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(54) Titre : MODIFICATION NON GENETIQUE DE VIRUS A ENVELOPPE
 (54) Title: NON-GENETIC MODIFICATION OF ENVELOPED VIRUSES

Anti-tumour peptides anchored into viral envelope by CPP or Cholesterol moieties induces extensive anti-tumour immunity and protects treated mice from tumour rechallenge

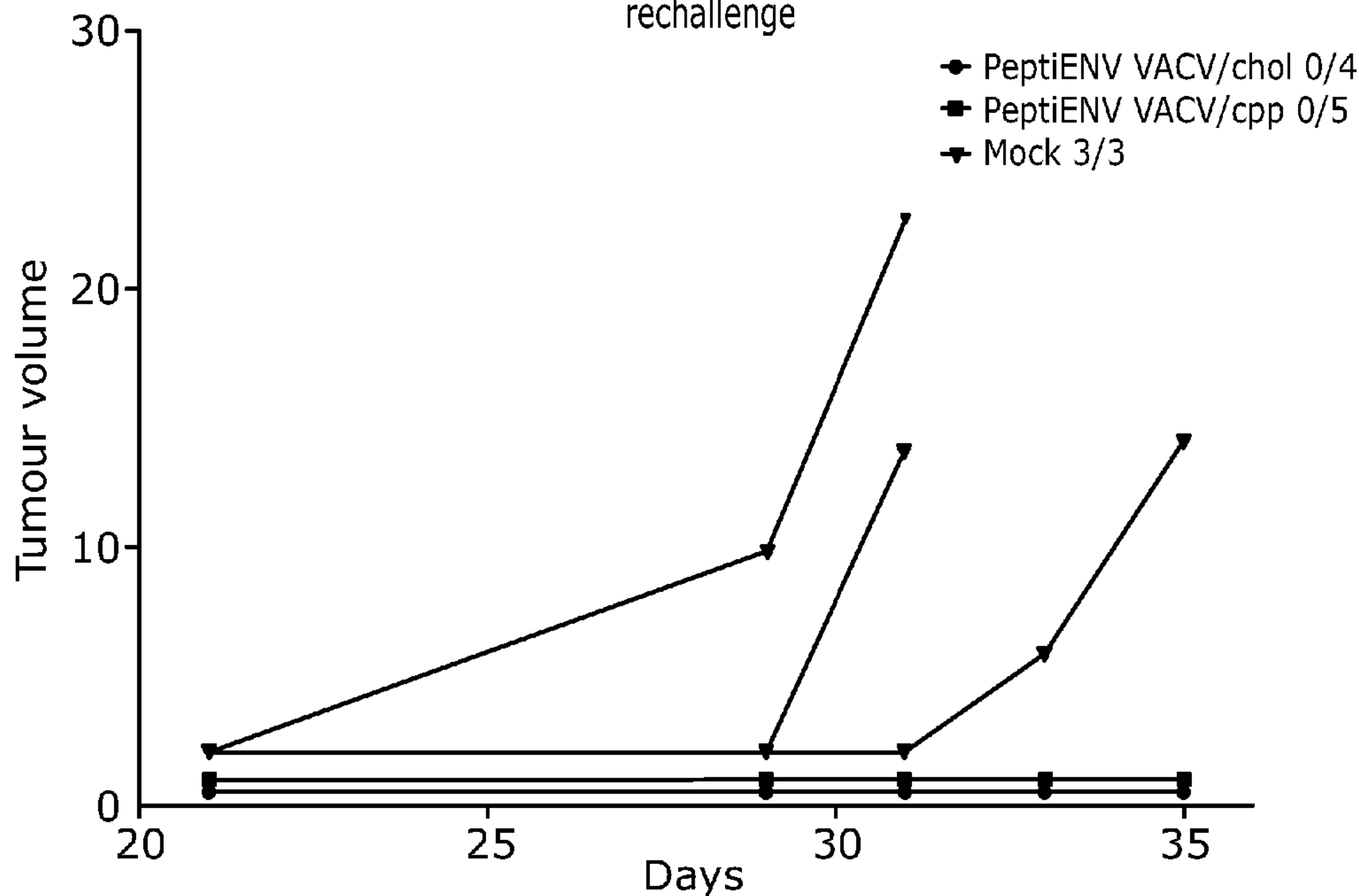


Figure 11

(57) **Abrégé/Abstract:**

The invention concerns a modified enveloped virus wherein said virus has at least one anti-tumor, tumor-specific peptide non-genetically attached to or inserted in/through the viral envelope; a pharmaceutical composition comprising same; and a method of treating cancer using same.

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Anti-tumour peptides anchored into viral envelope by CPP or Cholesterol moieties induces extensive anti-tumour immunity and protects treated mice from tumour rechallenge

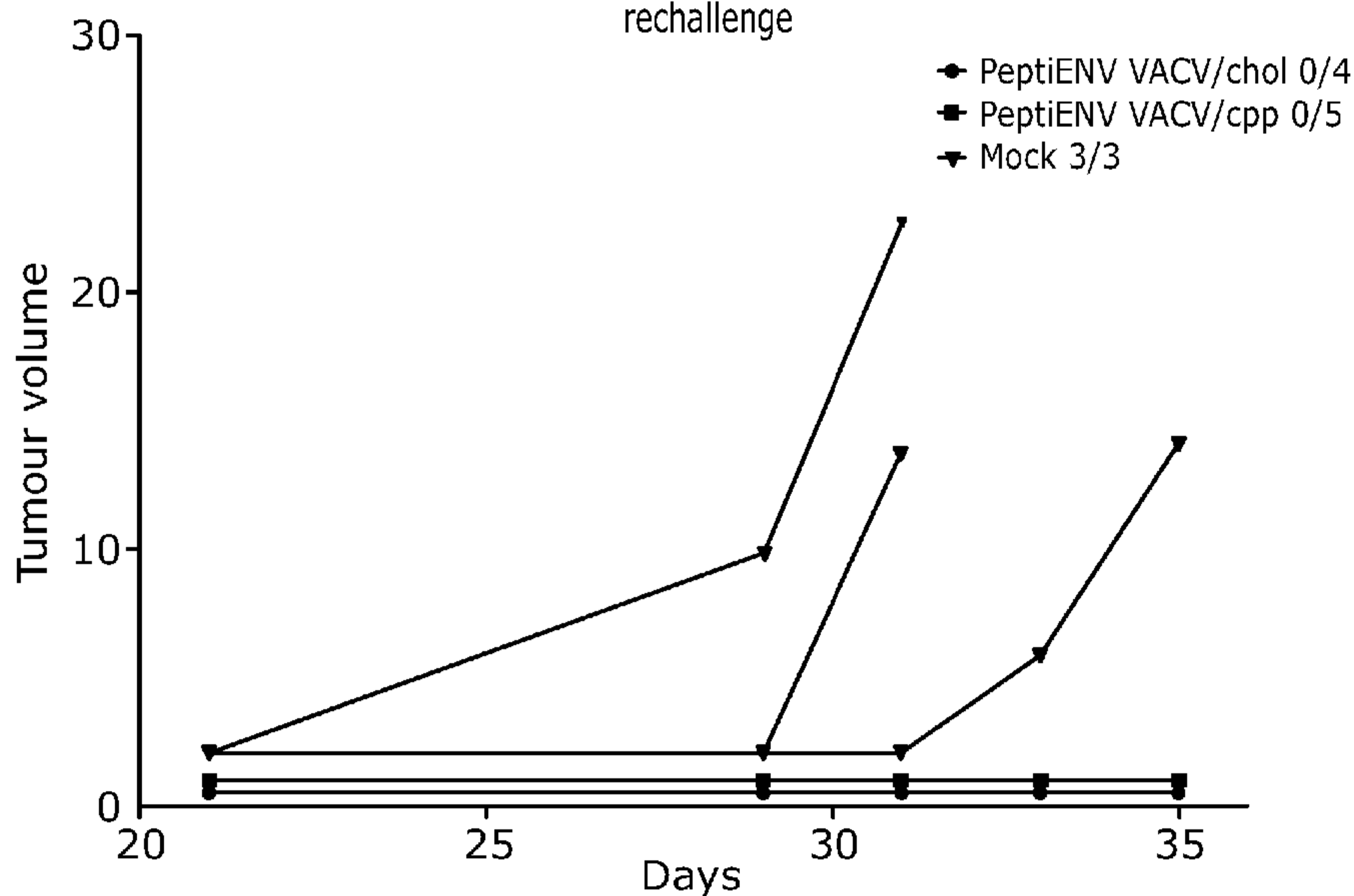


Figure 11

(57) Abstract: The invention concerns a modified enveloped virus wherein said virus has at least one anti-tumor, tumor-specific peptide non-genetically attached to or inserted in/through the viral envelope; a pharmaceutical composition comprising same; and a method of treating cancer using same.

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Non-genetic modification of enveloped viruses

Field of the Invention

The invention concerns a modified enveloped virus wherein said virus has at least one anti-tumor, tumor-specific peptide non-genetically attached to or inserted in/through the viral envelope; a pharmaceutical composition comprising same; and a method of treating cancer using same.

Background of the Invention

The current paradigm for effective cancer therapeutics is the enhancement of the activity and increase in the amount of tumor infiltrating tumor-specific T-effector cells. These T-effector cells, named CD8+ T cells, specifically cytotoxic T cell lymphocytes (CTLs) are a critical component of the protective immune response against tumors. These tumor-specific CTLs can be found in tumor tissue and there is a clear correlation between the amount of tumor-infiltrated CTLs and patient survival.

A recent approval of antibodies targeting immune checkpoint molecules such as PD-1, PD-L1 and CTLA-4, which function to interrupt the negative feedback systems within the tumor microenvironment to enhance pre-existing anti-tumor immune responses, have been met with tremendous clinical excitement. The use of these immune checkpoint inhibitor antibodies can create durable responses in 10-20% of cancer patients. However, the remaining 80-90% of patients are not responding due to the lack of anti-tumor immune responses or other immune suppressive aspects of the tumor microenvironment. To broaden the patient population responding to checkpoint inhibitor-therapy we have developed an enveloped viral vector platform called PeptiENV for augmenting or generating a broad anti-tumor immunity and to recruit tumor-specific T-effector cells into the tumor microenvironment.

Advantageously, by treating patients with a combination of immunostimulatory PeptiENV viruses and immune checkpoint inhibitor antibodies we expect to increase the amount of responders to the immune checkpoint inhibitor therapies.

This patent application describes a PeptiENV platform, which includes a novel method for coating and inserting immunomodulatory peptides onto a viral envelope which can then be readily cross-presented on antigen presenting cells. Currently there are no methods for non-

genetically attaching peptides onto the viral envelope with a view to activating the immune system. WO2005/060541 teaches anti-viral peptides that are inserted into a viral coat for the purposes of disrupting the viral membrane and destroying same.

5 Some viruses have viral envelopes covering their protective protein capsids. The envelopes are typically derived from portions of the host cell membranes (phospholipids and proteins), but include some viral glycoproteins. They may help viruses avoid the host immune system. Glycoproteins on the surface of the envelope serve to identify and bind to receptor sites on the host's membrane. The viral envelope then fuses with the host's membrane, allowing the
10 capsid and viral genome to enter and infect the host. In addition to entering the host cell via fusion of the viral and host cell membranes, some viruses can alternatively use endocytosis as an entry mechanism.

Essentially, we have found a novel way of boosting anti-tumor immunity at the expense of
15 anti-viral immunity using known therapeutic and clinically approved viruses.

Statements of the Invention

According to a first aspect, there is provided a modified enveloped virus selected from the group comprising Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2),
20 Vaccinia Vesicular stomatitis Indiana virus (VSV), Measles Virus (MeV), Maraba virus and New Castle Disease (NDV) virus wherein said virus has at least one anti-tumor, tumor-specific peptide non-genetically attached to or inserted in/through the viral envelope.

Reference here in to a modified enveloped virus, is to a virus that is modified non-genetically
25 to include said at least one anti-tumor, tumor-specific peptide in its viral envelope. For the avoidance of doubt said virus may or may not include any other genetic modification(s) that make(s) it suitable for its purpose but the attachment of said at least one anti-tumor, tumor-specific peptide to or through the viral envelope is undertaken non-genetically.

30 Those skilled in the art will realise that some viruses have viral envelopes covering their protective protein capsids. The envelopes are typically derived from portions of the host cell membranes (phospholipids and proteins), but include some viral glycoproteins. They may help viruses avoid the host immune system. Glycoproteins on the surface of the envelope serve to identify and bind to receptor sites on the host's membrane. During infection, the
35 viral envelope fuses with the host's membrane, allowing the capsid and viral genome to

enter and infect the host. Peptides attached to or inserted in or taken through the viral envelope can thus be used as antigens to trigger an immune response.

5

Reference herein to an anti-tumor peptide is to a peptide that can elicit an immune response against a tumor.

10

Reference herein to a tumor-specific peptide is to a peptide that can elicit an immune response against a particular one or more tumour(s).

In a preferred embodiment said peptide is patient-identified or patient-specific.

15

As will be apparent to those skilled in the art the exact nature of the peptide can vary having regard to the nature of the tumour to be treated, indeed the specificity of the technology means that different anti-tumor, tumor-specific peptides will be used to treat individuals presenting with different types of cancer and even different anti-tumor, tumor-specific peptides can be used to treat individuals presenting with the same type of cancer.

20

In yet a further embodiment of the invention said peptide is between 8-50 amino acids long, ideally 15-35 amino acids long. Most ideally said peptide has a length selected from the group comprising: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 and 50 amino acids.

25

Most ideally a plurality of said peptides are attached to or inserted in/through the viral envelope. These peptides may be identical or represent the same antigen with only a minor modification i.e. greater than 90% sequence identity with each other and most ideally greater than 92%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with each other.

30

Alternatively, a number of different anti-tumor, tumor-specific peptides are non-genetically attached to or inserted in/through the viral envelope.

35

More preferably, said peptide also comprises at least one cleavage site, such as, without limitation, a cathepsin cleavage site or a furin cleavage site. More preferably still, said peptide comprises at least one immunoproteasome processing site. Examples of these sites and their relative positioning with respect to the structure of the conjugated peptide are

shown in Figure 7. Ideally the one anti-tumor, tumor-specific peptide is positioned between a pair of immunoproteasome processing sites and upstream thereof is at least one cleavage site, ideally a furin cleavage site followed (upstream) by a cathepsin cleavage site.

- 5 Advantageously, we have found that these peptides when attached to or inserted in/through selected enveloped viruses (HSV-1 and -2, Vaccinia, VSV, MeV, Maraba virus and NDV) can trigger increased tumor-specific immune responses and drastically enhance anti-tumor efficacy by converting the anti-viral immunity into an anti-tumor immunity.
- 10 The elegance of this platform, compared to others, is that by attaching or inserting the patient-identified anti-tumor inducing tumor-specific peptides non-genetically to/in/through the viral envelope we can make clinical use of a medically approved virus. This means one can react very quickly to changes in a patients' tumor antigens that are presented on MHC-I simply by coating the virus with a new set of tumor-specific peptides derived from said
- 15 patient.

Another important feature of the invention is that the virus chosen for this platform needs to go through rigorous quality control and approval stages only once, thus saving time and money when compared to other platforms where viruses, having genetically introduced

20 modifications, need to go through checking stages every time a new modification or a peptide is introduced, thus making it virtually impossible to use these platforms in personalized medicine.

In a further preferred embodiment of the invention said peptide(s) is/are attached to or

25 inserted in/through said viral envelope using either a cell penetrating peptide or a cholesterol-conjugated peptide (purchased from PepScan or Ontores).

As is known to those skilled in the art, cell penetrating peptides (CPPs) are short peptides that facilitate cellular intake/uptake of various molecular equipment (from nanosize particles

30 to small chemical molecules and large fragments of DNA). This "cargo" is associated with the peptides either through chemical linkage via covalent bonds or through non-covalent interactions. The function of the CPP is to deliver the cargo into cells, a process that commonly occurs through endocytosis.

35 CPPs typically have an amino acid composition that either contains a high relative abundance of positively charged amino acids such as lysine or arginine or has sequences

that contain an alternating pattern of polar/charged amino acids and non-polar, hydrophobic amino acids. These two types of structures are referred to as polycationic or amphipathic, respectively. A third class of CPPs are the hydrophobic peptides, containing only apolar residues, with low net charge or have hydrophobic amino acid groups that are crucial for cellular uptake.

The invention envisages the use of any known CCP when linked with said anti-tumor, tumor-specific peptide.

Without wishing to be bound by an explanation of the mechanism of action, we consider that although CPPs normally deliver their cargo through the lipid bilayer, a peptide consisting of a CPP sequence together with our immunogenic peptide, partially passes through the lipid bilayer while part of it appears to stick, possibly via physical hydrophobic/-philic interactions, on the membrane sufficiently, if not exclusively, for our purposes.

An example of a CPP when conjugated to said peptide is shown in Figure 7B.

As is also known to those skilled in the art, cholesterol-conjugated peptides are short peptides attached to cholesterol. We have discovered that they can enter the viral envelope and so anchor the conjugated peptide within the viral coat. These peptides can be N- or C-terminally cholesterol conjugated.

Again, without wishing to be bound by an explanation of the mechanism of action, we consider that since cholesterol is a component of a lipid membrane, cholesterol-conjugated peptides find their "normal" location in the membrane. In fact we speculate that the hydroxy group on cholesterol interacts with the polar head groups of the membrane phospholipids and sphingolipids, while the bulky steroid and the hydrocarbon chain are embedded in the membrane, alongside the nonpolar fatty-acid chain of the other lipids.

An example of a cholesterol-conjugated peptide is shown in Figure 7A.

In a preferred embodiment of the invention the peptide for attaching to or inserting in/through said envelope comprises:

GRKKRRQRRRPQ (SEQ ID NO: 1) CPP sequence in the N- or C-terminus of the said anti-tumor, tumor-specific peptide;

RQIKIWFQNRRMKWKK (SEQ ID NO: 2) CPP sequence in the N- or C-terminus of the said anti-tumor, tumor-specific peptide;

KLALKLALKALKAALKLA (SEQ ID NO: 3) CPP sequence in the N- or C-terminus of the said anti-tumor, tumor-specific peptide;

5 RRRRRRRRRR (SEQ ID NO: 4) CPP sequence in the N- or C-terminus of the said anti-tumor, tumor-specific peptide;

KETWWETWWTEWSQPKKKRKV (SEQ ID NO: 5) CPP sequence in the N- or C-terminus of the said anti-tumor, tumor-specific peptide;

10 AGYLLGKINLKALAALAKKIL (SEQ ID NO: 6) CPP sequence in the N- or C-terminus of the said anti-tumor, tumor-specific peptide;

AGLWRALWRLLRSLWRLWRA (SEQ ID NO: 7) CPP sequence in the N- or C-terminus of the said anti-tumor, tumor-specific peptide; or

a cholesterol moiety N- or C-terminus of the said anti-tumor, tumor-specific peptide.

15 In yet a further preferred embodiment of the invention the peptide for attaching to or inserting in/through said envelope comprises one of the following wherein the sequence SIINFEKL is simply representative of a MHC-I restricted epitope or peptide:

CPP peptides:

20 GRKKRRQRRRPQRVRRALISLEQLESSIINFEKLTEW (SEQ ID NO: 8)

25 RQIKIWFQNRRMKWKKRWEKISIINFEKLYKLK (SEQ ID NO:9)

KLALKLALKALKAALKLARWEKISIINFEKLYKLK (SEQ ID NO: 10)

30 RRRRRRRRRRWEKISIINFEKLYKLK (SEQ ID NO: 11)

RWEKISIINFEKLYKLRRRRRRRRR (SEQ ID NO: 12)

RWEKISIINFEKLYKLBETWWETWWTEWSQPKKKRKV (SEQ ID NO: 13)

35 RWEKISIINFEKLYKLAGYLLGKINLKALAALAKKIL (SEQ ID NO: 14)

AGLWRALWRLLRSLWRLWRA RWEKISIINFEKLYKLB (SEQ ID NO: 15)

GRKKRRQRRRPQRWEKISIINFEKLYKL (SEQ ID NO: 16)

40 GRKKRRQRRRPQRWEKISIINFEKL (SEQ ID NO: 17)

GRKKRRQRRRPQRRWEKISIINFEKLYKLRWEKISIINFEKL (SEQ ID NO: 18) (where RWEKI and YKLRWEKI are immunoproteasome processing sites).

Cholesterol-conjugated peptides:

5 LEQLESIINFFEKLTEWRVRRALISC-cholesterol (SEQ ID NO: 19)

cholesterol-CRVRRALISLEQLESIINFFEKLTEW (SEQ ID NO: 20)

10 cholesterol-CSIINFFEKL (SEQ ID NO: 21)

cholesterol-CRWEKISIINFFEKL (SEQ ID NO: 22) or

cholesterol-CRWEKISVYDFFVWLYKLRWEKISIINFFEKL (SEQ ID NO: 23)

15 Accordingly, most preferably said anti-tumor, tumor-specific peptide(s) is/are attached to or inserted in/through said viral envelope using either a cell penetrating peptide or a cholesterol-conjugated peptide.

Typically virus particles were complexed with said CPP-peptide or Cholesterol-conjugated peptide by incubating same for approximately 15 min at 37 °C.

20

In yet another preferred embodiment said modified enveloped virus is provided with at least one anti-tumor, tumor-specific peptide that is MHC-I specific and so elicits an immune response ideally via MHC-I presentation on antigen presenting cells (APCs) for the activation of T-effector cells, named CD8⁺ T cells, specifically cytotoxic T cell lymphocytes (CTLs).

25

Additionally or alternatively, said modified enveloped virus is provided with at least one anti-tumor, tumor-specific peptide that is MHC-II specific and so elicits an immune response ideally via MHC-II presentation on antigen presenting cells (APCs) for the activation of a CD4⁺ (T-helper) cell response.

30

Accordingly, this invention also enables the use of different MHC-II epitopes coated onto the viral envelope to boost, alone or in combination with MHC-I epitopes, the immune response of the individual.

35 Additionally or alternatively the invention also enables the use of a peptide that (s) comprise(s) a fusion molecule including a plurality of different antigens.

Yet more preferably, there is provided a combination of different of said modified enveloped viruses where said viruses are selected from the group comprising Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Vaccinia, Vesicular stomatitis Indiana virus (VSV), Measles Virus (MeV), Maraba virus and New Castle Disease (NDV) virus. Thus the invention concerns the use of at least two of any of the afore viruses and also extends to the use of a combination of any 3, 4, 5, 6 or 7 of the afore viruses.

Accordingly, the invention concerns the use of two different virus types or viral backbones enabling the Prime-Boost immunovirotherapy method where the treatment with a Prime (priming) virus of a particular species/genus coated with said peptides is followed by a treatment with Boost (boosting) virus of another particular species/genus (an immunologically distinct virus) coated with the same said peptides. The Prime-Boost immunovirotherapy method can dramatically further increase the tumor-specific T-cell immune responses by directing the majority of the immune responses created by the Prime-Boost method towards the said peptides.

When working the invention an enveloped virus modified as herein described may be used in the Prime-Boost immunovirotherapy in combination with another enveloped virus modified as herein described but presenting the same peptides or any other virus, such as an adenovirus, that has also been modified, including genetically or non-genetically, to present the same peptides.

Those skilled in the art will appreciate that since an enveloped virus, namely Herpes Simplex Virus 1 (HSV-1) is the most studied oncolytic enveloped virus and an engineered form of HSV-1 called T-VEC (Imlygic) is the first oncolytic virus showing efficacy in Phase III clinical trials and is the first oncolytic virus approved by both FDA and EMEA for the treatment of inoperable melanoma, it is critically important to introduce novel ways of boosting the anti-tumor immunity at the expense of the anti-viral immunity of these therapeutic and clinically approved viruses.

According to a second aspect of the invention there is therefore provided a pharmaceutical composition comprising:

1) a modified enveloped virus selected from the group comprising Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Vaccinia, Vesicular stomatitis Indiana virus (VSV), Measles Virus (MeV), Maraba virus and New Castle Disease (NDV) virus wherein

said virus has at least one anti-tumor, tumor-specific peptide non-genetically attached to or inserted in/through the viral envelope; and

2) a suitable carrier.

5 According to a third aspect of the invention there is therefore provided a method for treating a cancer comprising exposing an individual to a modified enveloped virus selected from the group comprising Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Vaccinia, Vesicular stomatitis Indiana virus (VSV), Measles Virus (MeV), Maraba virus and New Castle Disease (NDV) virus wherein said virus has at least one anti-tumor, tumor-specific peptide non-genetically attached to or inserted in/through the viral envelope.

10 More preferably still, said method involves, after a selected period, exposing said individual to another modified enveloped virus selected from the group comprising Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Vaccinia, Vesicular stomatitis Indiana virus (VSV), Measles Virus (MeV), Maraba virus and New Castle Disease (NDV) virus wherein said virus has at least one anti-tumor, tumor-specific peptide non-genetically attached to or inserted in/through the viral envelope and further wherein said virus is different from the one used for the prior exposure. Alternatively, in the Prime-Boost immunovirotherapy said method involves, after a selected period, exposing said individual to any another virus, such as an adenovirus, that has also been modified, including genetically or non-genetically, to present the same peptides. Alternatively, yet again, said method may be practiced by first using any virus that has been modified in any way, including genetically or non-genetically, to express selected peptides, followed by using the modified virus of the invention, having the same peptides, to provide the booster therapy.

25

In this way Prime-Boost immunovirotherapy can be practiced.

Ideally, said step of exposing an individual to a virus includes intratumoral, intranodal, intraperitoneal or intravenous injection.

30

Most preferably the cancer referred to herein includes any one or more of the following cancers: nasopharyngeal cancer, synovial cancer, hepatocellular cancer, renal cancer, cancer of connective tissues, melanoma, lung cancer, bowel cancer, colon cancer, rectal cancer, colorectal cancer, brain cancer, throat cancer, oral cancer, liver cancer, bone cancer, pancreatic cancer, choriocarcinoma, gastrinoma, pheochromocytoma, prolactinoma, T-cell leukemia/lymphoma, neuroma, von Hippel-Lindau disease, Zollinger-Ellison

35

syndrome, adrenal cancer, anal cancer, bile duct cancer, bladder cancer, ureter cancer, brain cancer, oligodendroglioma, neuroblastoma, meningioma, spinal cord tumor, bone cancer, osteochondroma, chondrosarcoma, Ewing's sarcoma, cancer of unknown primary site, carcinoid, carcinoid of gastrointestinal tract, fibrosarcoma, breast cancer, Paget's
5 disease, cervical cancer, colorectal cancer, rectal cancer, esophagus cancer, gall bladder cancer, head cancer, eye cancer, neck cancer, kidney cancer, Wilms' tumor, liver cancer, Kaposi's sarcoma, prostate cancer, lung cancer, testicular cancer, Hodgkin's disease, non-Hodgkin's lymphoma, oral cancer, skin cancer, mesothelioma, multiple myeloma, ovarian cancer, endocrine pancreatic cancer, glucagonoma, pancreatic cancer, parathyroid cancer,
10 penis cancer, pituitary cancer, soft tissue sarcoma, retinoblastoma, small intestine cancer, stomach cancer, thymus cancer, thyroid cancer, trophoblastic cancer, hydatidiform mole, uterine cancer, endometrial cancer, vagina cancer, vulva cancer, acoustic neuroma, mycosis fungoides, insulinoma, carcinoid syndrome, somatostatinoma, gum cancer, heart cancer, lip cancer, meninges cancer, mouth cancer, nerve cancer, palate cancer, parotid gland cancer,
15 peritoneum cancer, pharynx cancer, pleural cancer, salivary gland cancer, tongue cancer and tonsil cancer.

In a further embodiment of the invention, either after exposing an individual to a modified virus that expresses at least one anti-tumor, tumor-specific peptide or after exposing said
20 individual to an enveloped virus selected from the group comprising Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Vaccinia, Vesicular stomatitis Indiana virus (VSV), Measles Virus (MeV), Maraba virus and New Castle Disease (NDV) virus wherein said virus has the same or a majority of the same of said peptide(s) non-genetically attached to or inserted in/through the viral envelope, exposing said individual to a checkpoint inhibitor.
25 The check point inhibitor inhibits immune checkpoint molecules such as PD-1, PD-L1 and CTLA-4,

In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word
30 "comprises", or variations such as "comprises" or "comprising" is used in an inclusive sense i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

All references, including any patent or patent application, cited in this specification are
35 hereby incorporated by reference. No admission is made that any reference constitutes prior

art. Further, no admission is made that any of the prior art constitutes part of the common general knowledge in the art.

5 Preferred features of each aspect of the invention may be as described in connection with any of the other aspects.

Other features of the present invention will become apparent from the following examples. Generally speaking, the invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including the accompanying claims and drawings).
10 Thus, features, integers, characteristics, compounds or chemical moieties described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein, unless incompatible therewith.

15 Moreover, unless stated otherwise, any feature disclosed herein may be replaced by an alternative feature serving the same or a similar purpose.

Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used,
20 the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

An embodiment of the present invention will now be described by way of example only with reference to the following wherein:

25 **Figure 1** is a summary of the data we have obtained when the anti-tumor, tumor-specific peptide is attached to different viral envelopes using either a CPP or a cholesterol-conjugated peptide;

Figure 2 shows CPP-containing peptides can be attached to the envelope of Herpes
30 Simplex Virus 1. CPP containing and FITC-labelled peptide was complexed with HSV-1 and a sandwich ELISA was used for detection of the complexes. An anti-HSV-1 antibody was coated to the bottom of 96-well plate and PeptiENV complexes were incubated in the wells. After washing the unbound fraction, an anti-FITC HRP-conjugated antibody was used for the detection of the PeptiENV complex. Figure 2 lower panel shows CPP-containing peptides

have reduced diffusion time when complexed with HSV-1. Fluorescence correlation spectroscopy was used to analyze the diffusion kinetics of peptides complexed with HSV-1.

Figure 3 upper panel shows cholesterol-containing peptides can be attached to the envelope of Vaccinia virus. Cholesterol-containing and FITC-labelled peptides were complexed with Vaccinia virus. After the purification of the PeptiENV complexes by 36% sucrose cushion and ultracentrifugation, the purified complexes were analyzed by flow cytometry. A. Vaccinia virus without complexed peptides and B. Vaccinia virus complexed with cholesterol containing FITC-labelled peptides. Figure 3 middle panel shows cholesterol-containing peptides can be attached to the envelope of Vaccinia virus. Cholesterol-containing and FITC-labelled peptides were complexed with Vaccinia virus and a sandwich ELISA was used for the detection of the complexes. An anti-Vaccinia virus antibody was coated to the bottom of 96-well plate and PeptiENV complexes were incubated in the wells. After washing the unbound fraction, an anti-FITC HRP-conjugated antibody was used for the detection of the PeptiENV complexes. Figure 3 lower panel shows cholesterol-containing SIINFEKL-peptides are readily presented by dendritic cells. Mouse splenocytes were pulsed with cholesterol-containing SIINFEKL-peptides and the presentation of MHC I epitope SIINFEKL by CD11c positive DC-population was determined by flow cytometry.

Figure 4 upper panel shows CPP-containing peptides can be attached to the envelope of Vaccinia virus. CPP-containing and FITC-labelled peptides were complexed with Vaccinia virus. After the purification of the PeptiENV complexes by 36% sucrose cushion and ultracentrifugation, the purified complexes were analyzed by flow cytometry. A. HSV-1 without complexed peptides and B. HSV-1 complexed with CPP-containing FITC-labelled peptides. Figure 4 middle panel shows CPP-containing peptides can be attached to the envelope Vaccinia virus. CPP-containing and FITC-labelled peptide was complexed with Vaccinia virus and a sandwich ELISA was used for the detection of the complexes. An anti-Vaccinia virus antibody was coated to the bottom of 96-well plate and PeptiENV complexes were incubated in the wells. After washing the unbound fraction, an anti-FITC HRP-conjugated antibody was used for the detection of the PeptiENV complex. Figure 4 lower panel shows CPP-containing SIINFEKL-peptides are readily presented by dendritic cells. Mouse splenocytes were pulsed with CPP-containing SIINFEKL-peptides and the presentation of MHC I epitope SIINFEKL by CD11c positive DC-population was determined by flow cytometry.

Figure 5 upper panel shows PeptiENV can induce DC activation even with very low virus amount. Vaccinia virus was complexed with CPP- or cholesterol-containing SIINFEKL-

peptides and used for infection of mouse splenocytes. Two hours post-infection dendritic cells were analyzed by flow cytometry for the expression of DC activation markers. Figure 5 lower panel shows PeptiENV can induce the presentation of specific anti-tumor MHC class I epitopes by the CD11c-positive DCs even with very low virus amount. Vaccinia virus was
5 complexed with CPP- or cholesterol-containing SIINFEKL-peptides and used for infection of mouse splenocytes. After two hours the presentation of MHC I epitope SIINFEKL by CD11c positive DC-population was determined by flow cytometry.

Figure 6 shows PeptiENV virus-peptide complexation has no effect on viral infectivity. Infectivity of PeptiENV was compared with normal non-complexed virus and cell viability was
10 measured 3 days post-infection.

Figure 7 shows a schematic presentation of A) a cholesterol-conjugated immunomodulatory peptide and B) an immunomodulatory peptide having N-terminal cell penetrating peptide
15 sequence. Color code for different functional sequences of the entities: In dark gray: cholesterol (A.) or cell penetrating sequence (B.). In red: cathepsin D/E cleavage site. In blue: furin cleavage site. In green: immunoproteasome processing sites. In black: the MHC-I restricted epitope.

Figure 8 shows that a variety of different CPP-sequences can be used to attach the anti-tumor peptides into the viral envelope. CPP-containing and FITC-labelled peptides were
20 complexed with Vaccinia virus and a sandwich ELISA was used for the detection of the complexes. An anti-Vaccinia virus antibody was coated to the bottom of 96-well plate and PeptiENV complexes were incubated in the wells. After washing the unbound fraction, an
25 anti-FITC HRP-conjugated antibody was used for the detection of the PeptiENV complexes.

Figure 9 shows CPP-containing peptides do not have any antiviral effects and can be safely attached to the viral envelope without loss of infectivity or oncolytic effect. Four different cell
lines, A549, MDMBA436, B16F10 and B16-OVA, were tested for viral infectivity and
30 PeptiENV was compared to non-modified virus.

Figure 10 shows anti-tumour peptides anchored into viral envelope by CPP- or Cholesterol moieties induce extensive anti-tumor immunity leading to enhanced control of tumor growth
and longer survival. A. Comparison of tumor growth between groups of Mock (treated with
35 injection media only), Vaccinia virus only, PeptiENV with anti-tumor peptides attached to the viral envelope with a cholesterol moiety (PeptiENV VACV/chol) and PeptiENV with anti-

tumor peptides attached to the viral envelope with a CPP moiety (PeptiENV VACV/cpp). B. Kaplan-Meier survival curve of groups of mice treated with PeptiENV VACV/cpp, Vaccinia virus only, anti-tumor peptide alone (without virus) or Mock. C. Flow cytometric analysis of tumor-specific T-cells in treated tumor. PeptiENV can induce extensive filtration of tumor-specific effector T-cells in to the tumor microenvironment. D. Flow cytometric analysis of virus-specific T-cells in the tumor microenvironment. PeptiENV induces anti-viral immunity comparable to non-modified Vaccinia virus, while specifically inducing extensive anti-tumor immunity not presented by the non-modified Vaccinia virus.

Figure 11 shows PeptiENV VACV/chol and PeptiENV VACV/cpp treated mice elicit strong anti-tumor immune response and are protected from tumor rechallenge while Mock-treated mice are not protected. 500 000 B16-OVA cells were injected into the opposite flank of the previous tumor implantation and mice were followed for 14 days. No tumor growth was observed in mice treated with PeptiENV while the tumor incidence was 100% in the Mock treated group.

Figure 12 shows anti-tumor peptides anchored into Herpes simplex virus 1 (HSV-1) envelope by CPP moiety induce strong anti-tumor immunity leading to enhanced control of tumor growth. A. Tumor growth curve of PeptiENV HSV-1/cpp, HSV-1 only and Mock treated groups. B. Flow cytometric analysis of tumor-specific T-cells in treated tumor. PeptiENV can induce extensive filtration of tumor-specific effector T-cells in to the tumor microenvironment.

Figure 13 shows surface plasmon resonance (SPR) measurements to confirm the high affinity of CPP-containing anti-tumor peptides to the viral envelope. Two-site binding kinetic fit model was used for the analysis of the SPR data.

Specific description

Materials and methods:

Peptides:

30

Peptides used in this study are listed below and were all purchased from PepScan:

CPP peptides:

35 GRKKRRQRRRPQRVRRALISLEQLESIIINFEKLTEW (SEQ ID NO: 8)

GRKKRRQRRRPQRVRRALISLEQLESIIINFEKLTEW-FITC (SEQ ID NO: 24)

RQIKIWFQNRRMKWKKRWEKISIINFEEKLYKLIK-FITC (SEQ ID NO: 25)

KLALKLALKALKAALKLARWEKISIINFEEKLYKLIK-FITC (SEQ ID NO: 26)

5

RRRRRRRRRRRWEKISIINFEEKLYKLIK-FITC (SEQ ID NO: 27)

FITC -RWEKISIINFEEKLYKLRRRRRRRRR (SEQ ID NO: 28)

10 FITC-RWEKISIINFEEKLYKLIKETWWETWWTEWSQPKKKRKV (SEQ ID NO: 29)

FITC-RWEKISIINFEEKLYKLAGYLLGKINLKALAALAKKIL (SEQ ID NO: 30)

15 GLWRALWRLLRSLWRLLRWRARWEKISIINFEEKLYKLIK-FITC (SEQ ID NO: 31)

15

GRKKRRQRRRPQRWEKISIINFEEKL (SEQ ID NO: 17)

GRKKRRQRRRPQRWEKISIINFEEKLYKLRWEKISIINFEEKL (SEQ ID NO: 18)

20

Cholesterol-conjugated peptides:

25

LEQLESIINFEEKLTEWRVRRALISC-cholesterol (SEQ ID NO: 19)

FITC-LEQLESIINFEEKLTEWRVRRALISC-cholesterol (SEQ ID NO: 32)

30 cholesterol-CRVRRALISLEQLESIINFEEKLTEW (SEQ ID NO: 20)

cholesterol-CRVRRALISLEQLESIINFEEKLTEW-FITC (SEQ ID NO: 33)

cholesterol-CSIINFEEKL (SEQ ID NO: 21)

35

cholesterol-CRWEKISIINFEEKL (SEQ ID NO: 22)

cholesterol-CRWEKISVYDFFVWLYKLRWEKISIINFEEKL (SEQ ID NO: 23)

40 Cell lines

Human lung carcinoma cell line A549, African Green monkey kidney epithelial cell line Vero (B) and Murine melanoma cell lines B16/OVA and B16-F10 were cultured in DMEM with 10% foetal calf serum (FBS) (Life Technologies) 1% L-glutamine and 1% penicillin/streptomycin at 37 °C/ 5% CO₂. Human triple negative breast cancer cell line

45 MDMBA436 was cultured in RPMI with 10% foetal calf serum (FBS) (Life Technologies) 1% L-glutamine and 1% penicillin/streptomycin at 37 °C/ 5% CO₂.

Production of viruses

Herpes simplex virus 1 was produced in Vero cells and purified with sucrose gradient ultracentrifugation and eluted in 20 mM MES, 100 mM NaCl, 30 mM Tris-HCl (pH 7.2).

Western reserve strain of Vaccinia virus (VVDD-mDAI-RFP) was produced in A549 cells and purified through 36% sucrose cushion ultracentrifugation and eluted in 1mM Tris (pH 9.0).

5

ELISAs

2,5x10⁷ Vaccinia virus particles were complexed with 8ug of either CPP-peptide-FITC or cholesterol-conjugated peptide-FITC in 100ul of DMEM for 15 min at 37 °C. After complexation, unbound peptides were removed by ultracentrifugation (20.000g, 40-80mins) through 36% sucrose cushion in 1mM Tris (pH 9.0). For ELISA, anti-Vaccinia polyclonal antibody (Abcam) was coated o/n at 4 °C into maxisorb 96-well immunoplates at the concentration of 2ug/ml. Vaccinia-peptide complexes were incubated for 30-60min at 37 °C or RT and washed with 1xPBS for three times. Complexes were detected with anti-FITC antibody conjugated to horseradish peroxidase (Abcam) (1:5000 dilution in 2%BSA-PBS).

2,5x10⁷ Herpes simplex 1 virus particles were complexed with 8ug of either CPP-peptide-FITC or cholesterol-conjugated peptide-FITC in 100ul of DMEM for 15 min at 37 °C. For ELISA, anti-HSV-1 polyclonal antibody (Abcam) was coated o/n at 4 °C into maxisorb 96-well immunoplates at the concentration of 2ug/ml. HSV-1-peptide complexes were incubated for 30-60min at 37 °C or RT and washed with 1xPBS for three times. Complexes were detected with anti-FITC antibody conjugated to horseradish peroxidase (Abcam) (1:5000 dilution in 2%BSA-PBS).

Flow cytometry

5x10⁷ Vaccinia virus particles were complexed with 24ug of either CPP-peptide-FITC or cholesterol-conjugated peptide-FITC in 200ul of DMEM for 15 min at 37 °C. After complexation, unbound peptides were removed by ultracentrifugation (20.000g, 40-80mins) through 36% sucrose cushion in 1mM Tris (pH 9.0) and eluted to 2%Formalin in PBS. After fixing, formalin was removed with another ultracentrifugation (20.000g, 40-80mins) through 36% sucrose cushion and pellet was eluted to 1x ultrapure PBS (Gibco). Flow cytometry was performed with Apogee A50 Micro Flow Cytometer (Apogee) and FITC detection was used for assessing the complexes.

Cross-presentation experiments

2x10⁶ spleenocytes in 800 µL of 10% RPMI-1640 culture media were incubated with 200 µL of GRKKRRQRRRPQRVRRALISLEQLESIINFELTEW (SEQ ID NO: 8),

LEQLESIINFEKLTIEWRVRRALISC-cholesterol (SEQ ID NO: 19) or cholesterol-CRVRRALISLEQLESIINFEKLTIEW (SEQ ID NO: 20) peptide dilution (0.19 µg/µL).

5 The vaccinia-peptide complexes were prepared as described for ELISAs. After 2 h of incubation cells were washed and stained with either APC anti-mouse H-2Kb bound to SIINFEKL or APC Mouse IgG1, κ Isotype Ctrl (BioLegend, San Diego, CA, USA), and the samples were analyzed by flow cytometry.

Cell Viability Assay

10 Cell viability was measured using the CellTiterGlo 96 Aqueous One Solution Cell Proliferation Assay (Promega), and a multi-well plate reader (Varioscan; ThermoLabsystems) to determine the luminescence of the samples.

Surface plasmon resonance

15 Measurements were performed using a multi-parametric SPR Navi™ 220A instrument (Bionavis Ltd, Tampere, Finland). Phosphate buffered saline (PBS) (pH 7.4) was used as a running buffer. A constant flow rate of 20 µL/min was used throughout the experiments, and temperature was set to +20°C. Laser light with a wavelength of 670 nm was used for surface plasmon excitation.

20 A sensor slide with a silicon dioxide surface was activated by 5 min of plasma treatment followed by coating with APTES ((3-aminopropyl)triethoxysilane) by incubating the sensor in 50 mM APTES in isopropanol for 4 h. The sensor was then washed and placed into the SPR device, and viruses were immobilized *in situ* on the sensor surface of the two test channels by injecting 1.1×10^7 pfu of VACV in PBS (pH 7.4) for approximately 12 min, followed by a 3-
25 min wash with PBS. CPP-containing anti-tumor peptide or peptide without CPP sequence (non-interacting control) was then injected into both flow channels of the flow cell in parallel, with increasing peptide concentrations ranging from 1.23µM to 100µM.

30 Animal experiments

C57BL/6JOlaHsd-mouse strain was used in all animal experiment. 350000 B16-OVA-cells were injected in the right flank of mice (in rechallence experiment, cells were injected in to the left flank) and when the tumor size reached approximately 50mm³ (10-12 days after injection) mice were treated with non-modified viruses, PeptiENV-platform, peptides only or
35 injection media only (Mock). Mice were treated on day 0, 2 and then a booster treatment

was given on day 8-10. Tumors were measured every second day until the tumor size reached the maximum allowed.

Claims

1. A modified enveloped virus selected from the group comprising Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Vaccinia, Vesicular stomatitis Indiana virus (VSV), Measles Virus (MeV), Maraba virus and New Castle Disease (NDV) virus wherein said virus has at least one anti-tumor, tumor-specific peptide non-genetically attached to or inserted in/through the viral envelope.
5
2. The modified enveloped virus according to claim 1 wherein said peptide has a length selected from the group comprising: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 and 50 amino acids.
10
3. The modified enveloped virus according to claim 1 or claim 2 wherein a plurality of said peptides are non-genetically attached to or inserted in/through the viral envelope.
4. The modified enveloped virus according to claim 3 wherein said peptides are identical; said peptides have greater than 90%, 91%, 92%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with each other; or said peptides are representative of a number of different antigens.
15
5. The modified enveloped virus according to any one of the preceding claims wherein said peptide(s) is/are MHC-I restricted.
6. The modified enveloped virus according to any one of the preceding claims wherein said peptide(s) is/are MHC-II restricted.
20
7. The modified enveloped virus according to any one of claims 3-6 wherein said peptides comprise a mix of MHC-I restricted peptides and MHC-II restricted peptides.
8. The modified enveloped virus according to any one of the preceding claims wherein said peptide(s) comprise(s) a fusion molecule including a plurality of different antigens.
25
9. The modified enveloped virus according to any one of claims 1-8 wherein said peptide also comprises at least one cleavage site.
10. The modified enveloped virus according to any one of claims 1-9 wherein said peptide also comprises at least one immunoproteasome processing site.
30
11. The modified enveloped virus according to any one of claims 1-10 wherein said peptide is positioned between a pair of immunoproteasome processing sites and upstream or downstream thereof is at least one cleavage site.
12. The modified enveloped virus according to any one of the preceding claims wherein said peptide(s) are non-covalently attached to or inserted in/through the viral envelope.
35

13. The modified enveloped virus according to any one of the preceding claims wherein said peptide(s) is/are non-genetically attached to or inserted in/through said viral envelope using either a cell penetrating peptide or a cholesterol-conjugated peptide.

14. The modified enveloped virus according to any one of the preceding claims wherein said peptide(s) is/are non-genetically attached to or inserted in/through said viral envelope using either a cell penetrating peptide or a cholesterol-conjugated peptide selected from the group comprising:

GRKKRRQRRRPQ (SEQ ID NO: 1), CPP sequence in the N- or C-terminus of the said anti-tumor, tumor-specific peptide;

RQIKIWFQNRRMKWKK (SEQ ID NO: 2), CPP sequence in the N- or C-terminus of the said anti-tumor, tumor-specific peptide;

KLALKLALKALKAALKLA (SEQ ID NO: 3), CPP sequence in the N- or C-terminus of the said anti-tumor, tumor-specific peptide;

RRRRRRRRRR (SEQ ID NO: 4), CPP sequence in the N- or C-terminus of the said anti-tumor, tumor-specific peptide;

KETWWETWWTEWSQPKKKRKV (SEQ ID NO: 5), CPP sequence in the N- or C-terminus of the said anti-tumor, tumor-specific peptide;

AGYLLGKINLKALAALAKKIL (SEQ ID NO: 6), CPP sequence in the N- or C-terminus of the said anti-tumor, tumor-specific peptide;

AGLWRALWRLLRSLWRLWRA (SEQ ID NO: 7), CPP sequence in the N- or C-terminus of the said anti-tumor, tumor-specific peptide; and

a cholesterol moiety N- or C-terminus of the said anti-tumor, tumor-specific peptide.

15. The modified enveloped virus according to claim 14 wherein said peptide(s) is/are selected from the group comprising:

GRKKRRQRRRPQRVRRALISLEQLESIIINFEKLTW (SEQ ID NO: 8);

RQIKIWFQNRRMKWKKRWEKISIINFEKLYKLYK (SEQ ID NO: 9);

KLALKLALKALKAALKLARWEKISIINFEKLYKLYK (SEQ ID NO: 10);

RRRRRRRRRRRWEKISIINFEKLYKLYK (SEQ ID NO: 11);

RWEKISIINFEKLYKLYKRRRRRRRRRR (SEQ ID NO: 12);

RWEKISIINFEKLYKLYKETWWETWWTEWSQPKKKRKV (SEQ ID NO: 13);

RWEKISIINFEKLYKLYKLAGYLLGKINLKALAALAKKIL (SEQ ID NO: 14);

AGLWRALWRLLRSLWRLWRA RWEKISIINFEKLYKLYK (SEQ ID NO: 15);

GRKKRRQRRRPQRWEKISIINFEKLYKLYK (SEQ ID NO: 16);

GRKKRRQRRRPQRWEKISIINFEKL (SEQ ID NO: 17);

GRKKRRQRRRPQRWEKISIINFEKLYKLYKLRWEKISIINFEKL (SEQ ID NO: 18);

LEQLESIIINFEKLTWVRRALISC-cholesterol (SEQ ID NO: 19);

cholesterol-CRVRRALISLEQLESIIINFEKLTEW (SEQ ID NO: 20);
 cholesterol-CIIINFEKL (SEQ ID NO: 21);
 cholesterol-CRWEKISIINFEKL (SEQ ID NO: 22);
 cholesterol-CRWEKISVYDFFVWLYKLRWEKISIINFEKL (SEQ ID NO: 23);
 5 GRKKRRQRRRPQRVRRALISLEQLESIIINFEKLTEW-FITC (SEQ ID NO: 24);
 RQIKIWFQNRRMKWKKRWEKISIINFEKLYKLYK-FITC (SEQ ID NO: 25);
 10 KLALKLALKALKAAALKLARWEKISIINFEKLYKLYK-FITC (SEQ ID NO: 26);
 RRRRRRRRRRRRWEKISIINFEKLYKLYK-FITC (SEQ ID NO: 27);
 FITC -RWEKISIINFEKLYKLYKRRRRRRRRRR (SEQ ID NO: 28);
 15 FITC-RWEKISIINFEKLYKLYKETWWETWWTEWSQPKKKRKV (SEQ ID NO: 29);
 FITC-RWEKISIINFEKLYKLAGYLLGKINLKALAALAKKIL (SEQ ID NO: 30) and
 20 GLWRALWRLLRSLWRLLRARWEKISIINFEKLYKLYK-FITC (SEQ ID NO: 31).

16. The modified enveloped virus according to any one of the preceding claims wherein there is provided a combination of different non-genetically modified enveloped viruses selected from the group comprising Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Vaccinia, Vesicular stomatitis Indiana virus (VSV), Measles Virus (MeV), Maraba virus and New Castle Disease (NDV) virus.
- 25 17. The modified enveloped virus according to claim 16 wherein said combination comprises any 2, 3, 4, 5, 6 or 7 of the afore viruses modified to comprise at least one anti-tumor, tumor-specific peptide non-genetically attached to or inserted in/through the viral envelope of said virus.
- 30 18. A pharmaceutical composition comprising: a modified enveloped virus according to any one of claims 1-17 and a suitable carrier.
19. A method for treating a cancer comprising exposing an individual to a modified enveloped virus selected from the group comprising Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Vaccinia, Vesicular stomatitis Indiana virus (VSV), Measles Virus (MeV), Maraba virus and New Castle Disease (NDV) virus
 35 wherein said virus has at least one anti-tumor, tumor-specific peptide non-genetically attached to or inserted in/through the viral envelope.
20. The method according to claim 19 wherein said method involves, after a selected period, exposing said individual to another modified enveloped virus selected from
 40 the group comprising Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Vaccinia, Vesicular stomatitis Indiana virus (VSV), Measles Virus (MeV),

Maraba virus and New Castle Disease (NDV) virus wherein said virus has at least one anti-tumor, tumor-specific peptide non-genetically attached to or inserted in/through the viral envelope and further wherein said virus is different from the one used for the prior exposure.

- 5 21. The method according to claim 20 wherein said another modified enveloped virus is coated with the same or a majority of the same said peptides as the first modified enveloped virus.
22. The method according to any one of claims 19-21 wherein after exposure to said one or more virus(es) said individual is further exposed to a checkpoint inhibitor.
- 10 23. A method for treating a cancer comprising exposing an individual to a modified virus that expresses at least one anti-tumor, tumor-specific peptide and then, after a selected period, exposing said individual to an enveloped virus selected from the group comprising Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Vaccinia, Vesicular stomatitis Indiana virus (VSV), Measles Virus (MeV), Maraba virus and New Castle Disease (NDV) virus wherein said virus has the same or a majority of the same of said peptide(s) non-genetically attached to or inserted in/through the viral envelope.
- 15 24. The method according to claim 23 wherein either after exposing an individual to a modified virus that expresses at least one anti-tumor, tumor-specific peptide or after exposing said individual to an enveloped virus selected from the group comprising Herpes Simplex Virus 1 (HSV-1), Herpes Simplex Virus 2 (HSV-2), Vaccinia, Vesicular stomatitis Indiana virus (VSV), Measles Virus (MeV), Maraba virus and New Castle Disease (NDV) virus wherein said virus has the same or a majority of the same of said peptide(s) non-genetically attached to or inserted in/through the viral envelope, exposing said individual to a checkpoint inhibitor.
- 20 25. The modified enveloped virus according to claims 1-17, the pharmaceutical composition according to claim 18 and the method according to claims 19-24 as substantially herein described.
- 25
- 30

SUMMARY OF THE DATA:

VIRUS	PEPTIDE ANCHORING TYPE	ANTI-TUMOUR PEPTIDE	ASSESSMENT OF COMPLEX FORMATION	ASSESSMENT OF COMPLEX FORMATION	ASSESSMENT OF COMPLEX FORMATION	ASSESSMENT OF MHC presentation (of Peptide) and immunogenicity of the Peptide	Peptide infectivity on AS49, B16-OVA, B16-F10, 4T1, MDMBA cancer cells	Peptide infectivity on AS49 cells	Peptide infectivity on 4T1, MDMBA cancer cells	Peptide platform in vivo anti-tumour effects
vaccinia virus	Cholesterol N-terminus	SIINFEKL	ELISA	Apogee	CD11c+ presenting SIINFEKL	CD86 and CD101	wt. like infectivity	wt. like infectivity	tested	tested
vaccinia virus	GRKKRRQRRPQ N-terminus	SIINFEKL	ELISA	Apogee	CD11c+ presenting SIINFEKL	CD86 and CD103	wt. like infectivity	tested	tested	tested
vaccinia virus	GLWRALWRLRLSLWRLLWRA N-terminus	SIINFEKL	ELISA							
vaccinia virus	AGYLLGKINLKAALAKKIL C-terminus	SIINFEKL	ELISA							
vaccinia virus	RQIKWFOVRRMKWKK N-terminus	SIINFEKL	ELISA							
vaccinia virus	KLALKALKALKALKLA N-terminus	SIINFEKL	ELISA							
vaccinia virus	RRRRRRRR N-terminus	SIINFEKL	ELISA							
vaccinia virus	RRRRRRRR C-terminus	SIINFEKL	ELISA							
vaccinia virus	KETWETWTEWSOPKKRKKV C-terminus	SIINFEKL	ELISA							
herpes virus	CPP	SIINFEKL	ELISA	fluorescence correlation spectroscopy	CD11c+ presenting SIINFEKL				tested	tested

Figure 1

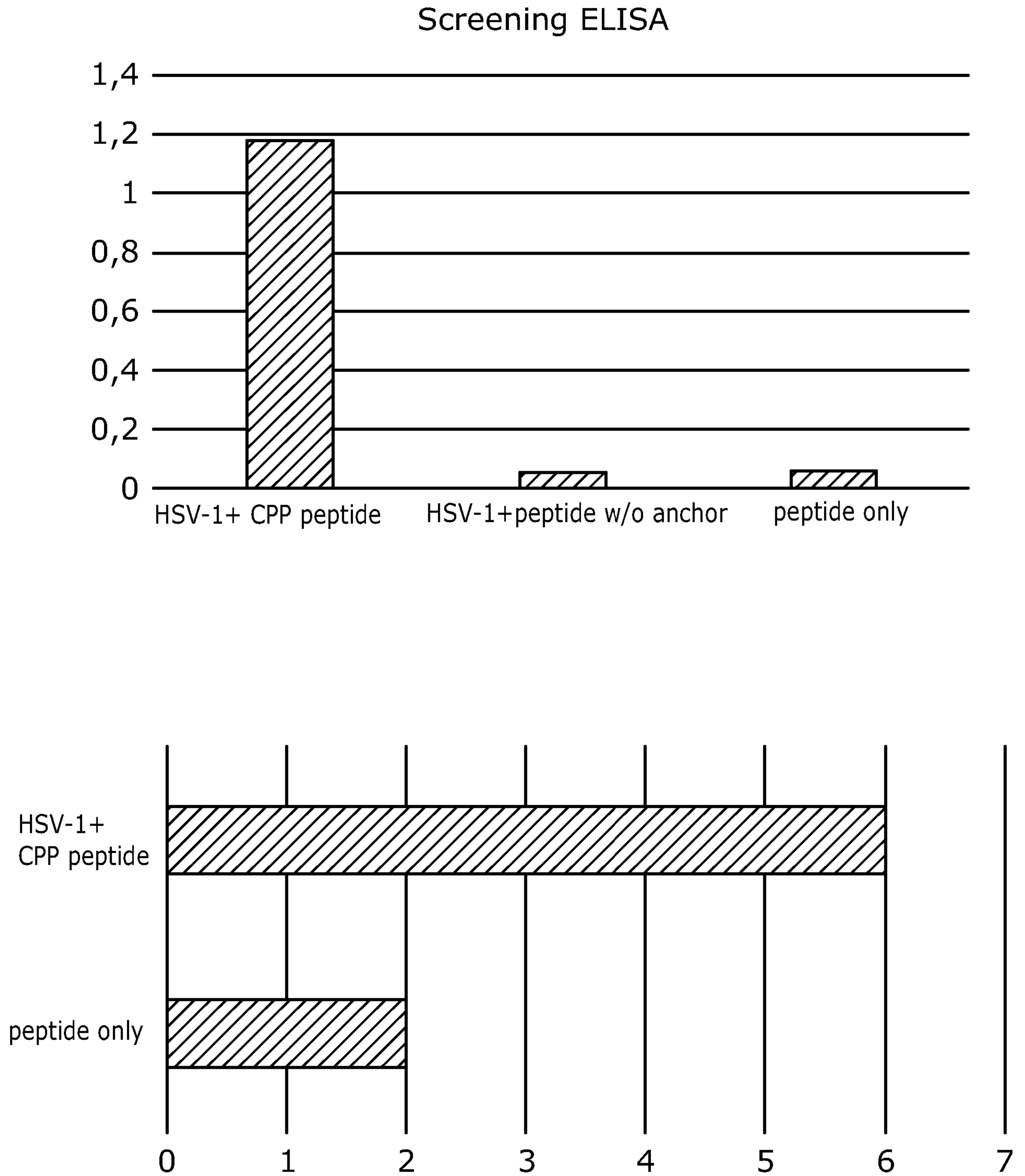
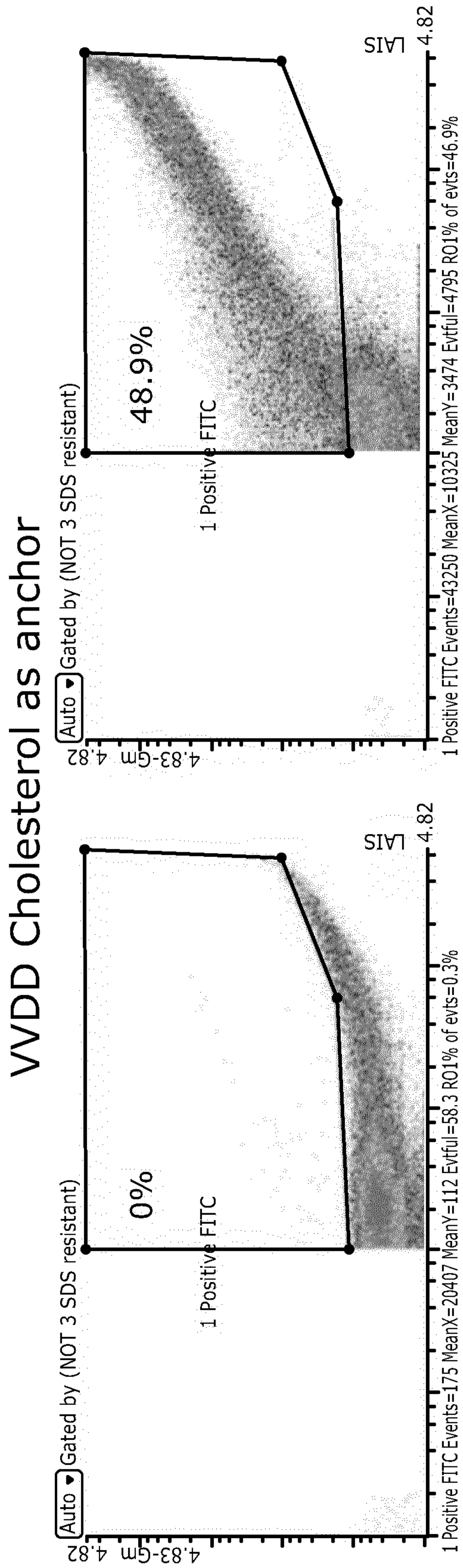


Figure 2



PeptiENV Vaccinia Virus coated with Cholesterol-conjugated Peptides

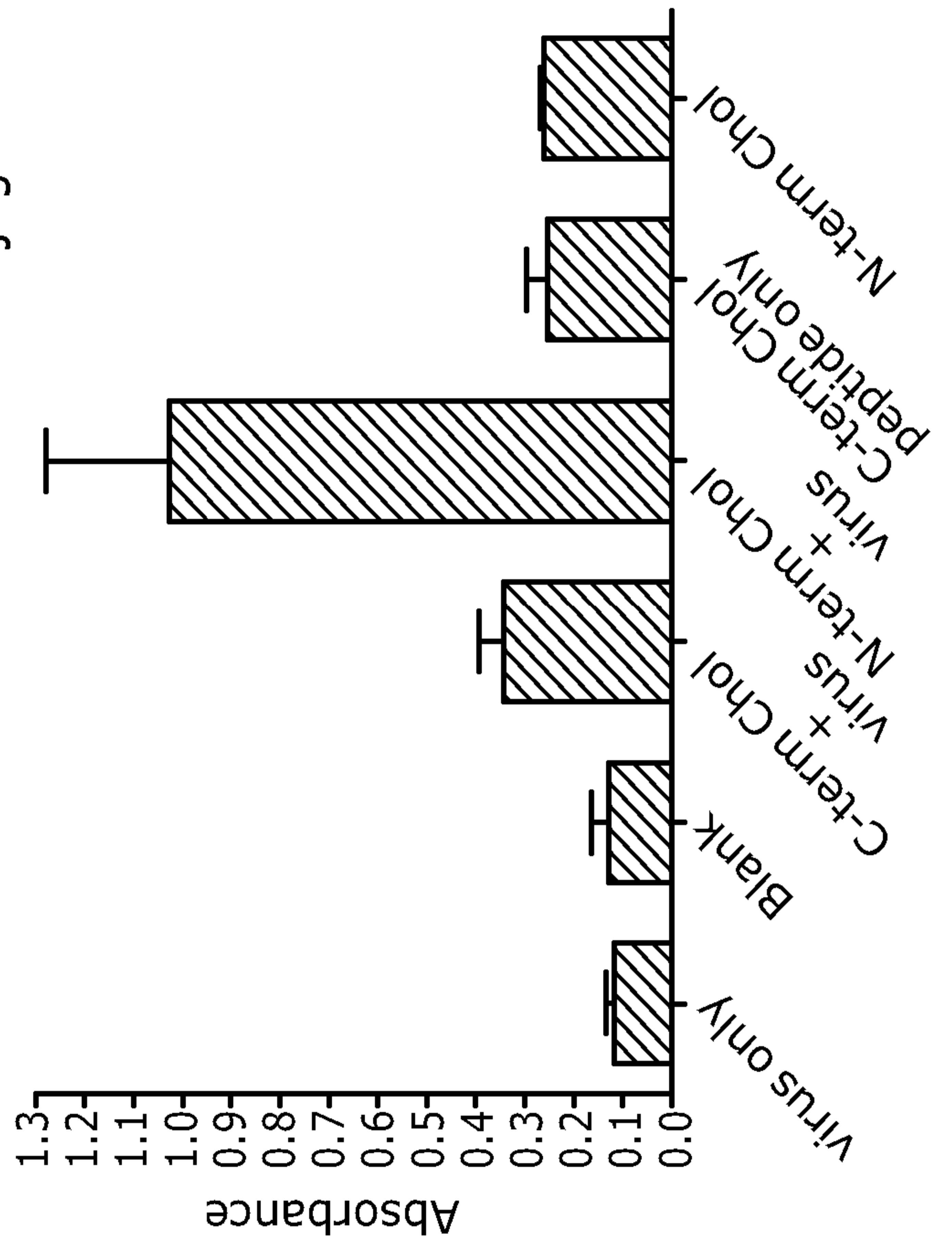


Figure 3

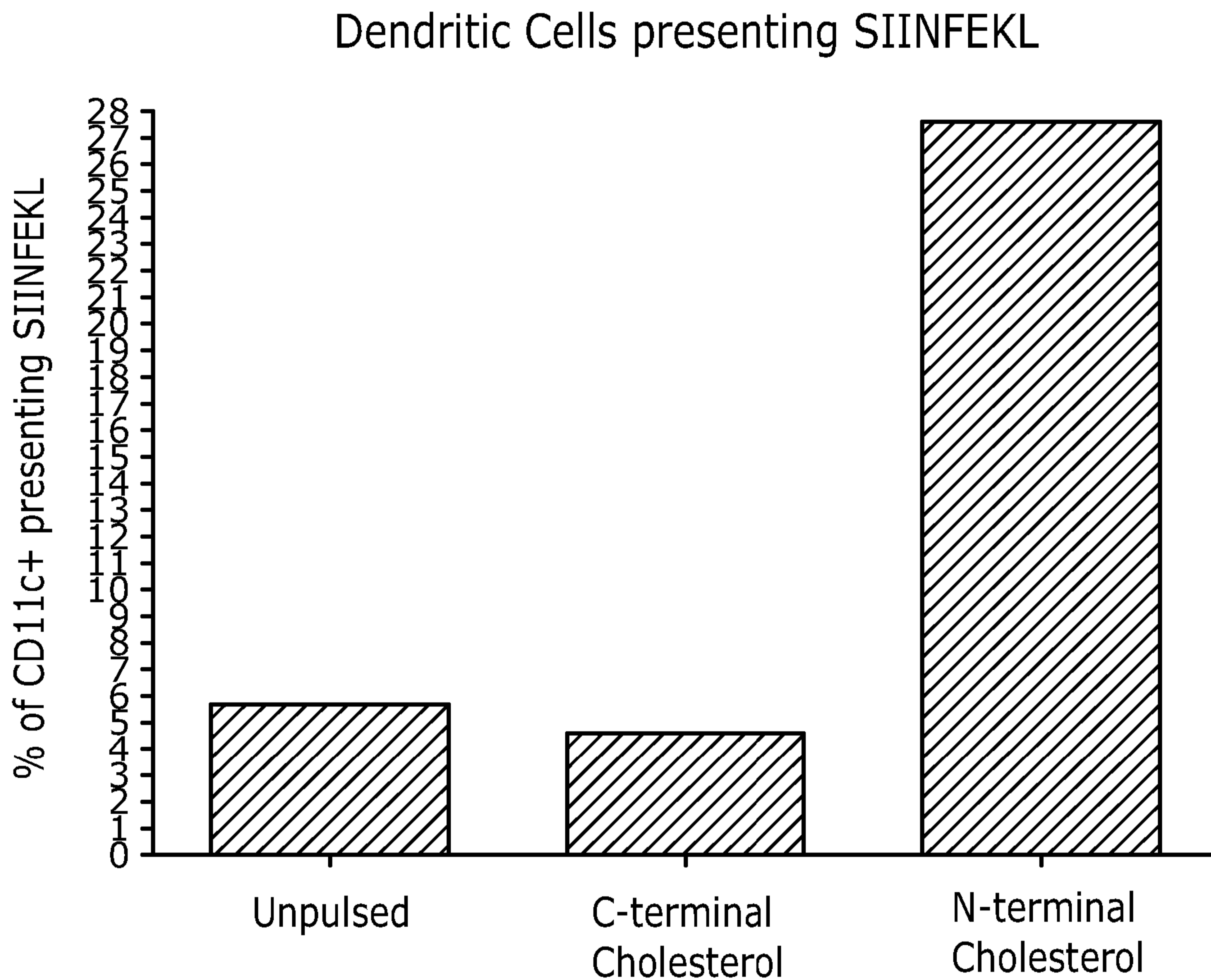


Figure 3
Continued

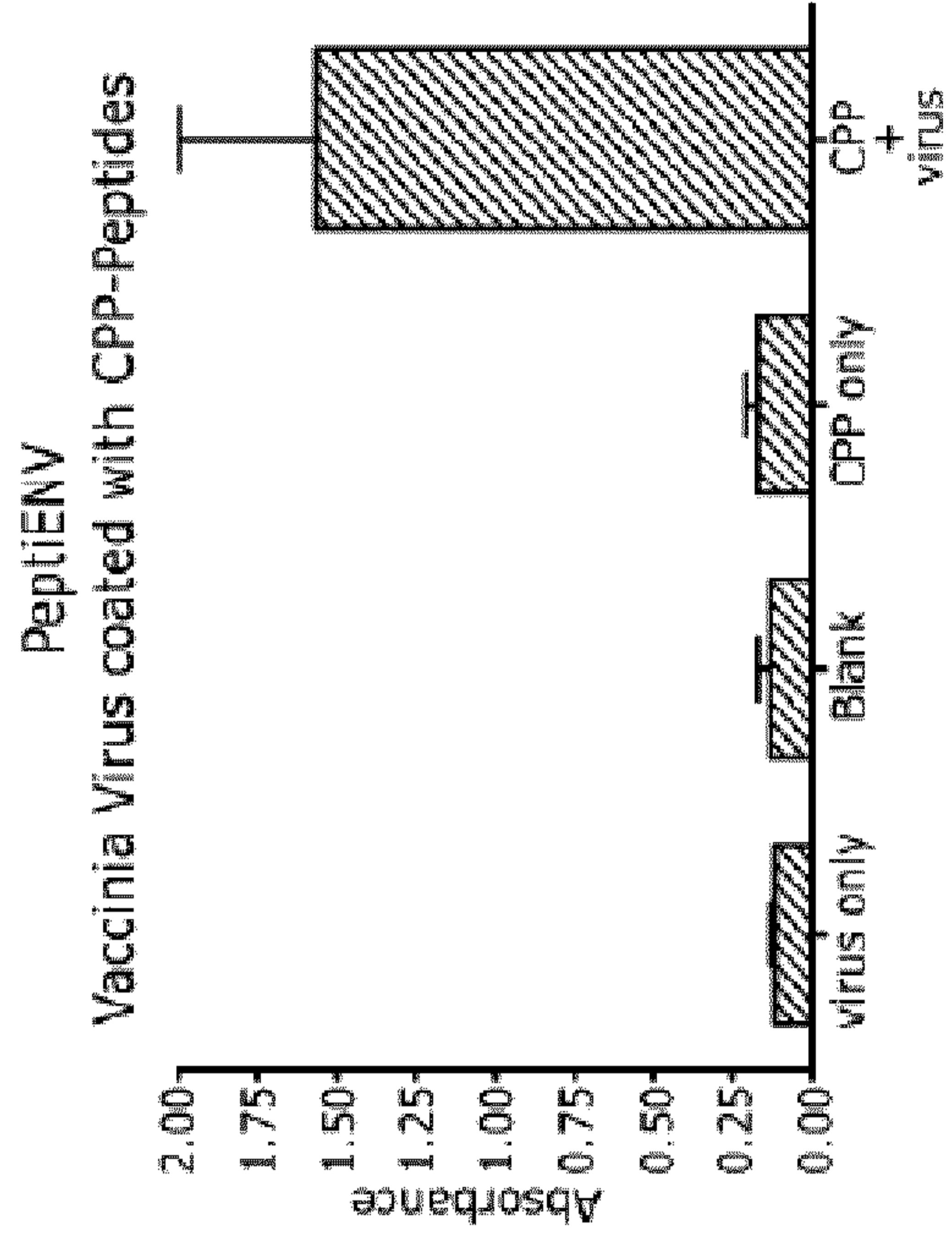
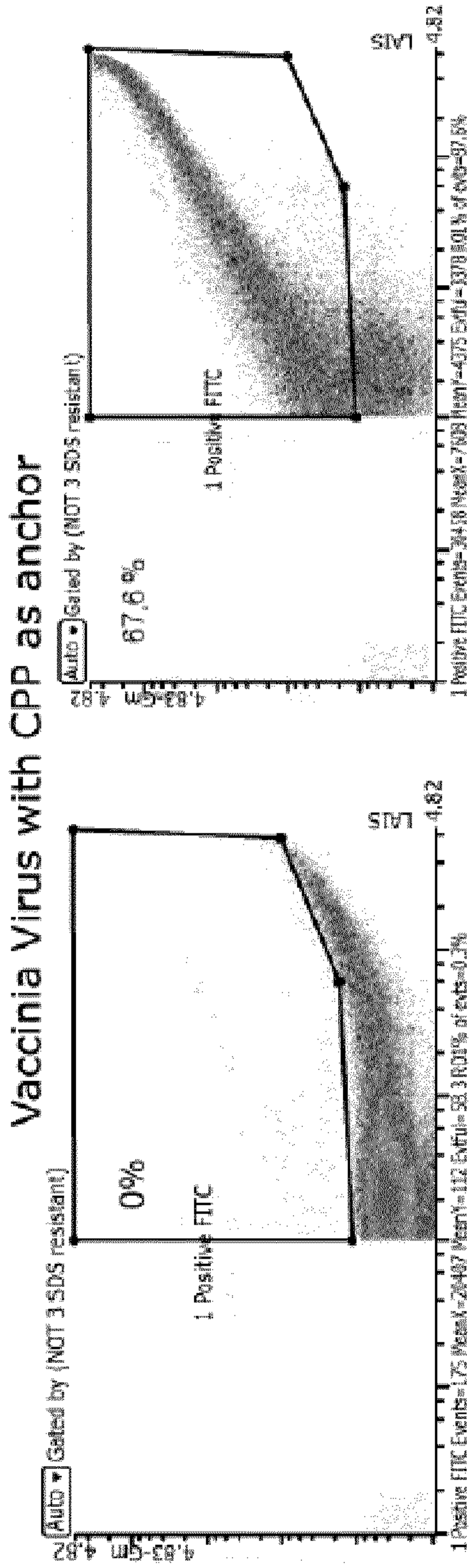


Figure 4

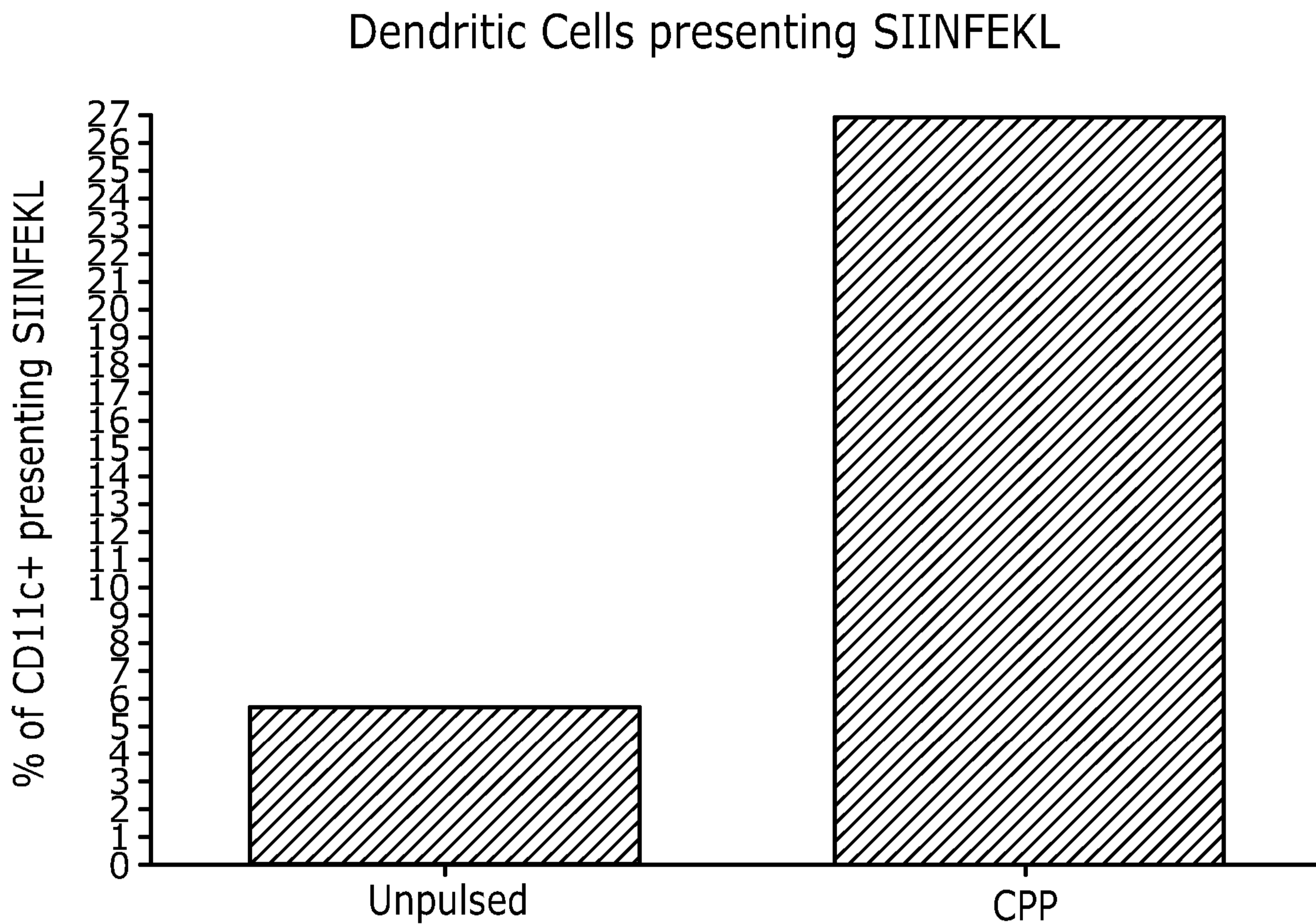
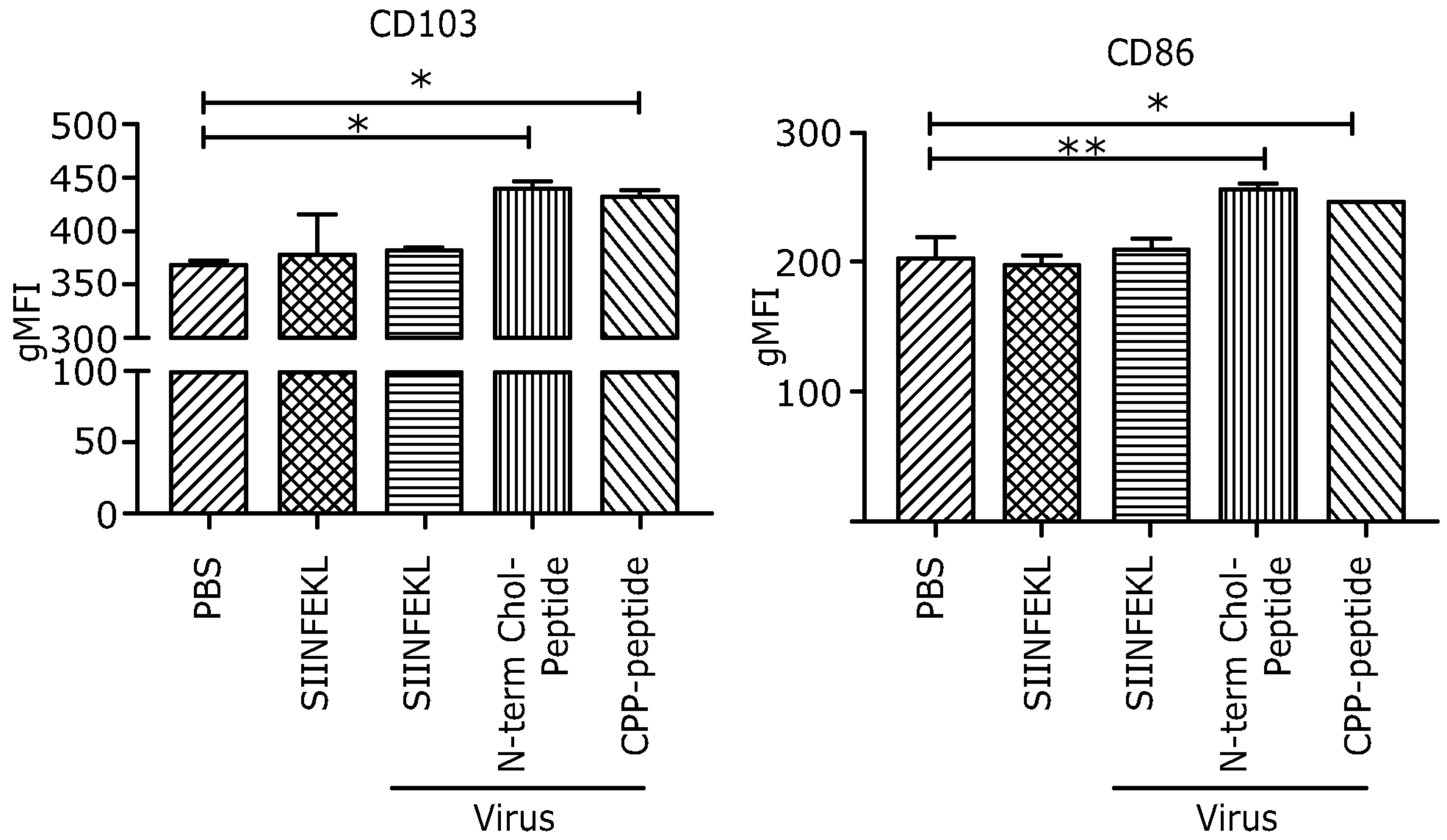


Figure 4
Continued



SIINFEKL presentation from PeptiENV platform on CD11c⁺ Dendritic Cells

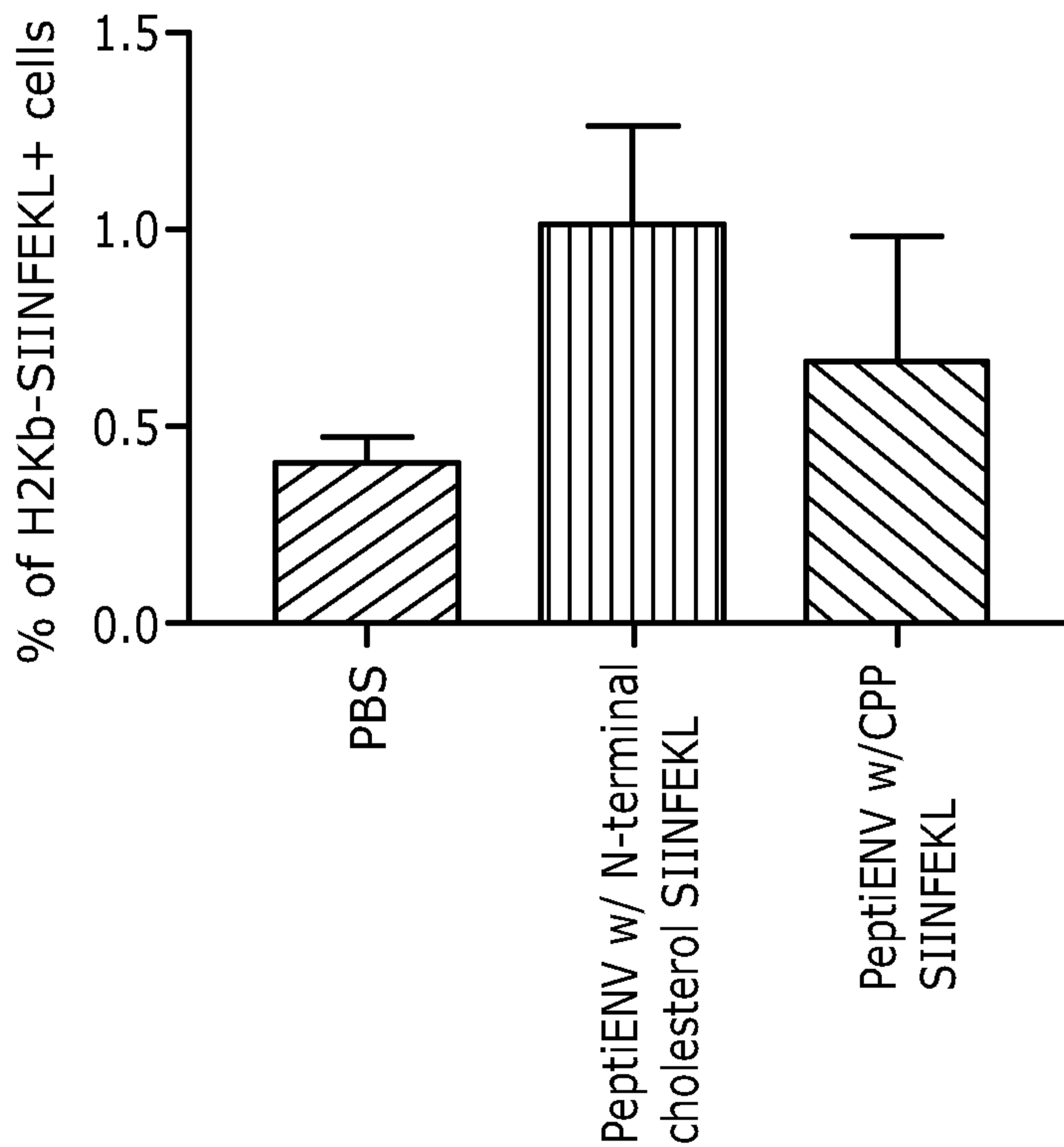


Figure 5

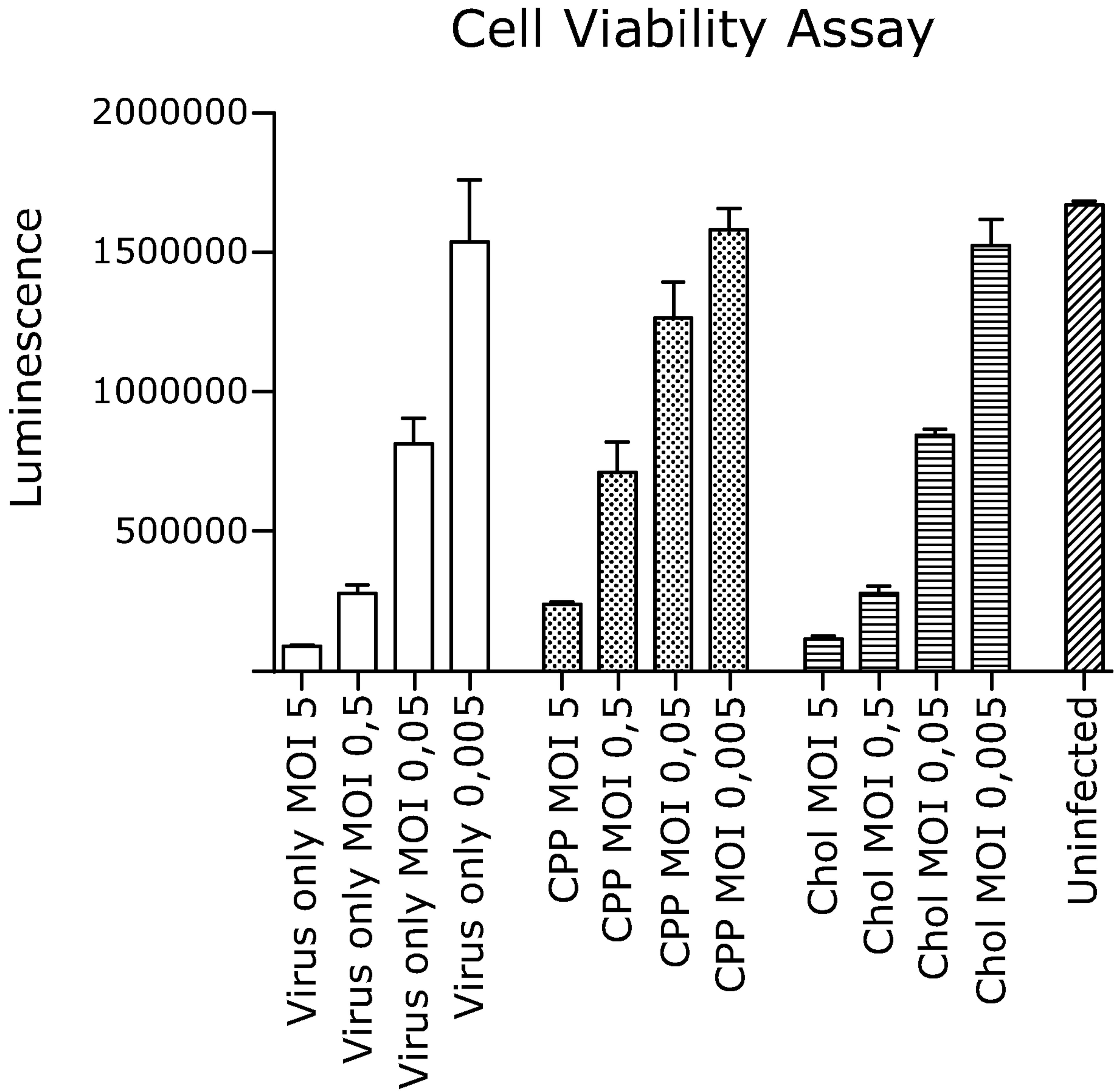


Figure 6

9/15

A

cholesterol-CRVRRALISLEQLESIINFEKLT EW (SEQ ID NO:20)

B

GRKKRRQRRRPQVRRALISLEQLESIINFEKLT EW
(SEQ ID NO:8)

Figure 7

A wide variety of different cell penetrating peptide (CPP) sequences can be used to attach peptides to the viral envelope

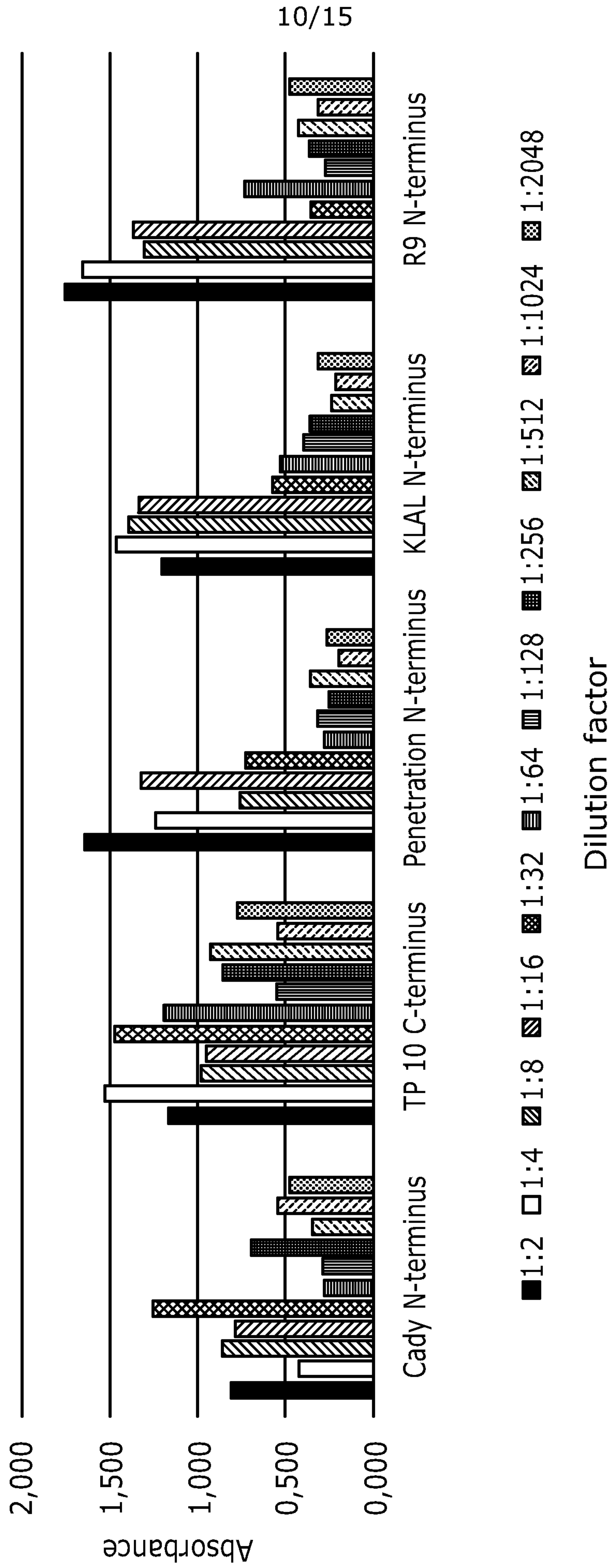


Figure 8

A wide variety of different cell penetrating peptide (CPP) sequences can be used to attach peptides to the viral envelope

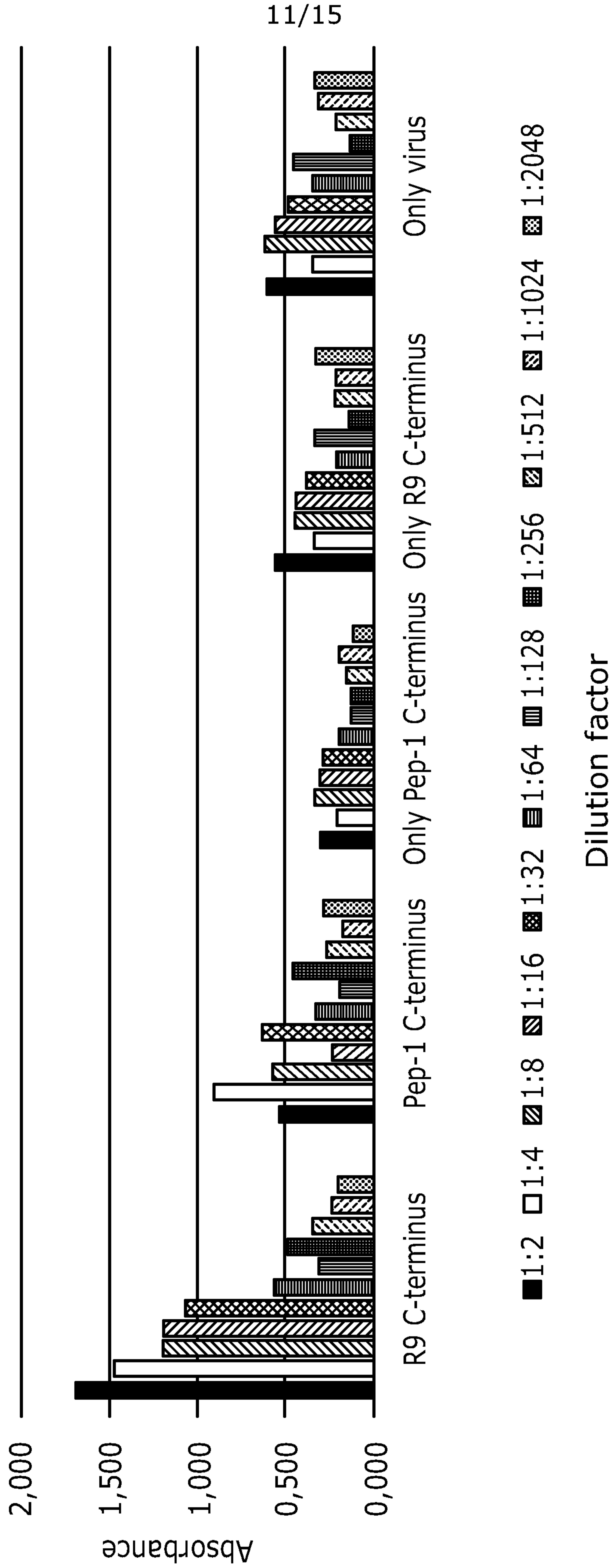


Figure 8
Continued

Attaching peptides into the viral envelope does not decrease viral infectivity

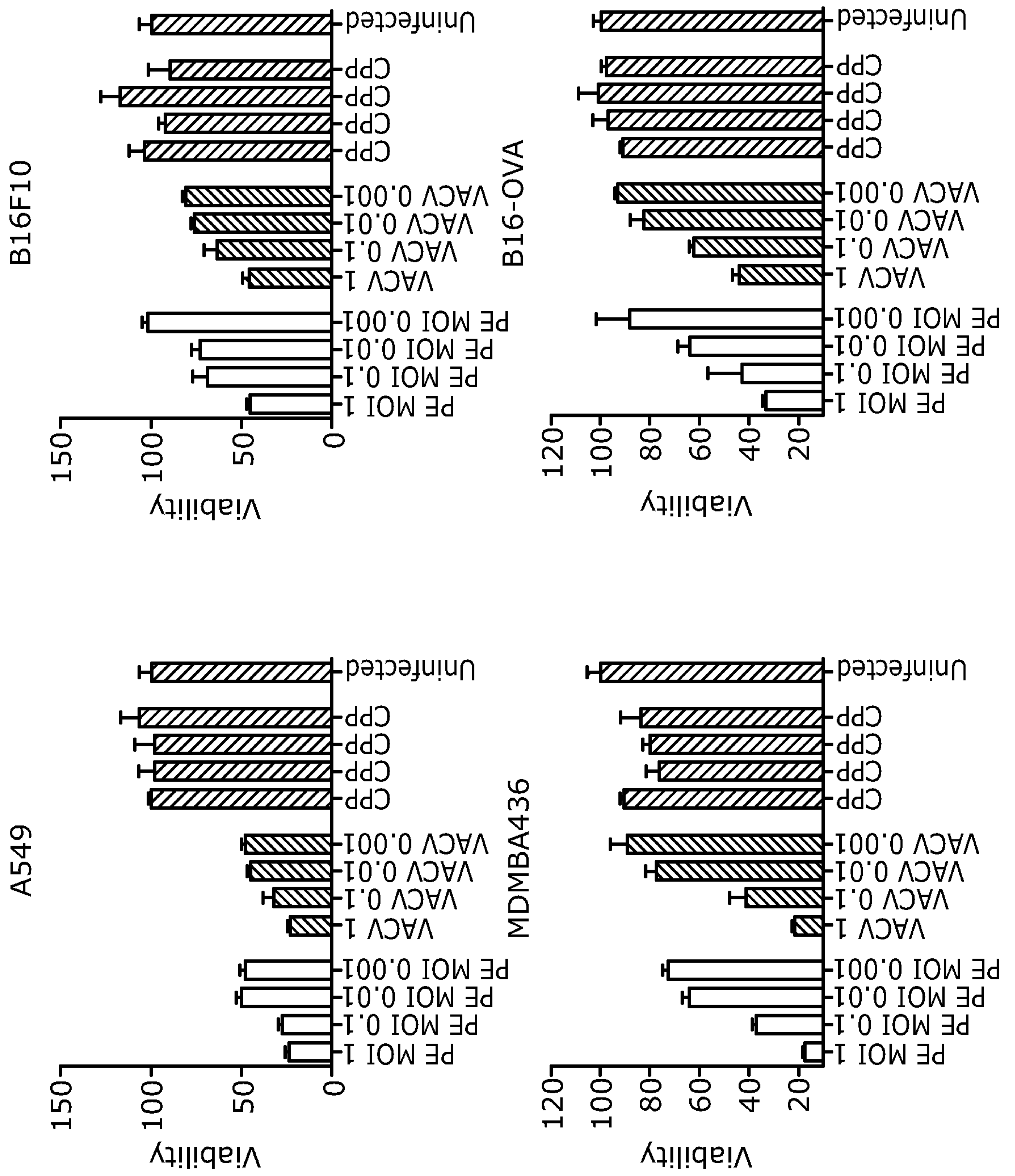


Figure 9

Anti-tumour peptides anchored into viral envelope by CPP or Cholesterol moieties induces extensive anti-tumour immunity leading to enhanced tumour control and longer survival

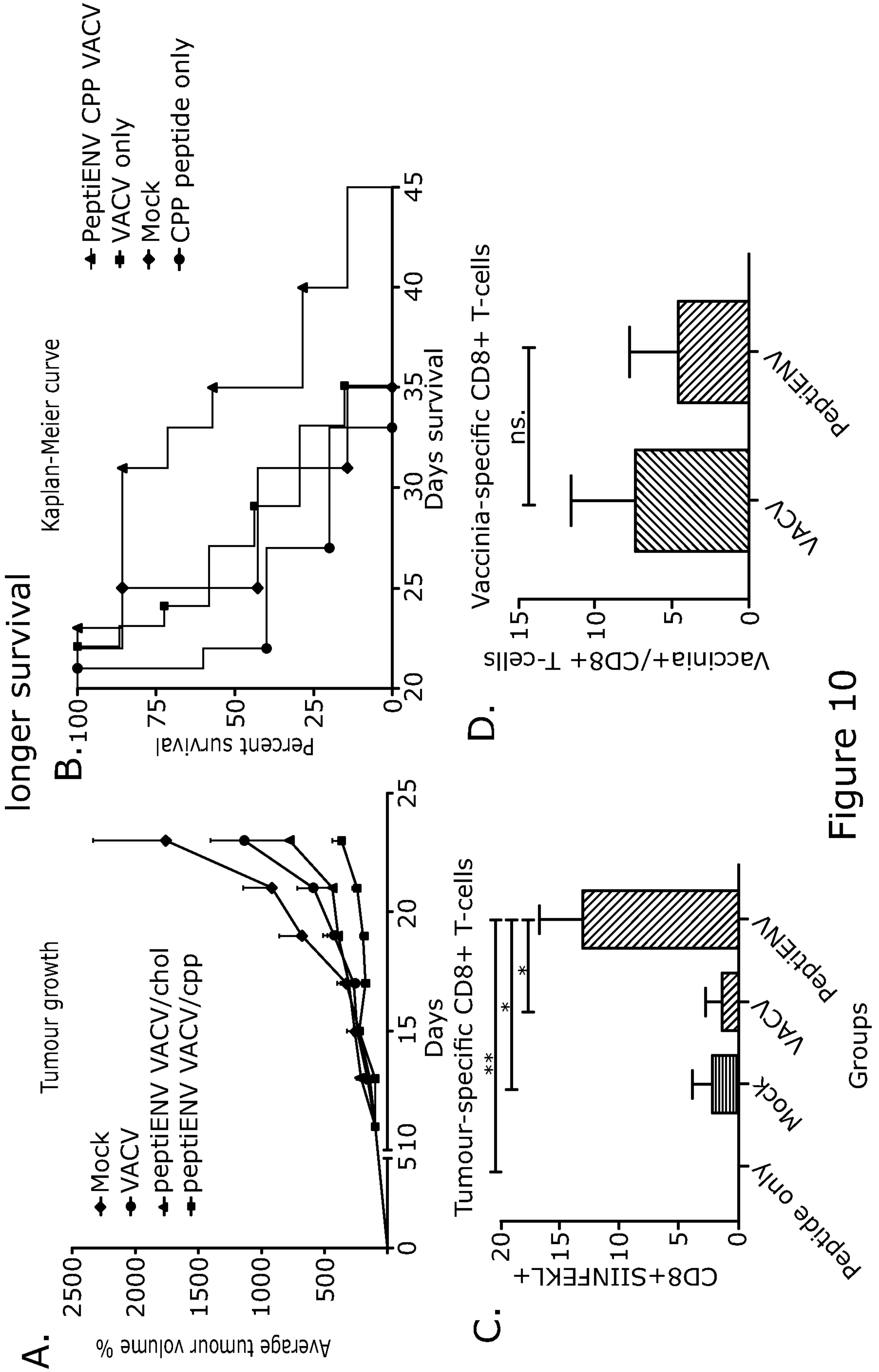


Figure 10

14/15

Anti-tumour peptides anchored into viral envelope by CPP or Cholesterol moieties induces extensive anti-tumour immunity and protects treated mice from tumour rechallenge

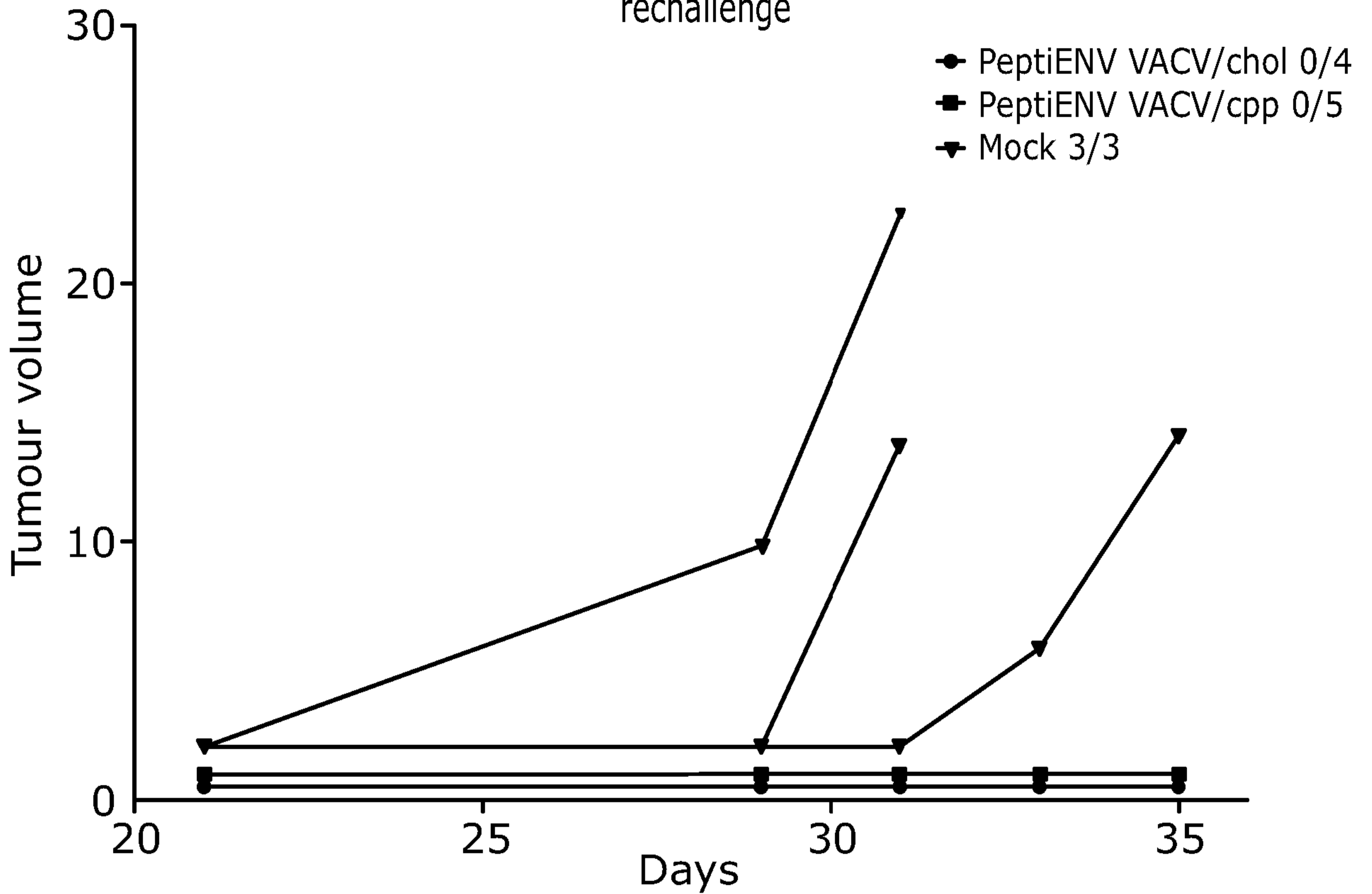


Figure 11

Anti-tumour peptides anchored into viral envelope by CPP moiety induces extensive anti-tumour immunity leading to enhanced tumour control

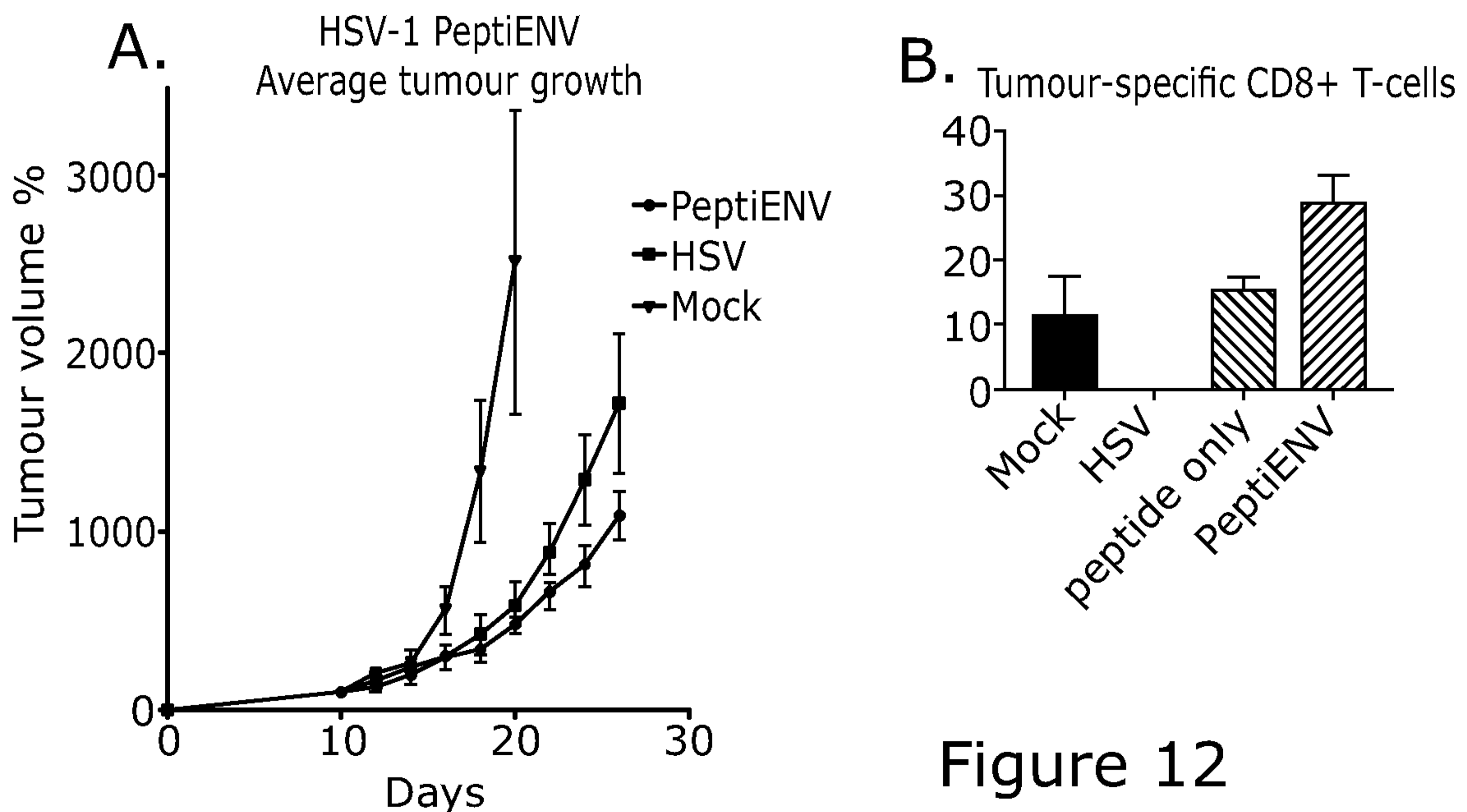


Figure 12

15/15

CPP-containing anti-tumour peptides have femtomolar affinity to viral envelope

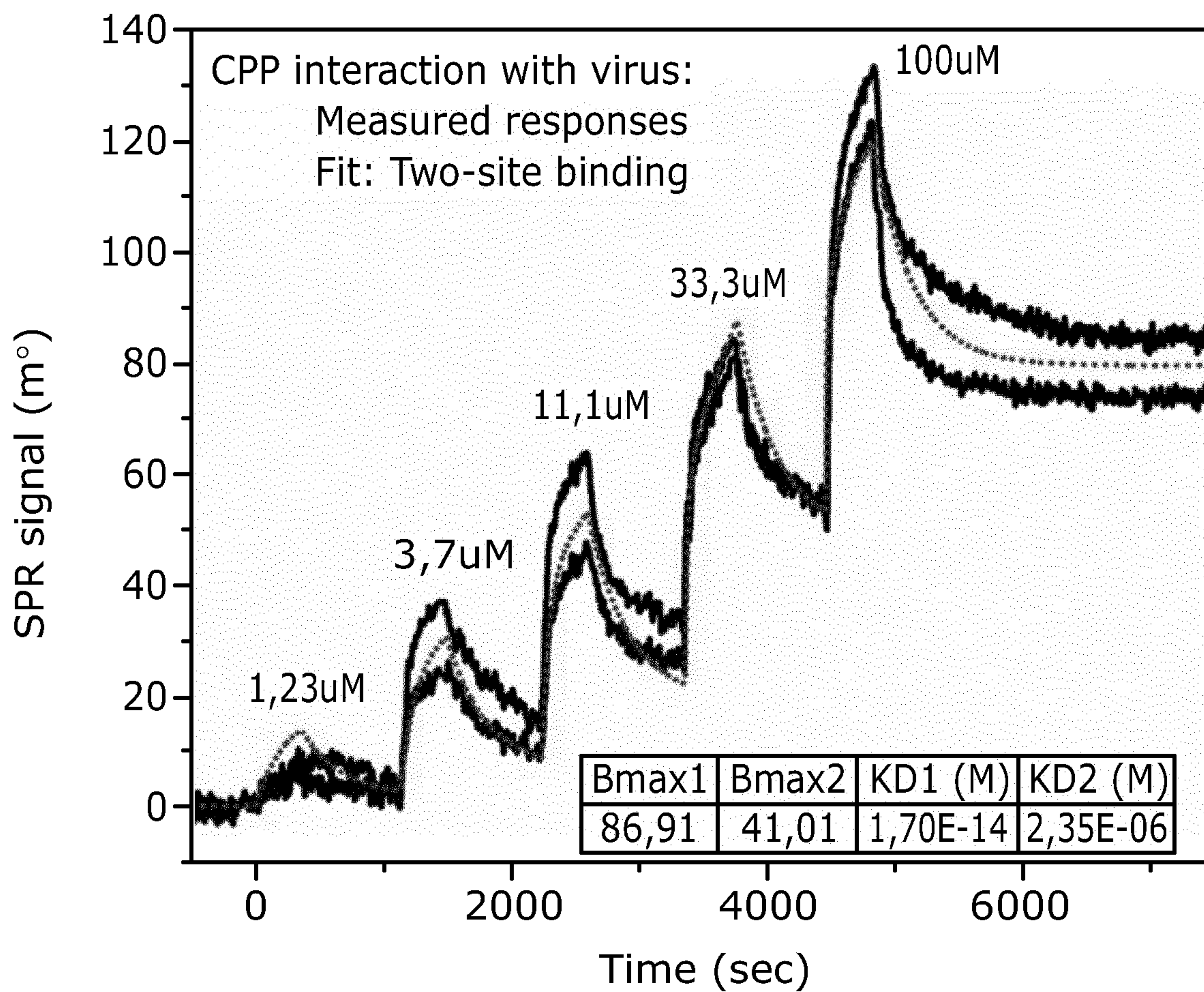


Figure 13

Anti-tumour peptides anchored into viral envelope by CPP or Cholesterol moieties induces extensive anti-tumour immunity and protects treated mice from tumour rechallenge

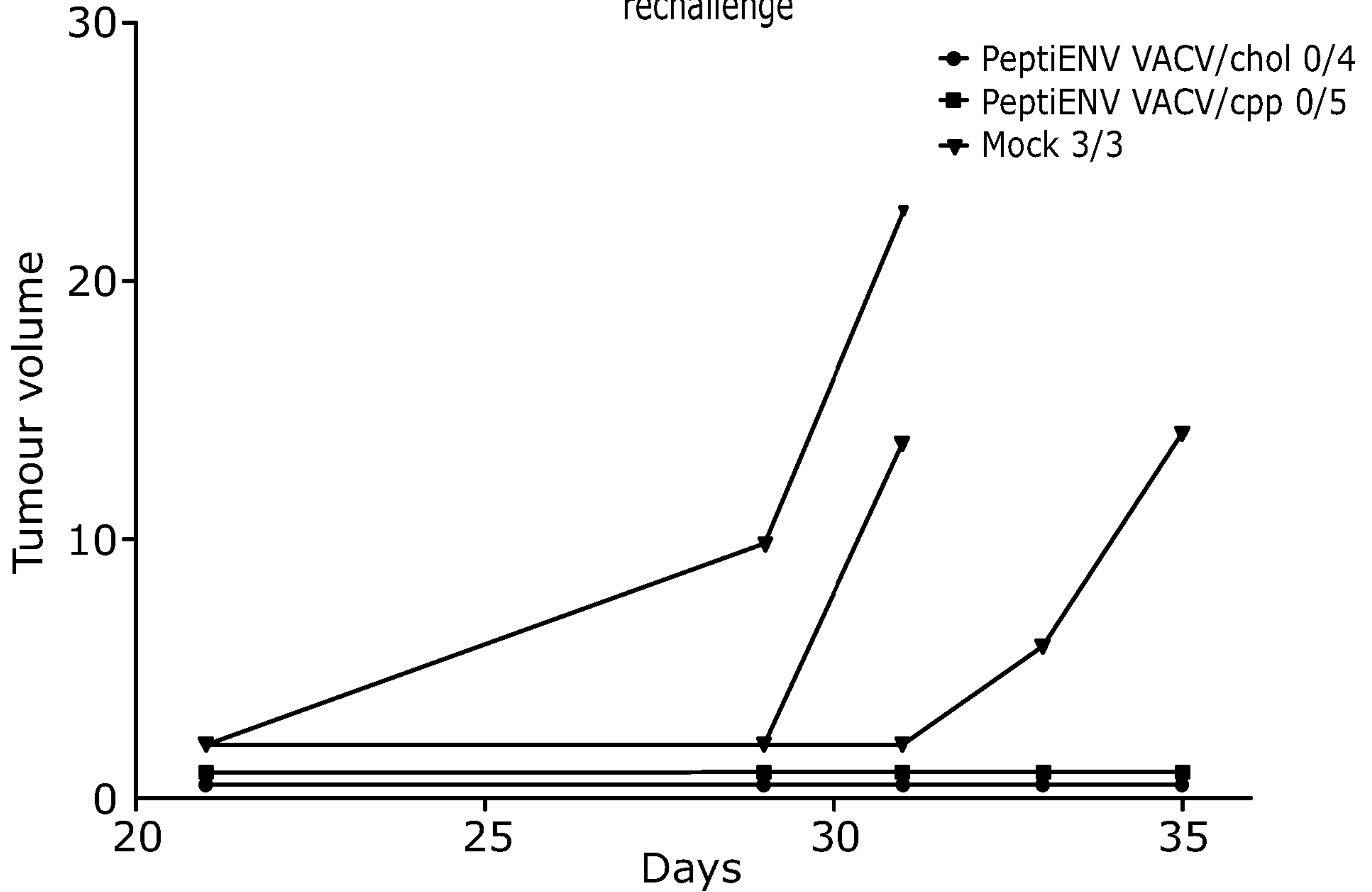


Figure 11