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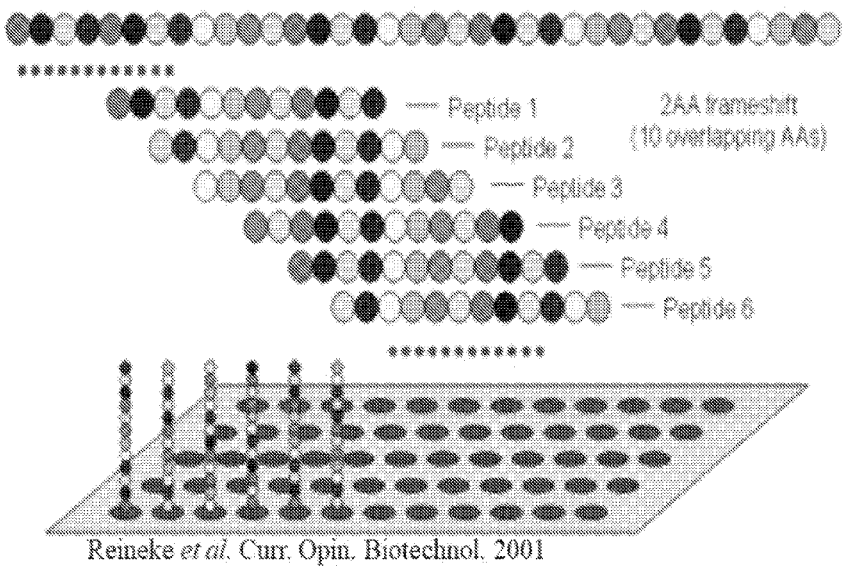
(54) Title: MUTANT VACCINIA VIRUSES AND USE THEREOF

Figure 1A



Reineke *et al.* Curr. Opin. Biotechnol. 2001

(57) Abstract: The present invention discloses recombinant vaccinia virus (VV) virions that are resistant to antiviral defenses and have enhanced anti-tumor activities. In one embodiment, the recombinant VV comprise one or more variant VV proteins that have mutations at one or more neutralizing antibody epitopes, thereby conferring viral escape from the neutralizing antibodies. In another embodiment, the recombinant VV is resistant to complement-mediated neutralization due to the expression of a regulator of complement activation (e.g. CD55). In another embodiment, the recombinant VV has enhanced anti-tumor activities due to the expression of bi-specific antibodies co-targeting cancer cells and immune effector cells, or the expression of a polypeptide blocking the PD-1 pathway. The recombinant vaccinia virus virions can be used to treat cancer in a subject.

WO 2020/086423 A1

[Continued on next page]

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INTERNATIONAL PATENT APPLICATION UNDER THE PATENT COOPERATION TREATY

MUTANT VACCINIA VIRUSES AND USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This Patent Cooperation Treaty Application claims the benefit of priority of U.S. Provisional Patent Application No. 62/749,102, filed on October 22, 2018, and U.S. Provisional Patent Application No. 62/912,344, filed on October 8, 2019. See further description in Summary of The Invention.

BACKGROUND OF THE INVENTION

[0002] Oncolytic viruses specifically infect, replicate in, and kill tumor cells while leaving normal cells undamaged. This preference for the transformed cells pegs oncolytic viruses as ideal candidates for the development of new cancer therapies. Various oncolytic viruses have been utilized to employ their tumor-specific killing activities by both direct (e.g. cell lysis due to viral replication and immune-mediated cytotoxicity), and indirect mechanisms (e.g. stimulation of the bystander cell killing, induction of cytotoxicity, etc). Oncolytic vaccinia virus (VV) is an appealing addition to the current treatment options, demonstrating efficacy and safety in animal models and in early clinical studies. In addition to infecting and killing tumor cells directly, VV may also induce a T-cell response against tumor antigens, increasing the efficiency of the killing. Whereas in some viruses this specificity toward cancer cells is naturally occurring (e.g. vesicular stomatitis virus, reovirus, mumps virus), other viruses can be genetically modified to improve their tumor specificity as well as to reduce their ability to induce antiviral immune response (e.g. adenovirus, measles virus, polio, and vaccinia virus). In addition, these viruses can be engineered to express genes that enhance antitumor immunity by recruitment of natural killer (NK) cells and T cells.

[0003] However, the effectiveness of oncolytic viruses is hindered by the strong immune response induced by the virus. Immune factors such as antibodies neutralize the virus by binding to it directly and preventing a successful infection of the cells or by marking it for destruction either by complement or by other immune cells. With each subsequent administration of the virus, the immune response is faster and stronger, which significantly

restricts the ability of the virus to persist long enough to reach the tumor. A direct injection of the virus into the tumor overcomes this limitation and delivers all the viral particles directly to the cancer cells. However, this approach may not be suitable for some tumors and does not take into the account cases in which the tumors may have metastasized to other locations. A more desirable systemic administration of the virus exposes it to the host immune system capable of recognizing and eliminating potential pathogens. Immune factors such as neutralizing antibodies (NAbs) recognize and bind viral glycoproteins with high affinity and prevent virus interaction with host cell receptors, leading to virus neutralization. Several oncolytic viruses, such as adenovirus, herpes simplex virus, and vesicular stomatitis virus, have been genetically attenuated to placate their ability to induce antiviral defenses and improve tumor specificity.

[0004] Oncolytic vaccinia virus (VV) is the most studied member of the *Poxviridae* and is a large, enveloped, dsDNA virus. Strains highly specific to the tumor cells have been reported. VV's ability for rapid replication results in efficient lysis of infected cells as well as spread to other tumor cells upon successive rounds of replication, leading to profound localized destruction of the tumor. The VV genome encodes ~ 250 genes and can accept as much as 20 kb of foreign DNA, making it ideal as a gene delivery vehicle. The recombinant VV vectors are being developed to deliver eukaryotic genes, such as tumor-associated antigens, to the tumors and thus facilitate an induction of the host immune system directed to kill the cancer cells. However, a limiting factor in the use of VVs as cancer treatment delivery vectors is the strong NAb response induced by the injection of VV into the bloodstream that limits the ability of the virus to persist and spread and prevents vector re-dosing. The NAbs recognize and bind viral glycoproteins embedded in the VV envelope, thus preventing virus interaction with host cell receptors. A number of VV glycoproteins involved in host cell receptor recognition have been identified. Among them, proteins H3L, L1R, A27L, D8L, A33R, and B5R have been shown to be targeted by NAbs, with A27L, H3L, D8L and L1R being the main NAb antigens presented on the surface of mature viral particles. A27L, H3L, and D8L are the adhesion molecules that bind to host glycosaminoglycans (GAGs) heparan sulfate (HS) (A27L and H3L) and chondroitin sulfate (CS) (D8L) and mediate endocytosis of the virus into the host cell. L1R protein is involved in virus maturation.

[0005] Vaccinia virus is the prototype virus of the orthopoxvirus genus in the family Poxviridae, which replicates in the cytoplasm of cells and encodes more than 200 open

reading frames (ORFs) in a 190-kb double-stranded DNA genome. Vaccinia virus infection produces multiple forms of infectious particles, namely, intracellular mature virions (IMV), intracellular enveloped virions (IEV), cell-associated enveloped virions (CEV), and extracellular enveloped virions (EEV). The IMV is the most abundant virion, with a single membrane in cells. IMVs are released only during cell lysis. Once released, IMVs efficiently infect neighboring cells via interactions between cell receptors and viral glycoproteins imbedded in the IMV membrane. A portion of the IMV is subsequently wrapped with two layers of Golgi membrane to form an IEV, which is transported through microtubules to the cell periphery and loses one membrane during virion egress to become a CEV. A small percentage (~ 5%) of the IMVs is moved toward the cell's periphery where it acquires an outer envelope via fusion with the cell plasma membrane and is subsequently released into the extracellular space as an EEV. Thus, EEV is composed of the viral DNA core, the intermediate IMV, and an outermost membrane. This outer membrane is fragile and can be easily lost, thus EEVs are easily converted to the IMVs exposing the IMV imbedded antigens. The IMV is robust and is known to be resistant to environmental and physical changes, whereas the CEV and EEV are very fragile, and the integrity of their outer membranes can be destroyed during purification procedures.

[0006] Many of the poxvirus genomes, including those of different strains of vaccinia virus, have been sequenced. The genome of the vaccinia virus Western Reserve (WR) strain contains 218 potential ORFs. Analysis of the proteins in the IMV showed that it contains 81 viral proteins, including structural proteins, enzymes, transcription factors, etc. The 81 viral proteins in IMV are A2.5L, A3L, A4L, A5R, A6L, A7L, A9L, A10L, A12L, A13L, A14L, A14.5L, A15L, A16L, A17L, A18R, A21L, A22R, A24R, A25L, A26L, A27L, A28L, A29L, A30L, A31R, A32L, A42R, A45R, A46R, B1R, C6L, D1R, D2R, D6R, D7R, D8L, D11L, D12L, D13L, E1L, E4L, E6R, E8R, E10R, E11L, F8L, F9L, F10L, F17R, G1L, G3L, G4L, G5R, G5.5R, G7L, G9R, H1L, H2R, H3L, H4L, H5R, H6R, I1L, I2L, I3L, I5L, I6L, I7L, I8R, J1R, J3R, J4R, K4L, L1R, L3L, L4R, L5R, O2L. Among these proteins, A27L, H3L, L1R, and D8L have been identified as major immunogenic proteins. IMV proteins A27L, H3L, and D8L are the adhesion molecules that bind to host glycosaminoglycans (GAGs) heparan sulfate (HS) and chondroitin sulfate (CS) (D8L) and mediate endocytosis of the virus into the host cell. IMV L1R protein is involved in virus maturation. These proteins are the main immunodominant antigens on the IMV.

[0007] VV H3L is the membrane protein tethered to the membrane of the mature viral particles post-translationally via its hydrophobic region in the C-terminus. It is expressed late during the infection and, together with A27L, recognizes the HS cell surface receptors and plays a major role in VV adhesion to the cells. H3L is an immunodominant antigen in the anti-VV Ab response and a direct target of NAbs in humans immunized by the smallpox vaccine. Strong immune responses to H3L have also been shown in mice and rabbits. To date, the exact epitopes on H3L that are recognized by the NAbs have not been elucidated.

[0008] D8L is the VV envelope protein expressed early in infection and is involved in viral adhesion to host cells. While A27L and H3L interact with the HS host cell receptors, D8L binds to the CS receptors via its N-terminal domain (between residues 1–234). As one of the main viral antigens, D8L elicits a strong NAb response with the NAbs targeting the CS-binding region on the D8L and blocking viral adhesion to the cells. Several Abs targeting the D8L protein have been described. One of these Abs neutralized VV in the presence of a complement and targeted a conformational epitope on D8 (between residues 41 to 220). Residues R44, K48, K98, K108, and R220, a region adjacent to the CS binding site on D8L, are also important for Ab binding. In addition, N9, E30, T34, T35, N46, F47, K48, G49, G50, Y51, N59, E60, L63, S64, D75, Y76, H95, W96, N97, K99, Y101, S102, S103, Y104, E105, E106, K108, H110, D112, Q122, L124, D126, K163, T187, P188, and N190 have been identified as D8 antibody binding sites. It is not known whether mutation of these residues will confer sufficient escape from neutralization antibodies. Furthermore, whether mutations of these residues will impair virus packaging and cell entry due to D8L's role in cell entry remain to be determined.

[0009] L1R is a transmembrane protein found on the surface of the mature VV particles. Its transmembrane domain lies in the C-terminal regions of the protein between residues 186 and 204. L1R is encoded by the L1R ORF, is highly conserved, and plays an essential role in viral entry and maturation. As one of the main targets of anti-VV NAb, L1R is included as a component of the poxvirus protein subunit and DNA vaccines. The NAb binding epitopes on the L1R protein have been characterized. An earlier study identified potent NAbs recognizing a linear epitope spanning residues 118-128 and a conformation epitope that partially overlapped with the linear peptide, specifically residues K125 and K127. A more recent study

identified a group of 3 anti-L1R monoclonal Abs that potently neutralized VV in an isotype- and complement-independent manner. These NAbs recognized a conformational epitope with D35 as the key residue. Viral clones that contained a single amino acid mutation at residue D35 (either D35N or D35Y substitution) were completely resistant to neutralization by all Abs, indicating that D35 is essential for NAb recognition of L1R and binding. However, it is not clear if D35N will induce new neutralization antibody responses against 35N. In addition to D35, residues E25, N27, Q31, T32, K33, S58, D60, and D62 have been identified to be directly involved in binding with the Ab. It is not known whether mutations of these residues will escape neutralization antibody sufficiently and impair virus packaging and cell entry due to L1R's role in cell entry.

[0010] A27L is a 14-kDa protein in the envelope of the intracellular mature virus (IMV) that functions in viral host cell recognition and entry. It binds to the HS receptor on the host cell surface via its N-terminal domain (residues 21 to 30) and is attached to the VV envelope by interacting with the envelope protein A17 through its C-terminal domain. A recent study has identified several linear epitopes on the A27L that are recognized by the anti-A27L Abs. The Abs were categorized into four different groups with the Abs in group I binding to the peptide (residues 31 to 40) adjacent to the HS binding site and showing potent virus neutralization in the presence of complement. Crystal structures of the full-length A27L in a complex with these Abs identified residues E33, I35, V36, K37, and D39 to be critical for binding. Alanine substitutions of these residues resulted in the decreased ability of the Abs to bind to the peptide. A further analysis of the structures showed that residues K27, A30, R32, A34, E40, R107, P108, and Y109, although not critical, also contribute to the A27L–Ab binding.

[0011] In view of the above, there is a need for improved or genetically attenuated vaccinia viruses that have reduced ability to induce antiviral defenses and have enhanced anti-tumor activities. For example, ways to reduce induction of antiviral defenses and enhance anti-tumor activities include strategies for resisting neutralizing antibodies, overcoming complement-mediated virus neutralization, arming vaccinia viruses with bi-specific polypeptides to boost virus therapy, and/or incorporating immune checkpoint molecules to boost virus therapy.

SUMMARY OF THE INVENTION

[0012] In one embodiment, the present invention provides mutant vaccinia viruses that are useful as viral vectors and vaccines.

[0013] Disclosed herein are recombinant vaccinia viruses comprising variant H3L, D8L, A27L and/or L1R viral proteins, including those of SEQ ID NOs:170 and 172. Further disclosed herein are recombinant vaccinia viruses comprising a heterologous nucleic acid encoding one of the following polypeptides: a domain of CD55 protein, a bi-specific polypeptide that binds to CD3e and FAP (fibroblast activation protein), a bi-specific polypeptide that binds to CD3e and BCMA (B-cell maturation antigen), and a fusion polypeptide comprising human PD-1 extracellular domain.

[0014] In one embodiment, the present invention provides mutant vaccinia viruses and uses thereof. In one embodiment, there is provided mutant vaccinia viruses having one or more mutation in the genes encoding proteins involved in binding neutralization antibodies or T cells. These mutations result in mutant vaccinia viruses having the ability to escape vaccinia virus-specific neutralization antibodies or T cells when compared to the wild-type virus.

[0015] In one embodiment, the present invention provides an isolated infectious recombinant vaccinia virus (VV) virion, the recombinant VV virion comprises a heterologous nucleic acid and one or more of:

(a) a variant vaccinia virus (VV) H3L protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:1;

(b) a variant vaccinia virus (VV) D8L protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:2;

(c) a variant vaccinia virus (VV) A27L protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:3;

(d) a variant vaccinia virus (VV) L1R protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:4;

(e) a variant vaccinia virus (VV) H3L protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:5;

(f) a variant vaccinia virus (VV) D8L protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:6 or SEQ ID NO:174;

(g) a variant vaccinia virus (VV) H3L protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:170; and

(h) a variant vaccinia virus (VV) D8L protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:172.

[0016] In one embodiment, the present invention provides recombinant vaccinia virus (VV) virions comprising a nucleic acid encoding a complement activation modulator such as part or all of CD55, CD59, CD46, CD35, factor H, and C4-binding protein, and the like, and uses thereof. Expression of the complement activation modulators results in recombinant vaccinia viruses having the ability to modulate complement activation and reduce complement-mediated virus neutralization when compared to the wild-type virus. In one embodiment, the CD55 protein comprises the amino acid sequence of SEQ ID NO:7.

[0017] In one embodiment, the present invention provides recombinant vaccinia virus (VV) virions comprising a bi-specific FAP-CD3 scFv that comprises an amino acid sequence having the sequence of SEQ ID NO:8.

[0018] In one embodiment, the present invention provides recombinant vaccinia virus (VV) virions comprising a bi-specific BCMA-CD3 scFv that comprises an amino acid sequence having the sequence of SEQ ID NO:9.

[0019] In one embodiment, the present invention provides recombinant vaccinia virus (VV) virions comprising a PD-1-ED-hIgG1-Fc fusion peptide that comprises an amino acid sequence having the sequence of SEQ ID NO:10.

[0020] In another embodiment, the present invention provides a method of delivering a gene product to an individual in need thereof, the method comprising administering to the individual an effective amount of an infectious recombinant vaccinia virus (VV) virion disclosed herein, wherein the gene product is encoded by the heterologous nucleic acid carried by the recombinant VV virion.

[0021] In one embodiment, there is provided a pharmaceutical composition comprising the recombinant vaccinia virus (VV) virion disclosed herein, and methods of using such composition to treat cancer.

[0022] In one embodiment, there is provided a library comprising one or more variant vaccinia virus (VV) virions, each of said variant VV virions comprises one or more variant VV protein, the variant VV protein comprises an amino acid sequence having at least one amino acid substitution relative to the amino acid sequence of a corresponding wild type VV protein.

[0023] In another embodiment, the present invention provides a method of delivering a gene product to an individual in need thereof, the method comprises administering to the individual an effective amount of infectious variant vaccinia virus (VV) virions derived from the above library, wherein the gene product is encoded by a nucleic acid carried by such variant VV virions.

[0024] In another embodiment, there is provided a pharmaceutical composition comprising variant vaccinia virus (VV) virions derived from the above library, and methods of using such composition to treat cancer.

[0025] In one embodiment, there is provided a recombinant vaccinia virus H3L protein that has at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to one of SEQ ID NOs:1, 5 or 170. In another embodiment, there is provided a recombinant vaccinia virus D8L protein that has at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NOs:6, 172 or 174.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] Figures 1A-C show neutralizing antibody (Nab**)** epitope determination of H3L – peptide arrays sequence analysis. Antibody 35219 was used for binding to the peptide array of the H3L sequence (Ab35219 is a rabbit polyclonal to VV; Immunogen: Native virus, Lister strain).

[0027] Figure 1A shows diagram of the SPOT-synthesis peptide array. **Figure 1B** shows autoradiograph of the H3L peptide array probed by ab35219. The peptide array consists of spots of 12-residue peptides in the H3L sequence, starting from the N terminus (spot 1) and ending with the C-terminal peptide (spot 69), with the N-terminal residue of the peptide in each spot shifted by 4 residues from the previous spot along the H3L sequence. **Figure 1C** are graphs showing signal intensity (*y* axis) of each spot (black bars) (*x* axis).

[0028] Figures 2A-B show NAb epitope mapping of H3L by linear peptide ELISA. **Figure 2A** shows ELISA results for H3L peptides 1 – 4. **Figure 2B** shows ELISA results for H3L peptides 5 – 9. Arrows indicate some examples of alanine-substituted residues that have an effect on antibody (Ab) binding. Alanine scan identified total of 29 residues positive for Ab binding: I14A, D15A, R16A, K33A, F34A, D35A, K38A, N40A, E45A, V52A, E131A, T134A, F135A, L136A, R137A, R154A, E155A, I156A, K161A, L166A, V167A, M168A, I198A, R227A, E250A, K253A, P254A, N255A, and F256A. A lower optical density (OD) indicates that the alanine-substituted peptide preincubated with the Ab binds sufficiently to prevent the Ab binding to plate-bound native peptide. A higher OD (arrows) indicates the decreased ability of the mutant peptide to interact with the Ab, signifying that the mutated residue is important for H3L binding to Ab.

[0029] Figures 3A-D show construction of modified H3L, D8L, L1R, and A27L plasmids.

[0030] Figure 3A shows a construct containing the H3L promoter, H3L ORF (with mutated nucleotides), and approximately ~ 250-bp flanking regions containing the H4L (left flank) and the H2R (right flank) ORF sequences was synthesized by GENEWIZ and cloned into the pUC57-Amp plasmid.

[0031] Figure 3B shows a construct containing the D8L promoter, D8L ORF (with mutated nucleotides), and approximately ~ 250-bp flanking regions containing the D9R (left flank)

and the D7R (right flank) ORF sequences was synthesized by GENEWIZ and cloned into the pUC57-Amp plasmid.

[0032] Figure 3C shows a construct containing the L1R promoter, L1R ORF (with mutated nucleotides), and approximately ~ 250-bp flanking regions containing the G9R (left flank) and the L2R (right flank) ORF sequences was synthesized by GENEWIZ and cloned into the pUC57-Amp plasmid.

[0033] Figure 3D shows a construct containing the A27L promoter, A27L ORF (with mutated nucleotides), and approximately ~ 250-bp flanking regions containing the A28-A29L (left flank) and the A26L (right flank) ORF sequences was synthesized by GENEWIZ and cloned into the pUC57-Amp plasmid. For all four constructs a green fluorescent protein (GFP) expression cassette under the control of the VV p7.5 promoter and flanked by LoxP sites was inserted immediately downstream of the stop codon before the right flank sequence.

[0034] Figure 4 shows identification of the correct H3L, D8L, L1R, and A27L recombinant clones. Single plaques were purified and correct gene insertions were confirmed by PCR.

[0035] Figure 5 shows plaque reduction neutralization tests (PRNTs) using polyclonal anti-VV Abs. A panel of five anti-VV polyclonal antibodies consisting of ab35219 (Abcam) – rabbit polyclonal to VV (Immunogen: Native virus, Lister strain), ab21039 (Abcam) – rabbit polyclonal to VV (Immunogen: Lister Strain (mixture of virions and infected cell polypeptides)), ab26853 (Abcam) – rabbit polyclonal to VV (Immunogen: Synthetic peptide containing amino acids on the predicted N terminus of A27L in VV), 9503-2057 (Bio-Rad) – rabbit polyclonal against VV Ab (Immunogen: Vaccinia virus, New York City Board of Health (NYCBOH) strain), and PA1-7258 (Invitrogen) – rabbit polyclonal against VV (Immunogen: NYCBOH strain and Lister strain) was used to test for neutralization escape *in vitro*. Rabbit polyclonal IgG ab37415 served as a control. Abs were preincubated with either the escape variant or the wt VV virus (control) in the presence of sterile baby rabbit complement. The mixture was then added to the CV-1 cells and 48 hrs later cells were stained and plaques counted. Whereas 83.3–95.5% of the control VV virus was neutralized across the panel, the escape variant (FAP-VVNEV) showed a significantly lower neutralization by the Abs (7.88–66.1%). Error bars are based on two or three data points per sample.

[0036] Figure 6 shows VV^{EM} (vaccinia virus escape mutant) *in vitro* plaque reduction neutralization test with anti-VV polyclonal Abs. VV^{EM} was isolated from the mutant VV library pool in the presence of anti-VV polyclonal antibodies. A panel of five anti-VV polyclonal antibodies consisting of ab35219, ab21039, ab26853, 9503-2057, and PA1-7258 was used to test VV^{EM} for neutralization escape *in vitro*. Rabbit polyclonal IgG ab37415 served as a control. Abs were preincubated with either the VV^{EM} or the wild type VV virus (control) in the presence of sterile baby rabbit complement. Whereas 77.7–96.4% of the control VV virus was neutralized across the panel, VV^{EM} showed a significantly lower (30.7-66.9%) neutralization by the Abs. Error bars are based on two or three data points per sample. VV^{EM} was further sequenced to identify the mutation within H3, L1, A27, or D8 that might be responsible for the Nab escape.

[0037] Figure 7 shows results of a recombinant virus replication assay. In a 24-well plate CV-1 cells were infected with duplicates of VV control and VV^{NEV} at MOI = 0.05. Prior to infection virus was preincubated with Ab 9503-2057 (40 µg/mL) for 1 hour at 37°C. Samples were collected at 24, 48, and 72 hours and titers were determined for each time point. The recombinant virus was significantly more efficient in replicating in the presence of Ab, compared to the control Ab, which was almost entirely inactivated.

[0038] Figure 8 shows anti-tumor efficiency of the recombinant virus. The recombinant virus and the control VV were preincubated with Ab 9503-2057 (see above) and used to infect transformed cells at MOI=1. Cells were incubated for 48 hours and cell viability was measured by MTS assay (colorimetric assessment of cell metabolic activity). Briefly, cells collected at 48 hours were washed once with PBST and resuspended at 1 × 105 cells/mL in complete DMEM. One hundred µL of each cell suspension was added to a 96-well (in triplicates). Twenty µl of CellTiter 96® AQueous One Solution Reagent (Promega, G358C) was added into each well of the 96-well assay plate containing the samples in 100µl of culture medium. The plate was incubated at 37°C for 2 hours (5% CO2). To measure the amount of soluble formazan produced by cellular reduction of MTS, the absorbance in each well was recorded at 490nm using a 96-well plate reader. In the presence of the Ab, the recombinant virus was able to efficiently kill the cells.

[0039] Figure 9 shows a recombinant VV^{NEV} *in vitro* plaque reduction neutralization test with anti-VV polyclonal Abs. Anti-VV polyclonal antibodies 9503-2057 and PA1-7258 were used to test VV^{EM} for neutralization escape *in vitro*. Rabbit polyclonal IgG ab37415 served as a control. Abs were preincubated with either the VV^{NEV} (right panel) or the wild type vaccinia virus (control, left panel) in the presence of sterile baby rabbit complement.

[0040] Figure 10 shows results of a recombinant virus replication assay. In a 24-well plate CV-1 cells were infected with duplicates of VV control and 3 single clones of VV^{NEV} at MOI = 0.05. Samples were collected at 24, 48, and 72 hours and titers were determined for each time point.

[0041] Figure 11 shows a CD55-A27-VV construct containing the A27 promoter, CD55-ED, A27, loxP-flanked tag, and flanking regions containing the A27L (left flank) and the A27R (right flank). ORF sequences was synthesized by GENEWIZ and cloned into the pUC57-Amp plasmid.

[0042] Figure 12 shows CD55-NEV escapes complement-mediated neutralization effectively in vitro.

[0043] Figure 13 shows CD55-NEV escapes neutralization antibody and complement-mediated neutralization effectively in vitro.

[0044] Figure 14 shows a FAP-TEA-NEV construct containing the F17R promoter, FAP-CD3 scFv, loxP-flanked tag, and flanking regions containing the TKL (left flank) and the TKR (right flank). ORF sequences was synthesized by GENEWIZ and cloned into the pUC57-Amp plasmid.

[0045] Figure 15 shows a FAP-TEA-NEV enhanced tumor lysis and human T cell proliferation in vitro (see circle, microscopy observation).

[0046] Figure 16 shows a FAP-TEA-NEV induced tumor cell apoptosis effectively (flow cytometry analysis).

[0047] Figure 17 shows MFI of apoptosis marker PI staining of gated U87 tumor cells.

[0048] Figure 18 shows a bispecific FAP-CD3 scFv expressed by FAP-TEA-NEV enhanced bystander tumor lysis in vitro (see circles, microscopy observation).

[0049] Figure 19 shows a BCMA-TEA-NEV construct containing the F17 promoter, BCMA-CD3 scFv, loxP-flanked GFP-tag, and flanking regions containing the TKL (left flank) and the TKR (right flank). ORF sequences was synthesized by GENEWIZ and cloned into the pUC57-Amp plasmid.

[0050] Figures 20A-B show flow cytometric analysis of co-culture of BCMA-positive RMPI-8226 MM and Jurkat T cells.

[0051] Figures 21A-B show ELISA measurement of IFNγ and IL2 expression by Jurkat T cells following 24 hours co-culture with BCMA-positive RMPI-8226 MM.

[0052] Figure 22 shows a PD-1-ED-hIgG1-Fc-VV construct containing the pE/L promoter, PD-1-ED-hIgG1-Fc, loxP-flanked GFP-tag, and flanking regions containing the TKL (left flank) and the TKR (right flank). A PD-1-ED-hIgG1-Fc-FAP-TEA-NEV construct containing the pE/L promoter, PD-1-ED-hIgG1-Fc, F17R promoter, FAP-CD3 scFv, loxP-flanked GFP-tag, and flanking regions containing the TKL (left flank) and the TKR (right flank) is also shown. ORF sequences was synthesized by GENEWIZ and cloned into the pUC57-Amp plasmid.

[0053] Figures 23A-B show flow cytometric analysis of co-culture of PD-L1-positive Raji cells and CD16-positive Jurkat T cells.

[0054] Figure 24A-B show ELISA measurement of IFNγ and IL2 expression by CD16-positive Jurkat T cells following 24 hours co-culture with PD-L1-positive Raji cells.

[0055] Figure 25 shows the luciferase activity measurement of CD16-positive Jurkat T cells following 24 hours co-culture with PD-L1-positive Raji cells.

DETAILED DESCRIPTION OF THE INVENTION

[0056] The present invention discloses the making and uses of variant vaccinia virus (VV) virions that have reduced ability to induce antiviral defenses and have enhanced anti-tumor activities.

Enhancing Resistance to Neutralizing Antibodies

[0057] In one embodiment, the variant vaccinia virus (VV) virions of the present invention have increased resistance to anti-VV neutralizing antibodies. For example, the variant vaccinia virus virions of the present invention comprise one or more variant VV proteins (such as H3L protein, D8L protein, A27L protein, and L1R protein) that have mutations at one or more neutralizing antibody epitopes, thereby conferring viral escape from the neutralizing antibodies.

[0058] The present specification discloses experiments studying variant VV protein H3L. The same experimental setup can be used to study other vaccinia virus viral proteins such as D8L protein, A27L protein, L1R protein etc. To identify possible regions on the viral protein that interact with neutralizing antibodies, peptide arrays encompassing the full-length viral protein was synthesized and screened for peptides that bound the anti-VV neutralizing antibodies. Peptides thus identified were further examined to elucidate the neutralizing antibody epitopes. In one embodiment, variants of the peptides identified by the peptide array were synthesized with alanine substitutions, and the neutralizing antibody epitopes were mapped using a series of ELISA binding assays. Once the neutralizing antibody epitopes were identified, mutations that destroy these epitopes can be introduced into the VV genome by genetic engineering.

[0059] The present invention discloses a number of neutralizing antibody epitopes on each of the vaccinia virus H3L protein, D8L protein, A27L protein, and L1R protein. Mutating or substituting amino acid(s) at these neutralizing antibody epitopes would confer viral escape from the neutralizing antibodies. Similarly, deleting amino acid(s) at these neutralizing antibody epitopes is also expected to confer viral escape from the neutralizing antibodies. Hence, it is expected that deletion of one or more amino acids within the H3L, D28L, A27L, L1R viral protein, or deletion of the whole H3L, D28L, A27L, or L1R viral protein could also confer escape from neutralizing antibody binding. H3L deletion mutant variants have been reported, indicating the feasibility of generating one or more amino acid deletion or whole

protein deletion virus mutants, even though the H3L deletion impaired the virus mutant's infectivity and replication capability.

[0060] In one embodiment, the present invention provides an isolated infectious recombinant vaccinia virus (VV) virion, comprising a heterologous nucleic acid and one or more of:

a) a variant vaccinia virus (VV) H3L protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:1;

b) a variant vaccinia virus (VV) D8L protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:2;

c) a variant vaccinia virus (VV) A27L protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:3;

d) a variant vaccinia virus (VV) L1R protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:4;

e) a variant vaccinia virus (VV) H3L protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:5;

f) a variant vaccinia virus (VV) D8L protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:6 or SEQ ID NO:174;

g) a variant vaccinia virus (VV) H3L protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:170; and

h) a variant vaccinia virus (VV) D8L protein that comprises an amino acid sequence having at least about 60%, 70%, 80%, 90%, or 95% amino acid sequence identity to SEQ ID NO:172.

[0061] In one embodiment, the above variant VV H3L protein comprises amino acid substitution or deletion at one or more of the following amino acid residues: 14, 15, 16, 33, 34, 35, 38, 40, 44, 45, 52, 131, 134, 135, 136, 137, 154, 155, 156, 161, 166, 167, 168, 198,

227, 250, 253, 254, 255, and 256 of SEQ ID NO:1. Any suitable amino acids can be used in the substitutions. For example, variant peptides can be synthesized with substitutions.

[0062] In one embodiment, the above variant VV D8L protein comprises amino acid substitution or deletion at one or more of the following amino acid residues: 44, 48, 98, 108, 117, and 220 of SEQ ID NO:2. Any suitable amino acids can be used in the substitutions. For example, variant peptides can be synthesized with substitutions.

[0063] In one embodiment, the above variant VV A27L protein comprises amino acid substitution or deletion at one or more of the following amino acid residues: 27, 30, 32, 33, 34, 35, 36, 37, 39, 40, 107, 108, and 109 of SEQ ID NO:3. Any suitable amino acids can be used in the substitutions. For example, variant peptides can be synthesized with substitutions.

[0064] In one embodiment, the above variant VV L1R protein comprises amino acid substitution or deletion at one or more of the following amino acid residues: 25, 27, 31, 32, 33, 35, 58, 60, 62, 125, and 127 of SEQ ID NO:4. Any suitable amino acids can be used in the substitutions. For example, variant peptides can be synthesized with substitutions.

[0065] In one embodiment, the above variant VV H3L protein comprises amino acid substitution or deletion at one or more of the following amino acid residues: 14, 15, 16, 33, 34, 35, 38, 40, 44, 45, 52, 131, 132, 134, 135, 136, 137, 154, 155, 156, 161, 166, 167, 168, 195, 198, 199, 227, 250, 251, 252, 253, 254, 255, 256, 258, 262, 264, 266, 268, 272, 273, 275, and 277 of SEQ ID NO:170. Any suitable amino acids can be used in the substitutions.

[0066] In one embodiment, the above variant VV D8L protein comprises amino acid substitution or deletion at one or more of the following amino acid residues: 43, 44, 48, 53, 54, 55, 98, 108, 109, 144, 168, 177, 196, 199, 203, 207, 212, 218, 220, 222, and 227 of SEQ ID NO:172. Any suitable amino acids can be used in the substitutions.

Overcoming Complement-Mediated Virus Neutralization

[0067] Complement is a key component of the innate immune system, targeting the virus for neutralization and clearance from the circulatory system. Complement could enhance neutralization antibody's neutralizing efficacy, and antibody-mediated protective immunity induced by smallpox vaccination was largely decreased in vitro in the absence of

complement, indicating the critical role of complement in the neutralization of vaccinia virus. Complement activation results in cleavage and activation of C3 and deposition of opsonic C3 fragments on surfaces. Subsequent cleavage of C5 leads to assembly of the membrane attack complex (C5b, 6, 7, 8, 9), which disrupts lipid bilayers.

[0068] Complement activation can be negatively regulated by several membrane regulator of complement activation (RCA). RCAs downregulate complement activation at different steps. First, CD35 (complement receptor 1) and CD55 (decay-accelerating factor) inhibit the formation and accelerate the decay of C3 convertases (C3-activating enzymes). Second, CD35 and CD46 (membrane cofactor protein) catabolizes C4b and C3b, inhibiting formation of the C3 convertases C4b2a and C3bBb. Third, CD59 prevents the formation of the membrane attack complex. Studies have shown that extracellular enveloped vaccinia virus (EEV) is resistant to complement because of incorporation of host RCA into its envelope. However, it is not known whether CD55 and/or other RCAs can be successfully expressed on the surface of the IMV of VV with the ability of overcoming complement-mediated neutralization, without affecting viral packaging and replication.

[0069] In one embodiment, the present invention provides recombinant vaccinia virus (VV) virions comprising a heterologous nucleic acid encoding a complement activation modulator such as CD55, CD59, CD46, CD35, factor H, C4-binding protein, or other identified complement activation modulators, and uses thereof. Expressing the complement activation modulators results in recombinant vaccinia viruses having the ability to modulate complement activation and reduce complement-mediated virus neutralization as compared to the wild-type virus. In one embodiment, the heterologous nucleic acid carried by the above recombinant vaccinia virus (VV) virion encodes a domain of human CD55, CD59, CD46, CD35, factor H, C4-binding protein, or other identified complement activation modulators. In another embodiment, the heterologous nucleic acid encodes a CD55 protein that comprises an amino acid sequence having the sequence of SEQ ID NO:7. In view of the disclosure presented herein, one of ordinary skill in the art would readily employ other complement activation modulators (e.g. CD59, CD46, CD35, factor H, C4-binding protein etc) in the recombinant vaccinia virus presented herein.

Incorporating Bi-Specific Antibodies To Boost Virus Therapy

[0070] Oncolytic virus can be armed to express bi-specific antibodies that bind to a first antigen on immune cells and a second antigen on tumor cells. Examples of the first antigen on immune cells include, but are not limited to, CD3, CD4, CD5, CD8, CD16, CD28, CD40, CD64, CD89, CD134, CD137, NKp46, and NKG2D, and the like. Examples of the second antigen on tumor cells include, but are not limited to, EphA2, HER2, GD2, Glypican-3, 5T4, 8H9, avb6 integrin, B7-H3, B7-H6, BCMA, CADC, CA9, CD19, CD20, CD22, kappa light chain, CD30, CD33, CD38, CD44, CD44v6, CD44v7 /8, CD70, CD123, CD138, CD171, CEA, CSPG4, EGFR, EGFRv111, EGP2, EGP40, EPCAM, ERBB3, ERBB4, ErbB3/4, FAP, FAR, FBP, fetal AchR, Folate Receptor a, GD2, GD3, HLA-AI MAGE Al, HLA-A2, IL11Ra, IL13Ra2, KDR, Lambda, Lewis-Y, MCSP, Mesothelin, Mucl, Muc16, NCAM, NKG2D ligands, NY-ESO-1, PRAME, PSCA, PSCl, PSMA, RORI, SURVIVIN, TAG72, TEMl, TEM8, VEGRR2, carcinoembryonic antigen, HMW-MAA, VEGF receptors, and other exemplary antigens that are present within the extracellular matrix of tumors, such as oncofetal variants of fibronectin, tenascin, or necrotic regions of tumors.

Targeting B-Cell Maturation Antigen (BCMA) To Treat Multiple Myeloma

[0071] Multiple myeloma (MM) is a malignancy of clonal plasma cells derived from the B-lymphocyte lineage that is part of a spectrum of diseases ranging from monoclonal gammopathy of undetermined significance (MGUS) to plasma cell leukemia. It is the second most common hematological cancer in the United States with an estimated 32,110 newly diagnosed cases and 12,960 deaths in 2019. MM currently accounts for 10% of hematological malignancies and 2.1% of all cancer-related deaths. Currently several treatments for MM are available, however no curative therapies have been defined and most patients will eventually relapse with a median survival of 3-5 years, regardless of treatment regimen or initial responses to treatment. Therapeutics with new mechanisms of action are therefore urgently needed to treat drug-resistant MM.

[0072] Oncolytic vaccinia virus (VV) emerged as a promising new class of agents with great potential for the treatment of MM. Live VV has been administered by WHO to over 200 million people to eradicate smallpox, giving VV an excellent history of safety in humans. While wild type VV has no tumor selectivity, double deletion of viral genes that are essential for viral replication in normal cells, such as thymidine kinase (TK) and vaccinia growth factor (VGF), have conferred a strict VV tumor specificity. Recent clinical trials of VV against solid tumors are reporting promising results. In vitro studies utilizing a strain double

deleted for TK and VGF showed that MM cell lines are susceptible to killing by VV. In those studies, viral replication was observed in primary MM cells, but not in normal peripheral blood mononuclear cells (PBMCs). The double deleted strain also reduced tumor volume and increased survival in a mouse xenograft model of MM. In addition, recently a TK-deleted VV strain that overexpresses two anti-tumor factors, miR-34a and Smac (frequently dysregulated in MM) showed increased efficacy against MM both in vitro and in vivo when compared to treatment with the parental virus, VV-miR-34a, or VV-Smac individually. However, the efficacy of VV therapy in current clinical studies is not optimized, indicating the need of further improvement of VV therapy.

[0073] VV can express T-cell engager targeting or co-targeting MM antigens, such as BCMA, CD19, CD26, CD38, CD44v6, CD56, CD138, CS1, EGFR, integrin beta7, KIRs, LIGHT/TNFSF14, NKG2D, PD-1/PD-L1, SLAMF7, TACI, and TGIT. B-cell maturation antigen (BCMA), a transmembrane glycoprotein in the tumor necrosis factor receptor superfamily 17 (TNFRSF17), is a promising target for MM therapy because it is expressed at significantly higher levels in all patient MM cells but not in normal tissues, except in plasma cells (PC). In recent clinical studies BCMA-targeted chimeric antigen receptor (CAR) T-cells showed significant clinical activities in patients with relapsed and refractory multiple myeloma (RRMM) who have undergone at least three prior treatments, including a proteasome inhibitor and an immunomodulatory agent. Anti-BCMA Ab–drug conjugate (ADC) also has achieved significant clinical responses in patients who failed at least three prior lines of therapy. Both BCMA-targeted CAR-T and ADC were granted breakthrough status for patients with RRMM by FDA in Nov 2017. As promising as these two therapies are there are several complicating factors for targeting BCMA. First, anti-BCMA treatment will potentially reduce the number of long-lived PCs and, since long-lived PCs play a critical role in maintaining humoral immunity, the impact of anti-BCMA therapy on immune function needs to be carefully and serially evaluated. Second, high serum levels of sBCMA, cleaved from BCMA by γ-secretase have been detected in MM patients, especially in the setting of progressive disease. Thus, it is necessary to develop a therapeutic strategy to deliver the BCMA-targeted treatment directly to BCMA+ MM cells.

[0074] As described herein, the present invention provides recombinant vaccinia virus (VV), BCMA-TEA-NEV, that overcomes the limitations discussed above because the BCMA-CD3 BiTE expression will be limited within the MM surrounding area while escaping the BCMA+

PCs and sBCMA. TEA-NEV encodes bi-specific scFvs that directs T cells to recognize and kill tumor cells that are not infected with VV (by-stander killing), resulting in enhanced tumor lysis. In addition, the CD3-scFv promotes T-cell infiltration into tumors and their activation, and the cytokines they release upon activation create a pro-inflammatory micro-environment that inhibites tumor growth. In addition, the TEA-NEV induces local production of T-cell engager that allows for higher concentrations of T cells at the target site while reducing systemic side effects. Thus, arming oncolytic VV with bi-specific scFvs is important to engage T cells for cancer therapy and produce the desired increase in anti-tumor activity of current VV by inducing by-stander killing.

[0075] In one embodiment, the heterologous nucleic acid carried by the above recombinant vaccinia virus (VV) virion encodes a bi-specific polypeptide that binds to a first antigen on immune cells and a second antigen, B-cell maturation antigen (BCMA), on multiple myeloma (MM). For example, the bi-specific polypeptide is a bi-specific scFvs, the first antigen is human CD3e, the second antigen is human BCMA (B-cell maturation antigen), and the bi-specific scFvs comprises an amino acid sequence of SEQ ID NO:9.

[0076] In another embodiment, VV can express T-cell engager targeting or co-targeting other MM antigens, such as CD19, CD38, SLAMF7, CD26, LIGHT/TNFSF14, integrin beta7, CD138, KIRs, EGFR, PD-1/PD-L1, TGIT, CD56, CS1, NKG2D, TACI, and CD44v6.

[0077] In another embodiment, the bi-specific polypeptide is a bi-specific scFvs, the first antigen is human CD3e and the second antigen is human FAP (fibroblast activation protein) that is overexpressed on most epithelial cancers. In one embodiment, the bi-specific FAP-CD3 scFv comprises the amino acid sequence of SEQ ID NO:8.

Incorporating Immune Checkpoint Molecules To Boost Virus Therapy

[0078] Increasing evidence has shown that T-cell immunotherapy has the ability to control tumor growth and prolong survival in cancer patients. However, tumor-specific T-cell responses are hard to achieve and sustain, likely due to the limitations of various immune escape mechanisms of tumor cells. Immune checkpoint molecules are proteins expressed on certain immune cells that need to be activated or inhibited to start an immune response, for example, to attack abnormal cells such as tumor cells in the body. The “immune escape” may include several activities by the tumor cells, such as down-regulation of co-stimulatory

molecule expression, such as stimulatory immune checkpoint molecules, and up-regulation of inhibitory molecule expression, such as inhibitory immune checkpoint molecules. Blockade of these inhibitory immune checkpoint molecules have shown very promising results in preclinical and clinical tests in cancer treatment. However, there are some unwanted side effects in some cases. For example, blocking these inhibitory immune checkpoint molecules (receptors or ligands) may lead to a disruption in immune homeostasis and self-tolerance, resulting in autoimmune/auto-inflammatory side effects.

[0079] Immune checkpoint molecules are well-known in the art. For example, the PD-1 (programmed cell death-1) receptor is expressed on the surface of activated T cells. Its ligands, PD-L1 and PD-L2, are commonly expressed on the surface of dendritic cells or tumor cells. PD-1 and PD-L1/PD-L2 belong to the family of inhibitory immune checkpoint proteins that can halt or limit the development of T cell response. PD-L1 expressed on the tumor cells could bind to PD-1 receptors on the activated T cells, which leads to inhibition of cytotoxic T cells. Hence, anti-tumor immune responses would be enhanced by blocking the interaction between PD-1 and its ligands.

[0080] In one embodiment, the present invention provides recombinant vaccinia virus (VV) virions that would block the inhibitory PD-1 pathway. In one embodiment, the present invention provides recombinant vaccinia virus (VV) virions comprising a heterologous nucleic acid encoding an extracellular domain of PD-1 fused to the constant (Fc) domain of immunoglobin-G1 (IgG1). In one embodiment, the PD-1 fusion protein (PD-1-ED-hIgG1-Fc) comprises the amino acid sequence of SEQ ID NO:10. In view of the disclosure presented herein, other immune checkpoint molecules can be readily incorporated into the recombinant vaccinia virus presented herein. The recombinant vaccinia viruses disclosed herein may comprise immune checkpoint molecules including, but not limited to, PD-1, PD-L1, PD-L2, CD47, CXCR4, CSF1R, LAG-3, TIM-3, HHLA2, BTLA, CTLA-4, TIGIT, VISTA, B7-H4, CD160, 2B4, and CD73.

[0081] In one embodiment, the present invention provides an isolated infectious recombinant vaccinia virus (VV) virion, the virion comprises a heterologous nucleic acid and one or more of:

a) a variant vaccinia virus (VV) H3L protein having at least about 60% amino acid sequence identity to SEQ ID NO:1;

b) a variant vaccinia virus (VV) D8L protein having at least about 60% amino acid sequence identity to SEQ ID NO:2;
c) a variant vaccinia virus (VV) A27L protein having at least about 60% amino acid sequence identity to SEQ ID NO:3;
d) a variant vaccinia virus (VV) L1R protein having at least about 60% amino acid sequence identity to SEQ ID NO:4;
e) a variant vaccinia virus (VV) H3L protein having at least about 60% amino acid sequence identity to SEQ ID NO:5;
f) a variant vaccinia virus (VV) D8L protein having at least about 60% amino acid sequence identity to SEQ ID NO:6 or SEQ ID NO:174;
g) a variant vaccinia virus (VV) H3L protein having at least about 60% amino acid sequence identity to SEQ ID NO:170; and
h) a variant vaccinia virus (VV) D8L protein having at least about 60% amino acid sequence identity to SEQ ID NO:172.

[0082] In one embodiment, the variant VV H3L protein comprises amino acid substitution or deletion at one or more of the following amino acid residues: 14, 15, 16, 33, 34, 35, 38, 40, 44, 45, 52, 131, 134, 135, 136, 137, 154, 155, 156, 161, 166, 167, 168, 198, 227, 250, 253, 254, 255, and 256 of SEQ ID NO:1.

[0083] In one embodiment, the variant VV D8L protein comprises amino acid substitution or deletion at one or more of the following amino acid residues: 44, 48, 98, 108, 117, and 220 of SEQ ID NO:2.

[0084] In one embodiment, the variant VV A27L protein comprises amino acid substitution or deletion at one or more of the following amino acid residues: 27, 30, 32, 33, 34, 35, 36, 37, 39, 40, 107, 108, and 109 of SEQ ID NO:3.

[0085] In one embodiment, the variant VV L1R protein comprises amino acid substitution or deletion at one or more of the following amino acid residues: 25, 27, 31, 32, 33, 35, 58, 60, 62, 125, and 127 of SEQ ID NO:4.

[0086] In one embodiment, the variant VV H3L protein comprises amino acid substitution or deletion at one or more of the following amino acid residues: 14, 15, 16, 33, 34, 35, 38, 40,

44, 45, 52, 131, 132, 134, 135, 136, 137, 154, 155, 156, 161, 166, 167, 168, 195, 198, 199, 227, 250, 251, 252, 253, 254, 255, 256, 258, 262, 264, 266, 268, 272, 273, 275, and 277 of SEQ ID NO:170.

[0087] In one embodiment, the variant VV D8L protein comprises amino acid substitution or deletion at one or more of the following amino acid residues: 43, 44, 48, 53, 54, 55, 98, 108, 109, 144, 168, 177, 196, 199, 203, 207, 212, 218, 220, 222, and 227 of SEQ ID NO:172.

[0088] In one embodiment, the heterologous nucleic acid carried by the recombinant VV encodes a domain of a regulator of complement activation. Examples of regulator of complement activation include, but are not limited to, CD55, CD59, CD46, CD35, factor H, and C4 -binding protein. In one embodiment, the heterologous nucleic acid encodes a CD55 polypeptide comprising the amino acid sequence of SEQ ID NO:7.

[0089] In another embodiment, the heterologous nucleic acid carried by the recombinant VV encodes a bi-specific polypeptide that binds to a first antigen on immune cells and a second antigen on tumor cells. In one embodiment, the first antigen on immune cells can be CD3, CD4, CD5, CD8, CD16, CD28, CD40, CD64, CD89, CD134, CD137, NKp46, or NKG2D. In one embodiment, the second antigen on tumor cells can be fibroblast activation protein (FAP), or tumor antigens on multiple myeloma.

[0090] In one embodiment, the bi-specific polypeptide is a bi-specific scFvs, the first antigen is human CD3e and the second antigen is human FAP. For example, this bi-specific polypeptide has the amino acid sequence of SEQ ID NO:8.

[0091] In another embodiment, the bi-specific polypeptide can target tumor antigens on multiple myeloma, e.g. B-cell maturation antigen (BCMA), CD19, CD38, SLAMF7, CD26, LIGHT/TNFSF14, integrin beta7, CD138, KIRs, EGFR, PD-1/PD-L1, TGIT, CD56, CS1, NKG2D, TACI, or CD44v6. In one embodiment, the bi-specific polypeptide is a bi-specific scFvs, the first antigen is human CD3e and the second antigen is human BCMA. For example, this bi-specific polypeptide has the amino acid sequence of SEQ ID NO:9.

[0092] In another embodiment, the heterologous nucleic acid carried by the recombinant VV encodes a fusion polypeptide comprising an immune checkpoint molecule. Examples of

immune checkpoint molecule include, but are not limited to, PD-1, PD-L1, PD-L2, CD47, CXCR4, CSF1R, LAG-3, TIM-3, HHLA2, BTLA, CTLA-4, TIGIT, VISTA, B7-H4, CD160, 2B4, and CD73. In one embodiment, the heterologous nucleic acid carried by the recombinant VV encodes a fusion polypeptide comprising human PD-1 extracellular domain and a human IgG1 Fc domain, e.g., this fusion polypeptide has the amino acid sequence of SEQ ID NO:10.

[0093] In one embodiment, the recombinant vaccinia virus (VV) virion disclosed herein exhibits resistance to neutralizing antibodies compared to the resistance exhibited by wild type VV. In another embodiment, the recombinant vaccinia virus (VV) virion disclosed herein exhibits increased transduction of mammalian cells in the presence of anti-VV neutralizing antibodies compared to transduction of mammalian cells by wild type VV.

[0094] In another embodiment, there is provided a method of delivering a gene product to a subject (human or animal) in need thereof. The method includes administering to the subject an effective amount of the recombinant vaccinia virus (VV) virion disclosed herein, wherein the gene product is encoded by the heterologous nucleic acid carried by the recombinant VV virion.

[0095] In another embodiment, there is provided a pharmaceutical composition comprising the recombinant vaccinia virus (VV) virions disclosed herein and a pharmaceutically acceptable carrier. In another embodiment, there is provided a method of using such pharmaceutical compositions to treat cancer in a subject. In one embodiment, the pharmaceutical compositions can be administered to the subject intravenously, or through injection, inhalant, infusion, implantation, parenteral administration, enteral administration (e.g. through the gastrointestinal tract), or other systemic administration approach generally known in the art. In one embodiment, the subject is a human. Alternatively, the present invention may also be used in administration to and treatment of animal subjects.

[0096] In another embodiment, there is provided a library comprising one or more variant vaccinia virus (VV) virions, each of the variant VV virions comprises one or more variant VV protein. The variant VV protein comprises an amino acid sequence having at least one amino acid substitution or deletion relative to the amino acid sequence of a corresponding wild type VV protein. In one embodiment, the variant VV protein can be variant H3L

protein, variant D8L protein, variant L1R protein, and/or variant A27L protein. In another embodiment, the variant VV protein comprises an amino acid sequence having at least one amino acid substitution or deletion relative to the amino acid sequence set forth in one of SEQ ID NOs:5, 6 or 174.

[0097] In another embodiment, there are provided variant vaccinia virus (VV) virions derived from the above library, the virions comprises a heterologous nucleic acid and one or more variant VV proteins, wherein at least one of the variant VV proteins comprises an amino acid sequence having at least one amino acid substitution or deletion relative to the amino acid sequence of a corresponding wild type VV protein. In one embodiment, the heterologous nucleic acid carried by such variant VV virions encodes a domain of a regulator of complement activation such as CD55, CD59, CD46, CD35, factor H, or C4-binding protein. For example, the heterologous nucleic acid encodes a CD55 protein that comprises the amino acid sequence of SEQ ID NO:7. In another embodiment, the heterologous nucleic acid encodes a bi-specific polypeptide that binds to a first antigen on immune cells and a second antigen on tumor cells. Examples of such first antigen and second antigen have been discussed above. In one embodiment, the bi-specific polypeptide is a bi-specific scFvs, the first antigen is human CD3e and the second antigen is human FAP, e.g. this bi-specific scFvs comprises the amino acid sequence of SEQ ID NO:8. In another embodiment, the bi-specific polypeptide is a bi-specific scFvs, the first antigen is human CD3e and the second antigen is human BCMA, e.g. this bi-specific scFvs comprises the amino acid sequence of SEQ ID NO:9. In yet another embodiment, the heterologous nucleic acid encodes a fusion polypeptide comprising an immune checkpoint molecule as discussed above. In one embodiment, the fusion polypeptide comprises human PD-1 extracellular domain and a human IgG1 Fc domain, the fusion polypeptide having the amino acid sequence of SEQ ID NO:10.

[0098] In one embodiment, the variant VV virions derived from the above library exhibit resistance to neutralizing antibodies compared to the resistance exhibited by wild type VV. In another embodiment, these variant VV virions exhibit increased transduction of mammalian cells in the presence of anti-VV neutralizing antibodies compared to transduction of mammalian cells by wild type VV.

[0099] In another embodiment, there is provided a method of using an effective amount of recombinant vaccinia virus (VV) virions derived from the above library to deliver a gene

product to a subject (human or animal) in need thereof, wherein the gene product is encoded by a nucleic acid carried by those variant VV virions.

[0100] In another embodiment, there is provided a pharmaceutical composition comprising variant vaccinia virus (VV) virions derived from the above library and a pharmaceutically acceptable carrier. In another embodiment, there is provided a method of using such pharmaceutical composition to treat cancer in a subject. In one embodiment, the pharmaceutical composition can be administered to the subject intravenously, or through injection, inhalant, infusion, implantation, parenteral administration, enteral administration (e.g. through the gastrointestinal tract), or other systemic administration approach generally known in the art. In one embodiment, the subject is a human, but the technology may also be used in administration to and treatment of animal subjects.

[0101] In another embodiment, there is provided a recombinant vaccinia virus (VV) H3L protein that has at least about 60% amino acid sequence identity to one of SEQ ID NOs:1, 5 or 170. In another embodiment, there is provided a recombinant vaccinia virus D8L protein that has at least about 60% amino acid sequence identity to one of SEQ ID NOs:2, 6, 172 or 174. These recombinant H3L or D8L proteins could confer viral resistance to anti-VV neutralizing antibodies.

[0102] The invention being generally described, will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

EXAMPLE 1

Materials And Methods

Materials

[0103] pUC57-Amp A27L, pUC57-Amp L1R, pUC57-Amp D8L, pUC57-Amp H3L, (GENEWIZ). CV-1 cells (ATCC, cat. # CCL-70). vSC20 Vaccinia virus stock. GeneJuice Transfection Reagent (Millipore, cat. # 2703870). DMEM media (GE Helathcare, cat. # SH30081.01), FBS (GE Healthcare, cat. # SH30070.03), DPBS (Sigma, cat. # 8537). Dry ice/ethanol bath, 6-well tissue culture plates, 12 × 75–mm polystyrene tubes, disposable scraper or plunger from a 1 ml syringe, sterile 2-ml sterile microcentrifuge tubes.

Cell Preparation And Infection With Wild-Type Vaccinia Virus

[0104] CV-1 cells (2×10^5/well) were seeded in wells of a 6-well tissue culture plate in complete DMEM medium and incubate to 50–80% confluency (37 °C, 5% CO_2 overnight). An aliquot of parental virus was thawed and sonicated (30 sec) in ice-water several times to remove the clumps (cool on ice between each sonication). Virus was diluted in complete DMEM to 0.5×10^5 pfu/ml. Medium was remove from confluent monolayer of cells and cells were infected with 0.5 ml diluted vaccinia virus (0.05 pfu/cell) and incubated 2 hrs at 37°C.

Transfection With pUC57-Amp Plasmid

[0105] For each well to be transfected, 100 µl serum-free medium was added into a sterile tube. Three µl GeneJuice was then added drop-wise directly to the serum-free medium and mixed thoroughly by vortexing and incubate at room temperature for 5 min. One µg of DNA was added to each tube and mixed by gentle pipetting (do not vortex) followed by incubation at room temperature for 5–15 min. Virus inoculum was then removed from monolayer of cells and washed twice with PBS. 0.5 mL of fresh complete DMEM medium was then added to the cells. The entire volume of GeneJuice/DNA mixture was then added drop-wise to cells in complete DMEM medium. The dish was gently rocked to ensure even distribution. Transfection mixture was removed after 4–8 hrs incubation and replaced with complete DMEM medium followed by incubation for 24–72 hrs at 37°C (5% CO2). After 24-72 hours, the cells were dislodged from the wells and transferred to a 2-ml sterile microcentrifuge tube. The cell suspension was then lysed by performing three freeze-thaw cycles, each time by freezing in a dry ice/ethanol bath, thawing in a 37°C water bath, and vortexing. The cell lysate was stored at −80°C until needed

Screening of Recombinant Virus Plaques

[0106] CV1 cells (5×10^5/well) were seeded in a 6-well tissue culture plate in complete DMEM medium (2mL/well) and incubate to >90% confluency (37 °C, 5% CO_2, 24 hrs). One hundred, 10, 1, or 0.1 µl of lysate were added to duplicate wells containing 1 ml complete DMEM medium and incubate 2 hrs at 37°C. The virus inoculum was then removed from the infected cells. 2 ml of complete DMEM medium containing 2.5% methylcellulose was added to each well with and incubated 2 days. Two days later, well-separated plaques were picked up by scraping and suction with a pipet tip. Fluorescent microscope was used to select GFP+ plaques that was transferred to a tube containing 0.5 ml complete DMEM medium. Each

virus-containing tube was vortexed followed by three freeze-thaw cycles, each time by freezing in a dry ice/ethanol bath, thawing in a 37°C water bath, and vortexing.

Several Rounds of GFP+ Plaque Purification

[0107] Wells of a 6-well tissue culture plate were seeded with 5×10^5 CV1 cells/well in complete DMEM medium (2mL/well). The cells were incubated to >90% confluency (37 ºC, 5% CO_2, 24 hrs). One 6-well plate is needed for each plaque isolate. One hundred, 10, 1, or 0.1 µl of lysate from each plaque were added to duplicate wells containing 1 ml complete DMEM medium, and incubated for 2 hrs. Medium was removed from the cell monolayers and overlay with complete DMEM containing 2.5% methylcellulose. The above steps were repeated for three or more rounds of plaque purification to ensure a clonally pure recombinant virus.

Single Plaque Purification Protocol

[0108] About 3-4 millions CV-1 cells were seeded and grown to 100% confluence in 24 well plate. The concentrated virus stock was diluted in 10-fold series dilutions with DMEM infection medium and added to each well. After 36-72 hour incubation, the wells that contain single plaque was marked and kept in the incubator until the whole well got infected, which takes about 4-5 days after initial infection. The infected cells were harvested and the recombination was confirmed by PCR assay. PCR conditions are listed below for each reaction.

TABLE 1

PCR setup	(µL)
Nuclease-free water	12
10XPCR Buffer	2
50mM MgCl	0.6
10mM dNTP Mix	0.4
Forward primer (5µM)	2
Reverse primer (5µM)	2
AccuStart Taq DNA polymerase	0.08
Template	1
Total	20.08

TABLE 2

Step	Temperature	Time	Note

1	94°C	1min	
2	94°C	20s	
3	60°C	30s	
4	72°C	30s	Go to step 2 for 34 cycles
5	4°C	Hold	

EXAMPLE 2

Neutralizing Antibody (Nab) Epitope Determination on H3L – Peptide Arrays Sequence Analysis

[0109] To identify possible regions on H3L that participate in the NAb interaction, peptide arrays encompassing full-length H3L were synthesized and screened for peptides that bound the anti-VV NAb. The array started at the N terminus of H3L and spanned the entire length of the protein sequence, with each successive spot containing 12 amino acids along the sequence shifted by 4 amino acids toward the C terminus, i.e., each spot in the array had an 8-residue overlap with the previous spot. Cellulose membrane containing synthesized H3L peptide array was then screened to identify peptides that bound to anti-VV polyclonal NAb (Abcam, ab35219). Briefly, the membrane was washed three times for 5 min in Millipore H_2O and blocked overnight at 4°C with 5% (wt/vol) milk–PBS (MPBS). Four μg/mL NAb was incubated with the membrane in MPBS for 3 h at room temperature with gentle agitation. After incubation, membrane was washed six times for 5 min with 20 mL PBS supplemented with 1% Tween 20 (PBST). The peptide-bound NAb was detected by incubating the membrane with 2 μg/ml of rabbit horseradish peroxidase (HRP)–conjugated secondary Ab (Abcam, ab6721) in MPBS for 4 h at 4°C with gentle agitation. The membrane was then washed three times for 5 min with PBST, incubated in 5 ml of the enhanced chemiluminescence (ECL) developing solution (Thermo Fisher, #32109). Peptides that are positive for binding appear as spots on the membranes (Fig. 1B). The signal was visualized, and the intensity of each spot was measured by a CCD camera (GE Healthcare, Amersham™ Imager 600). No oversaturation of the spots was detected and after integrating, the intensities of the spots were plotted (Fig. 1C). A signal of ≤ 110000 was considered background (determined by analysis of the membrane) and the spots showing a signal higher than 1100000 were considered to represent positive binding. Twenty six spots showed binding to ab35219 with higher than the cutoff intensity. To take into consideration that some positive signals could represent nonspecific binding, only those residues that were present in at least two spots that showed a binding intensity ≥1100000 were considered significant. In total 9

peptide sequences were identified positive for Ab binding (sequences appeared in multiple spots with positive binding signal, sequences with underlines shown below).

TABLE 3

Sequences of The Spots (And Their Corresponding Positions) Synthesized Onto A Peptide Array

1 MAAAKTPVIVVP (SEQ ID NO:20)	37 DKKIDILQMREI (SEQ ID NO:56)
2 KTPVIVVPVIDR (SEQ ID NO:21)	38 DILQMREIITGN (SEQ ID NO:57)
3 IVVPVIDRLPSE (SEQ ID NO:22)	39 MREIITGNKVKT (SEQ ID NO:58)
4 VIDRLPSETFPN (SEQ ID NO:23)	40 ITGNKVKTELVM (SEQ ID NO:59)
5 LPSETFPNVHEH (SEQ ID NO:24)	41 KVKTELVMDKNH (SEQ ID NO:60)
6 TFPNVHEHINDQ (SEQ ID NO:25)	42 ELVMDKNHAIFT (SEQ ID NO:61)
7 VHEHINDQKFDD (SEQ ID NO:26)	43 DKNHAIFTYTGG (SEQ ID NO:62)
8 INDQKFDDVKDN (SEQ ID NO:27)	44 AIFTYTGGYDVS (SEQ ID NO:63)
9 KFDDVKDNEVMP (SEQ ID NO:28)	45 YTGGYDVSLSAY (SEQ ID NO:64)
10 VKDNEVMPEKRN (SEQ ID NO:29)	46 YDVSLSAYIIRV (SEQ ID NO:65)
11 EVMPEKRNVVVV (SEQ ID NO:30)	47 LSAYIIRVTTEL (SEQ ID NO:66)
12 EKRNVVVVKDDP (SEQ ID NO:31)	48 IIRVTTELNIVD (SEQ ID NO:67)
13 VVVVKDDPDHYK (SEQ ID NO:32)	49 TTELNIVDEIIK (SEQ ID NO:68)
14 KDDPDHYKDYAF (SEQ ID NO:33)	50 NIVDEIIKSGGL (SEQ ID NO:69)
15 DHYKDYAFIQWT (SEQ ID NO:34)	51 EIIKSGGLSSGF (SEQ ID NO:70)
16 DYAFIQWTGGNI (SEQ ID NO:35)	52 SGGLSSGFYFEI (SEQ ID NO:71)
17 IQWTGGNIRNDD (SEQ ID NO:36)	53 SSGFYFEIARIE (SEQ ID NO:72)
18 GGNIRNDDKYTH (SEQ ID NO:37)	54 YFEIARIENEMK (SEQ ID NO:73)
19 RNDDKYTHFFSG (SEQ ID NO:38)	55 ARIENEMKINRQ (SEQ ID NO:74)
20 KYTHFFSGFCNT (SEQ ID NO:39)	56 NEMKINRQILDN (SEQ ID NO:75)
21 FFSGFCNTMCTE (SEQ ID NO:40)	57 INRQILDNAAKY (SEQ ID NO:76)
22 FCNTMCTEETKR (SEQ ID NO:41)	58 ILDNAAKYVEHD (SEQ ID NO:77)
23 MCTEETKRNIAR (SEQ ID NO:42)	59 AAKYVEHDPRLV (SEQ ID NO:78)
24 ETKRNIARHLAL (SEQ ID NO:43)	60 VEHDPRLVAEHR (SEQ ID NO:79)
25 NIARHLALWDSN (SEQ ID NO:44)	61 PRLVAEHRFENM (SEQ ID NO:80)
26 HLALWDSNFFTE (SEQ ID NO:45)	62 AEHRFENMKPNF (SEQ ID NO:81)
27 WDSNFFTELENK (SEQ ID NO:46)	63 FENMKPNFWSRI (SEQ ID NO:82)
28 FFTELENKKVEY (SEQ ID NO:47)	64 KPNFWSRIGTAA (SEQ ID NO:83)
29 LENKKVEYVVIV (SEQ ID NO:48)	65 WSRIGTAATKRY (SEQ ID NO:84)
30 KVEYVVIVENDN (SEQ ID NO:49)	66 GTAATKRYPGVM (SEQ ID NO:85)
31 VVIVENDNVIED (SEQ ID NO:50)	67 TKRYPGVMYAFT (SEQ ID NO:86)
32 ENDNVIEDITFL (SEQ ID NO:51)	68 PGVMYAFTTPLI (SEQ ID NO:87)
33 VIEDITFLRPVL (SEQ ID NO:52)	69 YAFTTPLISFFG (SEQ ID NO:88)
34 ITFLRPVLKAMH (SEQ ID NO:53)	
35 RPVLKAMHDKKI (SEQ ID NO:54)	

36 KAMHDKKIDILQ (SEQ ID NO:55)	

Sequences of The H3L Peptides (With Corresponding Residue Numbers) Identified By Peptide Array

[0110] PVIDRLP (aa 11-18) (SEQ ID NO:89), NDQKFDDVKDN (aa 30-40) (SEQ ID NO:90), PERKNVVVV (aa 44-52) (SEQ ID NO:91), NVIEDITFLR (aa 128-137) (SEQ ID NO:92), QMREI (aa 152-156) (SEQ ID NO:93), KVKTELVM (aa 161-168) (SEQ ID NO:94), NIVDEIIK (aa 197-204) (SEQ ID NO:95), KINRQI (aa 224-229) (SEQ ID NO:96), FENMKPNF (aa 249-265) (SEQ ID NO:97).

[0111] Ab-binding sites localized to the N-terminal domain (aa 11 to 52), the central (aa 128 to 168) and the C-terminal portions (aa 198 to 256) of H3L. Interestingly, the most C-terminal domain of the protein (aa 260 to 324) showed no binding to the Ab. This hydrophobic region of the H3L inserts into VV membrane post-translationally and would not be available for Ab binding in the context of the mature viral particle. The N-terminal domain is most likely involved in the binding of H3L to surface of cells, thus binding of the Ab to this region would interfere with the ability of the virus to infect the cells, supporting our array result of this region being involved in Ab binding. Additionally, an earlier study showed that H3L is a glycosyltransferase. Some viruses encode their own glycosyltransferases to aid in host immune response evasion. H3L binds the UDP-Glc via the D/ExD motif in its central domain and mutating this motif (aa 125 and 127, specifically) inhibited the binding. The peptide array showed a likely Ab binding site near the D/ExD motif (peptide NVIEDITFLR, aa 128-137 (SEQ ID NO:92)). Binding of the Ab in this region would interfere with the glycosyltransferase activity of the H3L, another possible mechanism of virus neutralization by the Ab.

EXAMPLE 3

NAb Epitope Determination of H3L – Alanine Scan of The Identified Peptides

[0112] To further map the NAb epitopes and to elucidate the key residues on the H3L peptides identified by our peptide array study, a series of ELISAs were performed with the 9 identified peptides and their alanine-substituted variants (Fig. 2). Variants of the 9 peptides identified by peptide array were synthesized with alanine substitutions (GenScript USA Inc. NJ, USA).

TABLE 4

Total of 80 variant peptides were synthesized

Peptide 1	Peptide 2	Peptide 3	Peptide 4	Peptide 5
PVIDRLP (SEQ ID NO:89)	**NDQKFDDVKDN** (SEQ ID NO:90)	**PEKRNVVVV** (SEQ ID NO:91)	**NVIEDITFLR** (SEQ ID NO:92)	**QMREI** (SEQ ID NO:93)
AVIDRLP (SEQ ID NO:98)	ADQKFDDVKDN (SEQ ID NO:105)	AKRNVVVV (SEQ ID NO:116)	AVIEDITFLR (SEQ ID NO:124)	AMREI (SEQ ID NO:134)
PAIDRLP (SEQ ID NO:99)	NAQKFDDVKDN (SEQ ID NO:106)	EARNVVVV (SEQ ID NO:117)	NAIEDITFLR (SEQ ID NO:125)	QAREI (SEQ ID NO:135)
PVADRLP (SEQ ID NO:100)	NDAKFDDVKDN (SEQ ID NO:107)	EKANVVVV (SEQ ID NO:118)	NVAEDITFLR (SEQ ID NO:126)	QMAEI (SEQ ID NO:136)
PVIARLP (SEQ ID NO:101)	NDQAFDDVKDN (SEQ ID NO:108)	EKRAVVVV (SEQ ID NO:119)	NVIADITFLR (SEQ ID NO:127)	QMRAI (SEQ ID NO:137)
PVIDALP (SEQ ID NO:102)	NDQKADDVKDN (SEQ ID NO:109)	EKRNAVVV (SEQ ID NO:120)	NVIEAITFLR (SEQ ID NO:128)	QMREA (SEQ ID NO:138)
PVIDRAP (SEQ ID NO:103)	NDQKFADVKDN (SEQ ID NO:110)	EKRNVAVV (SEQ ID NO:121)	NVIEDATFLR (SEQ ID NO:129)	
PVIDRLA (SEQ ID NO:104)	NDQKFDAVKDN (SEQ ID NO:111)	EKRNVVAV (SEQ ID NO:122)	NVIEDIAFLR (SEQ ID NO:130)	
	NDQKFDDAKDN (SEQ ID NO:112)	EKRNVVVA (SEQ ID NO:123)	NVIEDITALR (SEQ ID NO:131)	
	NDQKFDDVADN (SEQ ID NO:113)		NVIEDITFAR (SEQ ID NO:132)	
	NDQKFDDVKAN (SEQ ID NO:114)		NVIEDITFLA (SEQ ID NO:133)	
	NDQKFDDVKDA (SEQ ID NO:115)			

Peptide 6	Peptide 7	Peptide 8	Peptide 9
KVKTELVM (SEQ ID NO:94)	**NIVDEIIK** (SEQ ID NO:95)	**KINRQI** (SEQ ID NO:96)	**FENMKPNF** (SEQ ID NO:97)
AVKTELVM (SEQ ID NO:139)	AIVDEIIK (SEQ ID NO:147)	AINRQI (SEQ ID NO:155)	AENMKPNF (SEQ ID NO:161)
KAKTELVM (SEQ ID NO:140)	NAVDEIIK (SEQ ID NO:148)	KANRQI (SEQ ID NO:156)	FANMKPNF (SEQ ID NO:162)
KVATELVM (SEQ ID NO:141)	NIADEIIK (SEQ ID NO:149)	KIARQI (SEQ ID NO:157)	FEAMKPNF (SEQ ID NO:163)
KVKAELVM (SEQ ID NO:142)	NIVAEIIK (SEQ ID NO:150)	KINAQI (SEQ ID NO:158)	FENAKPNF (SEQ ID NO:164)
KVKTALVM (SEQ ID NO:143)	NIVDAIIK (SEQ ID NO:151)	KINRAI (SEQ ID NO:159)	FENMAPNF (SEQ ID NO:165)
KVKTEAVM (SEQ ID NO:144)	NIVDEAIK (SEQ ID NO:152)	KINRQA (SEQ ID NO:160)	FENMKANF (SEQ ID NO:166)
KVKTELAM (SEQ ID NO:145)	NIVDEIAK (SEQ ID NO:153)		FENMKPAF (SEQ ID NO:167)
KVKTELVA (SEQ ID NO:146)	NIVDEIIA (SEQ ID NO:154)		FENMKPNA (SEQ ID NO:168)

[0113] The native peptides (non-mutated, shown above in bold, SEQ ID Nos:89-97) were tagged with biotin (N-Terminal). 96-well Pierce™ NeutrAvidin coated plates (Thermo

Fisher, 15507) were rinsed with PBST and incubated overnight at 4°C in the MPBS (blocking buffer, 100 µL/well). Blocking buffer was discarded, and 100 µL of biotinylated peptides was added to the plate at 200 ng/mL and incubated for 90 min at 4°C. Simultaneously, anti-VV rabbit polyclonal NAb (Abcam, ab35219) was incubated with variant peptides. We used 30 µL/well of Ab at 800 ng/mL and incubated it with 30 µL/well of alanine-modified peptides at 100 µg/mL for 90 min at 4°C. After washing the plates with PBST, 50 µL of the Ab/alanine peptide mix was added to plate-bound peptides (in duplicate wells) and incubated for 60 min at 4°C. Plates were washed with PBST six times, and 100 µL/well of anti-rabbit horseradish peroxidase (HRP)–conjugated secondary Ab (Abcam, ab6721) diluted 1:1000 in MPBS was added. The plates were then incubated for 90 min at 4°C, washed with PBST four times and developed using 3,3',5,5',-Tetramethylbenzidine (TMB) (Sigma, T0440-100ML). The OD at 650 nm was read on Perkin Elmer Multimode Plate Reader (Corning). The intensity of each signal was measured and plotted using Kaleido™ 1.2 software. For each set of mutant peptides, a signal higher that the native control for that set was considered positive (Fig. 2). Control peptide for set 3 peptides (EKRNVVVV (SEQ ID NO:169)) showed a signal higher than the rest of the peptides in the set with only two other peptides in this set showing a signal above 0.07. The scan identified a total of 29 residues positive for Ab binding: I14, D15, R16, K33, F34, D35, K38, N40, E45, V52, E131, T134, F135, L136, R137, R154, E155, I156, K161, L166, V167, M168, I198, R227, E250, K253, P254, N255, and F256 (Fig. 2). The peptide arrays involve linear peptides and therefore may not represent the physiological confirmations of the residues in the context of the 3D protein structure. To analyze each identified residue in the context of the full-length H3L protein we mapped them onto the previously determined crystal structure of H3L. All but two residues (N40 and F135) mapped to the surface of the protein and therefore would potentially be available for interaction with the Abs. N40 and F135 mapped on the inside folds of the protein and therefore would be unlikely to interact with the Abs. An additional residue P44 was identified by a separate experiment (see below) and therefore was also included in our design. Lastly, the alanine scan identified 8 additional residues that showed a signal lower than the cutoff but higher than their respective controls, suggesting that they may also play a role in the Ab binding: K33, F34, D35, K161, L166, V167, and R227 (see Fig. 2).

[0114] In one embodiment, a mutant H3L protein comprises the following mutations: I14A, D15A, R16A, K33A, F34A, D35A, K38A, N40A, E45A, V52A, E131A, T134A, F135A, L136A, R137A, R154A, E155A, I156A, K161A, L166A, V167A, M168A, I198A, R227A,

E250A, K253A, P254A, N255A, and F256A. An example of mutant H3L amino acid sequence is shown in SEQ ID NO:1.

EXAMPLE 4

Homologous Recombination To Introduce Modified H3L, D8L, L1R, and A27L Genes Into The VV Genome

[0115] For each modified protein a DNA fragment containing the proteins' native promoter, ORF (with mutations in place), and approximately ~ 250-bp flanking regions for homologous recombination into the appropriate gene in the VV genome was synthesized by GENEWIZ and cloned into the pUC57-Amp plasmid. For all four constructs a green fluorescent protein (GFP) expression cassette under the control of the VV p7.5 promoter and flanked by LoxP sites was inserted immediately downstream of the stop codon before the right flank sequence (Fig. 3). The fluorescence marker expressed from the GFP cassette was used to screen for clones that had undergone homologous recombination and GFP was removed using the LoxP sites. The pUC57-Amp plasmids were transfected into the CV-1 cells and allowed to recombine with the VV genome. The fluorescence marker expressed from the GFP cassette was used to screen for clones that had undergone homologous recombination (HR) and GFP was removed using the LoxP sites. The correct gene insertion into the VV genome was verified by PCR.The plasmids were transfected into the CV-1 cells infected with the VV one at a time, starting with the L1R plasmid, following by A27L, D8L, and finally H3L. With the addition of each plasmid rounds of screening and purification were performed, followed by PCR and sequencing to make sure that the correct mutations were present. GFP was removed before the recombination with the next plasmid. The final variant contains modifications in all four proteins.

[0116] Nucleotide substitutions in a synthesized H3L construct result in the following amino acid mutations: I14A, D15A, R16A, K38A, P44A, E45A, V52A, E131A, T134A, L136A, R137A, R154A, E155A, I156A, M168A, I198A, E250A, K253A, P254A, N255A, and F256A**.** The mutant H3L amino acid sequence is shown in SEQ ID NO:11. Nucleotide sequences for such mutated *H3L* gene, containing left flank region, promoter region, p7.5 promoter, LoxP, GFP, LoxP, and right flank regions are shown in SEQ ID NO:12.

[0117] Nucleotide substitutions in a synthesized D8L construct result in the following amino acid mutations: R44A, K48A, K98A, K108A, K117A, and R220A. The mutant D8L amino

acid sequence is shown in SEQ ID NO:2. Nucleotide sequences for such mutated *D8L* gene, containing left flank region, promoter region, p7.5 promoter, LoxP, GFP, LoxP, and right flank regions are shown in SEQ ID NO:13.

[0118] Nucleotide substitutions in a synthesized A27L construct result in the following amino acid mutations: K27A, A30D, R32A, E33A, A34D, I35A, V36A, K37A, D39A, E40A, R107A, P108A, and Y109A. The mutant A27L amino acid sequence is shown in SEQ ID NO:3. Nucleotide sequences for such mutated *A27L* gene, containing left flank region, promoter region, p7.5 promoter, LoxP, GFP, LoxP, and right flank regions are shown in SEQ ID NO:14.

[0119] Nucleotide substitutions in a synthesized L1R construct result in the following amino acid mutations: E25A, N27A, Q31A, T32A, K33A, D35A, S58A, D60A, D62A, K125A, and K127A. The mutant L1R amino acid sequence is shown in SEQ ID NO:4. Nucleotide sequences for such mutated *L1R* gene, containing left flank region, promoter region, p7.5 promoter, LoxP, GFP, LoxP, and right flank regions are shown in SEQ ID NO:15.

EXAMPLE 5

In vitro Neutralization Assays With Anti-VV Polyclonal Antibodies

[0120] The ability of the anti-VV polyclonal Abs to neutralize the escape variants was investigated. A panel of anti-VV Abs consisting of ab35219 (Abcam), ab21039 (Abcam), ab26853 (Abcam), 9503-2057 (Bio-Rad), and PA1-7258 (Invitrogen) was used to test for neutralization escape *in vitro*. Rabbit polyclonal IgG ab37415 (Abcam) served as a control. CV-1 cells were seeded into 12-well plates and used within 2 days of reaching confluence. Forty μg/mL of Ab was preincubated with either the escape variant or the control VV at 1×10^3 pfu/sample for 1 hr at 37°C in the presence of 2% of sterile baby rabbit complement. The mixture was then added to the CV-1 cells and allowed to adhere for 2 hrs at 37°C / 5% CO_2 in 300 μL of serum free media. After 2 hrs, the inoculum was removed and 1mL of complete DMEM medium was added to the cells. The cells were then incubated at 37°C / 5% CO_2. After 48 hrs cells were fixed and stained with 1% crystal violet / 20% EtOH solution for 20 min at room temperature and plaques were counted. All five Abs reduced the control VV plaque numbers dramatically, showing a strong neutralizing ability (Fig. 5). On average 83.3–95.5% of the control VV virus was neutralized across the panel. In contrast, the L1R+A27L+D8L+H3 escape variant showed a significantly lower neutralization by the Abs,

with an average of 17.8 – 66.2% neutralization. Interestingly ab26853 neutralized 78% of control VV but almost completely failed to neutralize NEV variant (see Fig. 5). Based on these results, it is concluded that the escape variants disclosed herein can efficiently escape neutralization by anti-VV Abs in vitro.

[0121] Recombinant virus replication assay was performed (Fig 7). In a 24-well plate CV-1 cells were infected with duplicates of VV control, VVNEV, and VVEM at MOI = .05. Prior to infection virus was preincubated with Ab 9503-2057 (40 µg/mL) for 1 hr at 37°C. Samples were collected at 24, 48, and 72 hrs and titers were determined for each time point. The recombinant virus was significantly more efficient in replicating in the presence of Ab, compared to the control Ab, which was almost entirely inactivated.

[0122] Anti-tumor efficiency of the recombinant virus was evaluated (Fig 8). The recombinant virus and the control VV were preincubated with Ab 9503-2057 (see above) and used to infect transformed cells at MOI=1. Cells were incubated for 48 hrs and cell viability was measured by MTS assay (colorimetric assessment of cell metabolic activity). Briefly, cells collected at 48 hrs were washed once with PBST and resuspended at 1 × 105 cells/mL in complete DMEM. One hundred µL of each cell suspension was added to a 96-well (in triplicates). Twenty µl of CellTiter 96® AQueous One Solution Reagent (Promega, G358C) was added into each well of the 96-well assay plate containing the samples in 100µl of culture medium. The plate was incubated at 37°C for 2 hrs (5% CO2). To measure the amount of soluble formazan produced by cellular reduction of MTS, the absorbance in each well was recorded at 490nm using a 96-well plate reader. In the presence of the Ab, the recombinant virus was able to efficiently kill the cells.

EXAMPLE 6

Isolation of Neutralization Escape Mutant (VV^{EM})

[0123] To identify any additional key NAb epitope residues, VV mutants that resisted the neutralization by ab35219 and ab21039 were selected. Briefly, a stock of mutant VV was prepared from CV-1 cells that were infected with the Western Reserve strain of VV in the presence of ethyl methanesulfonate (EMS) to induce transition mutations in viral DNA. Polyclonal anti-VV ab35219 and ab21039 were then used to neutralize the mutated virus. EMS was present in the culture medium at 500 µg/mL. The mutant viral stock was incubated with the mixture of two polyclonal Abs at 50 µg/ml each (100 µg/ml total conc.) for 1 hr, and

then used to infect the CV-1 cells plated in the 12-well plates. After 2 hrs the inoculum was removed and fresh complete DMEM was added to the cells. Cells were then incubated at 37 °C, 5% CO_2 for 48 hrs. During the first round of infection, the titer of the mutant virus was significantly reduced by the Abs. After a multiple rounds of infections with constant Ab concentration and with the increasingly more purified virus than the previous round, the passaged viral stock was no longer significantly neutralized by the Abs. A clone of the escape mutant (VV^{EM}) was plaque purified and showed a significant escape of neutralization by a panel of five anti-VV Abs described above (Fig. 6). Whereas on average 77.7 – 96.4% of the control VV virus was neutralized across the panel, VV^{EM} showed an average of 30.7 – 66.9% neutralization by the Abs, significantly lower than the control. Viral DNA from pure virus was isolated and PCR was used to amplify the A27L, L1R, H3L, and D8L genes, the major Ab antigens of the VV. PCR products were sequenced and showed presence of the mutations in the genes encoding A27L, D8L, and H3L. D8L coding sequence contains the following mutations: V43F/L, R44W, G55W, A144T, T168S, S177Y, F199Y, L203S, P212T, N218C, P222L, and D227G. The A27L coding sequence showed two mutations at residues I35 and D39 that were previously determined to be involved in the NAb interaction with A27L and were included in our A27L plasmid design. The H3L sequence showed an amino acid substitution at residue P44, a residue immediately adjacent to the E45 residue identified by the peptide array as part of the Ab-binding peptide (peptide 3; Fig. 2A) and thus was also included in the H3L recombinant plasmid design. Other mutations identified in the H3 gene are: E250G, N255W (these two residues were also identified by the alanine scan), S258F, T262P, A264T, T265V, K266I, Y268C, M272K, Y273N, F275N, and T277A. All of these mutations are clustered in the flexible C-terminal region of the protein. SEQ ID NO:5 shows a mutant H3L amino acid sequence. SEQ ID NO:6 or SEQ ID NO:174 shows a mutant D8L amino acid sequence. Both SEQ ID NOs:6 and 174 were disclosed in parent application U.S. Provisional Patent Application No. 62/749,102 as SEQ ID NO:7.

EXAMPLE 7

Homologous Recombination To Introduce Modified H3L, D8L, L1R, and A27L Genes Into The VV Genome

[0124] A new recombinant VV was made to incorporate the mutations that were identified as above. In addition, structural analysis of the proteins also identified additional residues that were not identified by either the peptide arrays or the EM sequencing but were adjacent to the residues that were identified and could potentially play a role in Ab interactions. Those

residues were also included in the design. For each modified protein a DNA fragment containing the proteins' native promoter, ORF (with mutations in place), and approximately ~ 250-bp flanking regions for homologous recombination into the appropriate gene in the VV genome was synthesized by GENEWIZ and cloned into the pUC57-Amp plasmid. For all four constructs a green fluorescent protein (GFP) expression cassette under the control of the VV p7.5 promoter and flanked by LoxP sites was inserted immediately downstream of the stop codon before the right flank sequence (Fig. 3). The fluorescence marker expressed from the GFP cassette was used to screen for clones that had undergone homologous recombination and GFP was removed using the LoxP sites. The pUC57-Amp plasmids were transfected into the CV-1 cells and allowed to recombine with the VV genome. The fluorescence marker expressed from the GFP cassette was used to screen for clones that had undergone homologous recombination (HR) and GFP was removed using the LoxP sites. The correct gene insertion into the VV genome was verified by PCR.The plasmids were transfected into the CV-1 cells infected with the VV one at a time, starting with the L1R plasmid, following by A27L, D8L, and finally H3L. With the addition of each plasmid rounds of screening and purification were performed, followed by PCR and sequencing to make sure that the correct mutations were present. GFP was removed before the recombination with the next plasmid. The final variant contains modifications in all four proteins.

[0125] Nucleotide substitutions in a synthesized H3L construct result in the following amino acid mutations: I14A, D15A, R16A, K33A, F34A, D35A, K38A, N40A, P44A, E45A, V52A, E131A, D132A, T134A, F135A, L136A, R137A, R154A, E155A, I156A, K161A, L166A, V167A, M168A, E195A, I198A, V199A, R227A, E250A, N251A, M252A, K253A, P254A, N255A, F256A, S258A, T262P, A264T, K266I, Y268C, M272K, Y273N, F275N, and T277A**.** The mutant H3L amino acid sequence is shown in SEQ ID NO:170. Nucleotide sequences for such mutated *H3L* gene, containing left flank region, promoter region, p7.5 promoter, LoxP, GFP, LoxP, and right flank regions are shown in SEQ ID NO:171.

[0126] Nucleotide substitutions in a synthesized D8L construct result in the following amino acid mutations: V43A, R44A, K48A, S53A, G54A, G55A, K98A, K108A, K109A, A144G, T168A, S177A, L196A, F199A, L203A, N207A, P212A, N218A, R220A, P222A, and D227A. The mutant D8L amino acid sequence is shown in SEQ ID NO:172. Nucleotide

sequences for such mutated *D8L* gene, containing left flank region, promoter region, p7.5 promoter, LoxP, GFP, LoxP, and right flank regions are shown in SEQ ID NO:173.

[0127] Nucleotide substitutions in a synthesized A27L construct result in the following amino acid mutations: K27A, A30D, R32A, E33A, A34D, I35A, V36A, K37A, D39A, E40A, R107A, P108A, and Y109A. The mutant A27L amino acid sequence is shown in SEQ ID NO:3. Nucleotide sequences for such mutated *A27L* gene, containing left flank region, promoter region, p7.5 promoter, LoxP, GFP, LoxP, and right flank regions are shown in SEQ ID NO:14.

[0128] Nucleotide substitutions in a synthesized L1R construct result in the following amino acid mutations: E25A, N27A, Q31A, T32A, K33A, D35A, S58A, D60A, D62A, K125A, and K127A. The mutant L1R amino acid sequence is shown in SEQ ID NO:4. Nucleotide sequences for such mutated *L1R* gene, containing left flank region, promoter region, p7.5 promoter, LoxP, GFP, LoxP, and right flank regions are shown in SEQ ID NO:15.

EXAMPLE 8

In vitro Neutralization Assays With Anti-VV Polyclonal Antibodies

[0129] The ability of the anti-VV polyclonal Abs to neutralize the escape variants was investigated. Anti-VV Abs 9503-2057 (Bio-Rad) and PA1-7258 (Invitrogen) were used to test for neutralization escape *in vitro*. Rabbit polyclonal IgG ab37415 (Abcam) served as a control. CV-1 cells were seeded into 12-well plates and used within 2 days of reaching confluence. Forty µg/mL of Ab was preincubated with either the escape variant or the control VV at 1×10^3 pfu/sample for 1 hr at 37°C in the presence of 2% of sterile baby rabbit complement. The mixture was then added to the CV-1 cells and allowed to adhere for 2 hrs at 37°C / 5% CO_2 in 300 µL of serum free media. After 2 hrs, the inoculum was removed and 1mL of complete DMEM medium was added to the cells. The cells were then incubated at 37°C / 5% CO_2. After 48 hrs cells were fixed and stained with 1% crystal violet / 20% EtOH solution for 20 min at room temperature and plaques were counted. NAbs reduced the control VV plaque numbers dramatically, showing a strong neutralizing ability (Fig. 9). On average 86.1–92.1% of the control VV virus was neutralized across the panel. In contrast, the escape variant showed a significantly lower neutralization by the Abs, with an average of 20.8 - 23% neutralization. Based on these results, it is concluded that the escape variants disclosed herein can efficiently escape neutralization by anti-VV Abs in vitro. The replication of the escape

variant (3 single virus clones) and wild type VV were also compared in the absence of neutralization antibodies, the results suggested escape variants have similar replication capability compared to wild type virus, indicating that the mutation doeesn't impair the virus's entry and replication ability (Fig. 10).

EXAMPLE 9

Construction of VV Expressing CD55

[0130] The oncolytic vaccinia virus (VV) construct CD55-NEV was generated to human CD55 extracellular domain. Human CD55 extracellular domain fused to VV A27 were optimized and synthesized and cloned into a pMS shuttle plasmid (Fig. 11). Vaccinia viruses (Western Reserve strain) expressing CD55-A27 were generated by recombination of a version of pMS shuttle plasmid into the TK gene of the WR vaccinia virus (WR VV) or NEV. The inserted CD55 and A27 was expressed under the transcriptional control of the original A27 promoter. To construct the recombinant virus CD55-NEV, the shuttle vectors pMS were transfected into CV-1 or 293 cells. Cells were then infected with WR VV or NEV at a multiplicity of infection (MOI) of 0.1. After three rounds of plaque selection and amplification to confirm the expression of CD55, one of the corresponding clones was selected for amplification and purification.

[0131] In one embodiment, an amino acid sequence comprising the CD55-A27 fusion is shown in SEQ ID NO:7. An example of an optimized nucleotide sequence for CD55-A27, containing signal peptide, CD55, A27 and linker sequence is shown in SEQ ID NO:16.

EXAMPLE 10

In vitro Neutralization Assays With Complement or Complement/Anti-VV Polyclonal Antibodies

[0132] The ability of CD55-VV to escape complement-mediated neutralization was first investigated. To do this, CV-1 cells were seeded into 12-well plates and used within 2 days of reaching confluence. CD55-NEV or NEV control at 1×10^3 pfu/sample were added to the CV-1 cells at 37°C / 5% CO_2 in 300 μL of media in the presence of 1:10 human complement. Heat activated complement were used as control to calculate the escape rate. After 48 hrs, cells were fixed and stained with 1% crystal violet / 20% EtOH solution for 20 min at room temperature and plaques were counted. CD55-NEV escaped complement-mediated neutralization more effectively than NEV (Fig. 12). Around 59% of the CD55-NEV escaped

complement-mediated neutralization, while only around 18% of NEV escaped complement-mediated neutralization.

[0133] The ability of CD55-NEV to escape the neutralization of complement with anti-VV polyclonal Abs was further investigated. Two anti-VV Abs, 9503-2057 (Bio-Rad) and PA1-7258 (Invitrogen), were used to test for neutralization escape *in vitro*. CV-1 cells were seeded into 12-well plates and used within 2 days of reaching confluence. Forty μg/mL of Ab was preincubated with either CD55-NEV or the control VV at 1×10^3 pfu/sample for 1 hr at 37°C in the presence of 1:10 dilution of human complement. The mixture was then added to the CV-1 cells and allowed to adhere for 2 hrs at 37°C / 5% CO_2 in 300 μL of serum free media. After 2 hrs, the inoculum was removed and 1mL of complete DMEM medium was added to the cells. The cells were then incubated at 37°C / 5% CO_2. After 48 hrs cells were fixed and stained with 1% crystal violet / 20% EtOH solution for 20 min at room temperature and plaques were counted. The results suggested that CD55-NEV escaped the neutralization more effectively than NEV and VV in the absence or presence of complement (Fig. 13). Based on these results, it is concluded that the CD55-VV disclosed herein can efficiently escape complement/Nab mediated neutralization in vitro.

EXAMPLE 11

Construction of FAP-TEA-NEV

[0134] The oncolytic vaccinia virus (VV) construct FAP-TEA-NEV was generated to express a bispecific FAP-CD3 scFv targeting the FAP on cancer associated fibroblast (CAF) and CD3 on T cells. Bispecific FAP-CD3 scFv was optimized and synthesized and cloned into a pMS shuttle plasmid (FIG. 14). The mhFAP -cross reactive single chain variable fragment (scFv MO36) was previously generated by phage display from an immunized FAP/ knock-out mouse. Human CD3 scFv was derived from OKT3 clone. Vaccinia viruses (Western Reserve strain) expressing secretory bispecific FAP-CD3 scFv (FAP-TEA-NEV) were generated by recombination of a version of pMS shuttle plasmid into the TK gene of the WR VV or NEV. The inserted bispecific FAP-CD3 scFv was expressed under the transcriptional control of the F17R late promoter to allow for sufficient viral replication before T-cell activation. To construct the recombinant virus BCMA-TEA-NEV, the shuttle vectors pMS were transfected into CV-1 or 293 cells. Cells were then infected with WR VV or NEV at a multiplicity of infection (MOI) of 0.1. After three rounds of plaque selection and

amplification to confirm the expression of FAP-CD3, one of the corresponding clones was selected for amplification and purification.

[0135] In one embodiment, an amino acid sequence comprising the FAP-CD3 polypeptide is shown in SEQ ID NO:8. An example of an optimized nucleotide sequence for the FAP-CD3 polypeptide, containing signal peptide, FAP scFv, CD3 scFv and linker sequence is shown in SEQ ID NO:17.

EXAMPLE 12

Evaluation of FAP-TEA-NEV in vitro

[0136] Tumor lysis capacity of FAP-TEA-NEV was investigated. FAP-positive U87 tumor cells were seeded into 96-well plates at 5x10e4 cell number per well. The U87 tumor cells were then infected with FAP-TEA-NEV or NEV at MOI 1, and co-cultured with human T cells at ration of U87:T = 1:5. After 48 hrs, cells were observed under microscope. The microscope picture showed that FAP-TEA-VV induced U87 tumor cell lysis and human T cell proliferation effectively compared to NEV (Fig.15). Cells were stained with apoptosis marker PI and Flow analysis results suggested that FAP-TEA-VV induced U87 tumor apoptosis more effectively than NEV (Fig.16). Fig 17 showed the MFI of PI staining of gated U87 tumor cells.

[0137] The ability of FAP-TEA-NEV to induce bystander tumor lysis was also investigated. CV-1 cells were infected with FAP-TEA-VV at MOI 1, and the cell culture medium were collected at 24 hours and added to co-culture of FAP-positive U87 tumor cells and human T cells at ratio of U87:T = 1:5. U87 tumor cells were seeded into 96-well plates at 5x10e4 cell number per well. After 48 hrs, cells were observed under microscope. The microscope picture showed that FAP-TEA-VV induced U87 tumor cell lysis and human T cell proliferation effectively compared to NEV (Fig.18).

EXAMPLE 13

Construction of BCMA-TEA-NEV

[0138] The oncolytic vaccinia virus (VV) construct BCMA-TEA-NEV was generated to express a bispecific BCMA-CD3 scFv targeting the BCMA on multiple myeloma and CD3 on T cells. Bispecific BCMA-CD3 scFv was optimized and synthesized and cloned into a pMS shuttle plasmid (FIG. 19). BCMA scFV was derived from C11D5.3 clone

(US9034324B2). Human CD3 scFv was derived from OKT3 clone. Vaccinia viruses (Western Reserve strain) expressing secretory bispecific BCMA-CD3 scFv (BCMA-TEA-NEV) were generated by recombination of a version of pMS shuttle plasmid into the TK gene of the WR vaccinia virus (WR VV) or NEV. The inserted bispecific BCMA-CD3 scFv was expressed under the transcriptional control of the F17R late promoter to allow for sufficient viral replication before T-cell activation. To construct the recombinant virus BCMA-TEA-NEV, the shuttle vectors pMS were transfected into CV-1 or 293 cells. Cells were then infected with WR VV or NEV at a multiplicity of infection (MOI) of 0.1. After three rounds of plaque selection and amplification to confirm the expression of BCMA-CD3, one of the corresponding clones was selected for amplification and purification.

[0139] In one embodiment, an amino acid sequence comprising the BCMA-CD3 scFv is shown in SEQ ID NO:9. An example of an optimized nucleotide sequence for the BCMA-CD3 scFv, containing signal peptide, BCMA scFv, CD3 scFv and linker sequence is shown in SEQ ID NO:18.

EXAMPLE 14

Evaluation of BCMA-TEA-NEV in vitro

[0140] BCMA positive RPMI-8226 MM cell line was infected with BCMA-TEA-NEV or control NEV at MOI 2. After 24 hours, the virus infected RPMI-8226 cells were co-cultured with Jurkat T cells (Invivogen) at ratio of Jurkat T : RPMI-8226 = 2:1. After 24 hours of incubation, the cells were collected for counting the cell number and flow analysis of cell population. Flow analysis of the cell population suggested Jurkat T cells were significantly activated by BCMA-CD3 (Fig. 20A). Fig. 20B shows the cell number of the RPMI-8266 MM cells and activated Jurkat T cells. The results suggested that BCMA-TEA-NEV significantly induced Jurkat T cell activation and RPMI-8266 MM cell lysis compared to NEV control.

[0141] In the above experiment, after 24 hours of incubation, the cells were collected for measurement of cytokines IFNγ (Fig. 21A) and IL2 (Fig. 21B) secretion by ELISA. The results suggested that BCMA-TEA-NEV significantly induced IFNγ and IL2 expression by Jurkat T cells compared to NEV control.

EXAMPLE 15

Construction of PD-1-ED-hIgG1-Fc-NEV

[0142] The oncolytic vaccinia virus (VV) construct PD-1-ED-hIgG1-Fc-NEV was generated to express a recombinant protein with the extracellular domain of PD-1 fused to the constant (Fc) domain of immunoglobin-G1 (IgG1). FAP-CD3 is a bispecific molecule targeting the fibroblast activation protein on cancer associated fibroblast and CD3 on T cells. PD-1-ED-hIgG1-Fc was optimized and synthesized and cloned into a pMS shuttle plasmid (FIG. 22). Vaccinia viruses (Western Reserve strain) expressing secretory PD-1-ED-hIgG1-Fc (PD-1-ED-hIgG1-Fc-NEV) or co-expressing secretory PD-1-ED-hIgG1-Fc and FAP-CD3 (PD-1-ED-hIgG1-Fc-FAP-TEA-NEV) were generated by recombination of a version of pMS shuttle plasmid into the TK gene of the WR vaccinia virus (WR VV) or NEV. The inserted PD-1-ED-hIgG1-Fc was expressed under the transcriptional control of the pSE/L promoter. The inserted FAP-CD3 was expressed under the transcriptional control of the F17R late promoter to allow for sufficient viral replication before T-cell activation. To construct the recombinant virus PD-1-ED-hIgG1-Fc-NEV or PD-1-ED-hIgG1-Fc-FAP-TEA-NEV, the shuttle vectors pMS were transfected into CV-1 or 293 cells. Cells were then infected with WR VV or NEV at a multiplicity of infection (MOI) of 0.1. After three rounds of plaque selection and amplification to confirm the expression of PD-1-ED-hIgG1-Fc or FAP-CD3, one of the corresponding clones was selected for amplification and purification.

[0143] In one embodiment, an amino acid sequence comprising the PD-1-ED-hIgG1-Fc is shown in SEQ ID NO:10. An example of an optimized nucleotide sequence for the PD-1-ED-hIgG1-Fc, containing signal peptide, PD-1 extracellular domain, human IgG1 hinge and Fc domain is shown in SEQ ID NO:19.

EXAMPLE 16

Evaluation of PD1ED-NEV in vitro

[0144] Stable PD-L1-Raji (Invivogen) cell line was infected with PD1ED-NEV or control NEV at MOI 2. After 24 hours, the virus infected PD-L1-Raji cells were co-cultured with NFAT-CD16-Luc reporter Jurkat T cells (Invivogen) at ratio of Jurkat T : PD-L1-Raji = 2:1. To investigate the role of the secreted PD-1-ED-Fc, CV-1 cells were infected with BCMA-TEA-NEV at MOI2 and the cell culture medium was collected after 24 hours and added to the co-culture of Raji and Jurkat T cells. After 24 hours of incubation, the cells were collected for flow analysis (Fig 23A) and counting (Fig 23B). The results suggested secreted PD-1-ED-

Fc effectively induced Raji cell lysis compared to control group. PD-1-ED-Fc also induced significant Jurkat T cell exhaustion (Fig 19B). NEV infection of Raji has no effects likely because Raji is not susceptiable to VV infection. In the above experiment, after 24 hours of incubation, the cells were collected for measurement of cytokines IFNγ (Fig. 24A) and IL2 (Fig. 24B) secretion by ELISA. The results suggested that secreted PD1ED significantly induced IFNγ and IL2 expression by Jurkat T cells compared to NEV control.

[0145] In another experiment, stable PD-L1-Raji (Invivogen) cell line was infected with PD1ED-NEV or control NEV at MOI 2. After 24 hours, the virus infected PD-L1-Raji cells were co-cultured with NFAT-CD16-Luc reporter Jurkat T cells (Invivogen) at ratio of Jurkat T : PD-L1-Raji = 2:1. To investigate the role of the secreted PD-1-ED-Fc, CV-1 cells were infected with BCMA-TEA-NEV at MOI2 and the cell culture medium was collected after 24 hours and added to the co-culture of Raji and Jurkat T cells. After 6 hours of incubation, the supernatant was collected for luciferase measurement (Fig. 25). The results suggested secreted PD-1-ED-Fc effectively activated Jurkat T cells compared to control NEV or medium.

What is claimed is:

1. An isolated infectious recombinant vaccinia virus (VV) virion, comprising a heterologous nucleic acid and one or more of:

a) a variant vaccinia virus (VV) H3L protein having at least about 60% amino acid sequence identity to SEQ ID NO:1;

b) a variant vaccinia virus (VV) D8L protein having at least about 60% amino acid sequence identity to SEQ ID NO:2;

c) a variant vaccinia virus (VV) A27L protein having at least about 60% amino acid sequence identity to SEQ ID NO:3;

d) a variant vaccinia virus (VV) L1R protein having at least about 60% amino acid sequence identity to SEQ ID NO:4;

e) a variant vaccinia virus (VV) H3L protein having at least about 60% amino acid sequence identity to SEQ ID NO:5;

f) a variant vaccinia virus (VV) D8L protein having at least about 60% amino acid sequence identity to SEQ ID NO:6 or SEQ ID NO:174;

g) a variant vaccinia virus (VV) H3L protein having at least about 60% amino acid sequence identity to SEQ ID NO:170; and

h) a variant vaccinia virus (VV) D8L protein having at least about 60% amino acid sequence identity to SEQ ID NO:172.

2. The recombinant vaccinia virus (VV) virion of claim 1, wherein said variant VV H3L protein comprises amino acid substitution or deletion at one or more amino acid residues selected from the group consisting of 14, 15, 16, 33, 34, 35, 38, 40, 44, 45, 52, 131, 134, 135, 136, 137, 154, 155, 156, 161, 166, 167, 168, 198, 227, 250, 253, 254, 255, and 256 of SEQ ID NO:1.

3. The recombinant vaccinia virus (VV) virion of claim 1, wherein said variant VV D8L protein comprises amino acid substitution or deletion at one or more amino acid residues selected from the group consisting of 44, 48, 98, 108, 117, and 220 of SEQ ID NO:2.

4. The recombinant vaccinia virus (VV) virion of claim 1, wherein said variant VV A27L protein comprises amino acid substitution or deletion at one or more amino acid

residues selected from the group consisting of 27, 30, 32, 33, 34, 35, 36, 37, 39, 40, 107, 108, and 109 of SEQ ID NO:3.

5. The recombinant vaccinia virus (VV) virion of claim 1, wherein said variant VV L1R protein comprises amino acid substitution or deletion at one or more amino acid residues selected from the group consisting of 25, 27, 31, 32, 33, 35, 58, 60, 62, 125, and 127 of SEQ ID NO:4.

6. The recombinant vaccinia virus (VV) virion of claim 1, wherein said variant VV H3L protein comprises amino acid substitution or deletion at one or more amino acid residues selected from the group consisting of 14, 15, 16, 33, 34, 35, 38, 40, 44, 45, 52, 131, 132, 134, 135, 136, 137, 154, 155, 156, 161, 166, 167, 168, 195, 198, 199, 227, 250, 251, 252, 253, 254, 255, 256, 258, 262, 264, 266, 268, 272, 273, 275, and 277 of SEQ ID NO:170.

7. The recombinant vaccinia virus (VV) virion of claim 1, wherein said variant VV D8L protein comprises amino acid substitution or deletion at one or more amino acid residues selected from the group consisting of 43, 44, 48, 53, 54, 55, 98, 108, 109, 144, 168, 177, 196, 199, 203, 207, 212, 218, 220, 222, and 227 of SEQ ID NO:172.

8. The recombinant vaccinia virus (VV) virion of claim 1, wherein said heterologous nucleic acid encodes a domain of a regulator of complement activation.

9. The recombinant vaccinia virus (VV) virion of claim 8, wherein said regulator of complement activation is selected from the group consisting of CD55, CD59, CD46, CD35, factor H, and C4 -binding protein.

10. The recombinant vaccinia virus (VV) virion of claim 1, wherein said heterologous nucleic acid encodes a CD55 polypeptide comprising the amino acid sequence of SEQ ID NO:7.

11. The recombinant vaccinia virus (VV) virion of claim 1, wherein said heterologous nucleic acid encodes a bi-specific polypeptide that binds to a first antigen on immune cells and a second antigen on tumor cells.

12. The recombinant vaccinia virus (VV) virion of claim 11, wherein said first antigen on immune cells is selected from the group consisting of CD3, CD4, CD5, CD8, CD16, CD28, CD40, CD64, CD89, CD134, CD137, NKp46, and NKG2D.

13. The recombinant vaccinia virus (VV) virion of claim 11, wherein said second antigen on tumor cells is selected from the group consisting of fibroblast activation protein (FAP), and tumor antigens on multiple myeloma.

14. The recombinant vaccinia virus (VV) virion of claim 11, wherein the bi-specific polypeptide is a bi-specific scFvs, said first antigen is human CD3e, said second antigen is human FAP, and said bi-specific polypeptide having the amino acid sequence of SEQ ID NO:8.

15. The recombinant vaccinia virus (VV) virion of claim 13, wherein the tumor antigens on multiple myeloma are selected from the group consisting of B-cell maturation antigen (BCMA), CD19, CD38, SLAMF7, CD26, LIGHT/TNFSF14, integrin beta7, CD138, KIRs, EGFR, PD-1/PD-L1, TGIT, CD56, CS1, NKG2D, TACI, and CD44v6.

16. The recombinant vaccinia virus (VV) virion of claim 11, wherein the bi-specific polypeptide is a bi-specific scFvs, said first antigen is human CD3e, said second antigen is human BCMA, and said bi-specific polypeptide having the amino acid sequence of SEQ ID NO:9.

17. The recombinant vaccinia virus (VV) virion of claim 1, wherein said heterologous nucleic acid encodes a fusion polypeptide comprising an immune checkpoint molecule.

18. The recombinant vaccinia virus (VV) virion of claim 17, wherein said immune checkpoint molecule is selected from the group consisting of PD-1, PD-L1, PD-L2, CD47, CXCR4, CSF1R, LAG-3, TIM-3, HHLA2, BTLA, CTLA-4, TIGIT, VISTA, B7-H4, CD160, 2B4, and CD73.

19. The recombinant vaccinia virus (VV) virion of claim 1, wherein said heterologous nucleic acid encodes a fusion polypeptide comprising human PD-1 extracellular domain and a human IgG1 Fc domain, said fusion polypeptide having the amino acid sequence of SEQ ID NO:10.

20. The recombinant vaccinia virus (VV) virion of claim 1, wherein the VV exhibits resistance to neutralizing antibodies compared to that exhibited by wild type VV.

21. The recombinant vaccinia virus (VV) virion of claim 1, wherein the VV exhibits increased transduction of mammalian cells in the presence of VV neutralizing antibodies compared to transduction of mammalian cells by wild type VV.

22. A method of delivering a gene product to a subject in need thereof, comprising administering to the subject an effective amount of the recombinant vaccinia virus (VV) virion of claim 1, said gene product is encoded by said heterologous nucleic acid.

23. A pharmaceutical composition comprising the recombinant vaccinia virus (VV) virion of claim 1 and a pharmaceutically acceptable carrier.

24. A method of treating cancer in a subject, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 23.

25. The method of claim 24, wherein the pharmaceutical composition is administered to the subject systemically, intravenously, or through injection, inhalant, infusion, implantation, parenteral administration, or enteral administration.

26. The method of claim 24, wherein the subject is a human or an animal.

27. A library comprising one or more variant vaccinia virus (VV) virions, each of the one or more variant VV virions comprises one or more variant VV proteins, wherein at least one of said variant VV proteins comprises an amino acid sequence having at least one amino acid substitution or deletion relative to the amino acid sequence of a corresponding wild type VV protein.

28. The library of claim 27, wherein at least one of the one or more variant VV proteins is selected from the group consisting of H3L protein, D8L protein, A27L protein, and L1R protein.

29. The library of claim 27, wherein at least one of the one or more variant VV proteins comprises an amino acid sequence having at least one amino acid substitution or deletion relative to the amino acid sequence of one of SEQ ID No:5, SEQ ID No:6, or SEQ ID No:174.

30. A recombinant vaccinia virus (VV) virion derived from the library of claim 27, comprising a heterologous nucleic acid and one or more variant VV proteins, wherein at least one of said variant VV proteins comprises an amino acid sequence having at least one amino acid substitution or deletion relative to the amino acid sequence of a corresponding wild type VV protein.

31. The recombinant vaccinia virus (VV) virion of claim 30, wherein said heterologous nucleic acid encodes a domain of a regulator of complement activation.

32. The recombinant vaccinia virus (VV) virion of claim 31, wherein said regulator of complement activation is selected from the group consisting of CD55, CD59, CD46, CD35, factor H, and C4 -binding protein.

33. The recombinant vaccinia virus (VV) virion of claim 31, wherein said heterologous nucleic acid encodes a CD55 polypeptide comprising the amino acid sequence of SEQ ID NO:7.

34. The recombinant vaccinia virus (VV) virion of claim 30, wherein said heterologous nucleic acid encodes a bi-specific polypeptide that binds to a first antigen on immune cells and a second antigen on tumor cells.

35. The recombinant vaccinia virus (VV) virion of claim 34, wherein said first antigen on immune cells is selected from the group consisting of CD3, CD4, CD5, CD8, CD16, CD28, CD40, CD64, CD89, CD134, CD137, NKp46, and NKG2D.

36. The recombinant vaccinia virus (VV) virion of claim 34, wherein said second antigen on tumor cells is selected from the group consisting of fibroblast activation protein (FAP), and tumor antigens on multiple myeloma.

37. The recombinant vaccinia virus (VV) virion of claim 34, wherein the bi-specific polypeptide is a bi-specific scFvs, said first antigen is human CD3e, said second antigen is human FAP, and said bi-specific polypeptide having the amino acid sequence of SEQ ID NO:8.

38. The recombinant vaccinia virus (VV) virion of claim 36, wherein the tumor antigens on multiple myeloma are selected from the group consisting of B-cell maturation antigen (BCMA), CD19, CD38, SLAMF7, CD26, LIGHT/TNFSF14, integrin beta7, CD138, KIRs, EGFR, PD-1/PD-L1, TGIT, CD56, CS1, NKG2D, TACI, and CD44v6.

39. The recombinant vaccinia virus (VV) virion of claim 34, wherein the bi-specific polypeptide is a bi-specific scFvs, said first antigen is human CD3e, said second antigen is human BCMA, and said bi-specific polypeptide having the amino acid sequence of SEQ ID NO:9.

40. The recombinant vaccinia virus (VV) virion of claim 30, wherein said heterologous nucleic acid encodes a fusion polypeptide comprising an immune checkpoint molecule.

41. The recombinant vaccinia virus (VV) virion of claim 40, wherein said immune checkpoint molecule is selected from the group consisting of PD-1, PD-L1, PD-L2, CD47, CXCR4, CSF1R, LAG-3, TIM-3, HHLA2, BTLA, CTLA-4, TIGIT, VISTA, B7-H4, CD160, 2B4, and CD73.

42. The recombinant vaccinia virus (VV) virion of claim 40, wherein said heterologous nucleic acid encodes a fusion polypeptide comprising human PD-1 extracellular domain and a human IgG1 Fc domain, said fusion polypeptide having the amino acid sequence of SEQ ID NO:10.

43. The recombinant vaccinia virus (VV) virion of claim 30, wherein the VV virion exhibits resistance to neutralizing antibodies compared to wild type VV.

44. The recombinant vaccinia virus (VV) virion of claim 30, wherein the VV virion exhibits increased transduction of mammalian cells in the presence of VV neutralizing antibodies compared to transduction of mammalian cells by wild type VV.

45. A method of delivering a gene product to a subject in need thereof, comprising administering to the individual an effective amount of the recombinant vaccinia virus (VV) virion of claim 30, wherein the gene product is encoded by the heterologous nucleic acid carried by said variant VV virion.

46. A pharmaceutical composition comprising the recombinant vaccinia virus (VV) virion of claim 30 and a pharmaceutically acceptable carrier.

47. A method of treating cancer in a subject, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 46.

48. The method of claim 47, wherein the pharmaceutical composition is administered to the subject systemically, intravenously, or through injection, inhalant, infusion, implantation, parenteral administration, or enteral administration.

49. The method of claim 47, wherein the subject is a human or an animal.

50. A recombinant vaccinia virus H3L protein having at least about 60% amino acid sequence identity to one of SEQ ID NOs:1, 5 or 170.

51. A recombinant vaccinia virus D8L protein having at least about 60% amino acid sequence identity to one of SEQ ID NOs:6, 172 or 174.

Figure 1A

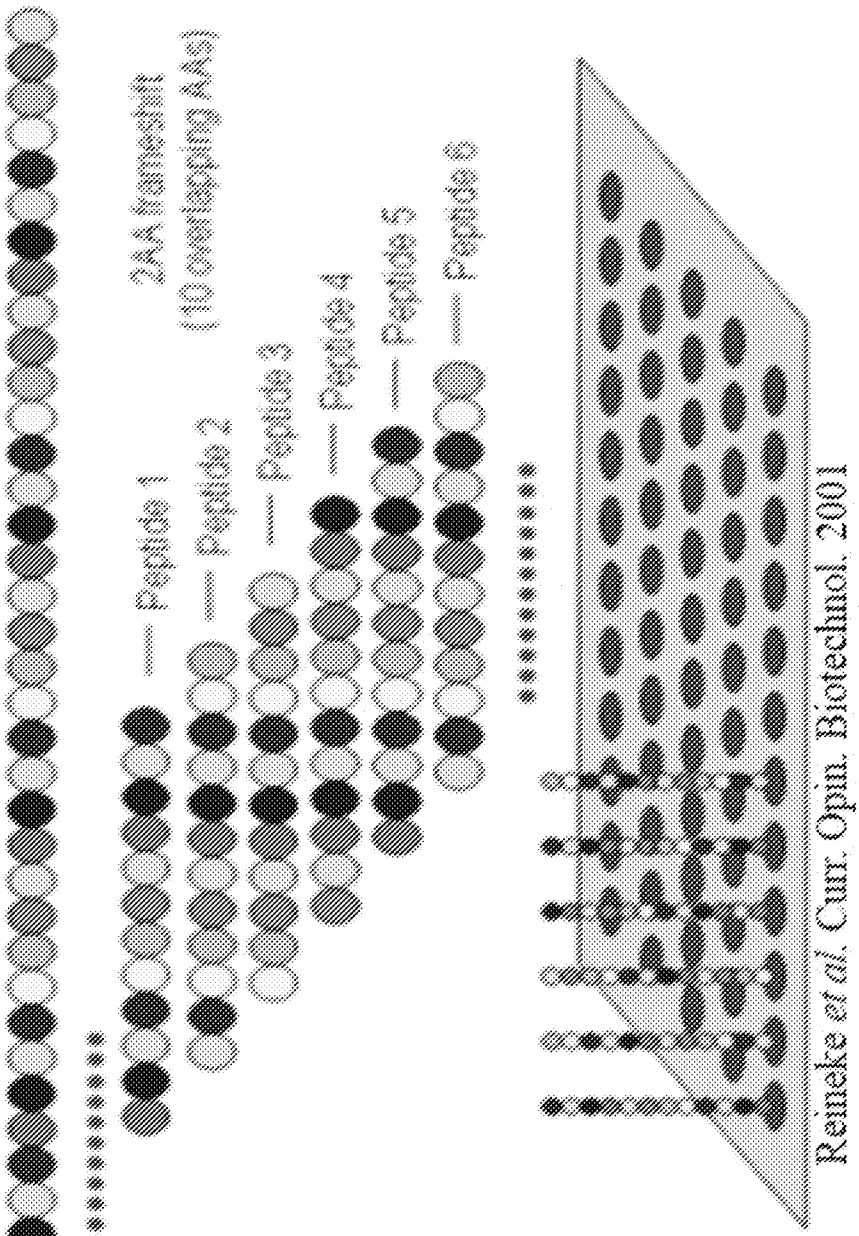

2AA transshift
(10 overlapping AAs)
Peptide 1
Peptide 2
Peptide 3
Peptide 4
Peptide 5
Peptide 6
Remeke et al. Curr. Opin. Biotechnol. 2001

Figure 1B

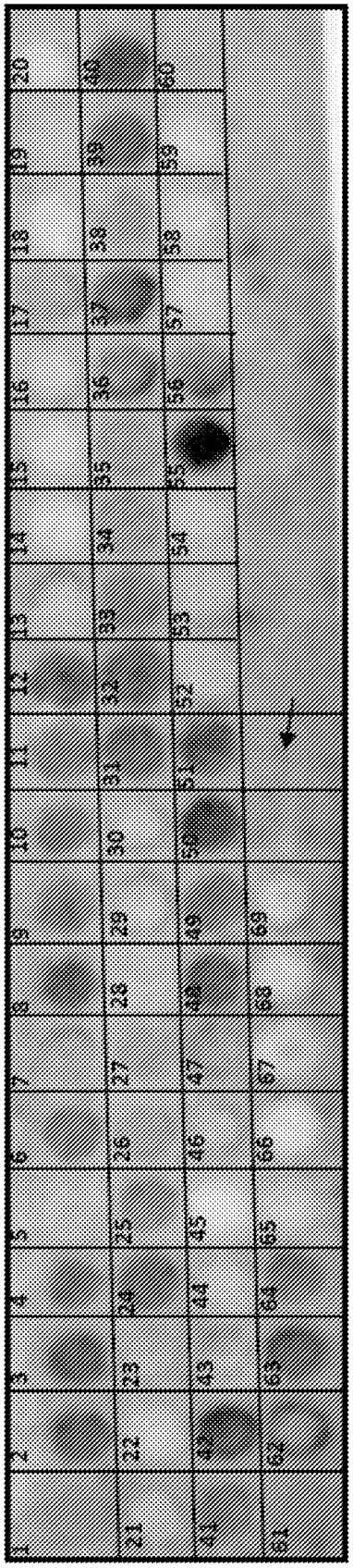

20
40
60
19
39
59
18
38
58
17
37
57
16
36
56
15
35
55
14
34
54
13
33
53
12
32
52
11
31
51
10
30
50
9
29
49
63
8
28
48
62
7
27
47
61
6
26
46
60
5
25
45
59
4
24
44
58
3
23
43
57
2
22
42
56
1
21
41
55

Figure 1C

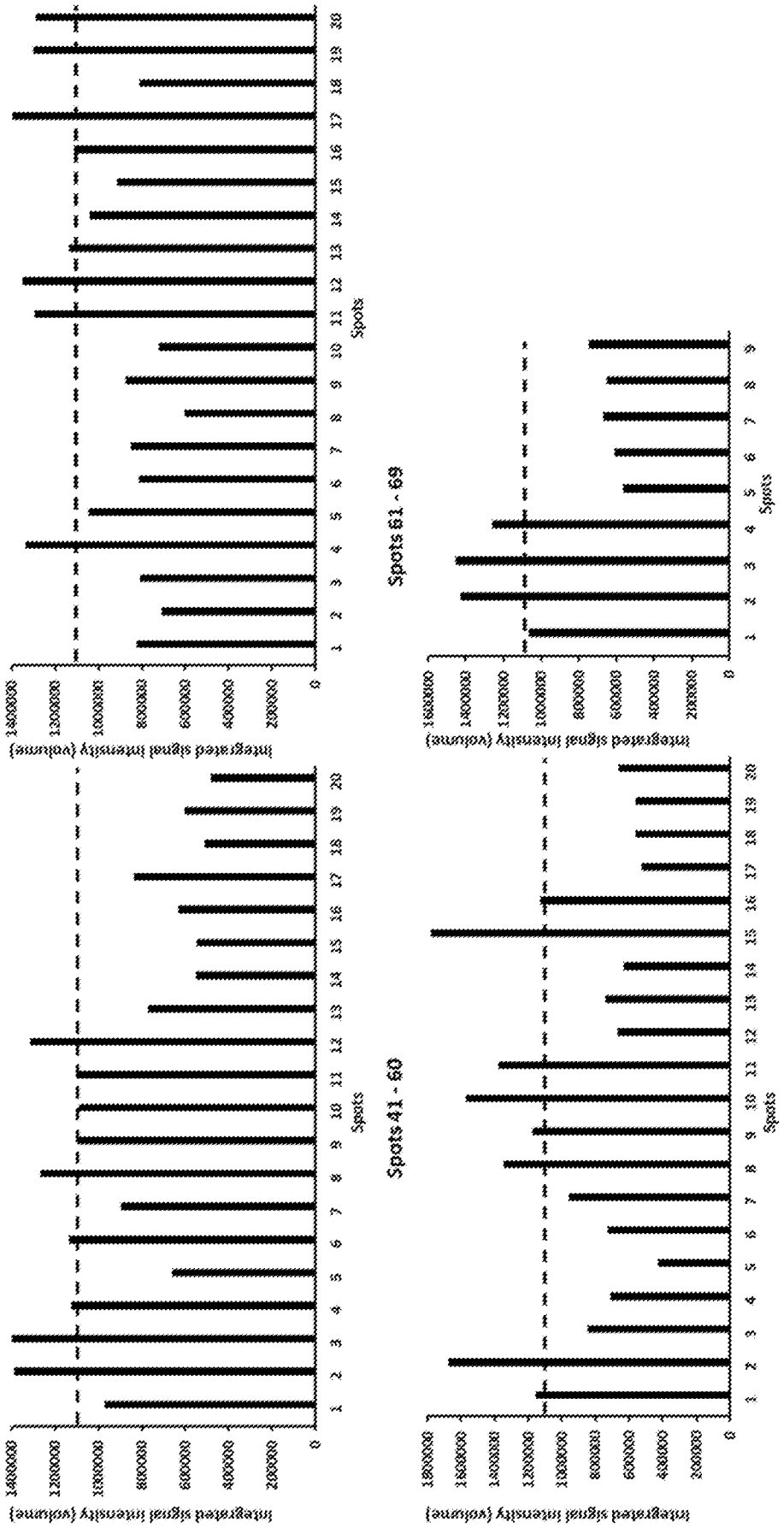

Spots 1 - 20
Spots 21 - 40
Spots 41 - 60
Spots 61 - 69
Integrated signal intensity (volume)
Spots

Figure 2A

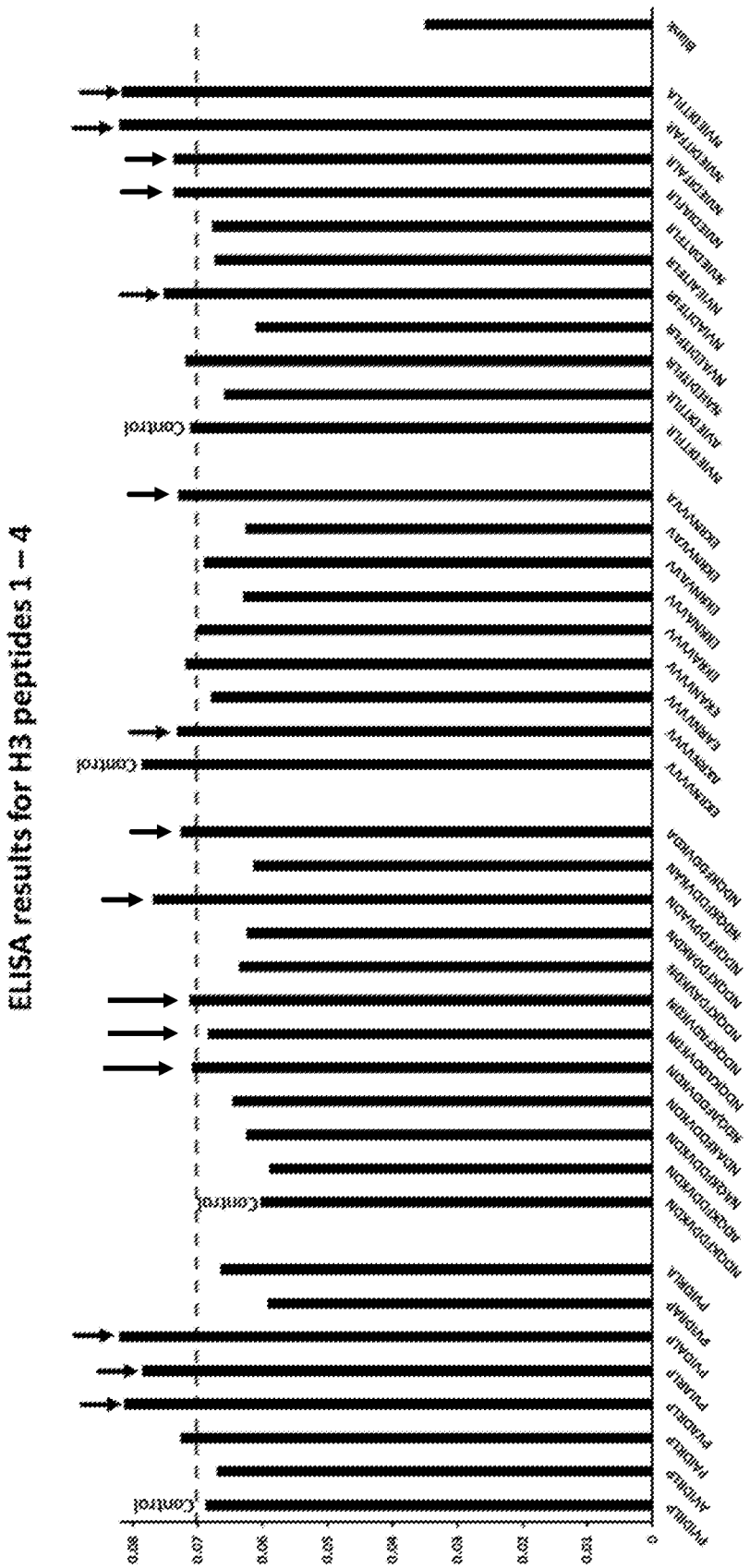

ELISA results for H3 peptides 1 – 4
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
control
control
control
control
control
Blank

Figure 2B

ELISA results for H3 peptides 5 – 9

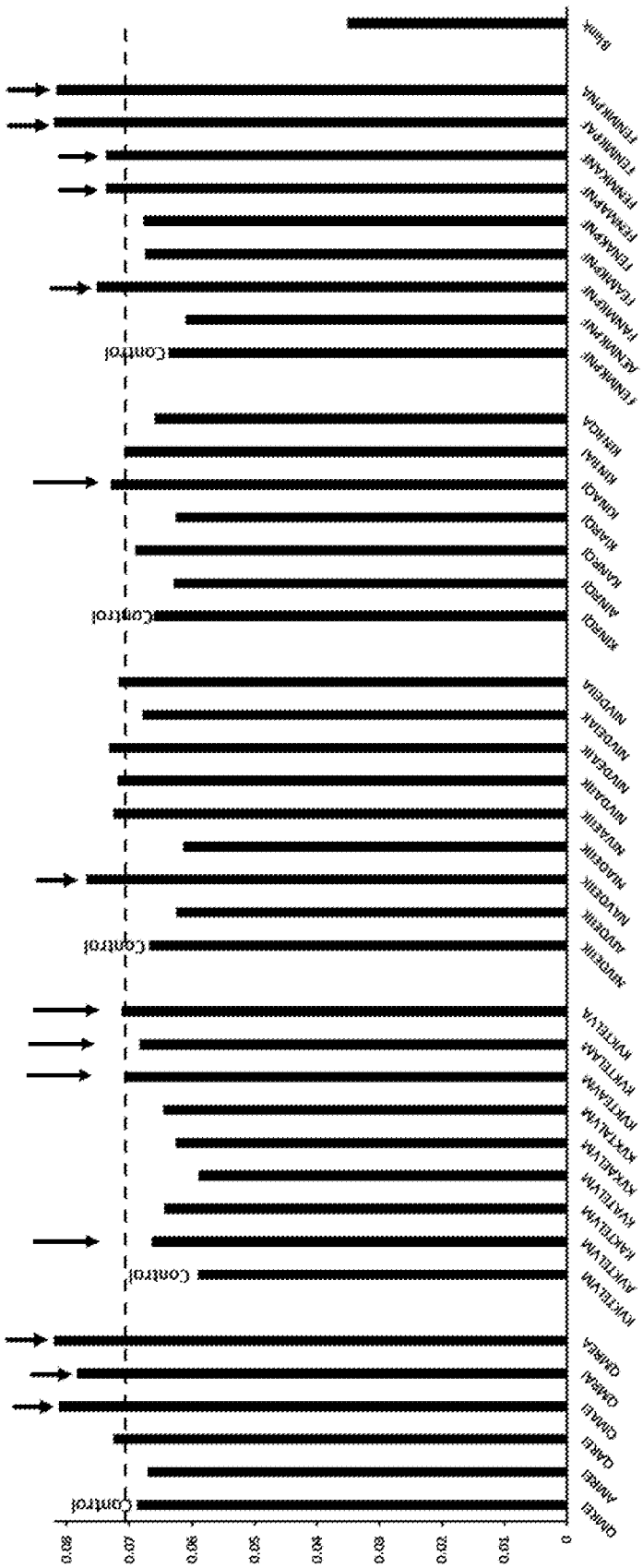


Figure 3A

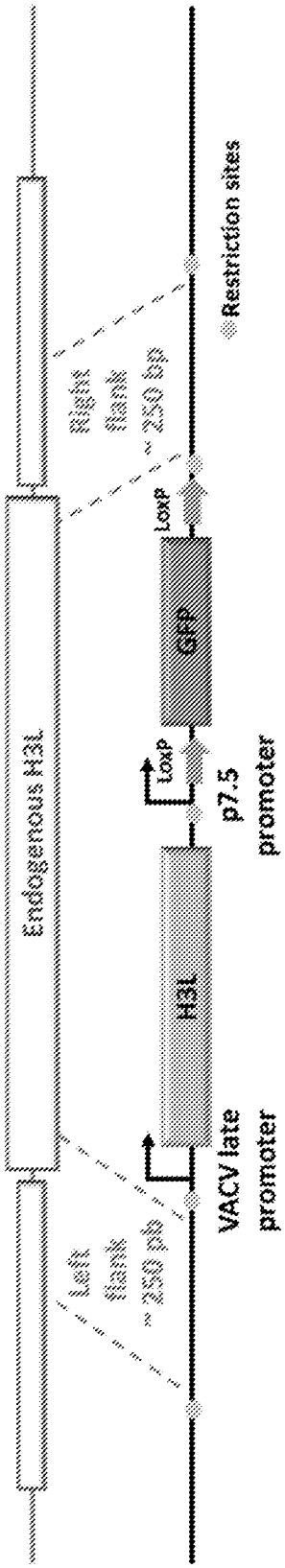

Endogenous H3L
Left flank ~ 250 pb
VACV late promoter
H3L
LoxP
p7.5 promoter
GFP
LoxP
Right flank ~ 250 bp
Restriction sites

Figure 3B

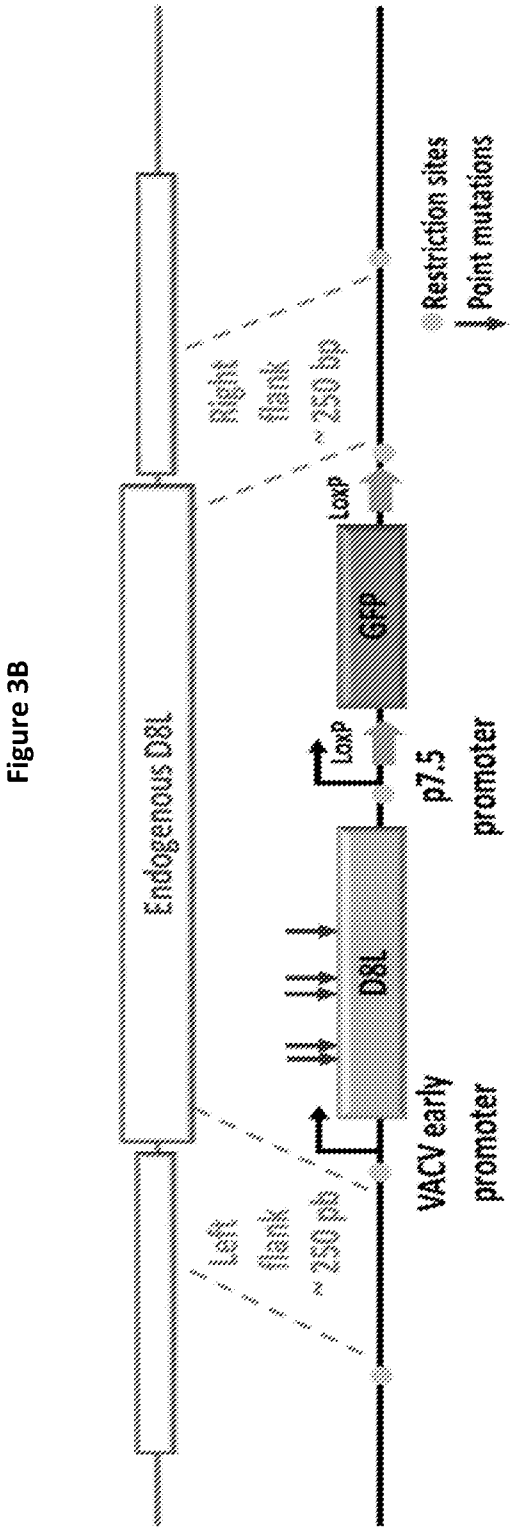

Restriction sites
Point mutations
Endogenous D8L
Left flank ~250 bp
Right flank ~250 bp
VACV early promoter
D8L
LoxP
p7.5 promoter
GFP
LoxP

Figure 3C

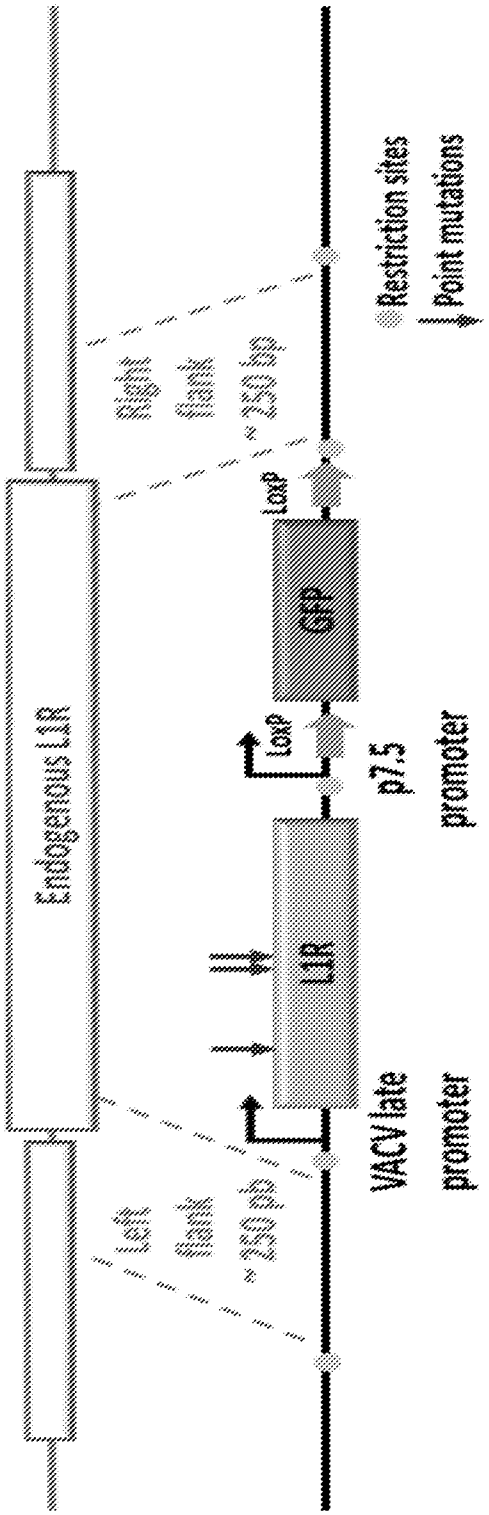
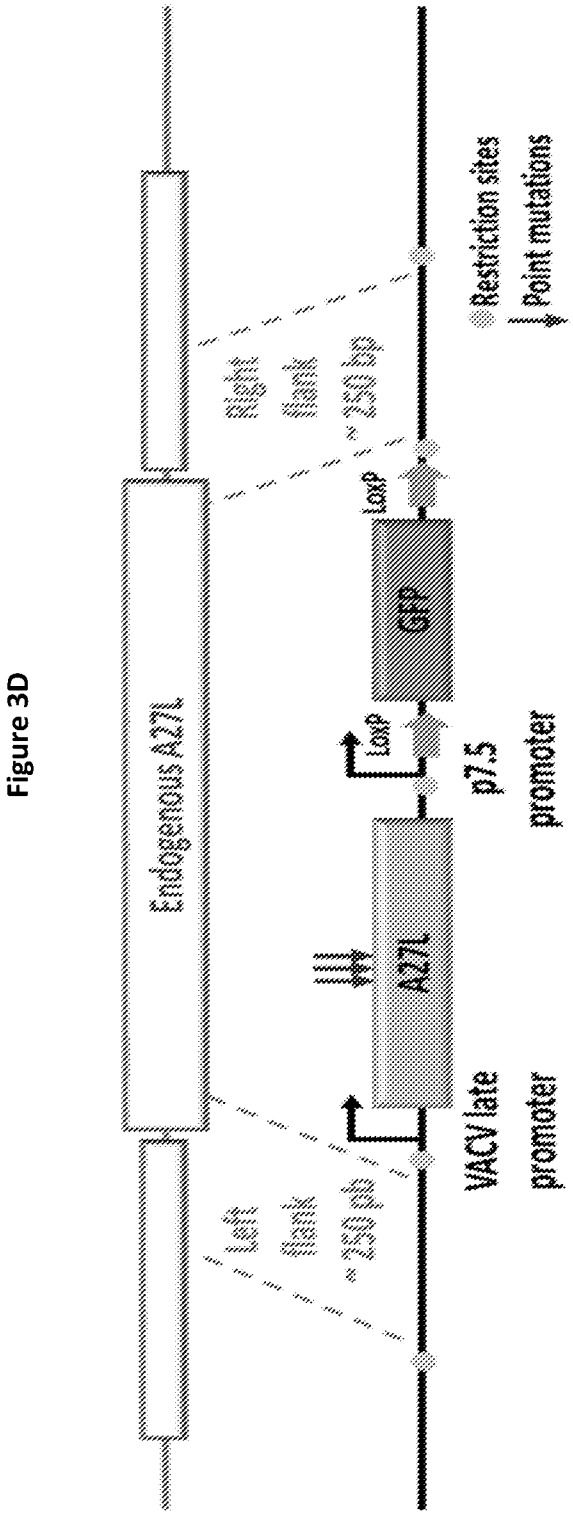

Endogenous L1R
Left flank ~250 bp
VACV late promoter
L1R
p7.5 promoter
LoxP
GFP
LoxP
Right flank ~250 bp
Restriction sites
Point mutations

Figure 3D


Restriction sites
Point mutations
Right flank ~750 bp
Left flank ~750 nb
Endogenous A27L
LoxP
GFP
LoxP
p7.5 promoter
A27L
VACV late promoter

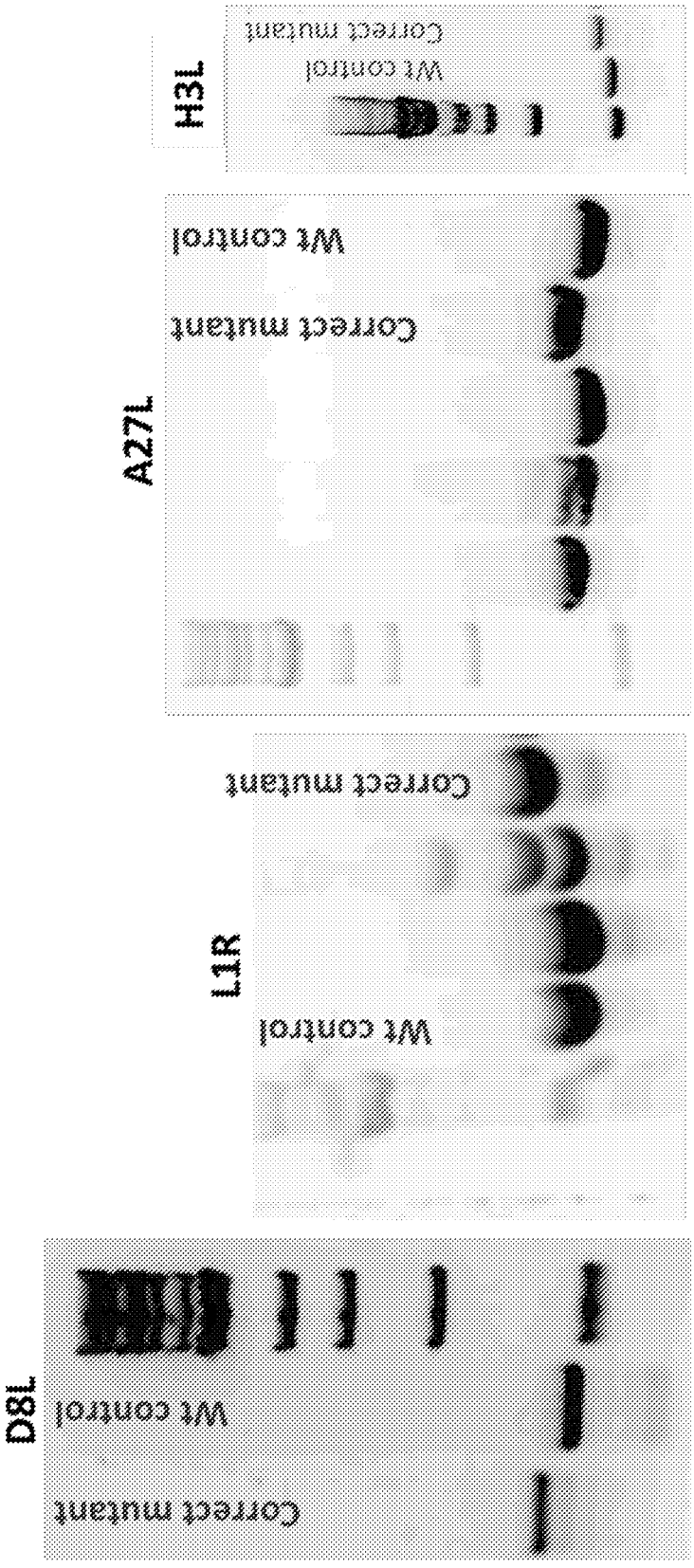

H3L
Wt control
Correct mutant
A27L
Wt control
Correct mutant
L1R
Wt control
Correct mutant
D8L
Wt control
Correct mutant

Figure 4

Figure 5

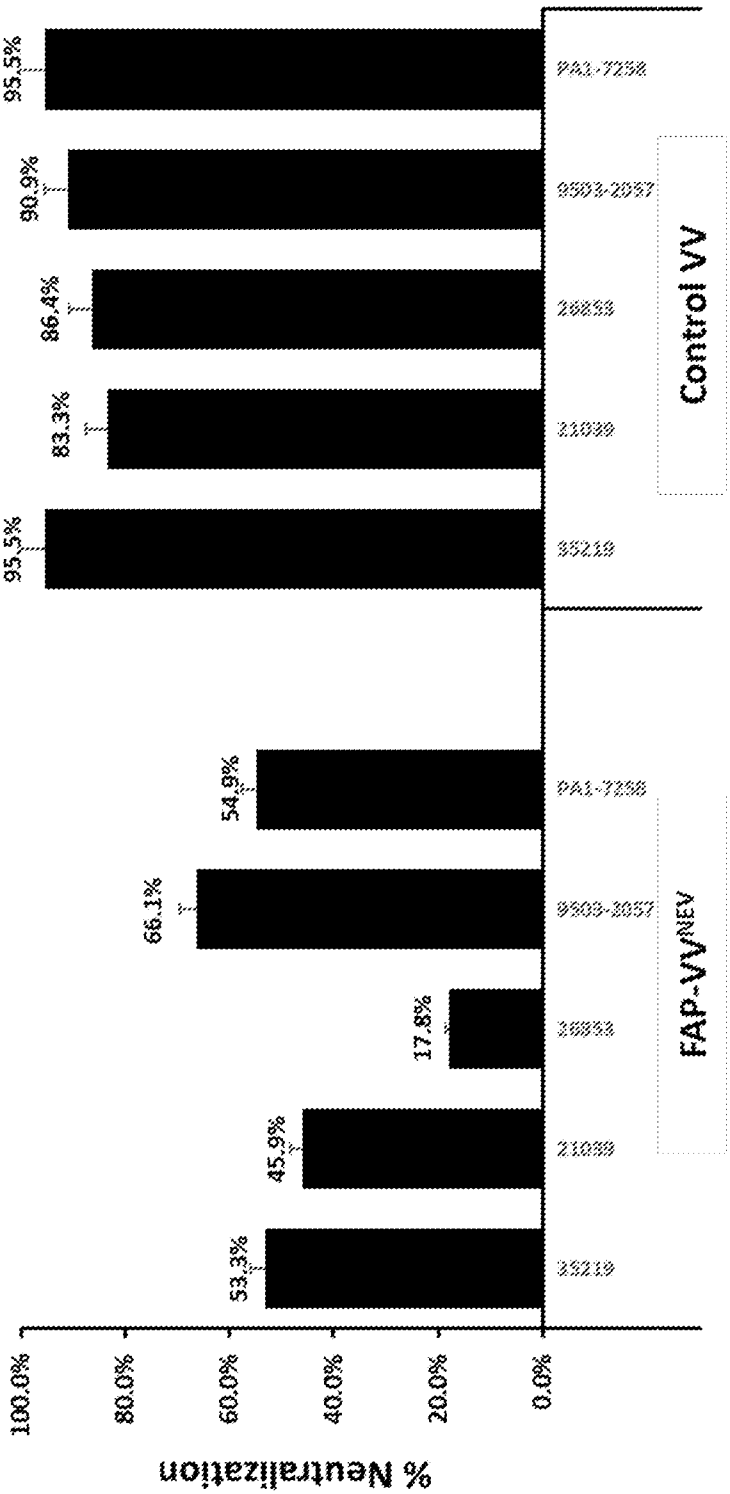

% Neutralization
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
53.3%
45.9%
17.8%
66.1%
54.8%
95.5%
83.3%
86.4%
90.9%
95.5%
35219
21009
28853
9503-2057
PA1-7258
FAP-VV^NEV
Control VV

Figure 6

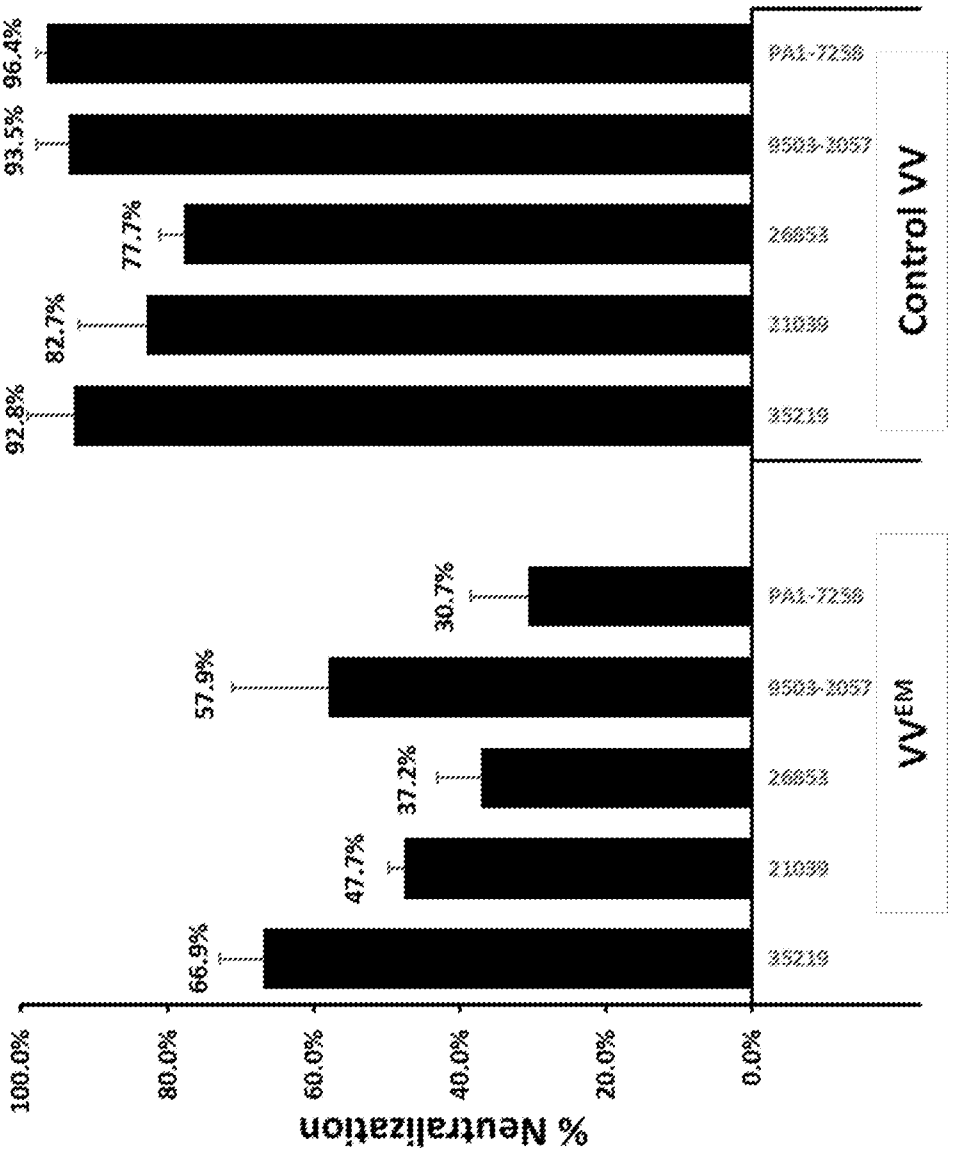

% Neutralization
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
66.9%
47.7%
37.2%
57.9%
30.7%
92.8%
82.7%
77.7%
93.5%
96.4%
35219
21009
26853
9503-3057
PA1-7238
VV^EM
Control VV

Figure 7

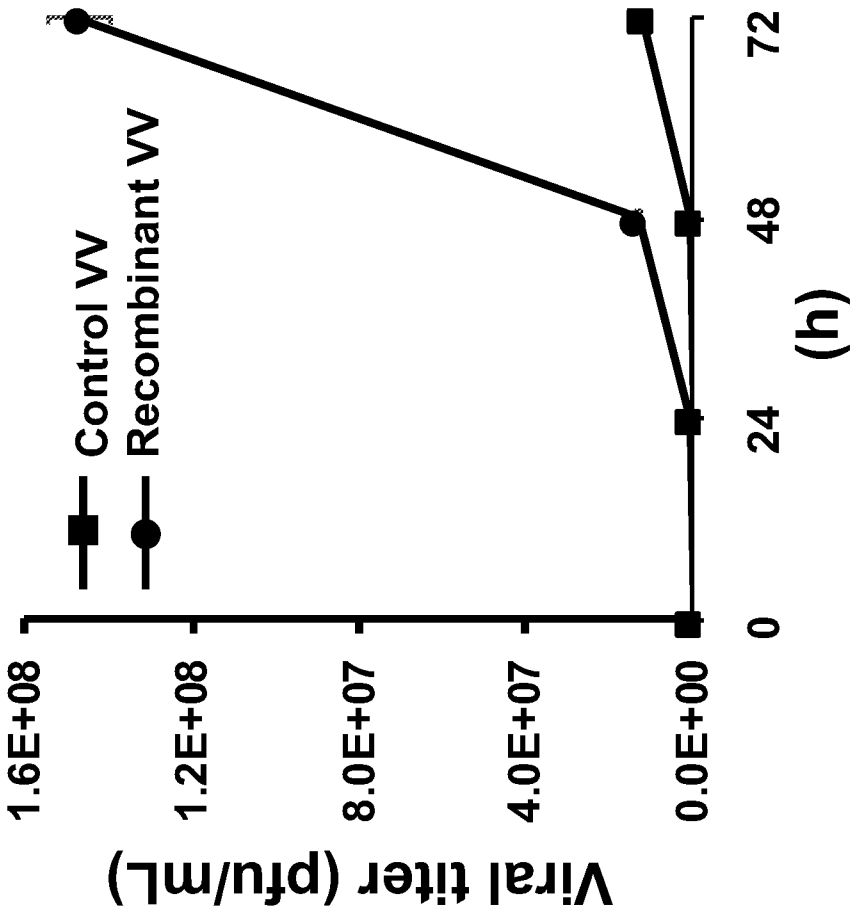

Viral titer (pfu/mL)
1.6E+08
1.2E+08
8.0E+07
4.0E+07
0.0E+00
0
24
48
72
(h)
Control VV
Recombinant VV

Figure 8

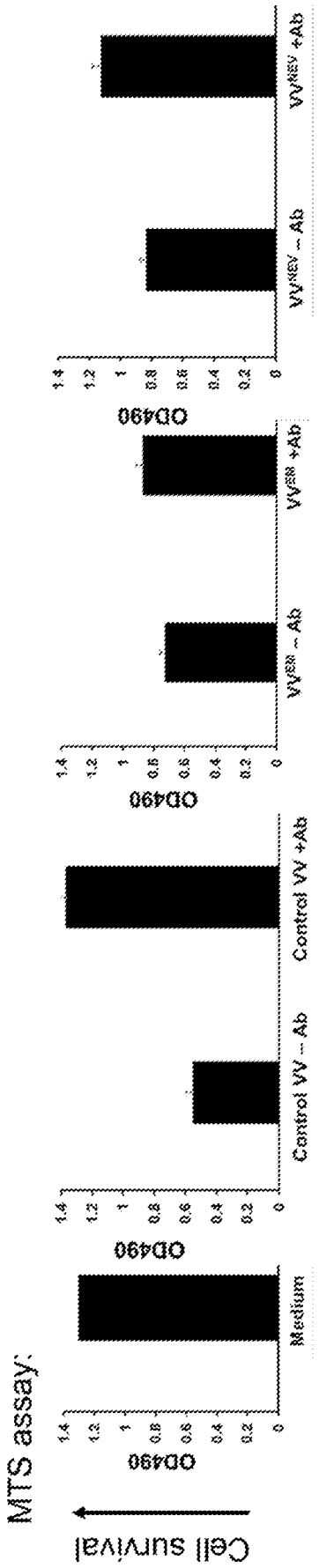

MTS assay:
Cell survival
OD490
Medium
Control VV – Ab
Control VV +Ab
VV^ER – Ab
VV^ER +Ab
VV^NEV – Ab
VV^NEV +Ab

Figure 9

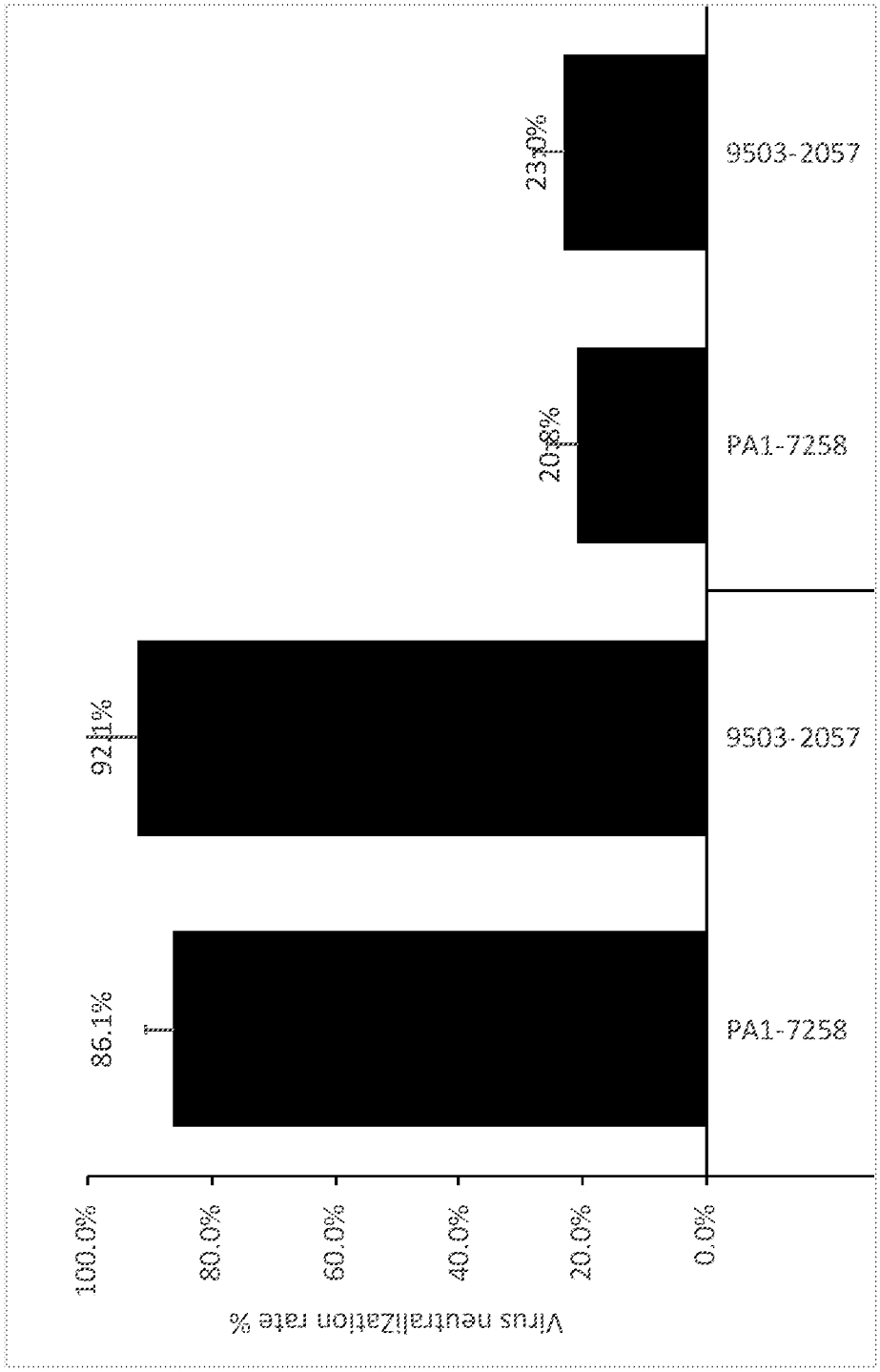

Virus neutralization rate %
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
86.1%
PA1-7258
92.1%
9503-2057
Wild type VV.
20.8%
PA1-7258
23.0%
9503-2057
VVNEV

Figure 10

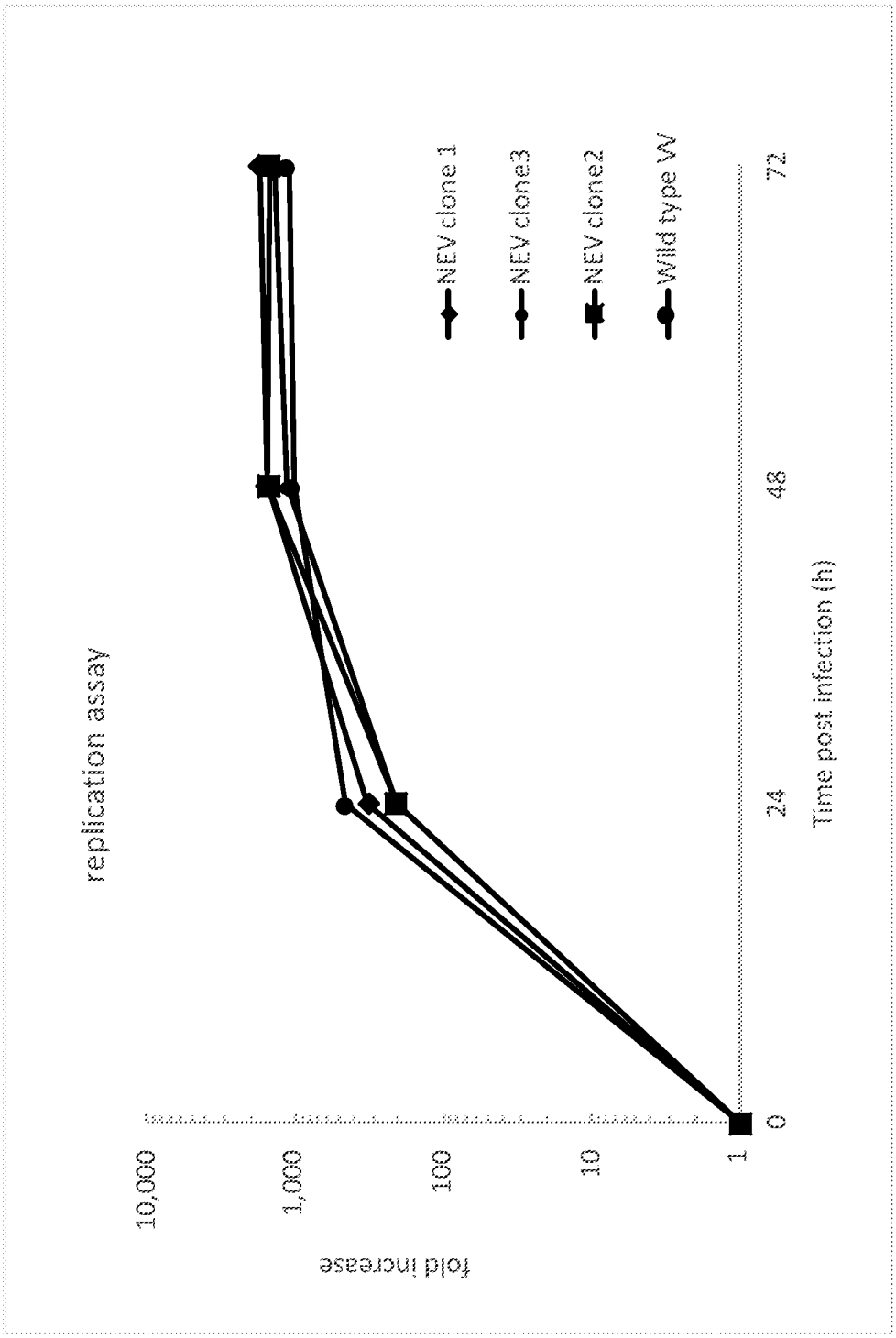
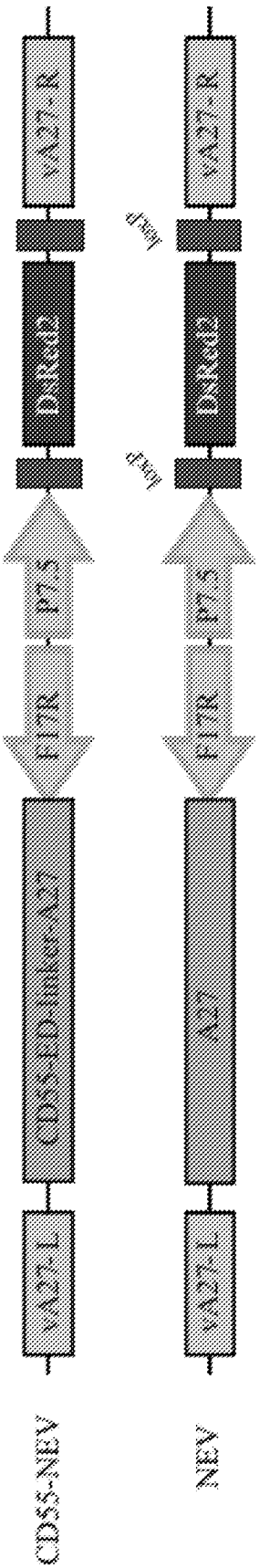

replication assay
fold increase
10,000
1,000
100
10
1
0
24
48
72
Time post infection (h)
NEV clone 1
NEV clone3
NEV clone2
Wild type W

Figure 11


CD55-NEV
NEV
hA27-L
CD55-LD linker-A27
A27
F-ITR
P7.5
DsRed2
hA27-R

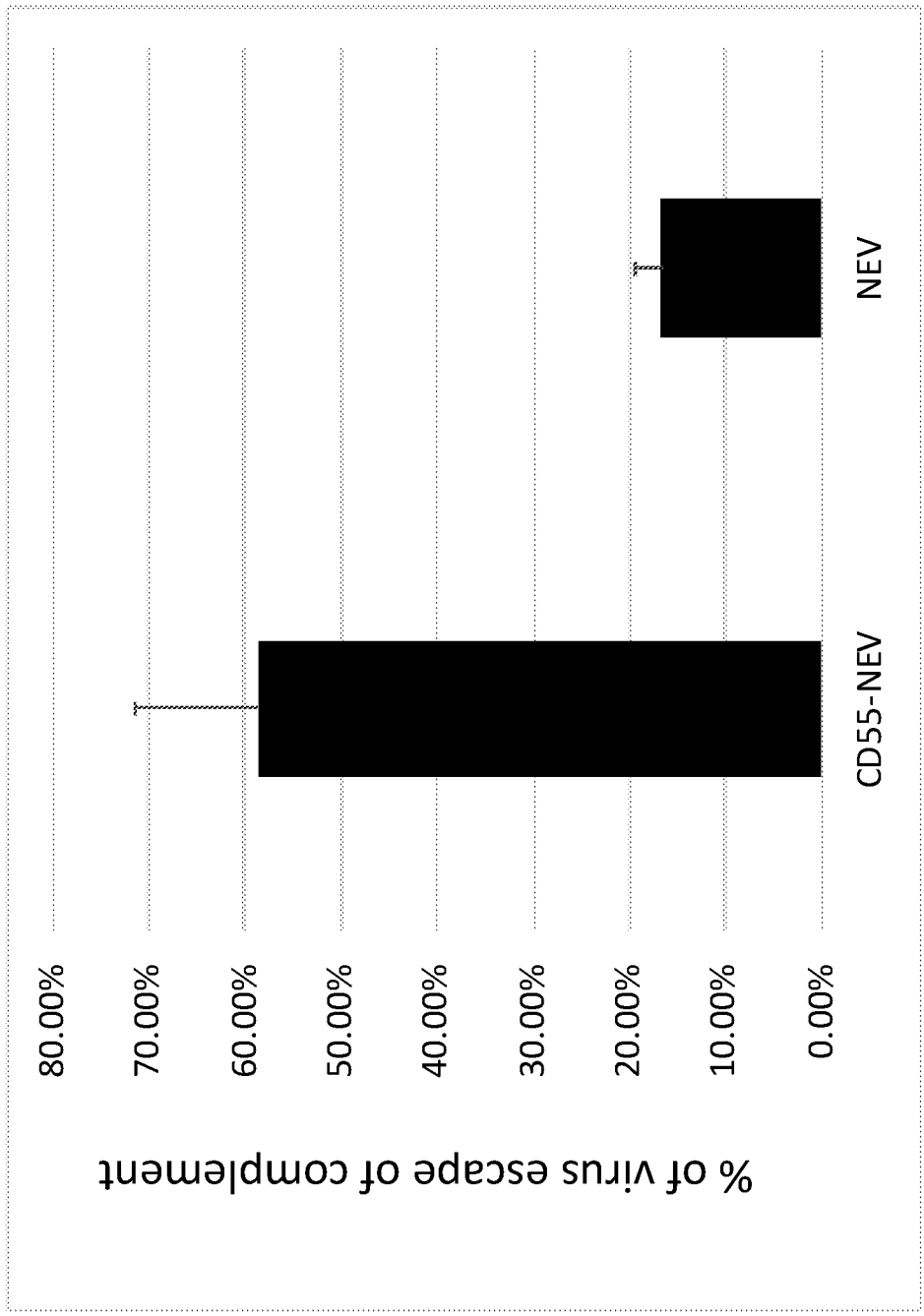

% of virus escape of complement
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
CD55-NEV
NEV

Figure 12

Figure 13

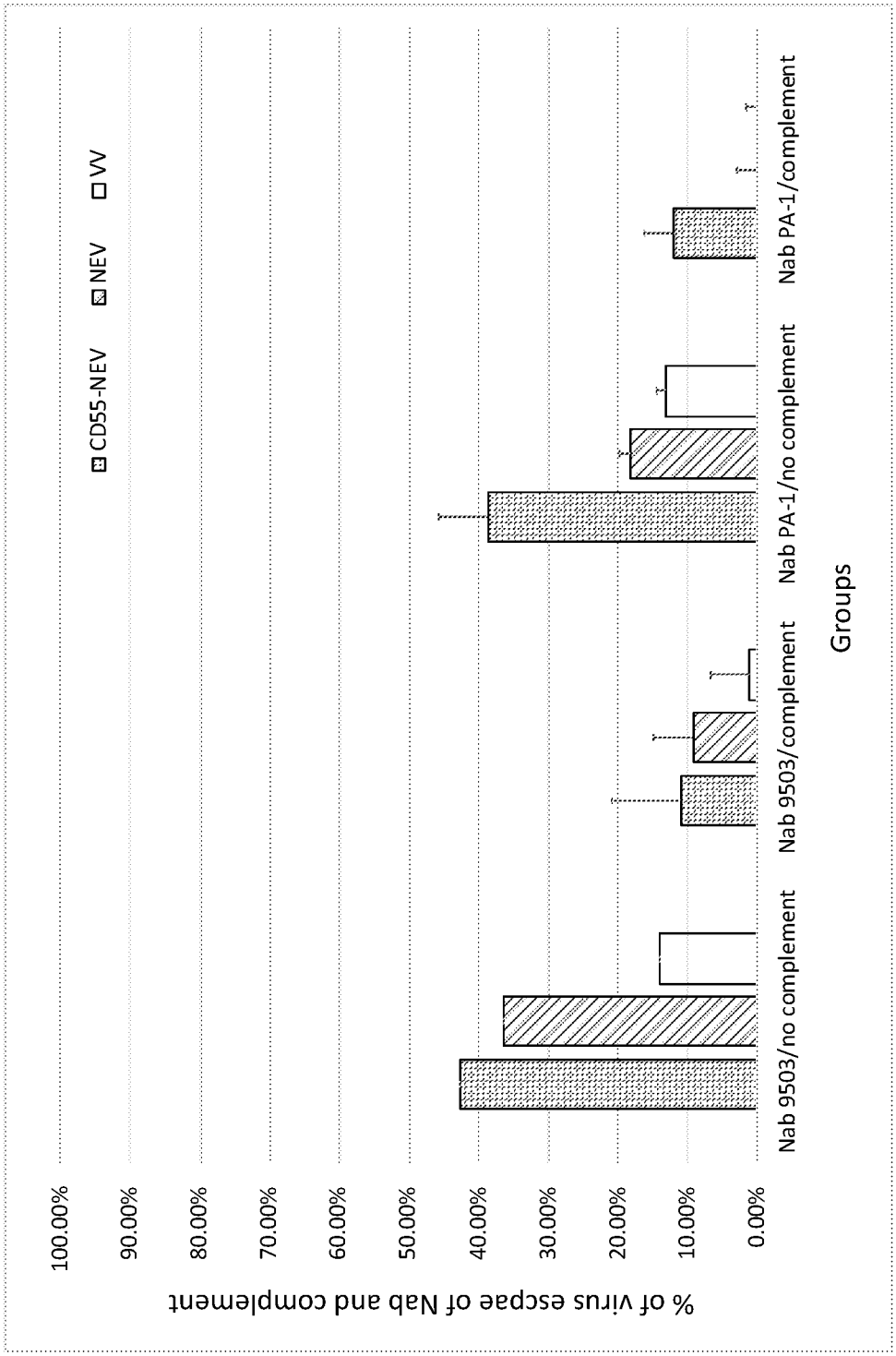
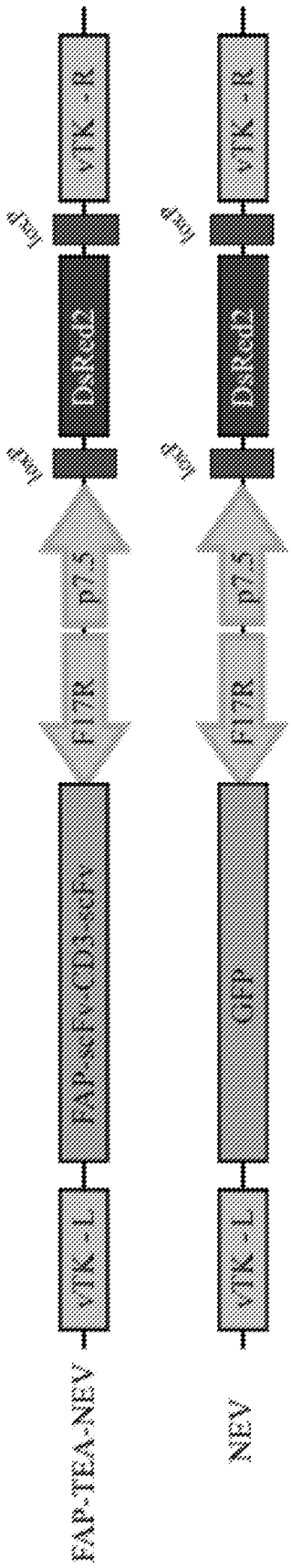

% of virus escape of Nab and complement
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
CD55-NEV
NEV
VV
Nab 9503/no complement
Nab 9503/complement
Nab PA-1/no complement
Nab PA-1/complement
Groups

Figure 14


VTK -R
linker
DARPin
linker
FITR
p2.5
FAP-scFv-CD3-scFv
VTK -L
FAP-TEA-NEV
VTK -R
linker
DARPin
linker
FITR
p2.5
GFP
VTK -L
NEV

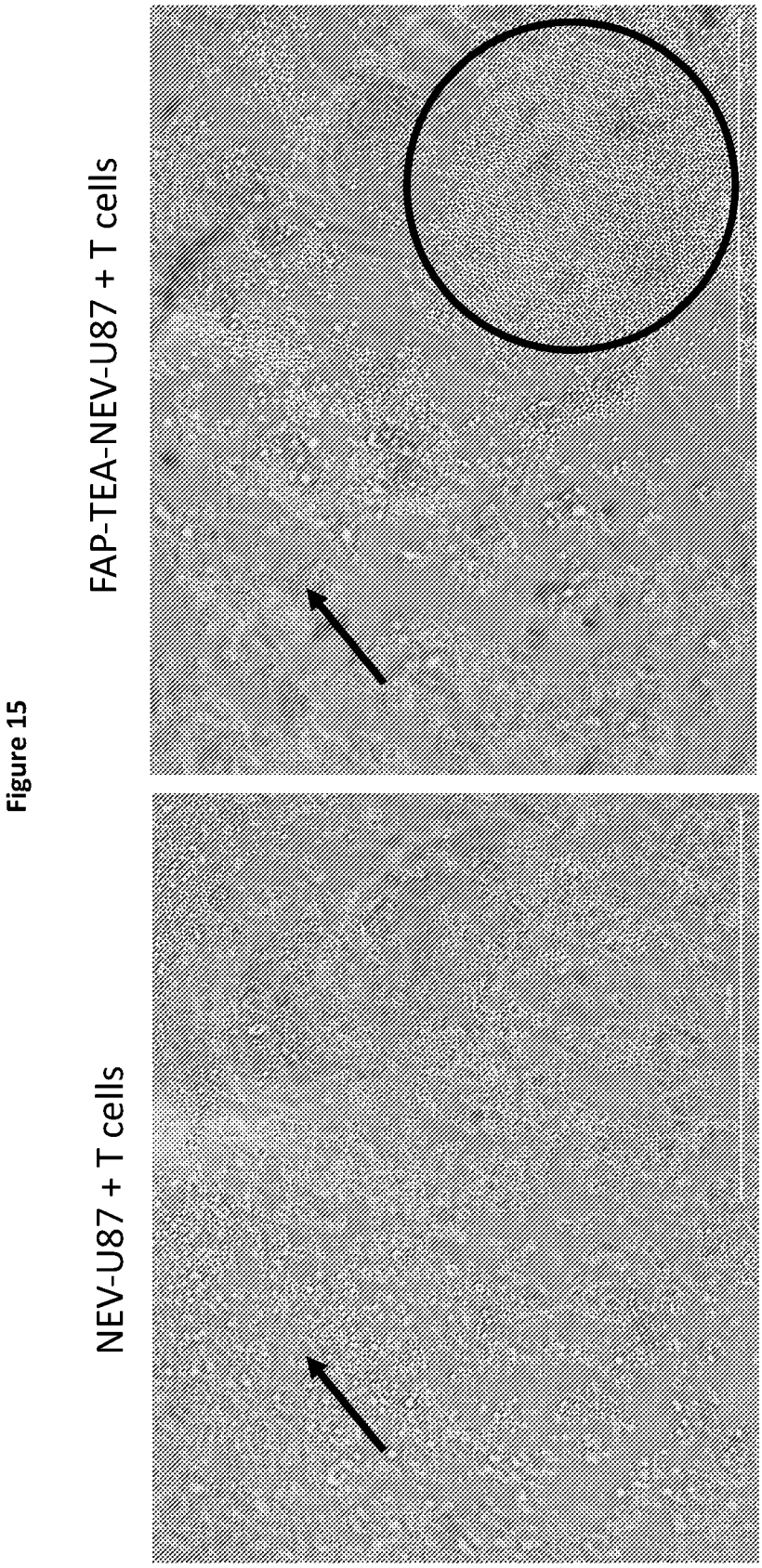


Figure 15

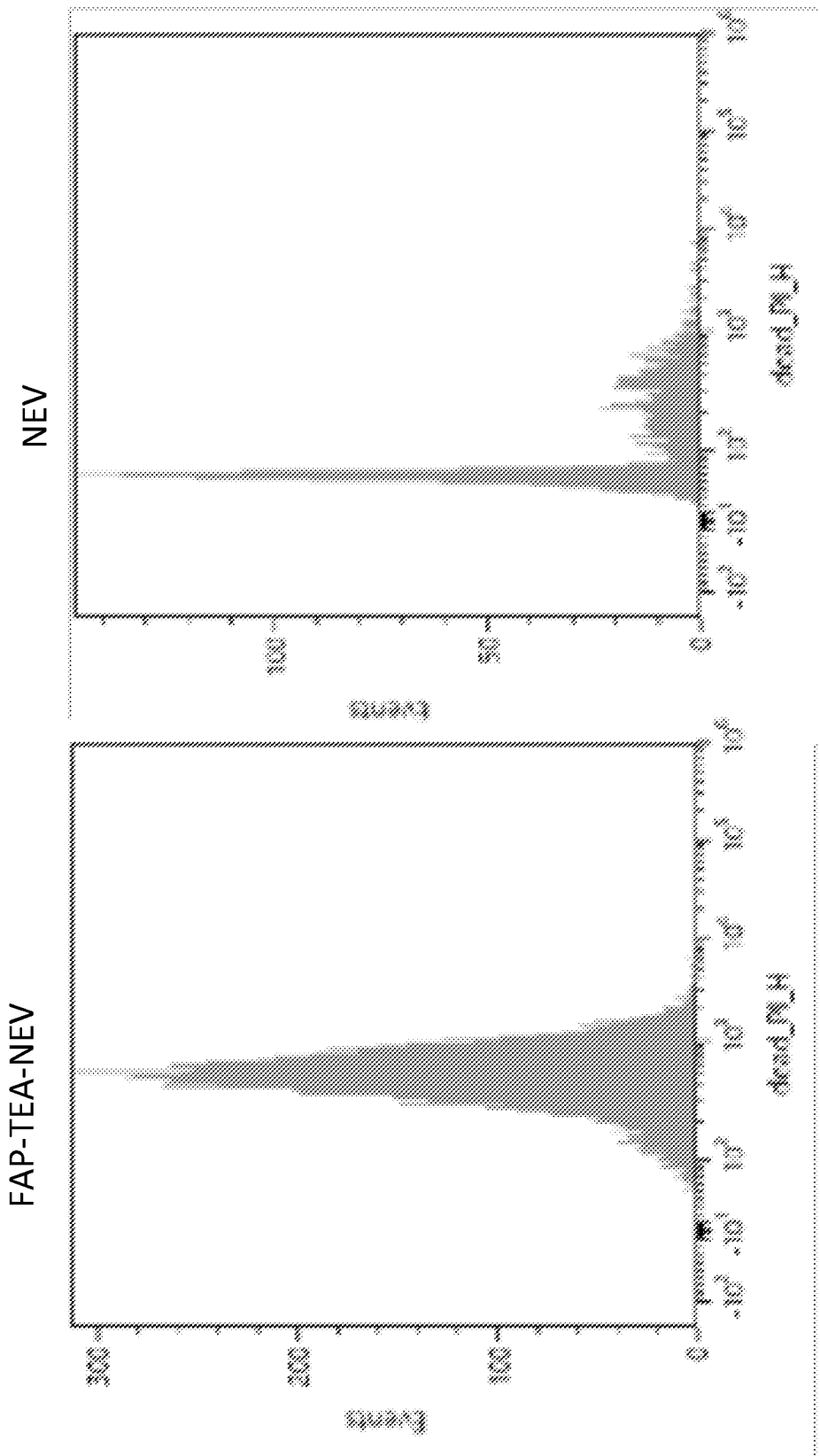


Figure 16

Figure 17

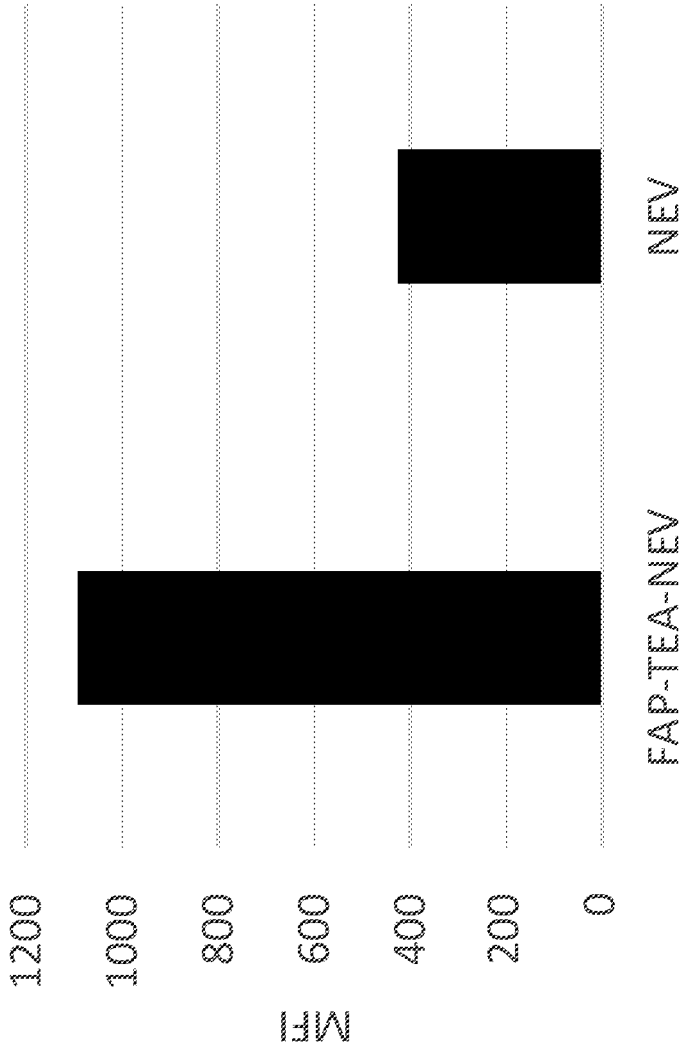
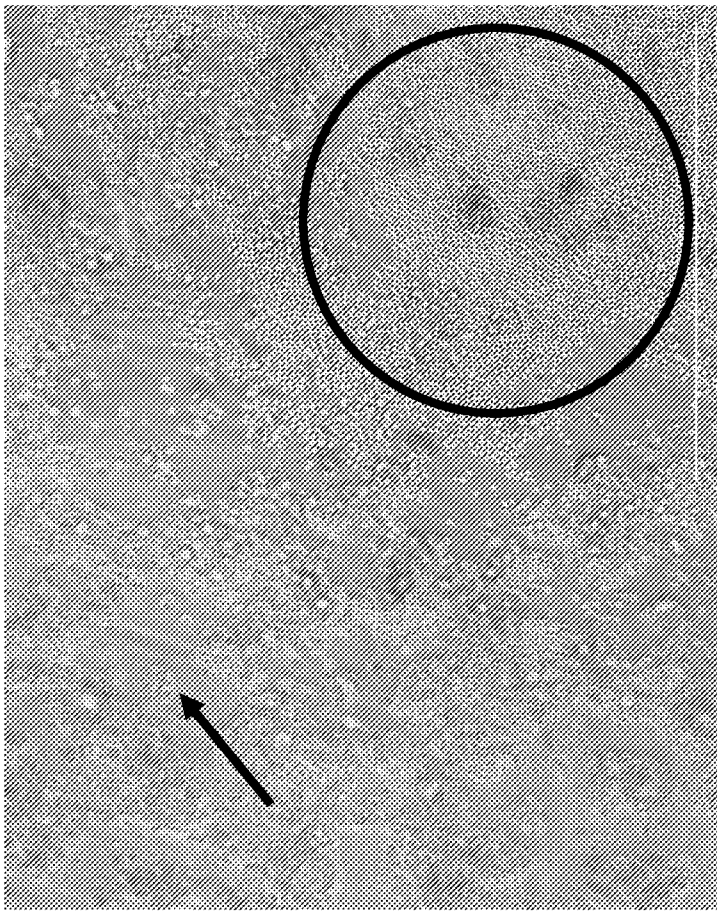


Figure 18



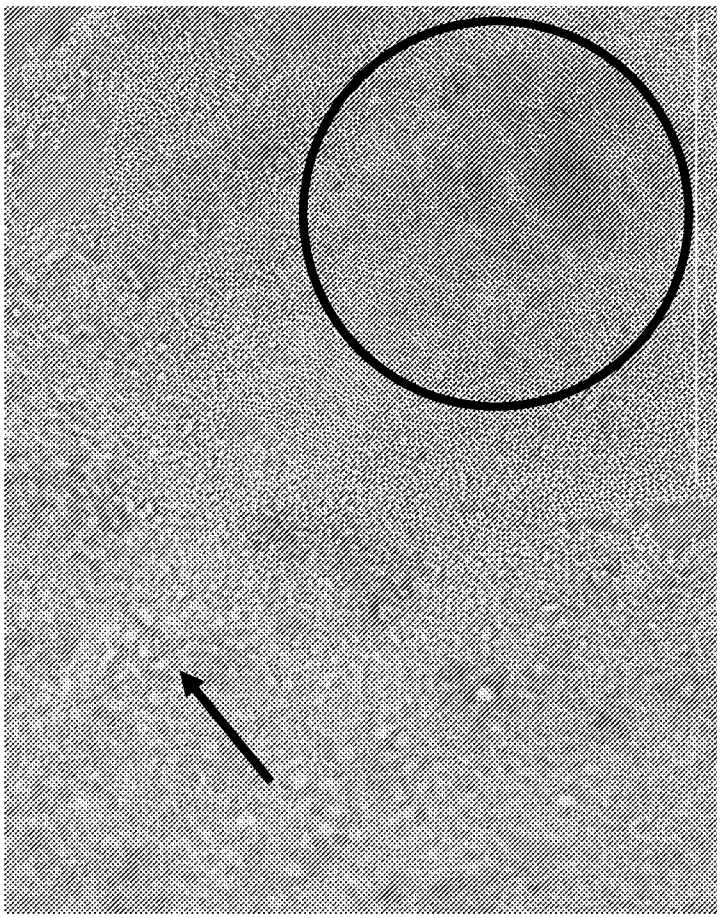


Figure 19

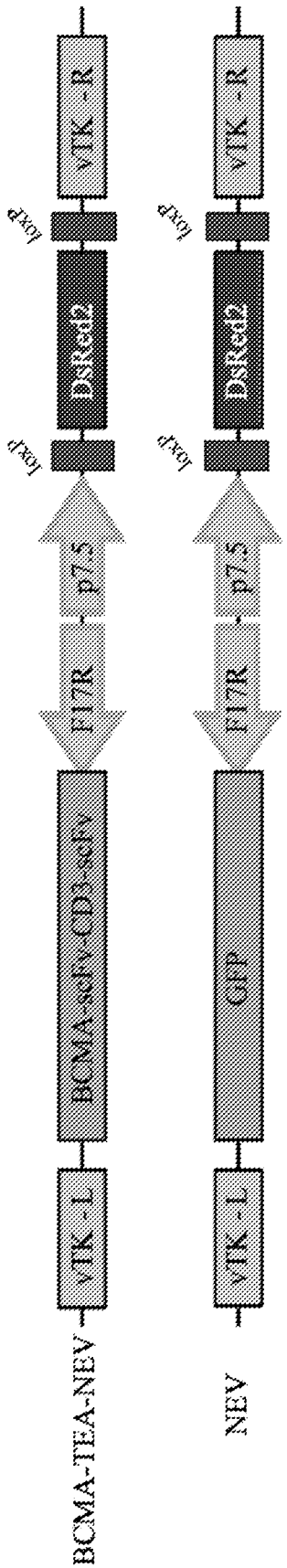

BCMA-TEA-NEV
NEV
vTK -L
BCMA-scfv-CD3-scfv
GFP
F17R
p7.5
loxP
DsRed2
loxP
vTK -R

Figure 20 A

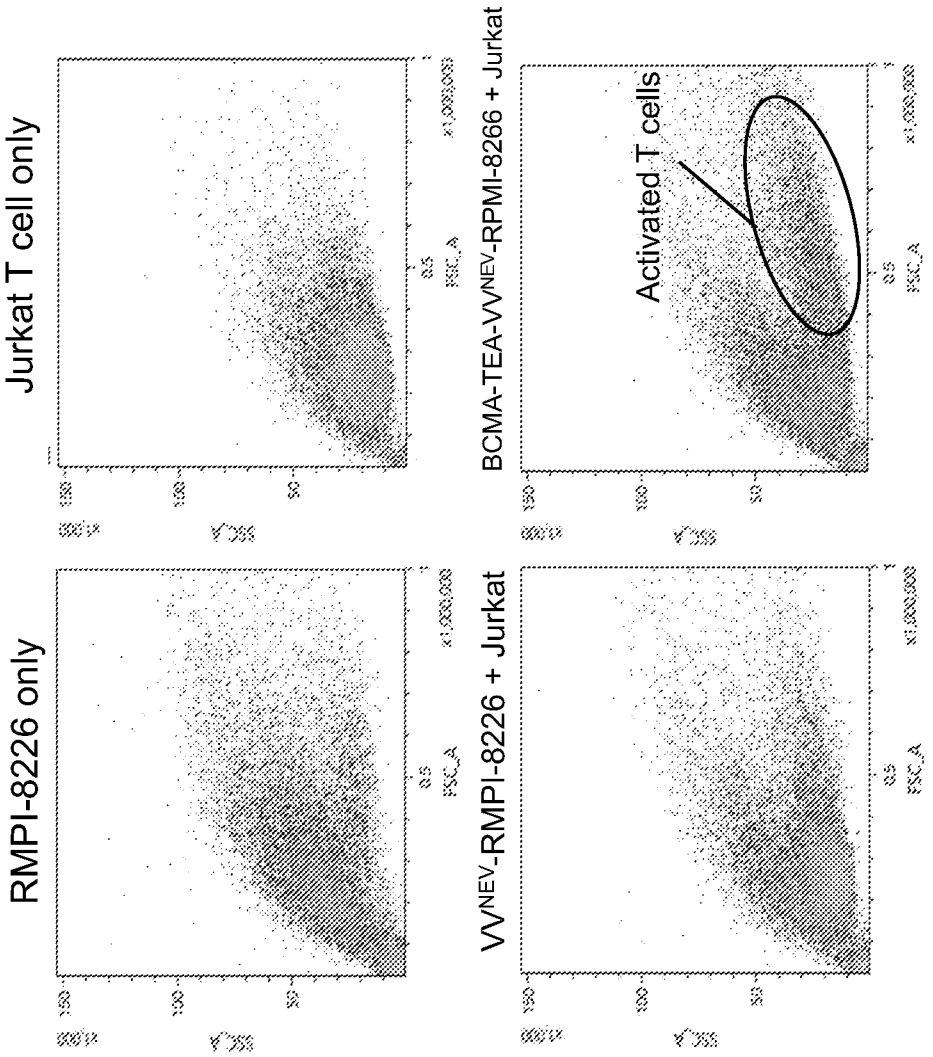

Jurkat T cell only
RMPI-8226 only
BCMA-TEA-VV^{NEV}-RPMI-8266 + Jurkat
VV^{NEV}-RMPI-8226 + Jurkat
Activated T cells
SSC_A
FSC_A

Figure 20 B

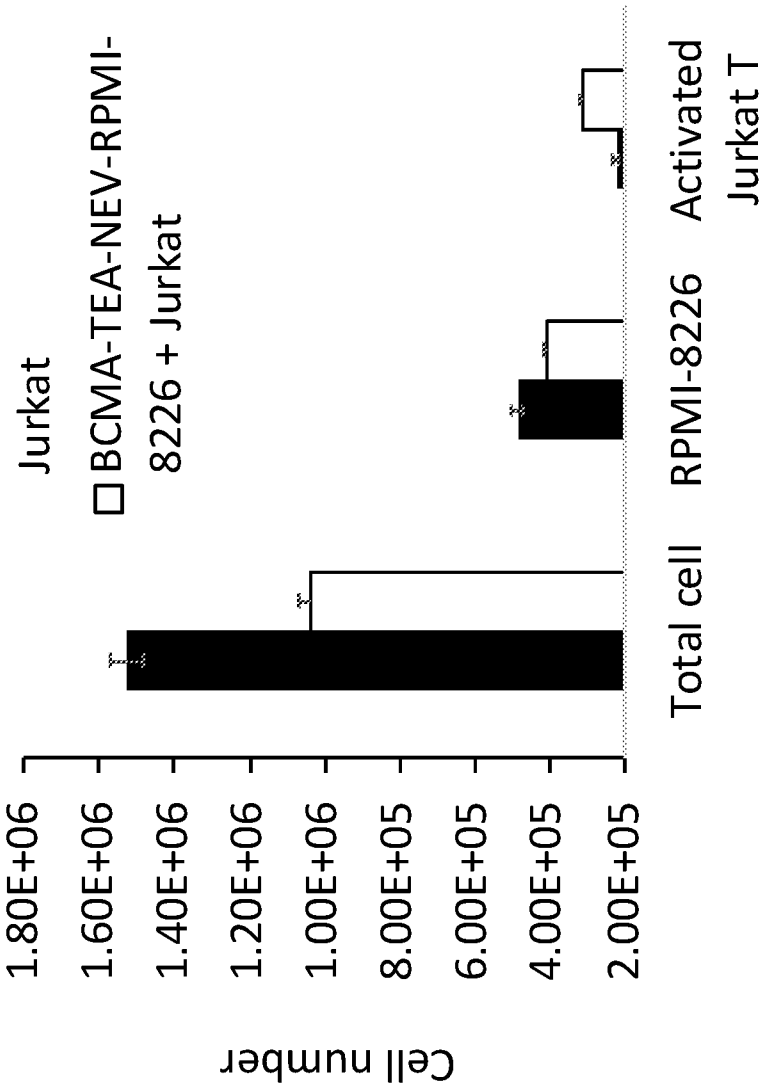

NEV-RPMI-8226 + Jurkat
BCMA-TEA-NEV-RPMI-8226 + Jurkat
Cell number
1.80E+06
1.60E+06
1.40E+06
1.20E+06
1.00E+06
8.00E+05
6.00E+05
4.00E+05
2.00E+05
Total cell
RPMI-8226
Activated Jurkat T

Figure 21 A

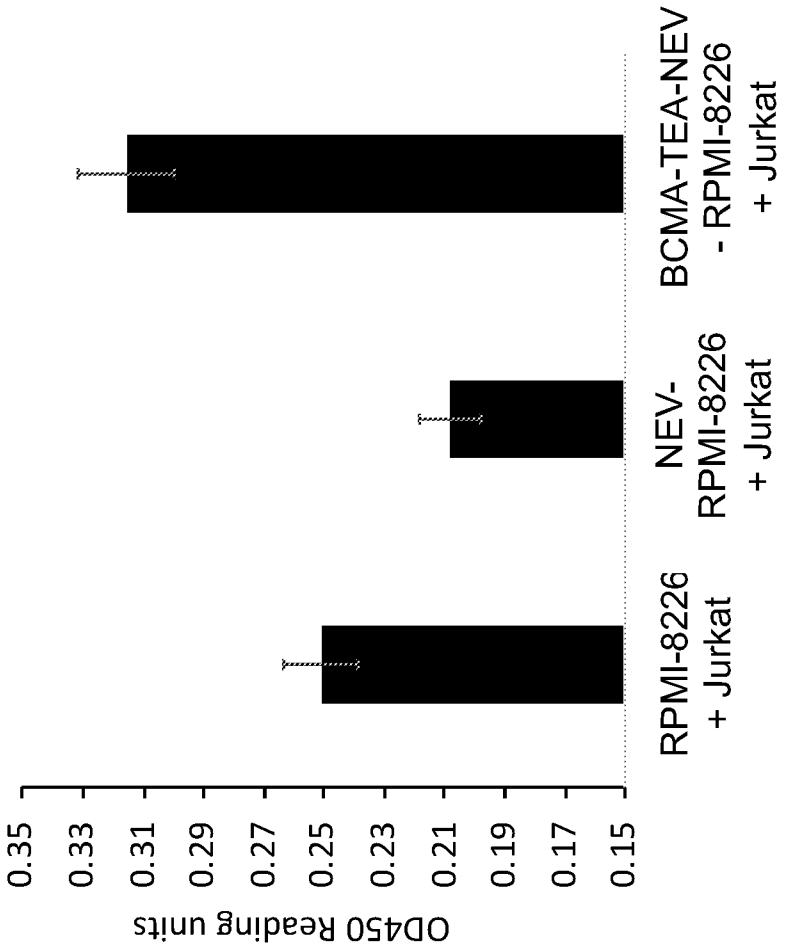

IFNg
OD450 Reading units
0.35
0.33
0.31
0.29
0.27
0.25
0.23
0.21
0.19
0.17
0.15
RPMI-8226 + Jurkat
NEV- RPMI-8226 + Jurkat
BCMA-TEA-NEV - RPMI-8226 + Jurkat

Figure 21 B

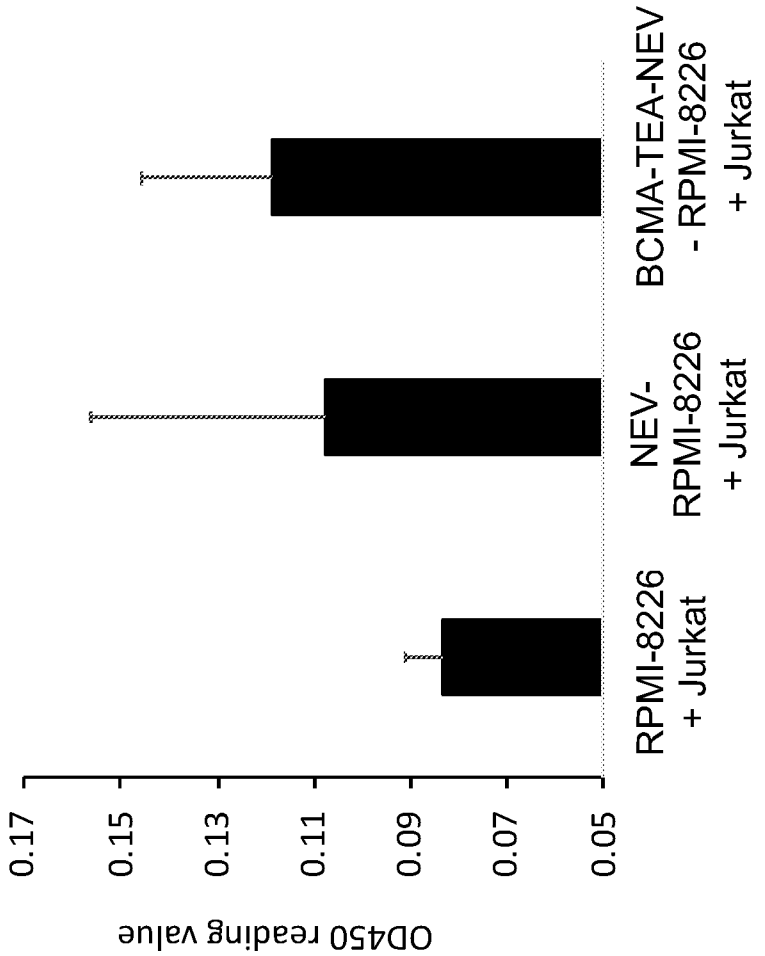

IL-2
OD450 reading value
0.17
0.15
0.13
0.11
0.09
0.07
0.05
RPMI-8226 + Jurkat
NEV- RPMI-8226 + Jurkat
BCMA-TEA-NEV - RPMI-8226 + Jurkat

Figure 22

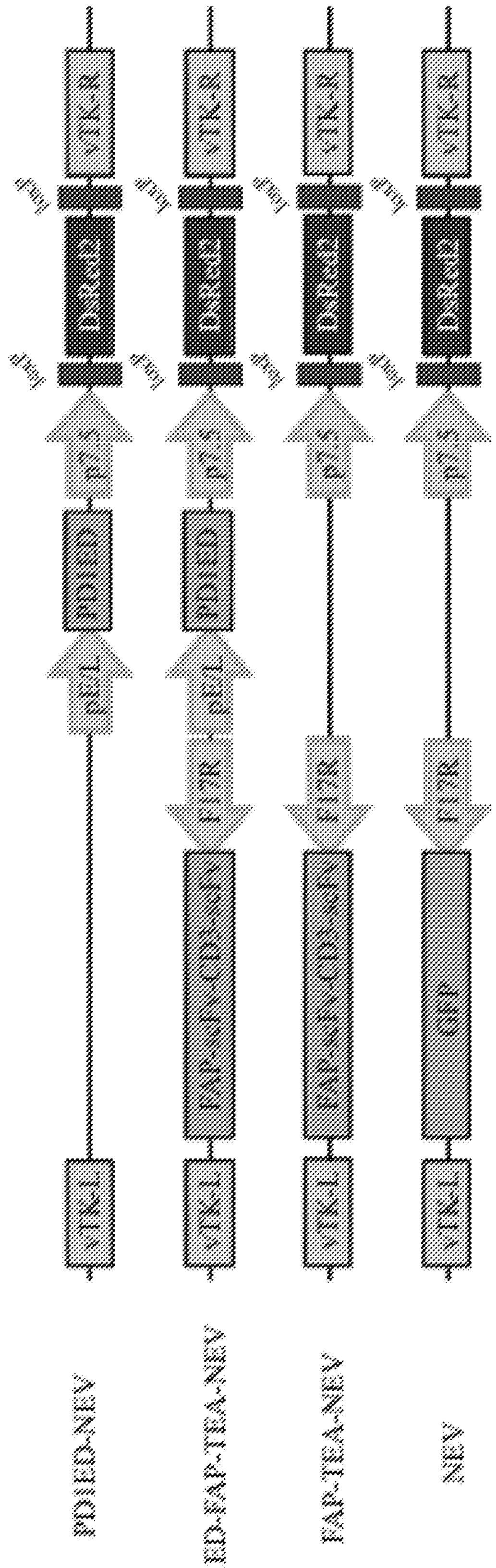
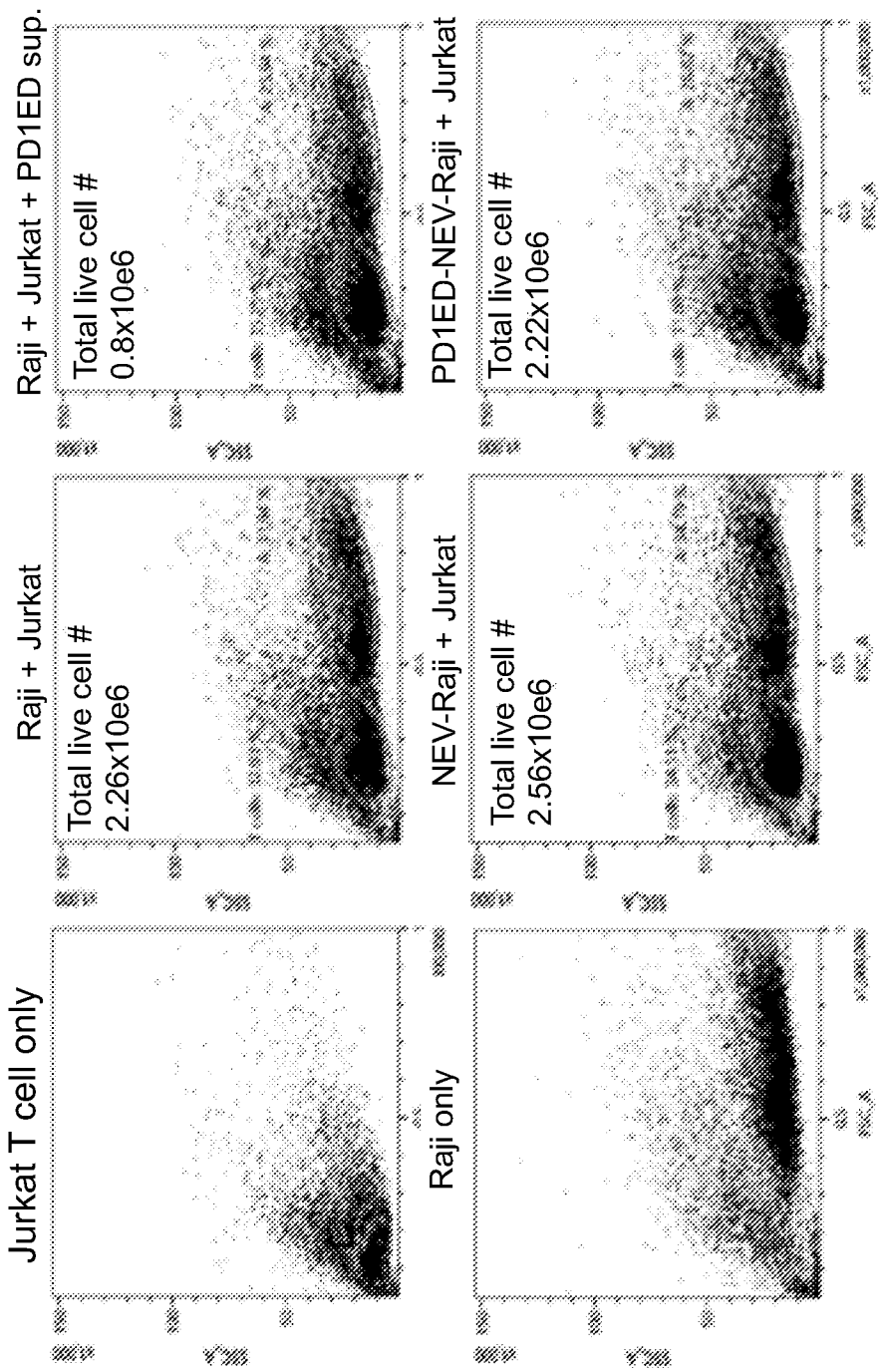


Figure 23 A


Jurkat T cell only
Raji only
Raji + Jurkat
Total live cell #
2.26x10e6
NEV-Raji + Jurkat
Total live cell #
2.56x10e6
Raji + Jurkat + PD1ED sup.
Total live cell #
0.8x10e6
PD1ED-NEV-Raji + Jurkat
Total live cell #
2.22x10e6

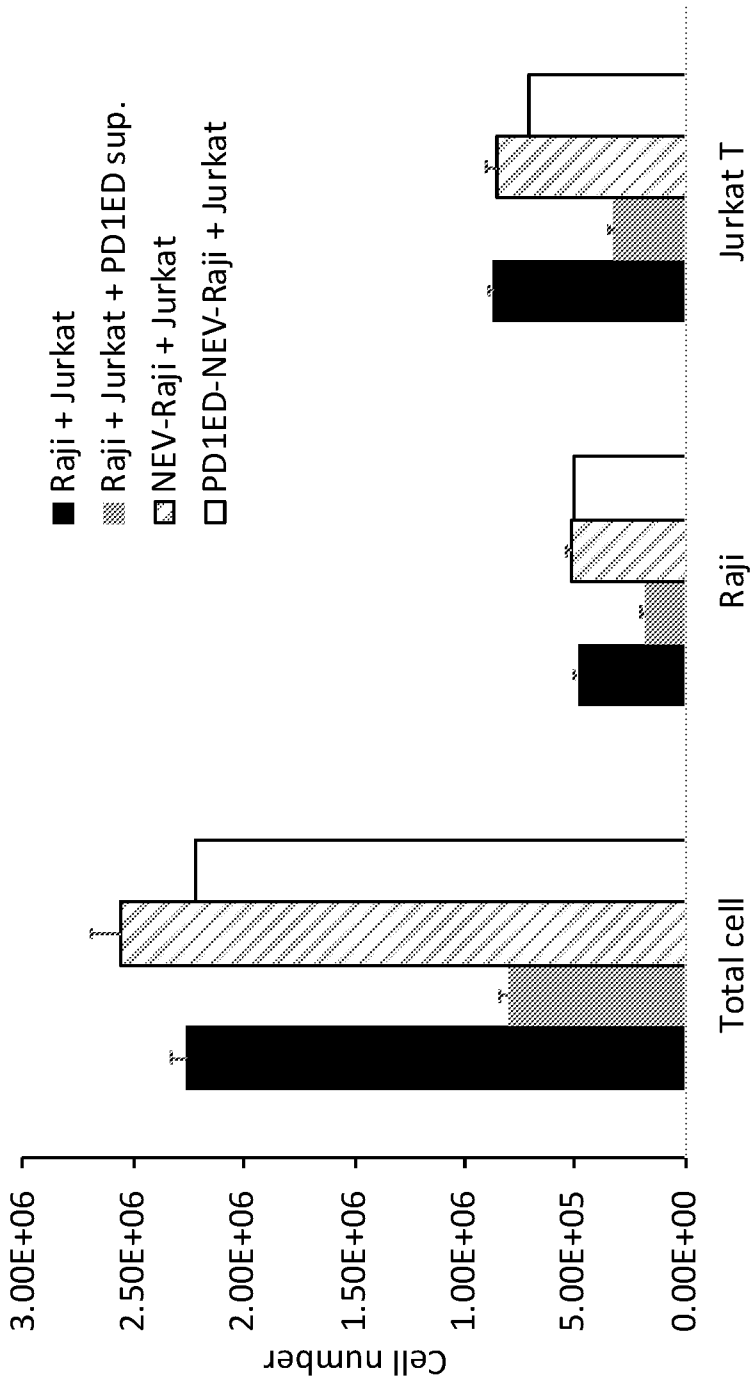

Raji + Jurkat
Raji + Jurkat + PD1ED sup.
NEV-Raji + Jurkat
PD1ED-NEV-Raji + Jurkat
Cell number
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
Total cell
Raji
Jurkat T

Figure 23 B

Figure 24 A

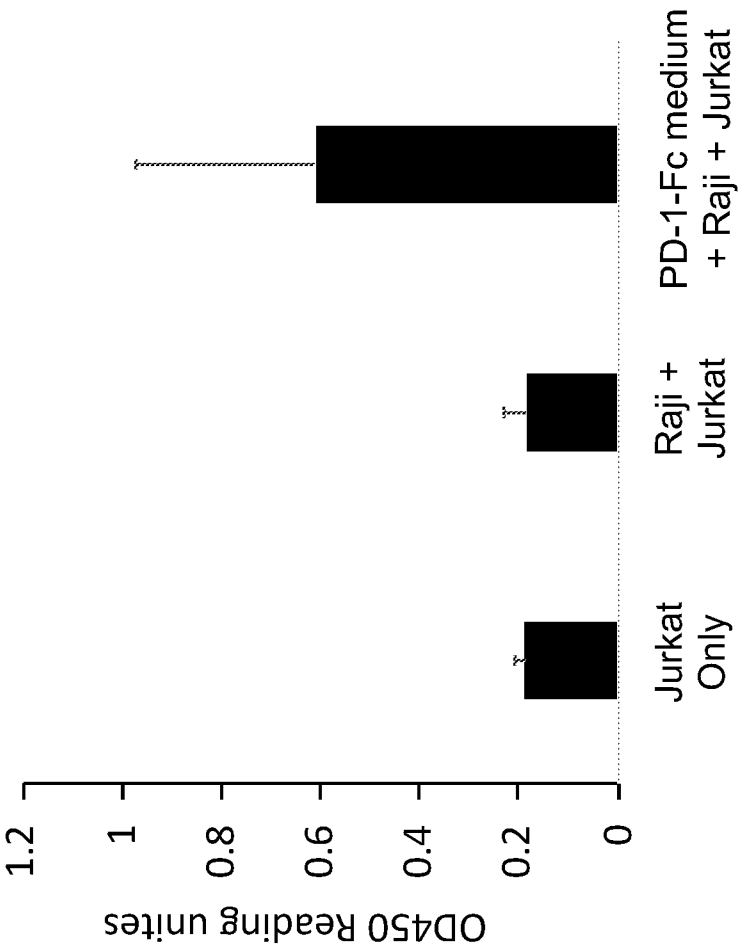
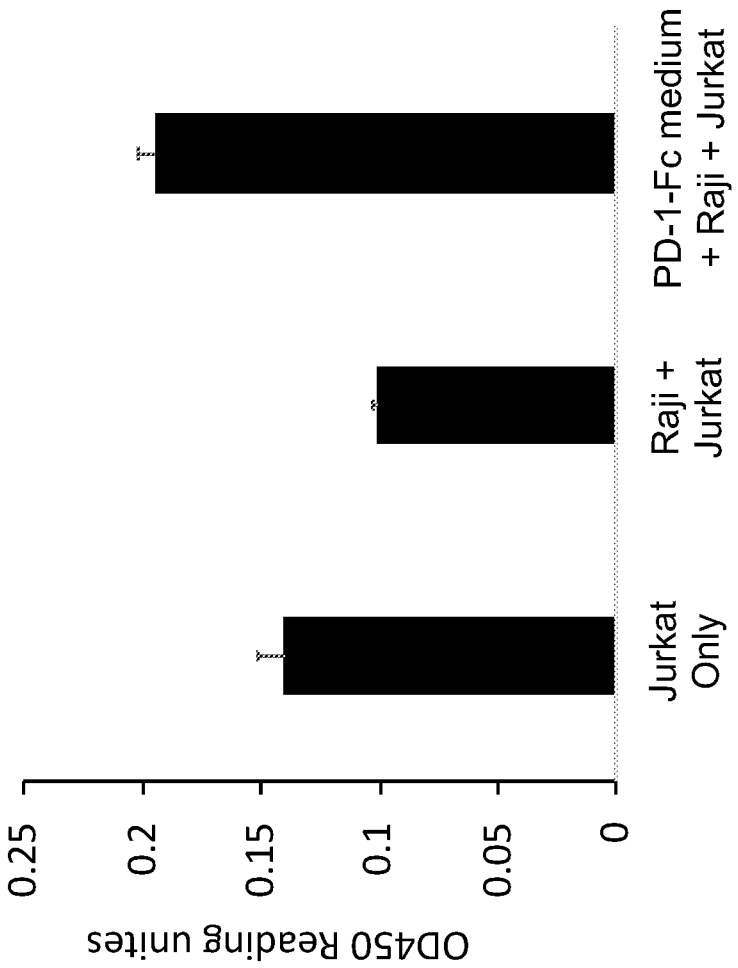

IFNg
OD450 Reading unites
1.2
1
0.8
0.6
0.4
0.2
0
Jurkat Only
Raji + Jurkat
PD-1-Fc medium + Raji + Jurkat

Figure 24 B


IL2
OD450 Reading unites
0.25
0.2
0.15
0.1
0.05
0
Jurkat Only
Raji + Jurkat
PD-1-Fc medium + Raji + Jurkat

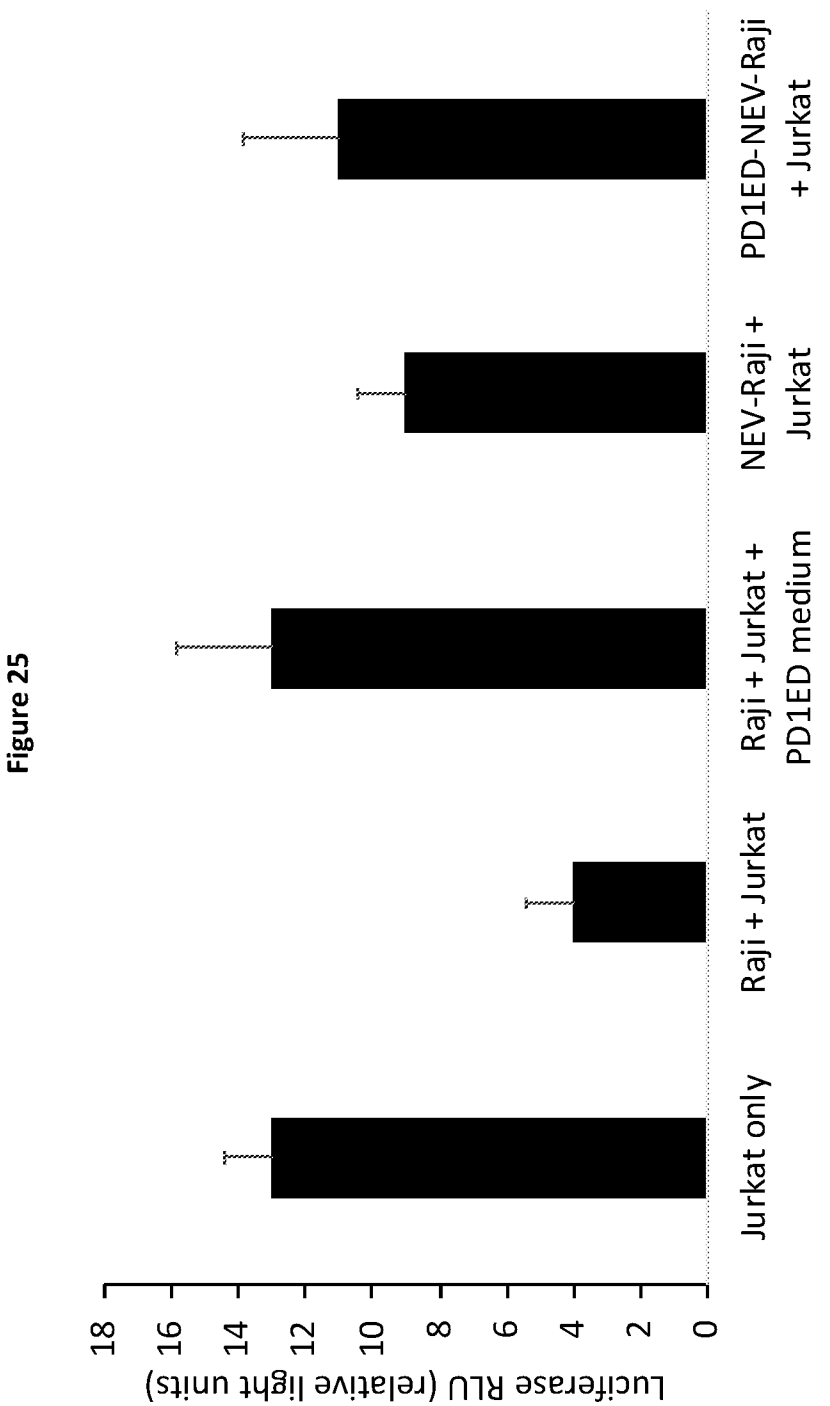

Luciferase RLU (relative light units)
0
2
4
6
8
10
12
14
16
18
Jurkat only
Raji + Jurkat
Raji + Jurkat + PD1ED medium
NEV-Raji + Jurkat
PD1ED-NEV-Raji + Jurkat

Figure 25

SEQUENCE LISTING

<110> Icell Kealex Therapeutics

<120> MUTANT VACCINIA VIRUSES AND USE THEREOF

<130> 190011001PCT

<160> 174

<170> PatentIn version 3.5

<210> 1
<211> 324
<212> PRT
<213> Artificial Sequence

<220>
<223> SEQ ID NO:1, Mutant H3L amino acid sequence

<400> 1

Met Ala Ala Ala Lys Thr Pro Val Ile Val Val Pro Val Ala Ala Ala
1 5 10 15

Leu Pro Ser Glu Thr Phe Pro Asn Val His Glu His Ile Asn Asp Gln
20 25 30

Ala Ala Ala Asp Val Ala Asp Ala Glu Val Met Ala Ala Lys Arg Asn
35 40 45

Val Val Val Ala Lys Asp Asp Pro Asp His Tyr Lys Asp Tyr Ala Phe
50 55 60

Ile Gln Trp Thr Gly Gly Asn Ile Arg Asn Asp Asp Lys Tyr Thr His
65 70 75 80

Phe Phe Ser Gly Phe Cys Asn Thr Met Cys Thr Glu Glu Thr Lys Arg
85 90 95

Asn Ile Ala Arg His Leu Ala Leu Trp Asp Ser Asn Phe Phe Thr Glu
100 105 110

Leu Glu Asn Lys Lys Val Glu Tyr Val Val Ile Val Glu Asn Asp Asn
115 120 125

Val Ile Ala Ala Ile Ala Phe Leu Ala Pro Val Leu Lys Ala Met His
130 135 140

Asp Lys Lys Ile Asp Ile Leu Gln Met Ala Glu Ala Ile Thr Gly Asn

```
145                 150                 155                 160

Ala Val Lys Thr Glu Ala Ala Ala Asp Lys Asn His Ala Ile Phe Thr
                165                 170                 175

Tyr Thr Gly Gly Tyr Asp Val Ser Leu Ser Ala Tyr Ile Ile Arg Val
            180                 185                 190

Thr Thr Ala Leu Asn Ile Ala Asp Glu Ile Ile Lys Ser Gly Gly Leu
        195                 200                 205

Ser Ser Gly Phe Tyr Phe Glu Ile Ala Arg Ile Glu Asn Glu Met Lys
    210                 215                 220

Ile Asn Ala Gln Ile Leu Asp Asn Ala Ala Lys Tyr Val Glu His Asp
225                 230                 235                 240

Pro Arg Leu Val Ala Glu His Arg Phe Ala Asn Met Ala Ala Ala Ala
                245                 250                 255

Trp Ser Arg Ile Gly Thr Ala Ala Thr Lys Arg Tyr Pro Gly Val Met
            260                 265                 270

Tyr Ala Phe Thr Thr Pro Leu Ile Ser Phe Phe Gly Leu Phe Asp Ile
        275                 280                 285

Asn Val Ile Gly Leu Ile Val Ile Leu Phe Ile Met Phe Met Leu Ile
    290                 295                 300

Phe Asn Val Lys Ser Lys Leu Leu Trp Phe Leu Thr Gly Thr Phe Val
305                 310                 315                 320

Thr Ala Phe Ile
```

<210> 2
<211> 304
<212> PRT
<213> Artificial Sequence

<220>
<223> SEQ ID NO:2, mutant D8L amino acid sequence

<400> 2

```
Met Pro Gln Gln Leu Ser Pro Ile Asn Ile Glu Thr Lys Lys Ala Ile
1               5                   10                  15
```

```
Ser Asn Ala Arg Leu Lys Pro Leu Asp Ile His Tyr Asn Glu Ser Lys
            20                  25                  30

Pro Thr Thr Ile Gln Asn Thr Gly Ala Leu Val Ala Ile Asn Phe Ala
        35                  40                  45

Gly Gly Tyr Ile Ser Gly Gly Phe Leu Pro Asn Glu Tyr Val Leu Ser
    50                  55                  60

Ser Leu His Ile Tyr Trp Gly Lys Glu Asp Asp Tyr Gly Ser Asn His
65                  70                  75                  80

Leu Ile Asp Val Tyr Lys Tyr Ser Gly Glu Ile Asn Leu Val His Trp
                85                  90                  95

Asn Ala Lys Lys Tyr Ser Ser Tyr Glu Glu Ala Ala Lys His Asp Asp
            100                 105                 110

Gly Leu Ile Ile Ile Ser Ile Phe Leu Gln Val Leu Asp His Lys Asn
        115                 120                 125

Val Tyr Phe Gln Lys Ile Val Asn Gln Leu Asp Ser Ile Arg Ser Ala
    130                 135                 140

Asn Thr Ser Ala Pro Phe Asp Ser Val Phe Tyr Leu Asp Asn Leu Leu
145                 150                 155                 160

Pro Ser Lys Leu Asp Tyr Phe Thr Tyr Leu Gly Thr Thr Ile Asn His
                165                 170                 175

Ser Ala Asp Ala Val Trp Ile Ile Phe Pro Thr Pro Ile Asn Ile His
            180                 185                 190

Ser Asp Gln Leu Ser Lys Phe Arg Thr Leu Leu Ser Ser Ser Asn His
        195                 200                 205

Asp Gly Lys Pro His Tyr Ile Thr Glu Asn Tyr Ala Asn Pro Tyr Lys
    210                 215                 220

Leu Asn Asp Asp Thr Gln Val Tyr Tyr Ser Gly Glu Ile Ile Arg Ala
225                 230                 235                 240

Ala Thr Thr Ser Pro Ala Arg Glu Asn Tyr Phe Met Arg Trp Leu Ser
```

245 250 255

Asp Leu Arg Glu Thr Cys Phe Ser Tyr Tyr Gln Lys Tyr Ile Glu Glu
260 265 270

Asn Lys Thr Phe Ala Ile Ile Ala Ile Val Phe Val Phe Ile Leu Thr
275 280 285

Ala Ile Leu Phe Phe Met Ser Arg Arg Tyr Ser Arg Glu Lys Gln Asn
290 295 300

<210> 3
<211> 110
<212> PRT
<213> Artificial Sequence

<220>
<223> SEQ ID NO:3, mutant A27L amino acid sequence

<400> 3

Met Asp Gly Thr Leu Phe Pro Gly Asp Asp Leu Ala Ile Pro Ala
1 5 10 15

Thr Glu Phe Phe Ser Thr Lys Ala Ala Lys Ala Pro Glu Asp Lys Ala
20 25 30

Ala Asp Ala Ala Ala Ala Ala Ala Asp Asp Asn Glu Glu Thr Leu Lys
35 40 45

Gln Arg Leu Thr Asn Leu Glu Lys Lys Ile Thr Asn Val Thr Thr Lys
50 55 60

Phe Glu Gln Ile Glu Lys Cys Cys Lys Arg Asn Asp Glu Val Leu Phe
65 70 75 80

Arg Leu Glu Asn His Ala Glu Thr Leu Arg Ala Ala Met Ile Ser Leu
85 90 95

Ala Lys Lys Ile Asp Val Gln Thr Gly Arg Ala Ala Ala Glu
100 105 110

<210> 4
<211> 250
<212> PRT
<213> Artificial Sequence

<220>

<223> SEQ ID NO:4, mutant L1R amino acid sequence

<400> 4

```
Met Gly Ala Ala Ala Ser Ile Gln Thr Thr Val Asn Thr Leu Ser Glu
1               5                  10                  15

Arg Ile Ser Ser Lys Leu Glu Gln Ala Ala Ala Ala Ser Ala Ala Ala
            20                  25                  30

Ala Cys Ala Ile Glu Ile Gly Asn Phe Tyr Ile Arg Gln Asn His Gly
        35                  40                  45

Cys Asn Leu Thr Val Lys Asn Met Cys Ala Ala Ala Ala Ala Ala Gln
    50                  55                  60

Leu Asp Ala Val Leu Ser Ala Ala Thr Glu Thr Tyr Ser Gly Leu Thr
65                  70                  75                  80

Pro Glu Gln Lys Ala Tyr Val Pro Ala Met Phe Thr Ala Ala Leu Asn
                85                  90                  95

Ile Gln Thr Ser Val Asn Thr Val Val Arg Asp Phe Glu Asn Tyr Val
            100                 105                 110

Lys Gln Thr Cys Asn Ser Ser Ala Val Val Asp Asn Ala Leu Ala Ile
        115                 120                 125

Gln Asn Val Ile Ile Asp Glu Cys Tyr Gly Ala Pro Gly Ser Pro Thr
    130                 135                 140

Asn Leu Glu Phe Ile Asn Thr Gly Ser Ser Lys Gly Asn Cys Ala Ile
145                 150                 155                 160

Lys Ala Leu Met Gln Leu Thr Thr Lys Ala Thr Thr Gln Ile Ala Pro
                165                 170                 175

Lys Gln Val Ala Gly Thr Gly Val Gln Phe Tyr Met Ile Val Ile Gly
            180                 185                 190

Val Ile Ile Leu Ala Ala Leu Phe Met Tyr Tyr Ala Lys Arg Met Leu
        195                 200                 205

Phe Thr Ser Thr Asn Asp Lys Ile Lys Leu Ile Leu Ala Asn Lys Glu
    210                 215                 220
```

```
Asn Val His Trp Thr Thr Tyr Met Asp Thr Phe Phe Arg Thr Ser Pro
225                 230                 235                 240


Met Val Ile Ala Thr Thr Asp Met Gln Asn
                245                 250


<210>  5
<211>  324
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  SEQ ID NO:5, mutant H3L amino acid sequence

<400>  5

Met Ala Ala Ala Lys Thr Pro Val Ile Val Val Pro Val Ile Asp Arg
1               5                   10                  15


Leu Pro Ser Glu Thr Phe Pro Asn Val His Glu His Ile Asn Asp Gln
            20                  25                  30


Lys Phe Asp Asp Val Lys Asp Asn Glu Val Met Ala Glu Lys Arg Asn
        35                  40                  45


Val Val Val Val Lys Asp Asp Pro Asp His Tyr Lys Asp Tyr Ala Phe
    50                  55                  60


Ile Gln Trp Thr Gly Gly Asn Ile Arg Asn Asp Asp Lys Tyr Thr His
65                  70                  75                  80


Phe Phe Ser Gly Phe Cys Asn Thr Met Cys Thr Glu Glu Thr Lys Arg
                85                  90                  95


Asn Ile Ala Arg His Leu Ala Leu Trp Asp Ser Asn Phe Phe Thr Glu
            100                 105                 110


Leu Glu Asn Lys Lys Val Glu Tyr Val Val Ile Val Glu Asn Asp Asn
        115                 120                 125


Val Ile Glu Asp Ile Thr Phe Leu Arg Pro Val Leu Lys Ala Met His
    130                 135                 140


Asp Lys Lys Ile Asp Ile Leu Gln Met Arg Glu Ile Ile Thr Gly Asn
145                 150                 155                 160
```

```
Lys Val Lys Thr Glu Leu Val Met Asp Lys Asn His Ala Ile Phe Thr
                165                 170                 175

Tyr Thr Gly Gly Tyr Asp Val Ser Leu Ser Ala Tyr Ile Ile Arg Val
            180                 185                 190

Thr Thr Ala Leu Asn Ile Val Asp Glu Ile Ile Lys Ser Gly Gly Leu
        195                 200                 205

Ser Ser Gly Phe Tyr Phe Glu Ile Ala Arg Ile Glu Asn Glu Met Lys
    210                 215                 220

Ile Asn Arg Gln Ile Leu Asp Asn Ala Ala Lys Tyr Val Glu His Asp
225                 230                 235                 240

Pro Arg Leu Val Ala Glu His Arg Phe Gly Trp Met Lys Pro Asn Phe
                245                 250                 255

Trp Phe Arg Ile Gly Pro Ala Thr Val Ile Arg Cys Pro Gly Val Lys
            260                 265                 270

Asn Ala Asn Thr Ala Pro Leu Ile Ser Phe Phe Gly Leu Phe Asp Ile
        275                 280                 285

Asn Val Ile Gly Leu Ile Val Ile Leu Phe Ile Met Phe Met Leu Ile
    290                 295                 300

Phe Asn Val Lys Ser Lys Leu Leu Trp Phe Leu Thr Gly Thr Phe Val
305                 310                 315                 320

Thr Ala Phe Ile
```

<210> 6
<211> 304
<212> PRT
<213> Artificial Sequence

<220>
<223> mutant D8L amino acid sequence (Alternate for this sequence: Seq No. 174 - only different at position 43)

<400> 6

```
Met Pro Gln Gln Leu Ser Pro Ile Asn Ile Glu Thr Lys Lys Ala Ile
1               5                   10                  15
```

```
Ser Asn Ala Arg Leu Lys Pro Leu Asp Ile His Tyr Asn Glu Ser Lys
            20                  25                  30

Pro Thr Thr Ile Gln Asn Thr Gly Lys Leu Phe Trp Ile Asn Phe Lys
        35                  40                  45

Gly Gly Tyr Ile Ser Gly Trp Phe Leu Pro Asn Glu Tyr Val Leu Ser
    50                  55                  60

Ser Leu His Ile Tyr Trp Gly Lys Glu Asp Asp Tyr Gly Ser Asn His
65                  70                  75                  80

Leu Ile Asp Val Tyr Lys Tyr Ser Gly Glu Ile Asn Leu Val His Trp
                85                  90                  95

Asn Lys Lys Lys Tyr Ser Ser Tyr Glu Glu Ala Lys Lys His Asp Asp
            100                 105                 110

Gly Leu Ile Ile Ile Ser Ile Phe Leu Gln Val Leu Asp His Lys Asn
        115                 120                 125

Val Tyr Phe Gln Lys Ile Val Asn Gln Leu Asp Ser Ile Arg Ser Thr
    130                 135                 140

Asn Thr Ser Ala Pro Phe Asp Ser Val Phe Tyr Leu Asp Asn Leu Leu
145                 150                 155                 160

Pro Ser Lys Leu Asp Tyr Phe Ser Tyr Leu Gly Thr Thr Ile Asn His
                165                 170                 175

Tyr Ala Asp Ala Val Trp Ile Ile Phe Pro Thr Pro Ile Asn Ile His
            180                 185                 190

Ser Asp Gln Leu Ser Lys Tyr Arg Thr Leu Ser Ser Ser Ser Asn His
        195                 200                 205

Asp Gly Lys Thr His Tyr Ile Thr Glu Cys Tyr Arg Asn Leu Tyr Lys
    210                 215                 220

Leu Asn Gly Asp Thr Gln Val Tyr Tyr Ser Gly Glu Ile Ile Arg Ala
225                 230                 235                 240

Ala Thr Thr Ser Pro Ala Arg Glu Asn Tyr Phe Met Arg Trp Leu Ser
                245                 250                 255
```

```
Asp Leu Arg Glu Thr Cys Phe Ser Tyr Tyr Gln Lys Tyr Ile Glu Glu
            260                 265                 270

Asn Lys Thr Phe Ala Ile Ile Ala Ile Val Phe Val Phe Ile Leu Thr
        275                 280                 285

Ala Ile Leu Phe Phe Met Ser Arg Arg Tyr Ser Arg Glu Lys Gln Asn
    290                 295                 300
```

<210> 7
<211> 375
<212> PRT
<213> Artificial Sequence

<220>
<223> SEQ ID NO:7, CD55-A27 amino acid sequence

<400> 7

```
Met Asp Cys Gly Leu Pro Pro Asp Val Pro Asn Ala Gln Pro Ala Leu
1               5                   10                  15

Glu Gly Arg Thr Ser Phe Pro Glu Asp Thr Val Ile Thr Tyr Lys Cys
            20                  25                  30

Glu Glu Ser Phe Val Lys Ile Pro Gly Glu Lys Asp Ser Val Ile Cys
        35                  40                  45

Leu Lys Gly Ser Gln Trp Ser Asp Ile Glu Glu Phe Cys Asn Arg Ser
    50                  55                  60

Cys Glu Val Pro Thr Arg Leu Asn Ser Ala Ser Leu Lys Gln Pro Tyr
65                  70                  75                  80

Ile Thr Gln Asn Tyr Phe Pro Val Gly Thr Val Val Glu Tyr Glu Cys
                85                  90                  95

Arg Pro Gly Tyr Arg Arg Glu Pro Ser Leu Ser Pro Lys Leu Thr Cys
            100                 105                 110

Leu Gln Asn Leu Lys Trp Ser Thr Ala Val Glu Phe Cys Lys Lys Lys
        115                 120                 125

Ser Cys Pro Asn Pro Gly Glu Ile Arg Asn Gly Gln Ile Asp Val Pro
    130                 135                 140
```

```
Gly Gly Ile Leu Phe Gly Ala Thr Ile Ser Phe Ser Cys Asn Thr Gly
145                 150                 155                 160

Tyr Lys Leu Phe Gly Ser Thr Ser Ser Phe Cys Leu Ile Ser Gly Ser
                165                 170                 175

Ser Val Gln Trp Ser Asp Pro Leu Pro Glu Cys Arg Glu Ile Tyr Cys
            180                 185                 190

Pro Ala Pro Pro Gln Ile Asp Asn Gly Ile Ile Gln Gly Glu Arg Asp
        195                 200                 205

His Tyr Gly Tyr Arg Gln Ser Val Thr Tyr Ala Cys Asn Lys Gly Phe
    210                 215                 220

Thr Met Ile Gly Glu His Ser Ile Tyr Cys Thr Val Asn Asn Asp Glu
225                 230                 235                 240

Gly Glu Trp Ser Gly Pro Pro Pro Glu Cys Arg Gly Gly Gly Gly Ser
                245                 250                 255

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Gly Thr Leu Phe Pro
            260                 265                 270

Gly Asp Asp Asp Leu Ala Ile Pro Ala Thr Glu Phe Phe Ser Thr Lys
        275                 280                 285

Ala Ala Lys Ala Pro Glu Asp Lys Ala Ala Asp Ala Ala Ala Ala Ala
    290                 295                 300

Ala Asp Asp Asn Glu Glu Thr Leu Lys Gln Arg Leu Thr Asn Leu Glu
305                 310                 315                 320

Lys Lys Ile Thr Asn Val Thr Thr Lys Phe Glu Gln Ile Glu Lys Cys
                325                 330                 335

Cys Lys Arg Asn Asp Glu Val Leu Phe Arg Leu Glu Asn His Ala Glu
            340                 345                 350

Thr Leu Arg Ala Ala Met Ile Ser Leu Ala Lys Lys Ile Asp Val Gln
        355                 360                 365

Thr Gly Arg Ala Ala Ala Glu
    370                 375
```

<210> 8
<211> 526
<212> PRT
<213> Artificial Sequence

<220>
<223> SEQ ID NO:8, FAP-CD3 amino acid sequence

<400> 8

```
Met Asp Trp Ile Trp Arg Ile Leu Phe Leu Val Gly Ala Ala Thr Gly
1               5                   10                  15


Ala His Ser Gln Val Gln Leu Lys Gln Ser Gly Ala Glu Leu Val Lys
            20                  25                  30


Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Thr Ser Gly Tyr Thr Phe
        35                  40                  45


Thr Glu Asn Ile Ile His Trp Val Lys Gln Arg Ser Gly Gln Gly Leu
    50                  55                  60


Glu Trp Ile Gly Trp Phe His Pro Gly Ser Gly Ser Ile Lys Tyr Asn
65                  70                  75                  80


Glu Lys Phe Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser
                85                  90                  95


Thr Val Tyr Met Glu Leu Ser Arg Leu Thr Ser Glu Asp Ser Ala Val
            100                 105                 110


Tyr Phe Cys Ala Arg His Gly Gly Thr Gly Arg Gly Ala Met Asp Tyr
        115                 120                 125


Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser
    130                 135                 140


Gly Gly Gly Gly Ser Gly Gly Ser Ala Gln Ile Leu Met Thr Gln Ser
145                 150                 155                 160


Pro Ala Ser Ser Val Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys
                165                 170                 175


Arg Ala Ser Lys Ser Val Ser Thr Ser Ala Tyr Ser Tyr Met His Trp
            180                 185                 190
```

```
Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala
        195                 200                 205

Ser Asn Leu Glu Ser Gly Val Pro Pro Arg Phe Ser Gly Ser Gly Ser
    210                 215                 220

Gly Thr Asp Phe Thr Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala
225                 230                 235                 240

Ala Thr Tyr Tyr Cys Gln His Ser Arg Glu Leu Pro Tyr Thr Phe Gly
                245                 250                 255

Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Gly Ser Gly Gly Gly Gly
            260                 265                 270

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Val Asp Asp Ile Lys
        275                 280                 285

Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys
    290                 295                 300

Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met His
305                 310                 315                 320

Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile
                325                 330                 335

Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Lys
            340                 345                 350

Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu
        355                 360                 365

Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Tyr
    370                 375                 380

Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu
385                 390                 395                 400

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
                405                 410                 415

Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile Met Ser Ala
            420                 425                 430
```

```
Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val
        435                 440                 445

Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg
    450                 455                 460

Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly Val Pro Tyr Arg Phe
465                 470                 475                 480

Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met
                485                 490                 495

Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn
            500                 505                 510

Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Ser
        515                 520                 525
```

<210> 9
<211> 515
<212> PRT
<213> Artificial Sequence

<220>
<223> SEQ ID NO:9, BCMA-CD3 scFv amino acid sequence

<400> 9

```
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Asp Ile Val Leu Thr Gln Ser Pro Pro Ser Leu
            20                  25                  30

Ala Met Ser Leu Gly Lys Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu
        35                  40                  45

Ser Val Thr Ile Leu Gly Ser His Leu Ile His Trp Tyr Gln Gln Lys
    50                  55                  60

Pro Gly Gln Pro Pro Thr Leu Leu Ile Gln Leu Ala Ser Asn Val Gln
65                  70                  75                  80

Thr Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe
                85                  90                  95
```

```
Thr Leu Thr Ile Asp Pro Val Glu Glu Asp Asp Val Ala Val Tyr Tyr
            100                 105                 110

Cys Leu Gln Ser Arg Thr Ile Pro Arg Thr Phe Gly Gly Gly Thr Lys
        115                 120                 125

Leu Glu Ile Lys Gly Ser Thr Ser Gly Ser Gly Lys Pro Gly Ser Gly
    130                 135                 140

Glu Gly Ser Thr Lys Gly Gln Ile Gln Leu Val Gln Ser Gly Pro Glu
145                 150                 155                 160

Leu Lys Lys Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly
                165                 170                 175

Tyr Thr Phe Thr Asp Tyr Ser Ile Asn Trp Val Lys Arg Ala Pro Gly
            180                 185                 190

Lys Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Glu Thr Arg Glu Pro
        195                 200                 205

Ala Tyr Ala Tyr Asp Phe Arg Gly Arg Phe Ala Phe Ser Leu Glu Thr
    210                 215                 220

Ser Ala Ser Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Tyr Glu Asp
225                 230                 235                 240

Thr Ala Thr Tyr Phe Cys Ala Leu Asp Tyr Ser Tyr Ala Met Asp Tyr
                245                 250                 255

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser
            260                 265                 270

Val Asp Asp Ile Lys Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro
        275                 280                 285

Gly Ala Ser Val Lys Met Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr
    290                 295                 300

Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu
305                 310                 315                 320

Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln
                325                 330                 335
```

```
Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr
            340                 345                 350

Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr
        355                 360                 365

Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly
    370                 375                 380

Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
385                 390                 395                 400

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser Pro
                405                 410                 415

Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg
            420                 425                 430

Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly
        435                 440                 445

Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Val Ala Ser Gly
    450                 455                 460

Val Pro Tyr Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
465                 470                 475                 480

Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
                485                 490                 495

Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu
            500                 505                 510

Leu Lys Ser
        515
```

<210> 10
<211> 402
<212> PRT
<213> Artificial Sequence

<220>
<223> SEQ ID NO:10, PD-1-ED-hIgG1-Fc amino acid sequence

<400> 10

```
Met Gln Ile Pro Gln Ala Pro Trp Pro Val Val Trp Ala Val Leu Gln
1               5                  10                  15

Leu Gly Trp Arg Pro Gly Trp Phe Leu Asp Ser Pro Asp Arg Pro Trp
            20                  25                  30

Asn Pro Pro Thr Phe Phe Pro Ala Leu Leu Val Val Thr Glu Gly Asp
        35                  40                  45

Asn Ala Thr Phe Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val
    50                  55                  60

Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala
65                  70                  75                  80

Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg
                85                  90                  95

Val Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg
            100                 105                 110

Ala Arg Arg Asn Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu
        115                 120                 125

Ala Pro Lys Ala Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val
    130                 135                 140

Thr Glu Arg Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro
145                 150                 155                 160

Arg Pro Ala Gly Gln Phe Gln Thr Leu Val Glu Pro Lys Ser Cys Asp
                165                 170                 175

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
            180                 185                 190

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
        195                 200                 205

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
    210                 215                 220

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
225                 230                 235                 240
```

```
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
                245                 250                 255

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
            260                 265                 270

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
        275                 280                 285

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
    290                 295                 300

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
305                 310                 315                 320

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
                325                 330                 335

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
            340                 345                 350

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
        355                 360                 365

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
    370                 375                 380

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
385                 390                 395                 400

Gly Lys
```

<210> 11
<211> 324
<212> PRT
<213> Artificial Sequence

<220>
<223> SEQ ID NO:11, Mutant H3L amino acid sequence

<400> 11

```
Met Ala Ala Ala Lys Thr Pro Val Ile Val Val Pro Val Ala Ala Ala
1               5                   10                  15
```

```
Leu Pro Ser Glu Thr Phe Pro Asn Val His Glu His Ile Asn Asp Gln
            20                  25                  30

Lys Phe Asp Asp Val Ala Asp Asn Glu Val Met Ala Ala Lys Arg Asn
        35                  40                  45

Val Val Val Ala Lys Asp Asp Pro Asp His Tyr Lys Asp Tyr Ala Phe
    50                  55                  60

Ile Gln Trp Thr Gly Gly Asn Ile Arg Asn Asp Asp Lys Tyr Thr His
65                  70                  75                  80

Phe Phe Ser Gly Phe Cys Asn Thr Met Cys Thr Glu Glu Thr Lys Arg
                85                  90                  95

Asn Ile Ala Arg His Leu Ala Leu Trp Asp Ser Asn Phe Phe Thr Glu
            100                 105                 110

Leu Glu Asn Lys Lys Val Glu Tyr Val Val Ile Val Glu Asn Asp Asn
        115                 120                 125

Val Ile Ala Ala Ile Ala Phe Leu Ala Pro Val Leu Lys Ala Met His
    130                 135                 140

Asp Lys Lys Ile Asp Ile Leu Gln Met Ala Glu Ala Ile Thr Gly Asn
145                 150                 155                 160

Lys Val Lys Thr Glu Leu Val Ala Asp Lys Asn His Ala Ile Phe Thr
                165                 170                 175

Tyr Thr Gly Gly Tyr Asp Val Ser Leu Ser Ala Tyr Ile Ile Arg Val
            180                 185                 190

Thr Thr Ala Leu Asn Ile Ala Asp Glu Ile Ile Lys Ser Gly Gly Leu
        195                 200                 205

Ser Ser Gly Phe Tyr Phe Glu Ile Ala Arg Ile Glu Asn Glu Met Lys
    210                 215                 220

Ile Asn Arg Gln Ile Leu Asp Asn Ala Ala Lys Tyr Val Glu His Asp
225                 230                 235                 240

Pro Arg Leu Val Ala Glu His Arg Phe Ala Asn Met Ala Ala Ala Ala
                245                 250                 255
```

Trp Ser Arg Ile Gly Thr Ala Ala Thr Lys Arg Tyr Pro Gly Val Met
260 265 270

Tyr Ala Phe Thr Thr Pro Leu Ile Ser Phe Phe Gly Leu Phe Asp Ile
275 280 285

Asn Val Ile Gly Leu Ile Val Ile Leu Phe Ile Met Phe Met Leu Ile
290 295 300

Phe Asn Val Lys Ser Lys Leu Leu Trp Phe Leu Thr Gly Thr Phe Val
305 310 315 320

Thr Ala Phe Ile

<210> 12
<211> 2548
<212> DNA
<213> Artificial Sequence

<220>
<223> SEQ ID NO:12, Nucleotide sequences for mutated H3L gene

<400> 12
gaagaactca tagatcacga acatgtgcaa tacaaaataa attgttacaa tattctaaga
60

tatcatttat tgccagacag tgacgtgttt gtatatttta gtaattcatt aaacagagaa
120

gcattggaat acgcatttta tatctttttg tcgaaatatg taaatgtgaa acaatggata
180

gacgaaaata taactcgtat taaagagttg tatatgatta atttcaataa ctaaatggcg
240

gcggcgaaaa ctcctgttat tttaatttat tatgatattt aaatatcgcc taatatggcg
300

gcggcgaaaa ctcctgttat tgttgtgcca gttgctgctg cacttccatc agaaacattt
360

cctaatgttc atgagcatat taatgatcag aagttcgatg atgtagcgga caacgaagtt
420

atggcagcaa aaagaaatgt tgtggtagcc aaggatgatc cagatcatta caaggattat
480

gcgtttatac agtggactgg aggaaacatt agaaatgatg acaagtatac tcacttcttt
540

tcagggtttt gtaacactat gtgtacagag gaaacgaaaa gaaatatcgc tagacattta
600

gccctatggg attctaattt ttttaccgag ttagaaaata aaaaggtaga atatgtagtt
660

attgtagaaa acgataacgt tattgcggct attgcgtttc ttgctcccgt cttgaaggca
720

atgcatgaca aaaaaataga tatcctacag atggcagaag ctattacagg caataaagtt
780

aaaaccgagc ttgtagcgga caaaaatcat gccatattca catatacagg agggtatgat
840

gttagcttat cagcctatat tattagagtt actacggcgc tgaacatcgc agatgaaatt
900

ataaagtctg gaggtctatc atcgggattt tattttgaaa tagccagaat tgaaaacgaa
960

atgaagatca ataggcagat actggataat gccgccaaat atgtagaaca cgatccccga
1020

cttgttgcag aacaccgttt cgcaaacatg gcagcggctg cttggtctag aataggaacg
1080

gcagctacta aacgttatcc aggagttatg tacgcgttta ctactccact gatttcattt
1140

tttggattgt ttgatattaa tgttataggt ttgattgtaa ttttgtttat tatgtttatg
1200

ctcatcttta acgttaaatc taaactgtta tggttcctta caggaacatt cgttaccgca
1260

tttatctaat aatccaaacc cacccgcttt ttatagtaag tttttcaccc ataaataata
1320

aatacaataa ttaatttctc gtaaaagtag aaaatatatt ctaatttatt gcacggtaag
1380

gaagtagatc ataactcgag ataacttcgt ataatgtatg ctatacgaag ttattactag
1440

cgctaccggt cgccaatggt gagcaagggc gaggagctgt tcaccggggt ggtgcccatc
1500

ctggtcgagc tggacggcga cgtaaacggc cacaagttca gcgtgtccgg cgagggcgag
1560

ggcgatgcca cctacggcaa gctgaccctg aagttcatct gcaccaccgg caagctgccc
1620

gtgccctggc ccaccctcgt gaccaccctg acctacggcg tgcagtgctt cagccgctac
1680

cccgaccaca tgaagcagca cgacttcttc aagtccgcca tgcccgaagg ctacgtccag
1740

gagcgcacca tcttcttcaa ggacgacggc aactacaaga cccgcgccga ggtgaagttc

1800

gagggcgaca ccctggtgaa ccgcatcgag ctgaagggca tcgacttcaa ggaggacggc
1860

aacatcctgg ggcacaagct ggagtacaac tacaacagcc acaacgtcta tatcatggcc
1920

gacaagcaga agaacggcat caaggtgaac ttcaagatcc gccacaacat cgaggacggc
1980

agcgtgcagc tcgccgacca ctaccagcag aacaccccca tcggcgacgg ccccgtgctg
2040

ctgcccgaca accactacct gagcacccag tccgccctga gcaaagaccc caacgagaag
2100

cgcgatcaca tggtcctgct ggagttcgtg accgccgccg ggatcactct cggcatggac
2160

gagctgtaca agtaacttac tagcgctcaa taacttcgta taatgtatgc tatacgaagt
2220

tattaataca ggaacattcg ttaccgcatt tatctaacac tattccatat tactaaaatc
2280

ggaacaccaa tgcggtgaca taaaataacc gctataacct aattcattta acatctcatt
2340

accacaagta ataacattat tagacttgtg ttttatcaaa tactgacaaa attgttgagc
2400

agatggatcg acctttgccg ccttttaac catccacgcg tctccagtac ctcgcctaat
2460

agcttgcggc agatatgttt tcttatccaa tcgcatagct ataaaatagg cgccgaaatc
2520

cacacatttg aattcgaata tatcatcc
2548

<210> 13
<211> 2467
<212> DNA
<213> Artificial Sequence

<220>
<223> SEQ ID NO:13, Nucleotide sequences for mutated D8L gene

<400> 13
agaatctgaa ttttgttgag ataatatcgc ctggaacgcg aatgaagttc ttctagctcc
60

tattaacgga tatccgtcac ttgttataca cgcagcaaac acgtgcgtgt cttttgatct
120

tggaatatct tttattcgtt taatagatat taattctcta ggagtttcaa atatcacttc
180

ctcatccatt gtaattccca tactaagagc tatttttaaa cagttatcat ttcattttta
240

ctatgccgca acaactatct cctattaaat agaaactatt aatttattat gatatttaaa
300

tatcgcctaa tatgccgcaa caactatctc ctattaatat agaaactaaa aaagcaattt
360

ctaacgcgcg attgaagccg ttagacatac attataatga gtcgaaacca accactatcc
420

agaacactgg agcactagta gcgattaatt ttgcaggagg atatataagt ggagggtttc
480

tccccaatga atatgtgtta tcatcactac atatatattg gggaaaggaa gacgattatg
540

gatccaatca cttgatagat gtgtacaaat actctggaga gattaatctt gttcattgga
600

atgcgaaaaa atatagttct tatgaagagg cagcaaaaca cgatgatgga cttatcatta
660

tttctatatt cttacaagta ttggatcata aaaatgtata ttttcaaaag atagttaatc
720

aattggattc cattagatcc gccaatacgt ctgcaccgtt tgattcagta ttttatctag
780

acaatttgct gcctagtaag ttggattatt ttacatatct aggaacaact atcaaccact
840

ctgcagacgc tgtatggata atttttccaa cgccaataaa cattcattct gatcaactat
900

ctaaattcag aacactattg tcgtcgtcta atcatgatgg aaaaccgcat tatataacag
960

agaactatgc aaatccgtat aaattgaacg acgacacgca agtatattat tctggggaga
1020

ttatacgagc agcaactacc tctccagcgc gcgagaacta ttttatgaga tggttgtccg
1080

atttgagaga gacatgtttt tcatattatc aaaaatatat cgaagagaat aaaacattcg
1140

caattattgc catagtattc gtgtttatac ttaccgctat tctctttttt atgagtcgac
1200

gatattcgcg agaaaaacaa aactagtaat ccaaacccac ccgcttttta tagtaagttt
1260

ttcacccata aataataaat acaataatta atttctcgta aaagtagaaa atatattcta
1320

atttattgca cggtaaggaa gtagatcata actcgagata acttcgtata atgtatgcta

1380

tacgaagtta ttactagcgc taccggtcgc caatggtgag caagggcgag gagctgttca
1440

ccggggtggt gcccatcctg gtcgagctgg acggcgacgt aaacggccac aagttcagcg
1500

tgtccggcga gggcgagggc gatgccacct acggcaagct gaccctgaag ttcatctgca
1560

ccaccggcaa gctgcccgtg ccctggccca ccctcgtgac caccctgacc tacggcgtgc
1620

agtgcttcag ccgctacccc gaccacatga agcagcacga cttcttcaag tccgccatgc
1680

ccgaaggcta cgtccaggag cgcaccatct tcttcaagga cgacggcaac tacaagaccc
1740

gcgccgaggt gaagttcgag ggcgacaccc tggtgaaccg catcgagctg aagggcatcg
1800

acttcaagga ggacggcaac atcctggggc acaagctgga gtacaactac aacagccaca
1860

acgtctatat catggccgac aagcagaaga acggcatcaa ggtgaacttc aagatccgcc
1920

acaacatcga ggacggcagc gtgcagctcg ccgaccacta ccagcagaac acccccatcg
1980

gcgacggccc cgtgctgctg cccgacaacc actacctgag cacccagtcc gccctgagca
2040

aagaccccaa cgagaagcgc gatcacatgg tcctgctgga gttcgtgacc gccgccggga
2100

tcactctcgg catggacgag ctgtacaagt aacttactag cgctcaataa cttcgtataa
2160

tgtatgctat acgaagttat taaatagtat tcgtgtttat acttaccgct attctctttt
2220

ttatgagtcg acgatattcg cgagaaaaac aaaactagat tcgatacctt gttgagcctc
2280

cattagaacg gcagtgactt cgctgccatt gtcatacgca ttaccatttc gaaaaaagca
2340

gtactttgaa tcgctaaatg atacagtacc cgaatctcta cttagtttac agattaaatc
2400

tccacattga atagttacat ttgattcatc ttcgatgttt aatgttcctc tgactatatc
2460

cccaacg
2467

<210> 14
<211> 1897
<212> DNA
<213> Artificial Sequence

<220>
<223> SEQ ID NO:14, Nucleotide sequences for mutated A27L gene

<400> 14
aaaagtggag atgtgtggtt tatccaggaa acggttttgt atccgcttcc atatttggat
60

ttcaggcaga agttggaccc aataatacta gatccattag aaaatttaac acgatgcaac
120

aatgtataga ctttacattt tctgatgtta ttaacatcga tatttataat ccatgtgttg
180

taccaaatat aaataacgca gagtgtcagt ttctaaaatc tgtactttaa atggacggaa
240

ctcttttccc cggagatgac ttaatatttt gttaattaaa attatattta taaaatatta
300

tataataaat ggacggaact cttttccccg gagatgacga tcttgcaatt ccagcaactg
360

aatttttttc tacaaaggct gctaaagcgc cagaggataa agccgcagac gctgctgcag
420

ccgctgcaga cgacaatgag gaaactctca aacaacggct aactaatttg gaaaaaaaga
480

ttactaatgt aacaacaaag tttgaacaaa tagaaaagtg ttgtaaacgc aacgatgaag
540

ttctatttag gttggaaaat cacgctgaaa ctctaagagc ggctatgata tctctggcta
600

aaaagattga tgttcagact ggacgggccg cagctgagta ataatccaaa cccacccgct
660

ttttatagta agtttttcac ccataaataa taaatacaat aattaatttc tcgtaaaagt
720

agaaaatata ttctaattta ttgcacggta aggaagtaga tcataactcg agataacttc
780

gtataatgta tgctatacga agttattact agcgctaccg gtcgccaatg gtgagcaagg
840

gcgaggagct gttcaccggg gtggtgccca tcctggtcga gctggacggc gacgtaaacg
900

gccacaagtt cagcgtgtcc ggcgagggcg agggcgatgc cacctacggc aagctgaccc
960

tgaagttcat ctgcaccacc ggcaagctgc ccgtgccctg gcccaccctc gtgaccaccc

1020

tgacctacgg cgtgcagtgc ttcagccgct accccgacca catgaagcag cacgacttct
1080

tcaagtccgc catgcccgaa ggctacgtcc aggagcgcac catcttcttc aaggacgacg
1140

gcaactacaa gacccgcgcc gaggtgaagt tcgagggcga caccctggtg aaccgcatcg
1200

agctgaaggg catcgacttc aaggaggacg gcaacatcct ggggcacaag ctggagtaca
1260

actacaacag ccacaacgtc tatatcatgg ccgacaagca gaagaacggc atcaaggtga
1320

acttcaagat ccgccacaac atcgaggacg gcagcgtgca gctcgccgac cactaccagc
1380

agaacacccc catcggcgac ggccccgtgc tgctgcccga caaccactac ctgagcaccc
1440

agtccgccct gagcaaagac cccaacgaga agcgcgatca catggtcctg ctggagttcg
1500

tgaccgccgc cgggatcact ctcggcatgg acgagctgta caagtaatag actagcgctc
1560

aataacttcg tataatgtat gctatacgaa gttatgttca gactggacgg cgcccatatg
1620

agtaataact taactctttt gttaattaaa agtatattca aaaaatgagt tatataaatg
1680

gcgaacatta taaatttatg gaacggaatt gtaccaacgg ttcaagatgt taatgttgcg
1740

agcattactg cgtttaaatc tatgatagat gaaacatggg ataaaaaaat cgaagcaaat
1800

acatgcatca gtagaaaaca tagaaacatt attcacgaag ttattaggga ctttatgaaa
1860

gcctatccta aaatggatga gaataaaaaa tctccat
1897

<210> 15
<211> 2244
<212> DNA
<213> Artificial Sequence

<220>
<223> SEQ ID NO:15, Nucleotide sequences for mutated L1R gene

<400> 15
aatattgtac gatgtaatac tagcgtgaac aacttacaga tggataaaac ttcctcatta
60

agattgtcat gtggattaag caatagtgat agattttcta ctgttcccgt caatagagca
120

aaagtagttc aacataatat taaacactcg ttcgacctaa aattgcattt gatcagttta
180

ttatctctct tggtaatatg gatactaatt gtagctattt aaatgggtgc cgcggcaagc
240

ttaatatttt gttaattaaa attatattta taaaatatta tataataaat gggtgccgcg
300

gcaagcatac agacgacggt gaatacactc agcgaacgta tctcgtctaa attagaacaa
360

gcagcggctg ctagtgctgc agcagcatgt gctatagaaa tcggaaattt ttatatccga
420

caaaaccatg gatgtaacct cactgttaaa aatatgtgcg ctgcggccgc ggctgctcag
480

ttggatgctg tgttatcagc cgctacagaa acatatagtg gattaacacc ggaacaaaaa
540

gcatacgtgc cagctatgtt tactgctgcg ttaaacattc agacgagtgt aaacactgtt
600

gttagagatt ttgaaaatta tgtgaaacag acttgtaatt ctagcgcggt cgtcgataac
660

gcattagcga tacaaaacgt aatcatagat gaatgttacg gagccccagg atctccaaca
720

aatttggaat ttattaatac aggatctagc aaaggaaatt gtgccattaa ggcgttgatg
780

caattgacga ctaaggccac tactcaaata gcacctaaac aagttgctgg tacaggagtt
840

cagttttata tgattgttat cggtgttata atattggcag cgttgtttat gtactatgcc
900

aagcgtatgt tgttcacatc caccaatgat aaaatcaaac ttattttagc caataaggaa
960

aacgtccatt ggactactta catggacaca ttctttagaa cttctccgat ggttattgct
1020

accacggata tgcaaaactg ataatccaaa cccacccgct ttttatagta agtttttcac
1080

ccataaataa taaatacaat aattaatttc tcgtaaaagt agaaaatata ttctaattta
1140

ttgcacggta aggaagtaga tcataactcg agataacttc gtataatgta tgctatacga
1200

agttattagc gctaccggtc gccaatggtg agcaagggcg aggagctgtt caccggggtg

1260

gtgcccatcc tggtcgagct ggacggcgac gtaaacggcc acaagttcag cgtgtccggc
1320

gagggcgagg gcgatgccac ctacggcaag ctgaccctga agttcatctg caccaccggc
1380

aagctgcccg tgccctggcc caccctcgtg accaccctga cctacggcgt gcagtgcttc
1440

agccgctacc ccgaccacat gaagcagcac gacttcttca agtccgccat gcccgaaggc
1500

tacgtccagg agcgcaccat cttcttcaag gacgacggca actacaagac ccgcgccgag
1560

gtgaagttcg agggcgacac cctggtgaac cgcatcgagc tgaagggcat cgacttcaag
1620

gaggacggca acatcctggg gcacaagctg gagtacaact acaacagcca caacgtctat
1680

atcatggccg acaagcagaa gaacggcatc aaggtgaact tcaagatccg ccacaacatc
1740

gaggacggca gcgtgcagct cgccgaccac taccagcaga acaccccat cggcgacggc
1800

cccgtgctgc tgcccgacaa ccactacctg agcacccagt ccgccctgag caaagacccc
1860

aacgagaagc gcgatcacat ggtcctgctg gagttcgtga ccgccgccgg gatcactctc
1920

ggcatggacg agctgtacaa gtaattgact agcgctcaat aacttcgtat aatgtatgct
1980

atacgaagtt atattgctac cacggatatg caaaactgaa aatatattga taatatttta
2040

atagattaac atggaagtta tcactgatcg tctagacgat atagtgaaac aaaatatagc
2100

ggatgaaaaa tttgtagatt ttgttataca cggtctagag catcaatgtc ctgctatact
2160

tcgaccatta attaggttgt ttattgatat actattattt gttatagtaa tttatatttt
2220

tacggtacgt ctagtaagta gaaa
2244

<210> 16
<211> 1131
<212> DNA
<213> Artificial Sequence

<220>
<223> SEQ ID NO:16, Nucleotide sequences for CD55-A27

<400> 16
atggattgtg gactgccccc cgacgtccct aacgctcaac ccgctctgga aggcagaaca
60

tccttccccg aagacaccgt gatcacctac aagtgtgagg agagcttcgt caagatcccc
120

ggcgagaagg atagcgtcat ctgtctgaag ggaagccaat ggtccgacat cgaagagttc
180

tgcaacagaa gctgtgaggt gcctaccaga ctgaacagcg cttctctgaa gcagccttac
240

atcacccaga actacttccc cgtgggcacc gtggtggagt acgagtgcag acccggatac
300

agaagagagc cttctctgag ccccaagctg acatgcctcc agaacctcaa gtggagcacc
360

gctgtggagt tttgcaagaa gaagagctgc cccaatcccg gcgagattag aaacggccag
420

attgacgtgc ccggcggcat tctgtttggc gccaccatca gcttcagctg caacaccggc
480

tacaagctgt ttggaagcac cagctccttc tgtctgatca gcggctccag cgtccagtgg
540

agcgatcctc tgcccgagtg tagggagatc tactgccccg cccctcctca aatcgacaac
600

ggcattatcc aaggcgagag ggatcactac ggctatagac agagcgtcac ctacgcttgc
660

aacaagggat tcaccatgat cggcgagcac tccatctact gcacagtcaa caacgacgag
720

ggagaatgga gcggccctcc tcccgagtgt aggggcggcg gcggcagcgg cggcggcggc
780

agcggcggcg gcggcagcga cggaactctt ttccccggag atgacgatct tgcaattcca
840

gcaactgaat ttttttctac aaaggctgct aaagcgccag aggataaagc cgcagacgct
900

gctgcagccg ctgcagacga caatgaggaa actctcaaac aacggctaac taatttggaa
960

aaaaagatta ctaatgtaac aacaaagttt gaacaaatag aaaagtgttg taaacgcaac
1020

gatgaagttc tatttaggtt ggaaaatcac gctgaaactc taagagcggc tatgatatct
1080

ctggctaaaa agattgatgt tcagactgga cgggccgcag ctgagtaata a

<210> 17
<211> 1581
<212> DNA
<213> Artificial Sequence

<220>
<223> SEQ ID NO:17, Nucleotide sequences for FAP-CD3

<400> 17
atggactgga tctggcgcat cctcttcctc gtcggcgctg ctaccggcgc tcattctcag
60

gtgcagctga agcagtctgg agctgaactg gtgaaacccg gggcatcagt gaagctgtcc
120

tgcaagactt ctggctacac cttcactgaa aatattatac actgggtaaa gcagaggtct
180

gggcagggtc ttgagtggat tgggtggttt caccctggaa gtggtagtat aaagtacaat
240

gagaaattca aggacaaggc cacattgact gcggacaaat cctccagcac agtctatatg
300

gagcttagta gattgacatc tgaagactct gcggtctatt tctgtgcaag acacggagga
360

actgggcgag gagctatgga ctactggggt caaggaacct cagtcaccgt ctcgagtggt
420

ggaggcggtt caggcggagg tggctctggc ggtagtgcac aaattctgat gacccagtct
480

cctgcttcct cagttgtatc tctggggcag agggccacca tctcatgcag ggccagcaaa
540

agtgtcagta catctgccta tagttatatg cactggtacc aacagaaacc aggacagcca
600

cccaaactcc tcatctatct tgcatccaac ctagaatctg gggtccctcc caggttcagt
660

ggcagtgggt ctgggacaga cttcaccctc aacatccacc ctgtggagga ggaggatgct
720

gcaacctatt actgtcagca cagtagggag cttccgtaca cgttcggagg ggggaccaag
780

ctggaaataa aacgggcggg atccggagga ggaggatctg gaggaggagg aagtggcggg
840

ggaggctcag tcgacgatat caagctgcag cagtctggag cagagctggc tagaccagga
900

gcatcagtga aaatgagctg taagacctcc ggctatacat tcactcgcta cacaatgcac
960

tgggtgaagc agcgacctgg gcagggactg gaatggatcg ggtacattaa tccaagcagg
1020

ggatacacca actacaacca gaagtttaaa gacaaggcta ctctgactac cgataagtca
1080

agctccaccg catacatgca gctgtctagt ctgacatcag aggacagcgc cgtgtactat
1140

tgcgctcgct actatgacga tcattattgt ctggattatt ggggacaggg gacaactctg
1200

acagtgtcaa gcggaggagg aggaagcgga ggaggcggct ccggcggagg aggctctgac
1260

atccagctga ctcagtctcc cgccattatg tcagcttccc ctggcgaaaa agtgaccatg
1320

acatgccggg cctcctctag tgtcagctat atgaactggt accagcagaa atccgggact
1380

tctccaaagc gatggatcta tgacacctct aaggtggcta gtggagtccc ctaccggttc
1440

tccggatctg gcagtgggac ttcatatagc ctgaccattt caagcatgga ggccgaagat
1500

gctgcaacct actattgtca gcagtggtcc tctaatcccc tgaccttcgg ggctgggact
1560

aaactggaac tgaaatcatg a
1581

<210> 18
<211> 1559
<212> DNA
<213> Artificial Sequence

<220>
<223> SEQ ID NO:18, Nucleotide sequences for BCMA-CD3 scFv

<400> 18
atgctataaa tggctctgcc cgtgacagct ctgctgctcc ctctggctct gctgctgcat
60

gctgctaggc ccgacatcgt gctgacccag tcccctccta gcctcgccat gtctctggga
120

aagagagcca ccatcagctg tagagcctcc gaaagcgtga ccattctcgg cagccatctg
180

atccactggt atcagcagaa gcccggccaa ccccctacac tgctgatcca gctggccagc
240

aatgtgcaga ccggagtgcc cgctagattt tccggatccg gatccagaac cgactttaca
300

ctgaccatcg accccgtgga agaggacgac gtggccgtgt actactgtct gcagtctaga
360

accatcccca gaacattcgg cggaggcaca aagctggaga tcaagggctc cacaagcggc
420

agcggcaaac ccggcagcgg agagggcagc acaaagggcc aaatccagct ggtgcagagc
480

ggccccgaac tcaagaagcc cggagaaacc gtgaagatca gctgcaaggc ctccggctac
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Glu Ile Ile Lys Ser Gly Gly Leu Ser Ser Gly Phe
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Ser Gly Gly Leu Ser Ser Gly Phe Tyr Phe Glu Ile
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Ser Ser Gly Phe Tyr Phe Glu Ile Ala Arg Ile Glu
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<223> Position 54 of the Peptide Array

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Tyr Phe Glu Ile Ala Arg Ile Glu Asn Glu Met Lys
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<210> 74
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Ala Arg Ile Glu Asn Glu Met Lys Ile Asn Arg Gln
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<210> 75
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Asn Glu Met Lys Ile Asn Arg Gln Ile Leu Asp Asn
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<210> 76
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<210> 77
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Ile Leu Asp Asn Ala Ala Lys Tyr Val Glu His Asp
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<210> 79
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<400> 79

Val Glu His Asp Pro Arg Leu Val Ala Glu His Arg
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Pro Arg Leu Val Ala Glu His Arg Phe Glu Asn Met
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Ala Glu His Arg Phe Glu Asn Met Lys Pro Asn Phe
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Phe Glu Asn Met Lys Pro Asn Phe Trp Ser Arg Ile
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<210> 84
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<400> 84

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<210> 85
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<400> 85

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<210> 86

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<220>
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<400> 86

Thr Lys Arg Tyr Pro Gly Val Met Tyr Ala Phe Thr
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<210> 87
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<400> 87

Pro Gly Val Met Tyr Ala Phe Thr Thr Pro Leu Ile
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<220>
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Tyr Ala Phe Thr Thr Pro Leu Ile Ser Phe Phe Gly
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<400> 89

Pro Val Ile Asp Arg Leu Pro
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Asn Asp Gln Lys Phe Asp Asp Val Lys Asp Asn
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Pro Glu Arg Lys Asn Val Val Val Val
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<400> 94

Lys Val Lys Thr Glu Leu Val Met
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<210> 95
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<223> aa 197-204 of the H3L Peptides

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Asn Ile Val Asp Glu Ile Ile Lys
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<210> 96
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<400> 96

Lys Ile Asn Arg Gln Ile
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<210> 97
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<400> 97

Phe Glu Asn Met Lys Pro Asn Phe
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<210> 98
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Ala Asp Gln Lys Phe Asp Asp Val Lys Asp Asn
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<210> 106
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<400> 107

Asn Asp Ala Lys Phe Asp Asp Val Lys Asp Asn
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Asn Asp Gln Ala Phe Asp Asp Val Lys Asp Asn
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<400> 109

Asn Asp Gln Lys Ala Asp Asp Val Lys Asp Asn
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<220>
<223> Variant of Peptide 2 (Seq No. 90)

<400> 111

Asn Asp Gln Lys Phe Asp Ala Val Lys Asp Asn
1   5   10

<210> 112
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 2 (Seq No. 90)

<400> 112

Asn Asp Gln Lys Phe Asp Asp Ala Lys Asp Asn
1   5   10

<210> 113
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 2 (Seq No. 90)

<400> 113

Asn Asp Gln Lys Phe Asp Asp Val Ala Asp Asn
1   5   10

<210> 114
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 2 (Seq No. 90)

<400> 114

Asn Asp Gln Lys Phe Asp Asp Val Lys Ala Asn
1   5   10

<210> 115
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 2 (Seq No. 90)

<400> 115

Asn Asp Gln Lys Phe Asp Asp Val Lys Asp Ala
1 5 10

<210> 116
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 3 (Seq No. 91)

<400> 116

Ala Lys Arg Asn Val Val Val Val
1 5

<210> 117
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 3 (Seq No. 91)

<400> 117

Glu Ala Arg Asn Val Val Val Val
1 5

<210> 118
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 3 (Seq No. 91)

<400> 118

Glu Lys Ala Asn Val Val Val Val
1 5

<210> 119
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 3 (Seq No. 91)

<400> 119

Glu Lys Arg Ala Val Val Val Val

1 5

<210> 120
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 3 (Seq No. 91)

<400> 120

Glu Lys Arg Asn Ala Val Val Val
1 5

<210> 121
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 3 (Seq No. 91)

<400> 121

Glu Lys Arg Asn Val Ala Val Val
1 5

<210> 122
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 3 (Seq No. 91)

<400> 122

Glu Lys Arg Asn Val Val Ala Val
1 5

<210> 123
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 3 (Seq No. 91)

<400> 123

Glu Lys Arg Asn Val Val Val Ala
1 5

<210> 124
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 4 (Seq No. 92)

<400> 124

Ala Val Ile Glu Asp Ile Thr Phe Leu Arg
1 5 10

<210> 125
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 4 (Seq No. 92)

<400> 125

Asn Ala Ile Glu Asp Ile Thr Phe Leu Arg
1 5 10

<210> 126
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 4 (Seq No. 92)

<400> 126

Asn Val Ala Glu Asp Ile Thr Phe Leu Arg
1 5 10

<210> 127
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 4 (Seq No. 92)

<400> 127

Asn Val Ile Ala Asp Ile Thr Phe Leu Arg
1 5 10

<210> 128
<211> 10
<212> PRT

<213> Artificial Sequence

<220>
<223> Variant of Peptide 4 (Seq No. 92)

<400> 128

Asn Val Ile Glu Ala Ile Thr Phe Leu Arg
1 5 10

<210> 129
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 4 (Seq No. 92)

<400> 129

Asn Val Ile Glu Asp Ala Thr Phe Leu Arg
1 5 10

<210> 130
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 4 (Seq No. 92)

<400> 130

Asn Val Ile Glu Asp Ile Ala Phe Leu Arg
1 5 10

<210> 131
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 4 (Seq No. 92)

<400> 131

Asn Val Ile Glu Asp Ile Thr Ala Leu Arg
1 5 10

<210> 132
<211> 10
<212> PRT
<213> Artificial Sequence

<220>

<223> Variant of Peptide 4 (Seq No. 92)

<400> 132

Asn Val Ile Glu Asp Ile Thr Phe Ala Arg
1   5   10

<210> 133
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 4 (Seq No. 92)

<400> 133

Asn Val Ile Glu Asp Ile Thr Phe Leu Ala
1   5   10

<210> 134
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 5 (Seq No. 93)

<400> 134

Ala Met Arg Glu Ile
1   5

<210> 135
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 5 (Seq No. 93)

<400> 135

Gln Ala Arg Glu Ile
1   5

<210> 136
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 5 (Seq No. 93)

<400> 136

Gln Met Ala Glu Ile
1 5

<210> 137
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 5 (Seq No. 93)

<400> 137

Gln Met Arg Ala Ile
1 5

<210> 138
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 5 (Seq No. 93)

<400> 138

Gln Met Arg Glu Ala
1 5

<210> 139
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 6 (Seq No. 94)

<400> 139

Ala Val Lys Thr Glu Leu Val Met
1 5

<210> 140
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 6 (Seq No. 94)

<400> 140

Lys Ala Lys Thr Glu Leu Val Met
1 5

<210> 141
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 6 (Seq No. 94)

<400> 141

Lys Val Ala Thr Glu Leu Val Met
1 5

<210> 142
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 6 (Seq No. 94)

<400> 142

Lys Val Lys Ala Glu Leu Val Met
1 5

<210> 143
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 6 (Seq No. 94)

<400> 143

Lys Val Lys Thr Ala Leu Val Met
1 5

<210> 144
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 6 (Seq No. 94)

<400> 144

Lys Val Lys Thr Glu Ala Val Met
1 5

<210> 145

<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 6 (Seq No. 94)

<400> 145

Lys Val Lys Thr Glu Leu Ala Met
1 5

<210> 146
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 6 (Seq No. 94)

<400> 146

Lys Val Lys Thr Glu Leu Val Ala
1 5

<210> 147
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 7 (Seq No. 95)

<400> 147

Ala Ile Val Asp Glu Ile Ile Lys
1 5

<210> 148
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 7 (Seq No. 95)

<400> 148

Asn Ala Val Asp Glu Ile Ile Lys
1 5

<210> 149
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 7 (Seq No. 95)

<400> 149

Asn Ile Ala Asp Glu Ile Ile Lys
1   5

<210> 150
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 7 (Seq No. 95)

<400> 150

Asn Ile Val Ala Glu Ile Ile Lys
1   5

<210> 151
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 7 (Seq No. 95)

<400> 151

Asn Ile Val Asp Ala Ile Ile Lys
1   5

<210> 152
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 7 (Seq No. 95)

<400> 152

Asn Ile Val Asp Glu Ala Ile Lys
1   5

<210> 153
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 7 (Seq No. 95)

<400> 153

Asn Ile Val Asp Glu Ile Ala Lys
1 5

<210> 154
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 7 (Seq No. 95)

<400> 154

Asn Ile Val Asp Glu Ile Ile Ala
1 5

<210> 155
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 8 (Seq No. 96)

<400> 155

Ala Ile Asn Arg Gln Ile
1 5

<210> 156
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 8 (Seq No. 96)

<400> 156

Lys Ala Asn Arg Gln Ile
1 5

<210> 157
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 8 (Seq No. 96)

<400> 157

Lys Ile Ala Arg Gln Ile
1 5

<210> 158
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 8 (Seq No. 96)

<400> 158

Lys Ile Asn Ala Gln Ile
1 5

<210> 159
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 8 (Seq No. 96)

<400> 159

Lys Ile Asn Arg Ala Ile
1 5

<210> 160
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 8 (Seq No. 96)

<400> 160

Lys Ile Asn Arg Gln Ala
1 5

<210> 161
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 9 (Seq No. 97)

<400> 161

Ala Glu Asn Met Lys Pro Asn Phe
1 5

<210> 162
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 9 (Seq No. 97)

<400> 162

Phe Ala Asn Met Lys Pro Asn Phe
1 5

<210> 163
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 9 (Seq No. 97)

<400> 163

Phe Glu Ala Met Lys Pro Asn Phe
1 5

<210> 164
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 9 (Seq No. 97)

<400> 164

Phe Glu Asn Ala Lys Pro Asn Phe
1 5

<210> 165
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 9 (Seq No. 97)

<400> 165

Phe Glu Asn Met Ala Pro Asn Phe
1 5

<210> 166
<211> 8

<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 9 (Seq No. 97)

<400> 166

Phe Glu Asn Met Lys Ala Asn Phe
1 5

<210> 167
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 9 (Seq No. 97)

<400> 167

Phe Glu Asn Met Lys Pro Ala Phe
1 5

<210> 168
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Variant of Peptide 9 (Seq No. 97)

<400> 168

Phe Glu Asn Met Lys Pro Asn Ala
1 5

<210> 169
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Control peptide for set 3 peptides

<400> 169

Glu Lys Arg Asn Val Val Val Val
1 5

<210> 170
<211> 324
<212> PRT
<213> Artificial Sequence

<220>
<223> Mutant H3L amino acid

<400> 170

Met Ala Ala Ala Lys Thr Pro Val Ile Val Val Pro Val Ala Ala Ala
1   5   10   15

Leu Pro Ser Glu Thr Phe Pro Asn Val His Glu His Ile Asn Asp Gln
            20   25   30

Ala Ala Ala Asp Val Ala Asp Ala Glu Val Met Ala Ala Lys Arg Asn
        35   40   45

Val Val Val Ala Lys Asp Asp Pro Asp His Tyr Lys Asp Tyr Ala Phe
    50   55   60

Ile Gln Trp Thr Gly Gly Asn Ile Arg Asn Asp Asp Lys Tyr Thr His
65   70   75   80

Phe Phe Ser Gly Phe Cys Asn Thr Met Cys Thr Glu Glu Thr Lys Arg
                85   90   95

Asn Ile Ala Arg His Leu Ala Leu Trp Asp Ser Asn Phe Phe Thr Glu
            100   105   110

Leu Glu Asn Lys Lys Val Glu Tyr Val Val Ile Val Glu Asn Asp Asn
        115   120   125

Val Ile Ala Ala Ile Ala Ala Ala Ala Pro Val Leu Lys Ala Met His
    130   135   140

Asp Lys Lys Ile Asp Ile Leu Gln Met Ala Ala Ala Ile Thr Gly Asn
145   150   155   160

Ala Val Lys Thr Glu Ala Ala Ala Asp Lys Asn His Ala Ile Phe Thr
                165   170   175

Tyr Thr Gly Gly Tyr Asp Val Ser Leu Ser Ala Tyr Ile Ile Arg Val
            180   185   190

Thr Thr Ala Leu Asn Ala Ala Asp Glu Ile Ile Lys Ser Gly Gly Leu
        195   200   205

Ser Ser Gly Phe Tyr Phe Glu Ile Ala Arg Ile Glu Asn Glu Met Lys
    210   215   220

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Ile Asn Ala Gln Ile Leu Asp Asn Ala Ala Lys Tyr Val Glu His Asp
225                 230                 235                 240

Pro Arg Leu Val Ala Glu His Arg Phe Ala Ala Ala Ala Ala Ala Ala
                245                 250                 255

Trp Ala Arg Ile Gly Pro Ala Thr Thr Ile Arg Cys Pro Gly Val Lys
            260                 265                 270

Asn Ala Asn Thr Ala Pro Leu Ile Ser Phe Phe Gly Leu Phe Asp Ile
        275                 280                 285

Asn Val Ile Gly Leu Ile Val Ile Leu Phe Ile Met Phe Met Leu Ile
    290                 295                 300

Phe Asn Val Lys Ser Lys Leu Leu Trp Phe Leu Thr Gly Thr Phe Val
305                 310                 315                 320

Thr Ala Phe Ile
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<210> 171
<211> 978
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequences for Mutant H3L

<400> 171
atggctgccg ccaaaacccc cgtgattgtg gtccccgtgg ccgctgctct gccttccgag
60

acattcccca acgtgcacga acacatcaat gaccaagctg ccgctgacgt ggccgacgcc
120

gaagtcatgg ccgctaagag aaacgtggtc gtggccaagg atgaccccga ccactacaag
180

gactatgcct tcatccagtg gactggtggc aacatcagaa acgacgacaa gtacacccat
240

ttcttcagcg gcttctgcaa caccatgtgt accgaggaga ccaagaggaa catcgctcgt
300

cacctcgccc tctgggactc caatttcttc accgagctgg agaacaagaa ggtcgagtac
360

gtggtgatcg tggagaacga caacgtgatc gccgctatcg ctgccgccgc tcccgtttta
420

aaagccatgc acgacaagaa gatcgacatt ttacagatgg ccgctgccat caccggaaac
480

gccgtcaaga ccgaggctgc cgccgataag aaccacgcca tcttcaccta caccggcgga
540

tatgacgtga gcctctccgc ttacatcatt agggtgacca ccgctttaaa cgccgccgac
600

gaaatcatca aatccggagg tttaagctcc ggcttctact tcgagatcgc tcgtatcgag
660

aatgaaatga agatcaatgc ccagatttta gataatgccg ccaaatacgt ggaacatgac
720

cctcgtctgg tggctgagca tcgttttgct gctgctgccg ctgctgcttg ggccagaatc
780

ggacccgcca ccaccattag atgccccggt gtgaaaaacg ccaacaccgc ccctttaatt
840

tccttcttcg gtttattcga catcaacgtg atcggcctca tcgtgatttt attcatcatg
900

ttcatgctga tcttcaacgt gaagtccaag ttattatggt ttttaactgg taccttcgtg
960

accgccttca tctgataa
978

<210> 172
<211> 304
<212> PRT
<213> Artificial Sequence

<220>
<223> Mutant D8L amino acid sequence

<400> 172

Met Pro Gln Gln Leu Ser Pro Ile Asn Ile Glu Thr Lys Lys Ala Ile
1 5 10 15

Ser Asn Ala Arg Leu Lys Pro Leu Asp Ile His Tyr Asn Glu Ser Lys
20 25 30

Pro Thr Thr Ile Gln Asn Thr Gly Lys Leu Ala Ala Ile Asn Phe Ala
35 40 45

Gly Gly Tyr Ile Ala Ala Ala Phe Leu Pro Asn Glu Tyr Val Leu Ser
50 55 60

Ser Leu His Ile Tyr Trp Gly Lys Glu Asp Asp Tyr Gly Ser Asn His

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65                  70                  75                  80

Leu Ile Asp Val Tyr Lys Tyr Ser Gly Glu Ile Asn Leu Val His Trp
                85                  90                  95

Asn Ala Lys Lys Tyr Ser Ser Tyr Glu Glu Ala Ala Ala His Asp Asp
            100                 105                 110

Gly Leu Ile Ile Ile Ser Ile Phe Leu Gln Val Leu Asp His Lys Asn
        115                 120                 125

Val Tyr Phe Gln Lys Ile Val Asn Gln Leu Asp Ser Ile Arg Ser Gly
    130                 135                 140

Asn Thr Ser Ala Pro Phe Asp Ser Val Phe Tyr Leu Asp Asn Leu Leu
145                 150                 155                 160

Pro Ser Lys Leu Asp Tyr Phe Ala Tyr Leu Gly Thr Thr Ile Asn His
                165                 170                 175

Ala Ala Asp Ala Val Trp Ile Ile Phe Pro Thr Pro Ile Asn Ile His
            180                 185                 190

Ser Asp Gln Ala Ser Lys Ala Arg Thr Leu Ala Ser Ser Ser Ala His
        195                 200                 205

Asp Gly Lys Ala His Tyr Ile Thr Glu Ala Tyr Ala Asn Ala Tyr Lys
    210                 215                 220

Leu Asn Ala Asp Thr Gln Val Tyr Tyr Ser Gly Glu Ile Ile Arg Ala
225                 230                 235                 240

Ala Thr Thr Ser Pro Ala Arg Glu Asn Tyr Phe Met Arg Trp Leu Ser
                245                 250                 255

Asp Leu Arg Glu Thr Cys Phe Ser Tyr Tyr Gln Lys Tyr Ile Glu Glu
            260                 265                 270

Asn Lys Thr Phe Ala Ile Ile Ala Ile Val Phe Val Phe Ile Leu Thr
        275                 280                 285

Ala Ile Leu Phe Phe Met Ser Arg Arg Tyr Ser Arg Glu Lys Gln Asn
    290                 295                 300
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<210> 173
<211> 918
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequences for Mutant D8L

<400> 173
atgccccagc aactgtctcc catcaacatc gagaccaaga aggccatttc caacgctcgt
60

ctgaagcctt tagacatcca ctacaatgag agcaagccca ccaccatcca gaacactggt
120

aagctggccg ccatcaactt tgccggcggc tacatcgccg ccgcctttct gcccaacgag
180

tacgtgctca gctctttaca catctattgg ggcaaagagg acgactacgg ctccaaccat
240

ttaatcgacg tctacaagta ttccggcgag atcaatttag tgcactggaa cgccaagaag
300

tactccagct acgaagaagc cgctgcccac gacgacggac tgatcatcat cagcatcttt
360

ctccaagttc tggaccacaa gaacgtgtac ttccagaaga tcgtcaacca gctcgacagc
420

attcgttccg gcaatacatc cgccccctttt gattccgtgt tctatttaga caatttactg
480

ccctccaagc tggactactt cgcctattta ggcaccacca tcaatcacgc cgccgatgct
540

gtgtggatca tcttccccac ccccattaac attcacagcg atcaagctag caaggccaga
600

actttagcct ccagcagcgc tcacgacggc aaggctcact acatcaccga ggcctatgcc
660

aacgcctaca agctcaacgc cgacacccaa gtttactact ccggtgagat cattagagct
720

gccacaacct cccccgctcg tgagaactac ttcatgaggt ggctgtccga tttaagagag
780

acttgtttct cctactatca gaaatacatc gaggagaaca agaccttcgc catcatcgcc
840

atcgtgttcg tgttcatttt aaccgccatt ttattcttca tgtctcgtag gtactctcgt
900

gagaagcaga attgataa
918

<210> 174

<211> 304
<212> PRT
<213> Artificial Sequence

<220>
<223> mutant D8L amino acid sequence (Alternate for Seq No. 6 - only different at position 43)

<400> 174

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Met Pro Gln Gln Leu Ser Pro Ile Asn Ile Glu Thr Lys Lys Ala Ile
1               5                  10                  15

Ser Asn Ala Arg Leu Lys Pro Leu Asp Ile His Tyr Asn Glu Ser Lys
            20                  25                  30

Pro Thr Thr Ile Gln Asn Thr Gly Lys Leu Leu Trp Ile Asn Phe Lys
        35                  40                  45

Gly Gly Tyr Ile Ser Gly Trp Phe Leu Pro Asn Glu Tyr Val Leu Ser
    50                  55                  60

Ser Leu His Ile Tyr Trp Gly Lys Glu Asp Asp Tyr Gly Ser Asn His
65                  70                  75                  80

Leu Ile Asp Val Tyr Lys Tyr Ser Gly Glu Ile Asn Leu Val His Trp
                85                  90                  95

Asn Lys Lys Lys Tyr Ser Ser Tyr Glu Glu Ala Lys Lys His Asp Asp
            100                 105                 110

Gly Leu Ile Ile Ile Ser Ile Phe Leu Gln Val Leu Asp His Lys Asn
        115                 120                 125

Val Tyr Phe Gln Lys Ile Val Asn Gln Leu Asp Ser Ile Arg Ser Thr
    130                 135                 140

Asn Thr Ser Ala Pro Phe Asp Ser Val Phe Tyr Leu Asp Asn Leu Leu
145                 150                 155                 160

Pro Ser Lys Leu Asp Tyr Phe Ser Tyr Leu Gly Thr Thr Ile Asn His
                165                 170                 175

Tyr Ala Asp Ala Val Trp Ile Ile Phe Pro Thr Pro Ile Asn Ile His
            180                 185                 190

Ser Asp Gln Leu Ser Lys Tyr Arg Thr Leu Ser Ser Ser Ser Asn His
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        195                 200                 205

Asp Gly Lys Thr His Tyr Ile Thr Glu Cys Tyr Arg Asn Leu Tyr Lys
    210                 215                 220

Leu Asn Gly Asp Thr Gln Val Tyr Tyr Ser Gly Glu Ile Ile Arg Ala
225                 230                 235                 240

Ala Thr Thr Ser Pro Ala Arg Glu Asn Tyr Phe Met Arg Trp Leu Ser
                245                 250                 255

Asp Leu Arg Glu Thr Cys Phe Ser Tyr Tyr Gln Lys Tyr Ile Glu Glu
            260                 265                 270

Asn Lys Thr Phe Ala Ile Ile Ala Ile Val Phe Val Phe Ile Leu Thr
        275                 280                 285

Ala Ile Leu Phe Phe Met Ser Arg Arg Tyr Ser Arg Glu Lys Gln Asn
    290                 295                 300
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