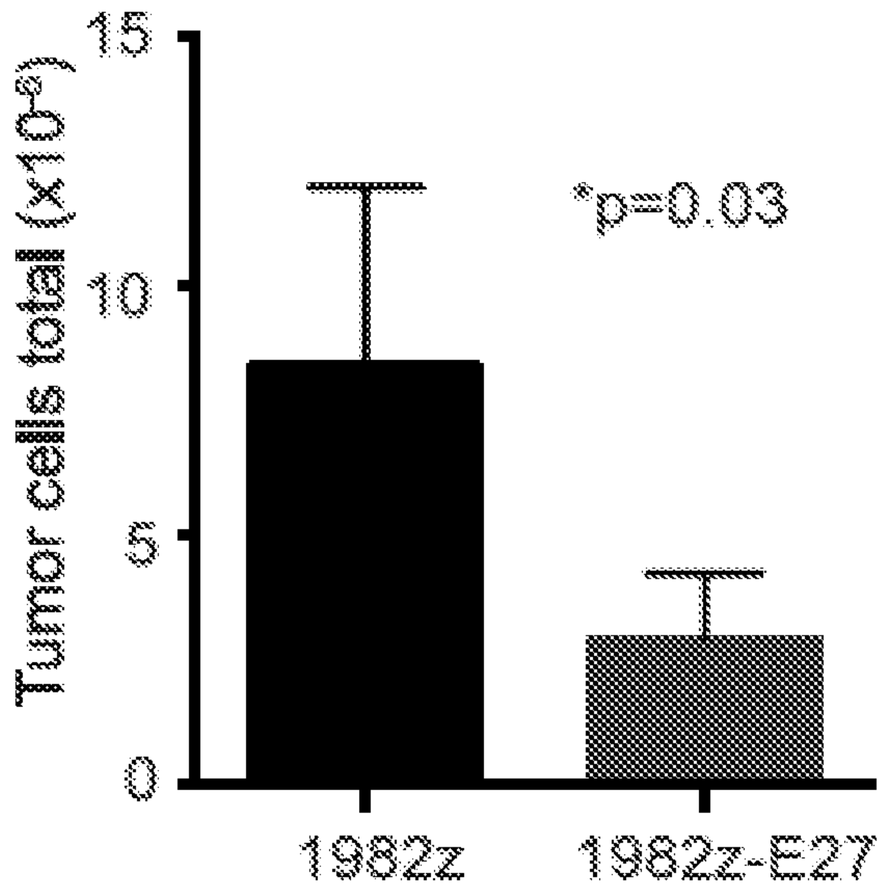


**FIGURE 17C**

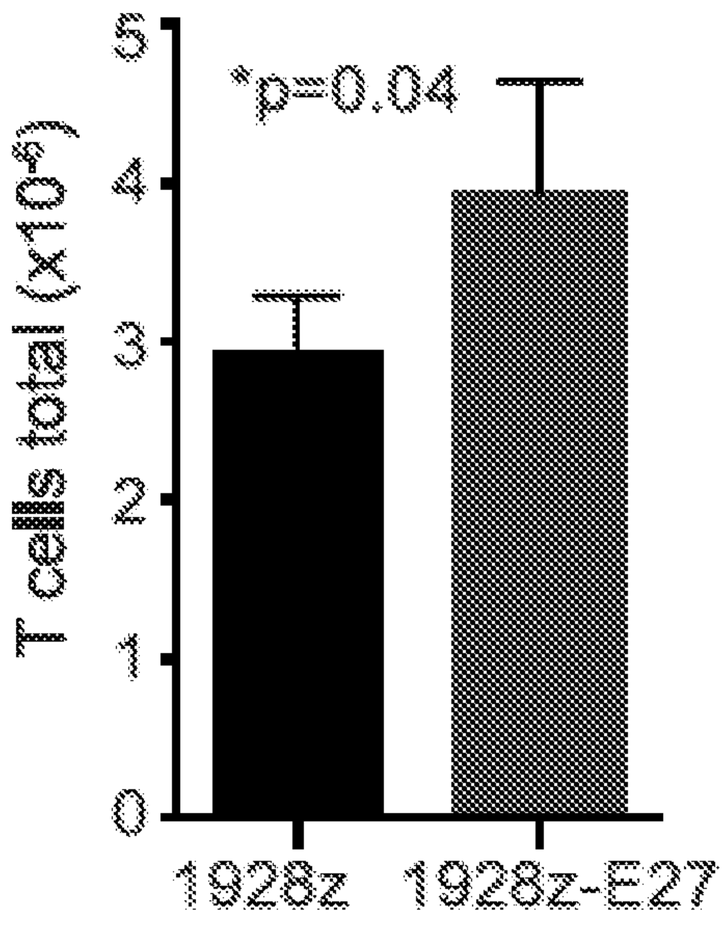


**FIGURE 17D**

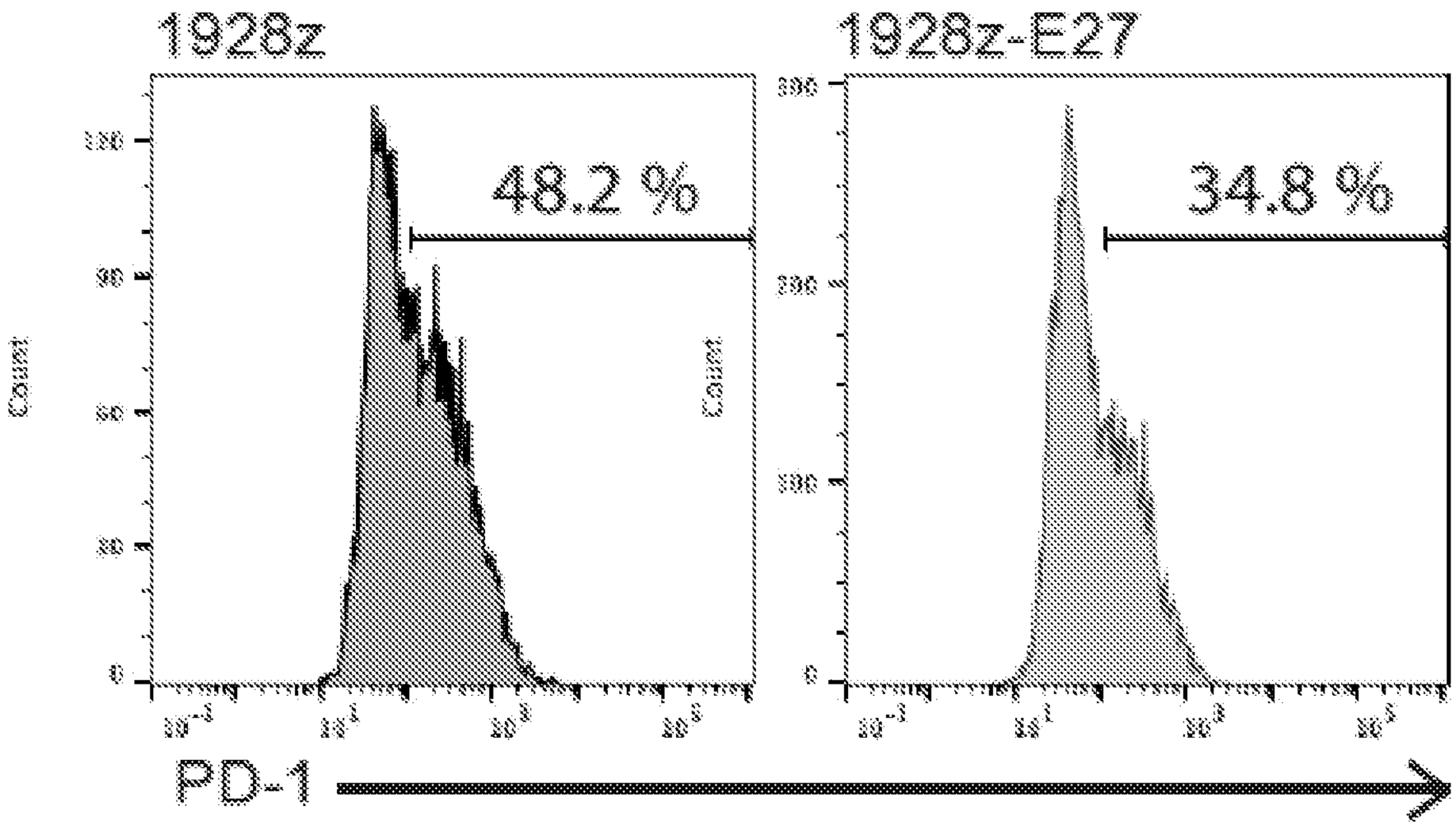


**FIGURE 17E**

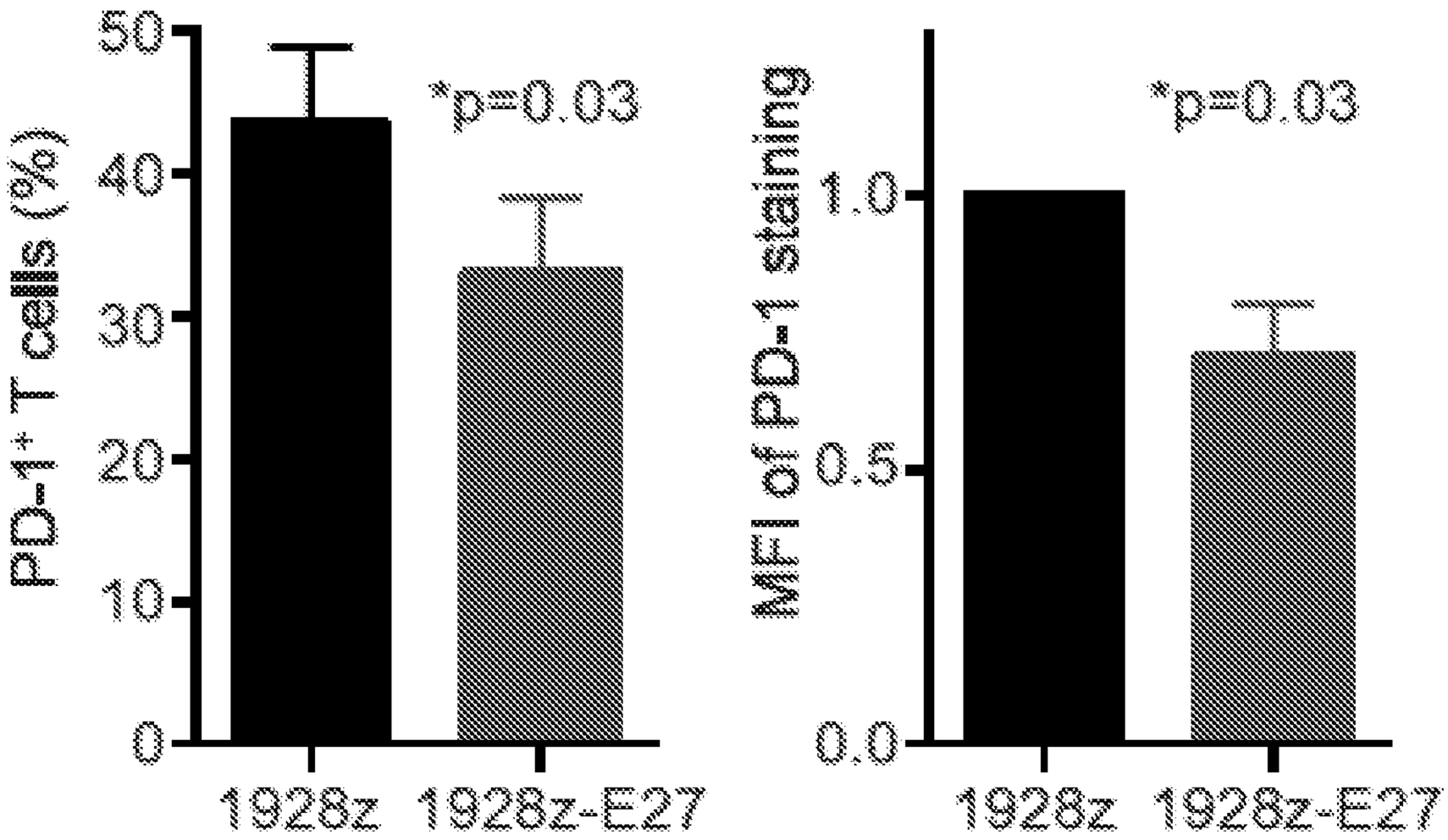
PD-1+ T cells (%)
50
40
30
20
10
0
*p=0.03
1928z
1928z-E27
MFI of PD-1 staing
1.0
0.5
0.0
*p=0.03
1928z
1928z-E27

**FIGURE 17F**

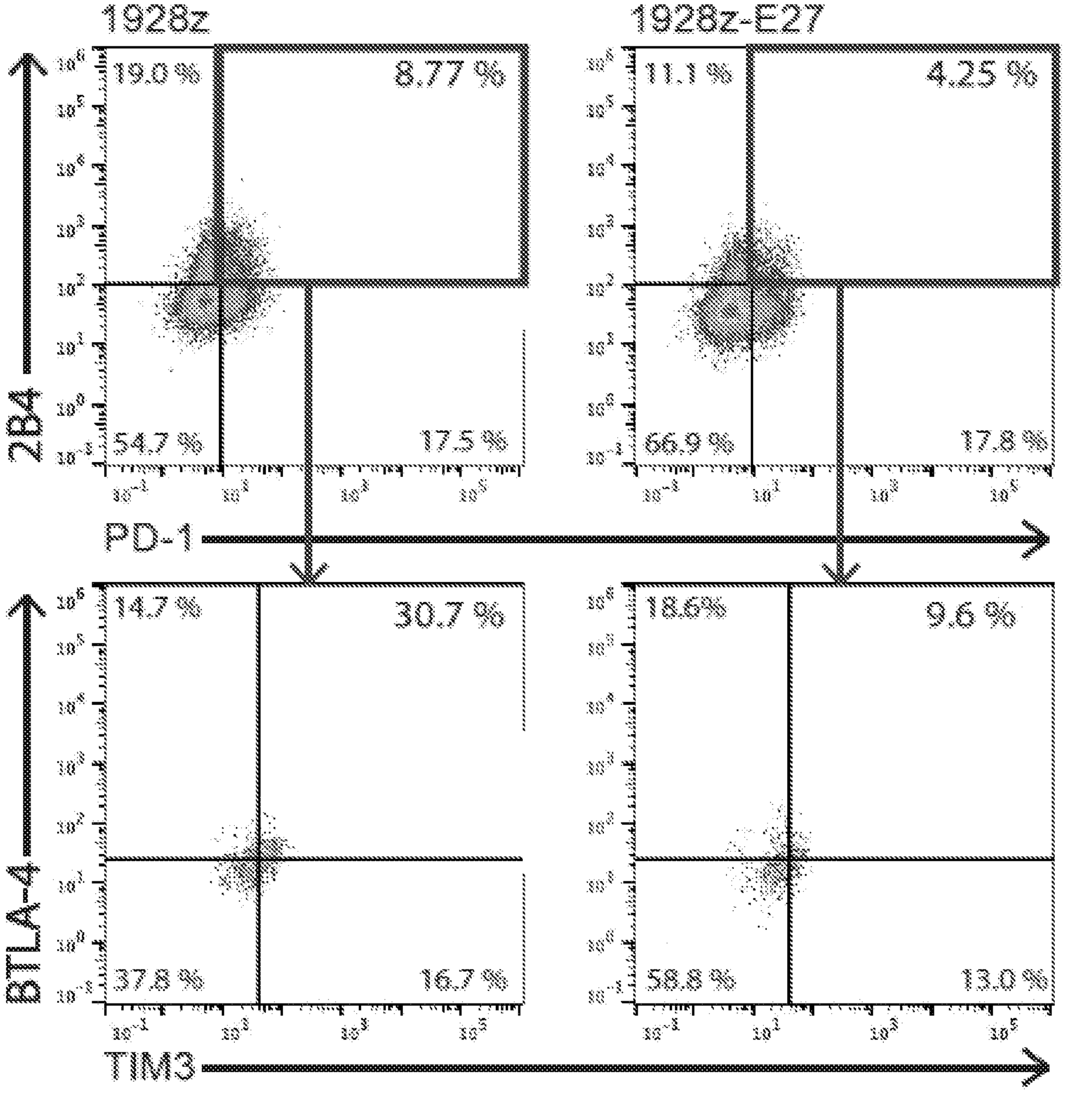
1928z
1928z-E27
19.0 %
8.77 %
54.7 %
17.5 %
11.1 %
4.25 %
66.9 %
17.8 %
2B4
PD-1
14.7 %
30.7 %
37.8 %
16.7 %
18.6%
9.6 %
58.8 %
13.0 %
BTLA-4
TIM3

**FIGURE 17G**

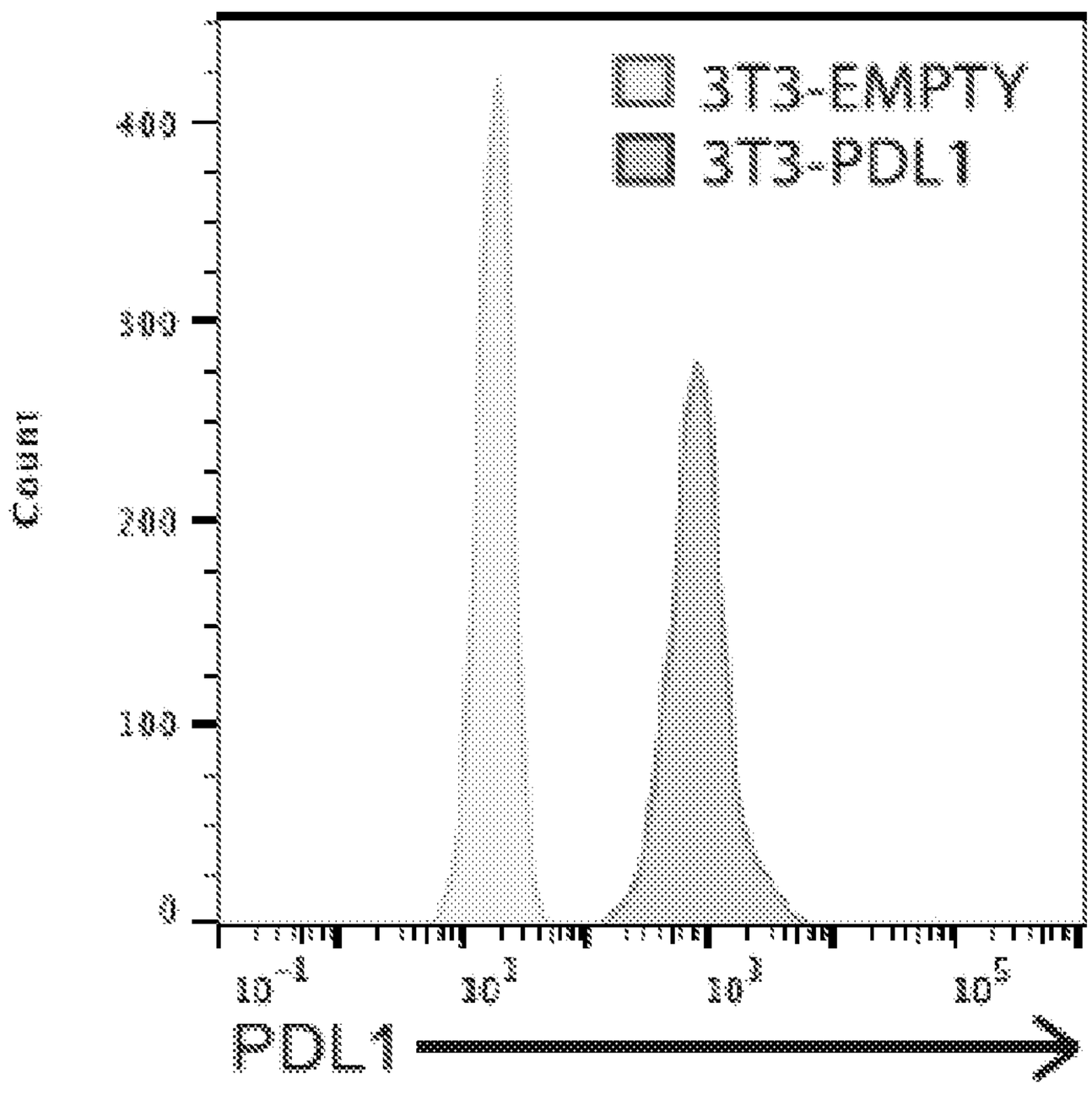


**FIGURE 18A**

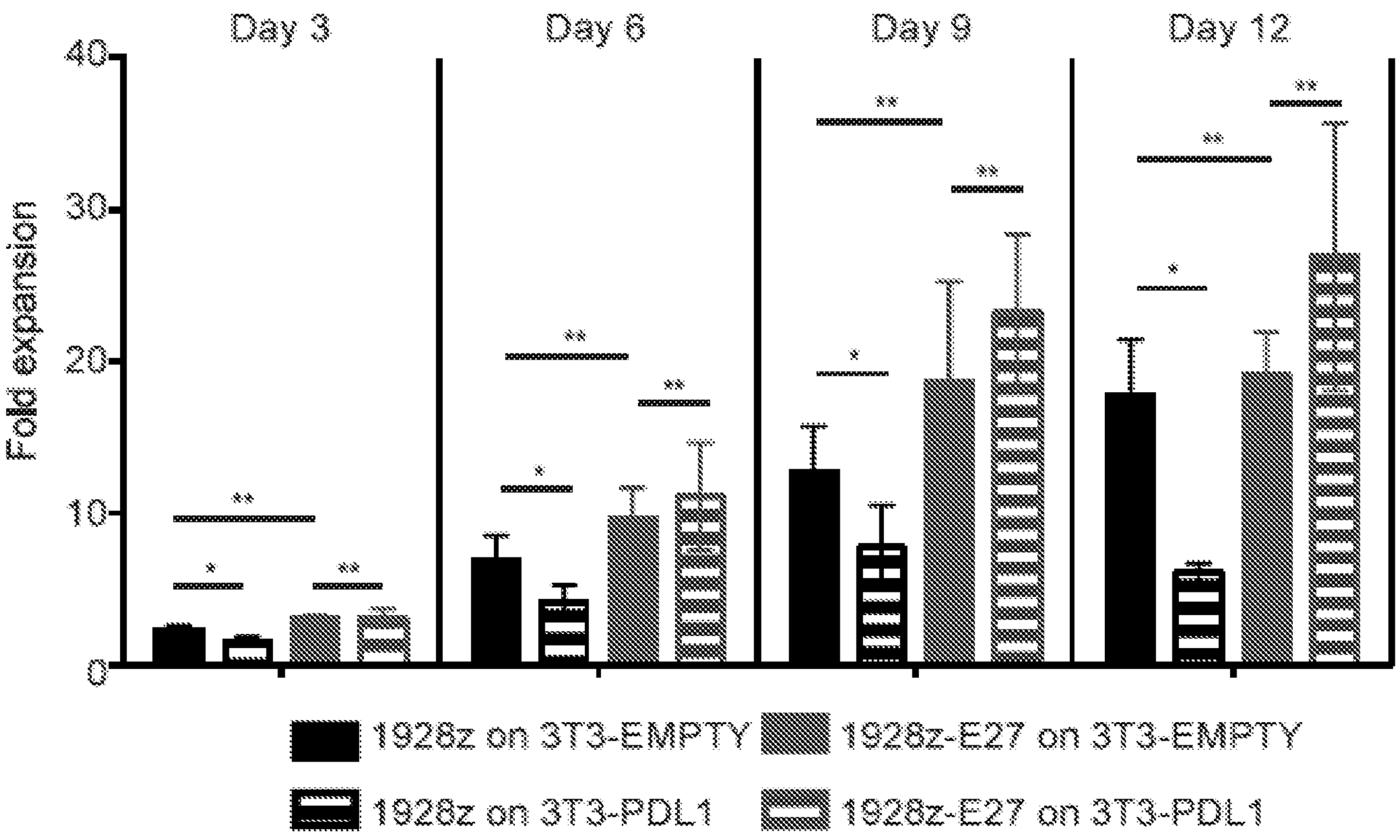
Day 3
Day 6
Day 9
Day 12
Fold expansion
40
30
20
10
0
1928z on 3T3-EMPTY
1928z-E27 on 3T3-EMPTY
1928z on 3T3-PDL1
1928z-E27 on 3T3-PDL1

**FIGURE 18B**

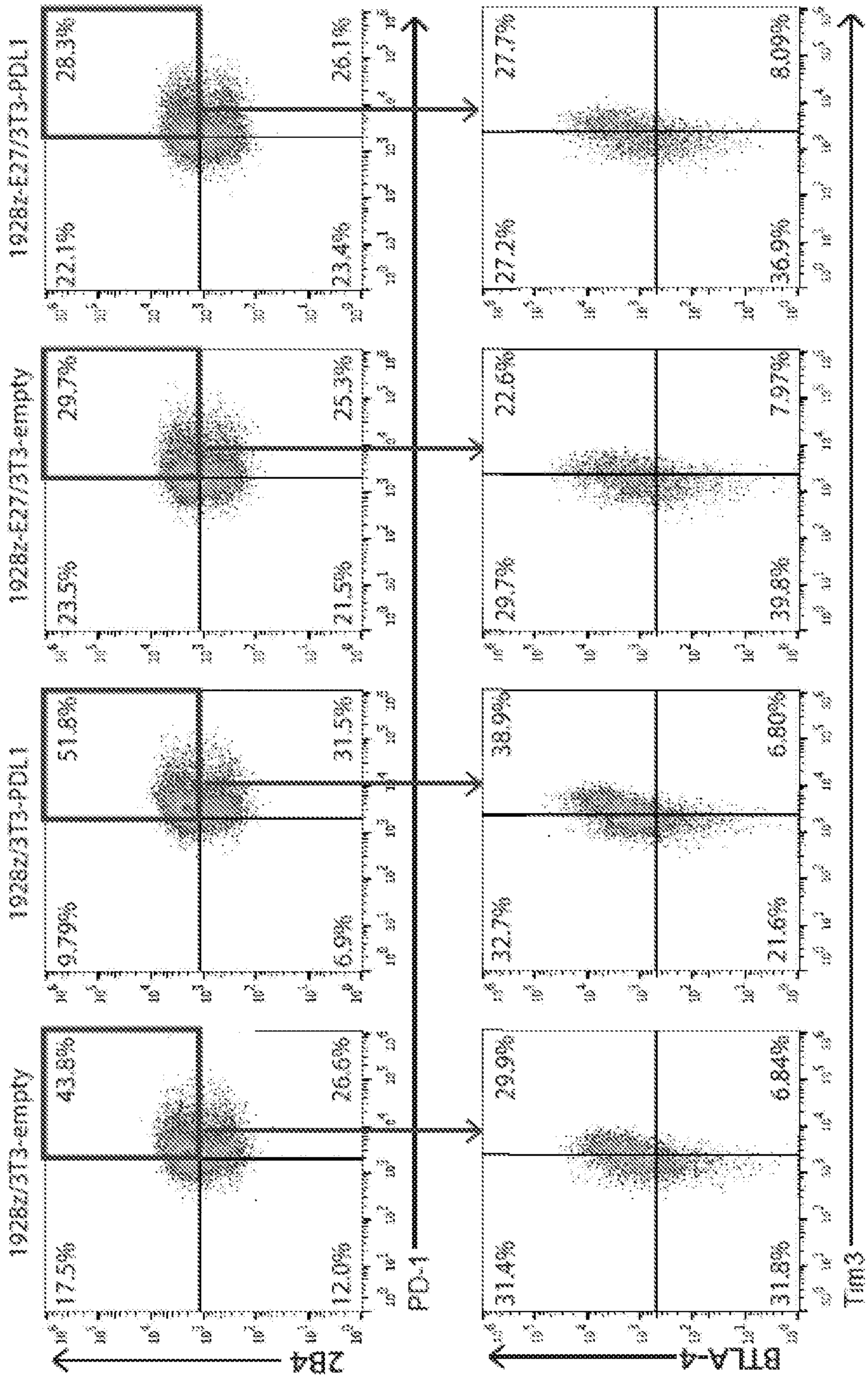
1928z-27/3T3-PDL1
28.3%
26.1%
27.7%
8.09%
22.1%
23.4%
27.2%
39.9%
1928z-27/3T3-empty
29.7%
25.3%
22.6%
7.97%
23.5%
21.5%
29.7%
39.8%
1928z/3T3-PDL1
51.8%
31.5%
38.9%
6.80%
9.79%
6.9%
32.7%
21.6%
1928z/3T3-empty
43.8%
26.6%
29.9%
6.84%
7.5%
12.0%
31.4%
31.8%
PD-1
CD28
BTLA-4
Tim3

**FIGURE 18C**

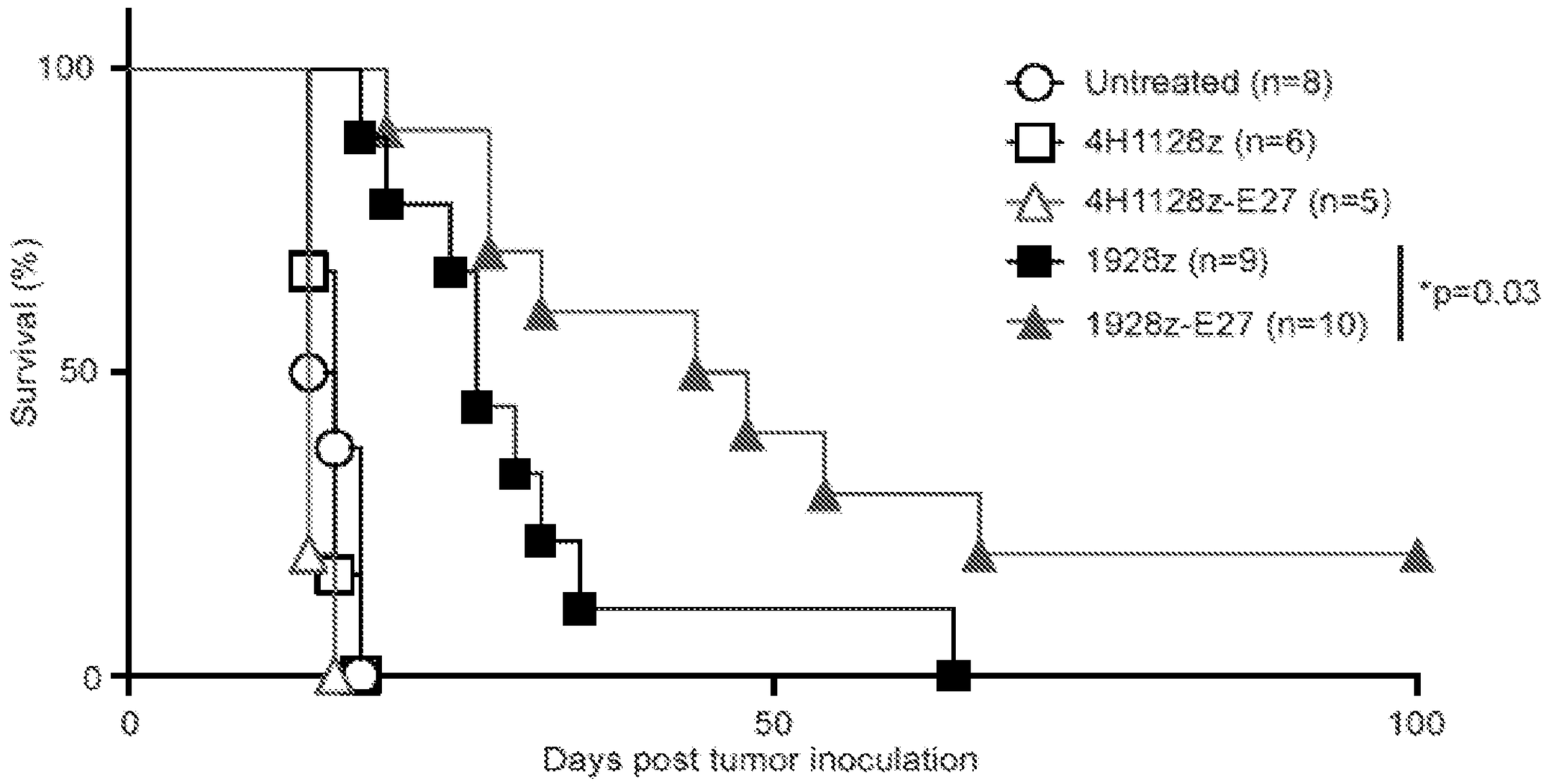
100
50
0
Survival (%)
0
50
100
Days post tumor inoculation
Untreated (n=8)
4H1128z (n=6)
4H1128z-E27 (n=5)
1928z (n=9)
1928z-E27 (n=10)
*p=0.03

**FIGURE 19**

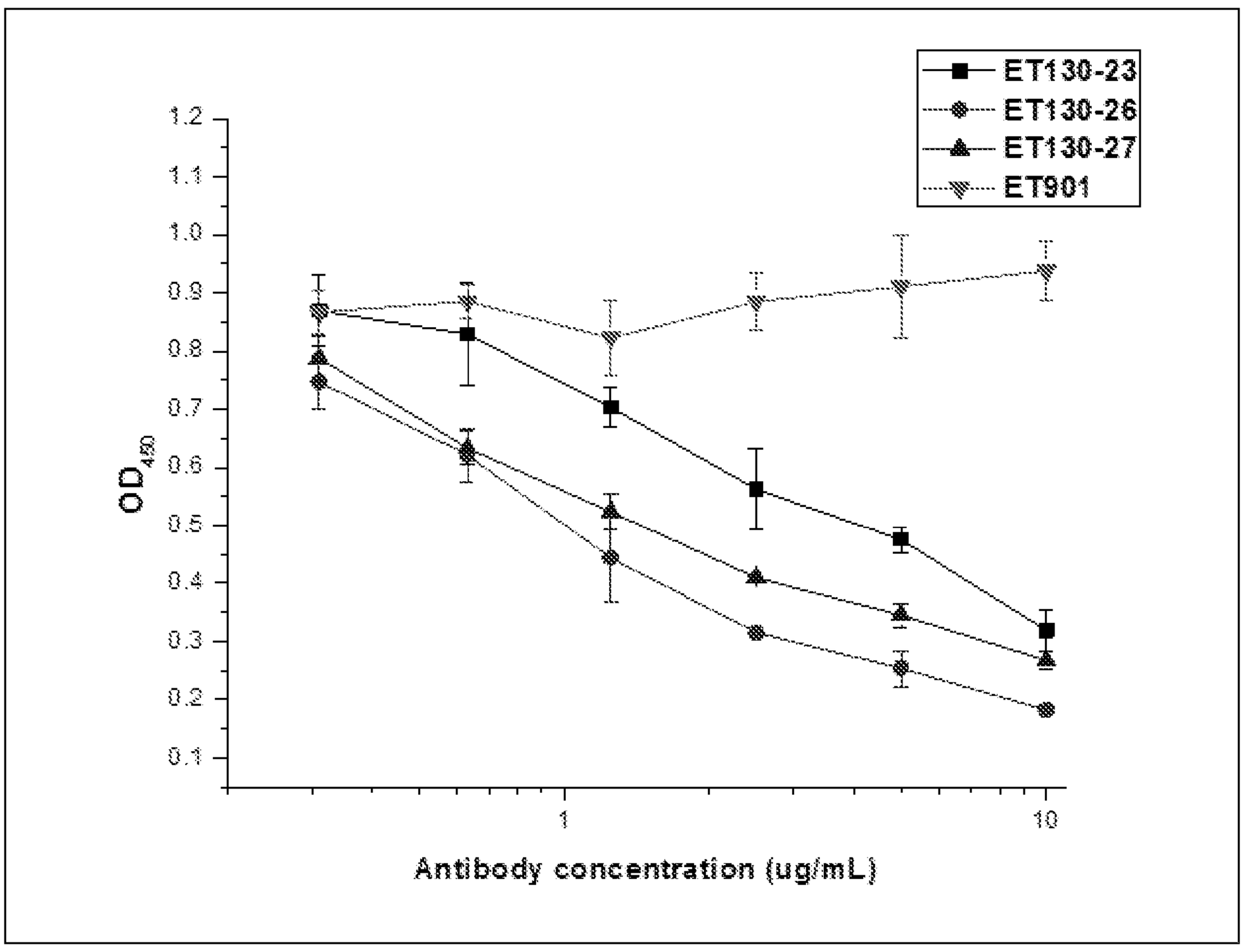
ET130-23
ET130-26
ET130-27
ET901
OD450
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
1
10
Antibody concentration (ug/mL)

**FIGURE 20**

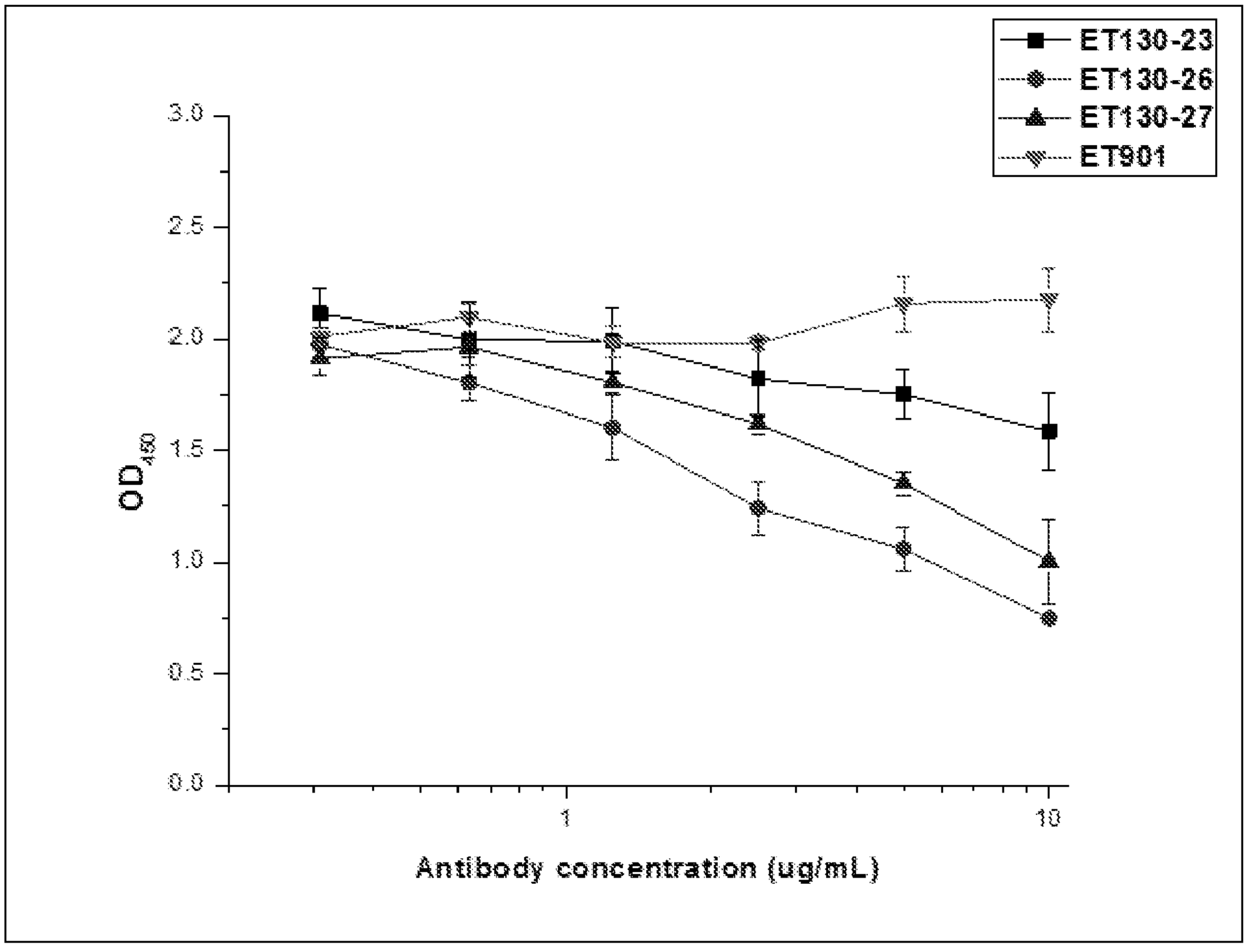
ET130-23
ET130-26
ET130-27
ET901
OD450
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
10
Antibody concentration (ug/mL)

**FIGURE 21**