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(54) **Titre : SEQUENCE POLYPEPTIDIQUE IMPLIQUEE DANS LA MODULATION DE L'EFFET IMMUNOSUPPRESSIF DE
PROTEINES VIRALES**

(54) **Title: POLYPEPTIDE SEQUENCE INVOLVED IN THE MODULATION OF THE IMMUNOSUPPRESSIVE EFFECT OF VIRAL
PROTEINS**

(57) **Abrégé/Abstract:**

The present invention relates to a polypeptide having a sequence of 7 to 20 amino acid residues, which is capable of modulating the immunosuppressive properties of a viral protein or a fragment thereof, against the host in which it is expressed (immunosuppression-modulatory sequence) when it substitutes the homologous sequence of said viral protein or fragment, said polypeptide comprising the minimum following consensus amino acid sequence: $X_1Y_9Y_{10}Y_{11}CY_{12}X_2$ wherein, X_1 and X_2 are selected to impact on said immunosuppressive properties, and Y_9 to Y_{12} represent variable amino acid residues.



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(54) Title: POLYPEPTIDE SEQUENCE INVOLVED IN THE MODULATION OF THE IMMUNOSUPPRESSIVE EFFECT OF
VIRAL PROTEINS

(57) Abstract: The present invention relates to a polypeptide having a sequence of 7 to 20 amino acid residues, which is capable of modulating the immunosuppressive properties of a viral protein or a fragment thereof, against the host in which it is expressed (immunosuppression-modulatory sequence) when it substitutes the homologous sequence of said viral protein or fragment, said polypeptide comprising the minimum following consensus amino acid sequence: X₁Y₉Y₁₀Y₁₁CY₁₂X₂ wherein, X₁ and X₂ are selected to impact on said immunosuppressive properties, and Y₉ to Y₁₂ represent variable amino acid residues.



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POLYPEPTIDE SEQUENCE INVOLVED IN THE MODULATION OF THE IMMUNOSUPPRESSIVE EFFECT OF VIRAL PROTEINS

FIELD OF THE INVENTION

5 The present invention relates to an amino acid sequence capable of modulating the immunosuppressive properties of a protein, especially from antigenic proteins. The invention also provides polypeptides, derived from an antigenic and immunosuppressive protein, having acquired modulated immunosuppressive properties with respect to
10 the protein from which it is derived, while substantially retaining its antigenic properties.

 The invention especially concerns the field of viral or retroviral infections, including the field of endogenous retroviruses, and provides means for the design of agents for the prophylaxis and/or treatment of
15 hosts susceptible to such viruses or retroviruses, including animal or human hosts.

 Polypeptides of the invention can especially be used in the generation of immunogenic compositions and in the production of attenuated viruses, for use in methods for prophylaxis and/or treatment of
20 viral infections or their detrimental consequences or for prophylaxis and/or treatment of the detrimental consequences of the induction of expression of endogenous retroviruses (ERV).

BACKGROUND OF THE INVENTION

25 Infectious agents, such as viruses, have evolved mechanisms and strategies to invade their hosts and to escape their immune response. Various publications have demonstrated the immunosuppressive properties of proteins encoded by viruses: the Epstein Barr human herpes virus 4
30 (Suzuki et al. 1995. J. Exp. Med.182, 477-486; Qin et al. 1996 J. Immunol. 156, 2316-2323), the Mason-Pfizer monkey virus (Blaise et al. 2001 J. Gen.

Virol. 82, 1597-1600), the Moloney murine leukaemia virus (Mangeney and Heidmann. 1998. Proc. Natl. Sci. USA. 95, 14920-14925) and others (see review Alcamì et al. 2002 EMBO reports. 3(10), 927-932). This may be confirmed by the fact that infection by retroviruses is frequently associated with dysfunctions of the immune system of the host.

These immunosuppressive effects include the inhibition of interleukin-2-dependent lymphocyte proliferation, of the cytolytic activity of human natural killer cells, and of monocyte-mediated tumor cell killing as well as modulation of cytokine synthesis.

In vivo tests demonstrated that inactivated viruses, as well as synthetic peptides similar to retrovirus envelope proteins have immunosuppressive properties (Oostendorp et al. 1993 Crit. Rev. Oncol. Hematol. 14, 189-206; Haraguchi et al. 1997 J. Leukocyte Biol. 61, 654-666). More recently, Mangeney et al. (1998. Proc. Natl. Sci. USA. 95, 14920-14925) showed that murine tumoral cells from C57BL/6 strain, expressing a retroviral envelope protein, form tumours when injected in Balb/c mice (allograft), whereas the same cells, which do not express the retroviral envelope protein, are rejected. By carrying out different deletions in the envelope protein, a domain responsible for the immunosuppressive function that was called ISU (for "immunosuppressive") domain, was identified.

The ISU domain was first identified in the transmembrane moiety of the envelope glycoprotein. The *env* (envelope) gene of retroviruses encodes a precursor polypeptide which is then cleaved into two proteins: the surface glycoprotein (SU) and the transmembrane subunit (TM). The SU protein is responsible for the recognition and the binding to the cellular receptor for the virus. The TM moiety is involved in anchoring the envelope complex (SU and TM) to the target cell membrane, and is directly responsible for cell membrane fusion and virus entry.

The structure of the TM subunit has been elucidated for many viruses, especially for the Moloney murine leukaemia virus (Mo-MuLV), the

human immunodeficiency virus 1 (HIV-1) and the human T-cell leukaemia virus type 1 (HTLV-1). A highly conserved organization in the envelope proteins has also been found in non-retroviral proteins, such as those of influenza virus and Ebola virus.

5 Immunosuppressive effects have also been discovered in another class of proteins, characterized in the ERVs, especially HERVs (Human Endogenous Retroviruses). HERVs comprise elements which are sequences of retroviral origin that have spread into the human genome, and represent proviral remnants of ancestral infections. Therefore, strong
10 similarities can be inferred between HERVs and retroviruses. Some of these HERV elements are still functional and can encode active proteins, i.e., viral-like proteins although most of them have accumulated mutations, deletions and/or truncations.

 A role for these functional HERVs has been proposed,
15 including a protection against retrovirus infection (Best et al. 1997 Trends Microbiol. 5, 313-318) or a protection of the foetus against the maternal immune system via immunosuppressive effects (Cianciolo et al. 1985 Science 230, 453-455; Mangeney and Heidmann 1998 Proc. Natl. Sci. USA. 95, 14920-14925). An HERV encoding an envelope protein having
20 immunosuppressive properties was identified by Mangeney et al. (2001 J. Gen. Virology 82, 2515-2518). This publication reports that the protein encoded by HERV-H allows the envelope-expressing cells to escape immune response and to proliferate, whereas the same cells transfected with empty vectors are normally rejected by engrafted mice.

25 Other ERVs, especially HERVs, encoding functional envelope proteins were identified, which have fusogenic properties, i.e. are able to form syncytia *in vitro* (multi-nucleate cells): they include HERV-FRD and HERV-W (Blond et al. 2000 J. Virol. 74, 3321-3329 ; Blaise et al. 2003 Proc. Natl. Acad. Sci. 22, 13013-13018). Moreover, *in vivo* experiments
30 have shown that when co-expressed with MoMLV viral particles deficient for the production of their own envelope protein, the HERV-W envelope

protein can form functional viral particles, capable of infecting human cells (Patience et al. 1998 J. Virol. 72, 2671-2676). In conclusion, HERV-W has conserved its fusogenic and infectiosity properties. Analog fusogenic and infectious properties have been observed for HERV-FRD.

5 The observed immunosuppressive effects may be related, depending on the context, on the one hand to a virulent viral infection and on the other hand to an active proliferation of tumour cells, in mammals and particularly in human. Active proliferation of tumour cells is especially a consequence of expression of ERV viral-like proteins. However, whereas
10 more insights are needed to completely understand the mechanisms of immunosuppression, the identification of these immunosuppressive proteins opens new perspectives for therapeutic, including vaccinal, strategies against viral infections, against induction of expression of endogenous retroviruses, or against their detrimental consequences in a
15 host.

 Vaccines currently used can especially be classified as follows:

 - live attenuated vaccines (bacteria or virus vaccine) consisting in an attenuated or weakened, modified pathogen. After
20 administration to the host, the modified pathogenic organism replicates in the host and stimulates an immune response. This type of vaccine generally produces a long-lasting immunity upon single dose administration, but may cause side effects, i.e. a mild case of the illness caused by said pathogen, and thus should not be given to people with a
25 weakened immune system.

 - inactivated or killed vaccines, consisting in killed or inactivated pathogen, especially as a result of heat and/or chemical treatments (whole organism). Such treated pathogens cannot replicate, and cannot cause the disease they normally raise. Therefore, they are
30 safe and can be administered even to hosts whose immune system is

weakened. However, they are not usually as effective as live vaccines and therefore require multiple dose administration.

- vaccines consisting in antigenic fractions of a pathogen organism, including whole proteins or antigenic determinants thereof, especially obtained by recombinant technologies, as a result of the expression of genes encoding the antigen. The expressed protein can be administered to a patient, or the gene encoding the protein can be inserted into an expression vector which is administered to the host. Such vaccines however are usually not as effective as live vaccines and therefore require multiple doses.

Principles applied for the design of compounds suitable for vaccine preparations capable of eliciting an immune response in a host, in order to protect a host from infection due to pathogens, including viruses, bacteria or others, have been transposed to the design of compounds suitable for treatment of established infections, by immunotherapy. Efficiency of such compounds has however not proved to be sufficient enough, especially in the field of anti-viral or anti-viral-like prophylaxis or immunotherapy. Moreover, the use of compounds still raises many issues regarding safety.

One drawback observed in the use of some retroviral envelope proteins for immunisation, either as vaccine principles or for immunotherapy, lies in their immunosuppressive properties which can prevent or affect the efficiency of the host's immune response. Consequently these proteins cannot be administered to a patient in their native form because of their potential inhibition of the immune response. A great challenge would hence be to suppress or modulate the immunosuppression properties of these proteins, without altering their antigenic properties and/or their properties related to host cell infection. However, attempts to mutate the envelope protein complex, have led to a strong alteration of its fusion and infection functions and therefore of their interest as active principle to raise an immune response (Delamarre et al.

1997 J. Virol. 71(1), 259-266; Rosenberg et al. 1999 J. Cell Biol. 145, 57-68).

5 This is a purpose of the present invention to identify determinants of the immunosuppressive properties of proteins, including to identify polypeptide sequences and amino acid residues involved in the modulation of the immunosuppressive properties of proteins, particularly viral or viral-like proteins, which substantially retain their antigenic properties of said immunosuppressive proteins.

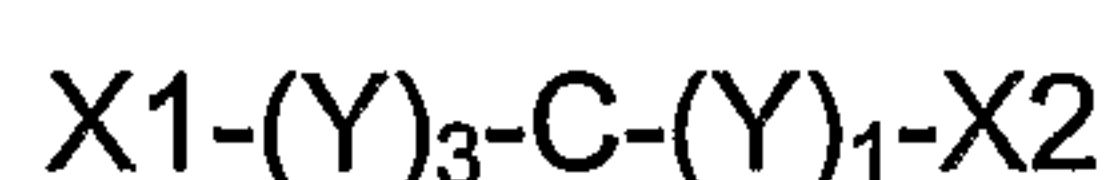
10 It is a further object of the invention, to identify such determinants of the immunosuppressive properties of the protein, and to use the same for the design of polypeptides having modified, i.e., modulated immunosuppressive properties.

15 Another object of the present invention is to provide such polypeptides, which are derived from an antigenic and immunosuppressive protein, which polypeptides are characterized by modulated immunosuppressive properties while retaining antigenic properties of the starting protein.

20 This is also an object of the present invention, to provide means to promote an efficient immune response against pathogen organisms, especially against viruses, i.e., a cell-mediated and/or humoral immune response which would be protective against infection by such pathogen organisms, especially viruses, or protective against their detrimental effects in the host, or protective against the detrimental consequences of expression of endogenous retroviruses in a host, with
25 reduced risks of immune system alteration. The invention also provides means suitable for treatment by immunotherapy, of patients infected with pathogen organisms including viruses, or for treatment of their detrimental effects, including malignant effects or for the treatment of patients suffering from pathologies associated with induction of the expression of
30 endogenous viruses which are normally silent in hosts.

SUMMARY OF THE INVENTION

In one aspect, the invention provides a polypeptide which is capable of modulating the immunosuppressive properties of a viral protein or a fragment thereof against the host in which it is expressed when it substitutes the homologous sequence of said protein or fragment, said polypeptide having the minimum following consensus amino acid sequence:



10

wherein, X1 and X2 are selected to impact on said immunosuppressive properties, Y represents variable amino acid residues, and 3 and 1 represent the number of variable amino acid residues respectively between X1 and C and between C and X2.

15

Said minimum consensus sequence is designated "immunosuppression-modulatory sequence".

20

In an embodiment, peptides replying to the above definition, comprising an immunosuppression-modulatory sequence, are derived from a viral including from a viral-like protein, especially a retroviral protein, in particular, a viral or retroviral envelope protein or an envelope protein from an endogenous retrovirus, especially from a human endogenous retrovirus (HERV).

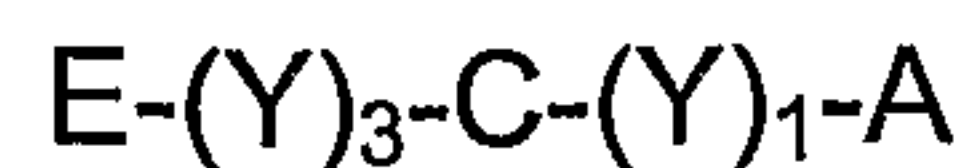
25

The amino acid sequences of several envelope proteins of viruses (including ERV) have been disclosed in Figure 3 of Benit et al (J Virol. December 2001, p. 11707-11719).

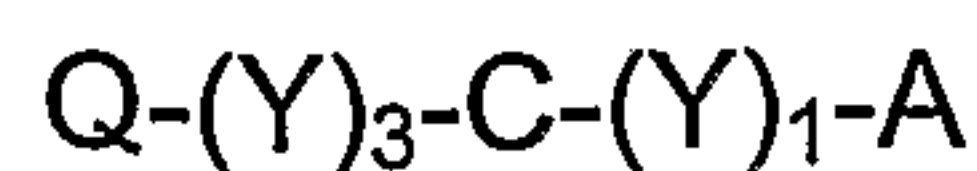
30

Particular pairs of amino acid residues impacting on the immunosuppressive properties in the context of a determined protein have been characterized, and accordingly sequences having the desired "immunosuppression-modulatory" properties have been identified and can be selected from the group consisting of:

a) sequences involved in the occurrence of immunosuppressive properties of a protein in which they are present comprise:

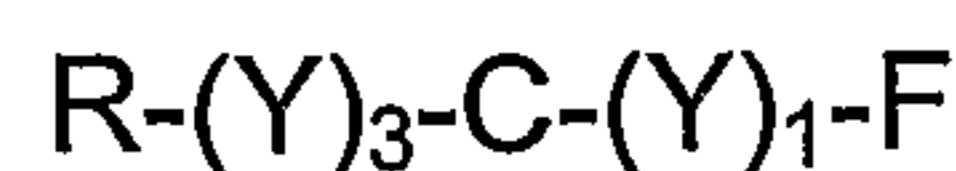


5



and b) sequences altering, e.g. decreasing or suppressing immunosuppressive properties of an immunosuppressive protein when they are present therein, comprise

10



In another aspect, the invention provides a polypeptide derived from a determined antigenic and immunosuppressive protein, said polypeptide comprising an amino acid sequence (so-called immunosuppression-modulatory sequence) represented by $\text{X1}-(\text{Y})_3-\text{C}-(\text{Y})_1-\text{X2}$ wherein in said polypeptide Y represents variable amino acid residues, 3 and 1 represent the number of variable amino acid residues Y respectively between X1 and C and between C and X2, and X1 and X2 are chosen to confer to said polypeptide altered immunosuppressive properties with respect to the immunosuppressive properties of said determined protein.

20

In a particular embodiment, the protein having antigenic and immunosuppressive properties is encoded by a gene derived from a virus, and especially by an env gene from a retrovirus.

25

Such protein comprises an immunosuppressive sequence determinant having the following consensus sequence: E/Q-G-G-L/T/I-C-A/K/L/M/V/I-A. The same protein wherein X1 (E/Q) and optionally X2 (A) residues are substituted can be devoid of immunosuppressive properties but retains its antigenic properties. An example of modified immunosuppression-modulatory sequence is R-G-G-L/T/I-C-A/K/L/M/V/I-F, which alters immunosuppressive properties and especially can give rise to a non-immunosuppressive polypeptide which contains said sequence. A

30

particular modified immunosuppression-modulatory sequence is selected from the group of:

	RGGLCAF (SEQ ID NO: 1)
	RGGLCKF (SEQ ID NO: 2)
5	RGGLCLF (SEQ ID NO: 3)
	RGGLCMF (SEQ ID NO: 4)
	RGGLCVF (SEQ ID NO: 5)
	RGGLCIF (SEQ ID NO: 6)
	RGGTCAF (SEQ ID NO: 7)
10	RGGTCKF (SEQ ID NO: 8)
	RGGTCMF (SEQ ID NO: 9)
	RGGTCIF (SEQ ID NO: 10)
	RGGICAF (SEQ ID NO: 11)
	RGGICKF (SEQ ID NO: 12)
15	RGGICLF (SEQ ID NO: 13)
	RGGICMF (SEQ ID NO: 14)
	RGGICVF (SEQ ID NO: 15)
	RGGICIF (SEQ ID NO: 16)

20 In a particular embodiment, the protein further has infectious and/or fusion properties. The modification of the immunosuppression-modulatory sequence, e.g. by substitution of X1 and optionally X2 amino acid residues can advantageously be carried out in a way that does not affect one of these or both supplementary properties.

25 In another aspect, the invention relates to compositions comprising such polypeptides or recombinant viral particles expressing these polypeptides. Such compositions or particles can be used in the prevention or treatment of a viral infection including for the prevention or treatment of its detrimental effects, or for prevention or treatment or the
30 consequences in a host, of the expression of an endogenous virus, especially an HERV, by the elicitation of an immune response in the host in which they are injected. They can also be used in the preparation of attenuated viruses.

35 In another aspect, the invention relates to methods to modulate the immunosuppressive properties of a protein by modifying the amino acid composition of the immunosuppression-modulatory sequence.

BRIEF DESCRIPTION OF THE DRAWINGS

5 **Figure 1: schematic representation of vectors containing the env nucleic acid of MoMLV or its derived polypeptides.**

Nucleic acids contained in these vectors encode the wild-type envelope protein of MoMLV (envMoMLV) or its derived polypeptides of the invention by substitutions of codons encoding X1 and/or X2.

Figure 1A represents the phCMV-envMOMLV vector.

10 Figure 1B represents the pDFG-envMoMLV-iresHygro vector.

Figure 2: schematic representation of vectors containing the env nucleic acid of MPMV or its derived polypeptides.

15 Nucleic acids contained in these vectors encode the wild-type envelope protein of MPMV (envMPMV) or its derived polypeptides of the invention by substitutions of codons encoding X1 and/or X2.

Figure 2A represents the phCMV-envMPMV vector

Figure 2B represents the pDFG-envMPMV-iresHygro vector

20 **Figure 3: schematic representation of vectors containing the HERV-W nucleic acid of HERV-W or its derived polypeptides.**

Nucleic acids contained in these vectors encode the wild-type envelope protein W (envW) or its derived polypeptides of the invention by substitutions of codons encoding X1 and/or X2.

25 Figure 3A represents the phCMV-envW vector

Figure 3B represents the pDFG-envW-iresHygro vector

Figure 4: Schematic representation of the cell-cell fusion assay.

30 The vector used comprises the nucleic acid encoding an envelope protein of interest (SU and TM subunits), a CMV promoter and a poly A nucleotide element (pA).

Figure 5: Schematic representation of the establishment of Envelope Expressing tumours cells and *in vivo* assay.

5 The vector used comprises the nucleic acid encoding an envelope protein of interest (env), the hygromycin gene (hygro) and an IRES (Internal Ribosome Entry Site). White boxes represent LTRs and the arrow indicates the start of transcription.

Figure 6: Results of infectious property assay.

10 The numbers 1 to 12 refer to lines used in the present specification. This diagram presents the results of infection for wild-type (wt) or mutant envelope proteins according to the invention.

Figure 7: Results of immunosuppressive property assay.

15 The diagram presents the results of immunosuppressive property assay of MCA205 cells expressing envelope when injected in allogenic balb/c mice. In insets, results of MCA205 cells expressing envelope protein injected in syngenic C57Bl/6 mice. Filled bars represent HERV-W envelope protein, white bars represent MPMV envelope protein and shaded bars represent
20 double-mutant (R44Q+F50A) HERV-W envelope protein.

Figure 8: Structural design of the TM subunit of the HERV-W ENV protein.

25 This structural design shows the position of the Arginine (X1) and Phenylalanine (X2) amino acid residues of the immunosuppression-modulatory sequence, as well as the two amino acid residues (Alanine and Threonine) not involved in such properties.

Figure 9: Examples of immunosuppression-modulatory sequence of different viruses and HERVs.

The first column indicates the common names of viruses or HERVs, the second column indicates the origin of the viruses or HERVs, the third column indicates the nucleotide sequences of identified immunosuppression-modulatory sequences (one letter amino acid used) and the last column indicates the Accession Number of the envelope protein. The X1 and X2 amino acid residues are in bold.

Figure 10: Nucleotide and amino acid sequences of wild-type envelope proteins.

In the amino acid sequences, the X1 and X2 positions have been underlined.

A and B represent the nucleotide and protein sequences of the envelope protein of MoMLV, C and D represent the nucleotide and protein sequences of the envelope protein of MPMV and E and F represent the nucleotide and protein sequences of the envelope protein of HERV-W (envW).

The nucleotide sequences (A, C and E) are the coding sequences of the envelope proteins, with the first codon (ATG) being the first codon of transcription and the last codon (TAG) being the termination codon.

For the protein sequences (B, D and F), the first letter amino acid code is used. The first M represents the first methionine of the protein, and the symbol "*" represent the termination codon.

Figure 11A, Figure 11B and Figure 11C: *In vitro* properties of the immunosuppression-defective FV envelope protein. Figure 11A, Infectivity of FV wild type (wt) envelope protein, E14R mutant envelope protein, A20F mutant envelope protein, and E14R+A20F double mutant (DM) envelope protein as expressed on the surface of a MLV viral

pseudotypes, using NIH 3T3 cells as a target. The vertical axis represents the infectivity (ffu/ml) Figure 11B, *In vivo* immunosuppressive activity (horizontal axis, immunosuppression index) of the wild-type (wt) and the double-mutant (DM) FV envelope protein. Figure 11C, Comparison of *in vitro* propagation rates of the wild-type (black circles) and immunosuppression-defective (gray circles) FV virions, using NIH 3T3 cells as a target. Viral load of cell supernatants (vertical axis, RNA copy number/mL) is assayed by quantitative RT-PCR. Horizontal axis represents the number of days after infection. The white circles represent a control.

Figure 12A and Figure 12B: *In vivo* effects of the loss of envelope-driven immunosuppression on FV infection. Serum viral loads (Vertical axis, RNA copy numbers/mL) of irradiated (Figure 12A) and non-irradiated (Figure 12B) Swiss mice after injection of the wild-type FV (black circles) or the non-immunosuppressive mutant FV (gray circles). The signal for PBS-injected mice was below detection threshold (white circles). Horizontal axis represents the days after injection.

Figure 13: Immunological detection of FV in infected mice. IgGs directed against the SU subunit of the FV envelope protein were quantitated (vertical axis, arbitrary units) in the sera of mice injected with the wild-type FV (black circles and line), the non-immunosuppressive mutant FV (gray circles and line) or PBS (white circles and dotted lines). The lines represent the geometric means of the IgG levels. Horizontal axis represents the days after injection.

Figure 14A and Figure 14B: Antigenicity of the wild-type and non-immunosuppressive mutant FV envelope proteins. Figure 14A, IgMs and IgGs directed against the TM subunit of the FV envelope protein were quantitated in the sera of mice injected with recombinant TM subunits of the FV envelope protein (left) or UV-inactivated FV viral particles (right). Black:

wild-type FV; gray: non-immunosuppressive mutant FV; white: adjuvant only. Mean $\pm$ standard deviation on 5 (left) or 14 (right) Swiss mice. The vertical axis represents the anti-TM ELISA signal in arbitrary units (a.u.). Figure 14B, same as in Figure 14A with mice injected with the wild type (wt) or double mutant (dm) recombinant TM subunits of MoMLV (left) and
5 HERV-W ENV (right) as described in Example 1. The vertical axis represents the IgG level in ng/mL.

Figure 15: Vaccination assays. Figure 15 represents the viral load
10 (Vertical axis, RNA copies/mL serum) of mice immunized with UV-inactivated wild-type or non-immunosuppressive double mutant Friend Virus (FV), with intact non-immunosuppressive double mutant Friend Virus (FV), or with CpG adjuvant only, and challenged with the wild-type FV. Immunization was performed on day 1, day 7 and day 14 before challenge
15 on day 21, and the corresponding viral loads are represented as grey dots. 5 days post-challenge viral loads are represented as black dots. The detection threshold is represented as a horizontal line at 2.10^3 RNA copies/mL. On top of the graph is indicated the number and the percentage of mice having a viral load below the detection level at 5 days post-
20 challenge. Horizontal bars represent the geometric means of the viral loads.

Figure 16A, Figure 16B and Figure 16C: Knockdown procedure and rationale of the assay. Figure 16A represents the procedure to knock
25 down ERV expression, a plncx-derived vector was constructed making use of the pSUPER vector to generate, under control of the H1-RNA promoter, short double-stranded transcripts for RNA interference. B16 cells were transduced with these expression vectors, submitted to G418 selection, and the resulting ERV^{KD} and control B16 cells were injected subcutaneously into the flank of the mice, whose tumor growth was monitored. Figure 16B,
30 predicted structure of the dsRNA generated by the ERV and control (gfp) vectors; numbers refer to nt positions within the respective targeted

sequences (see Methods). Figure 16C, Western blot analysis of Gag (anti-Gag) and Env (anti-Env) expression in the supernatant of ERV-knocked down (ERV^{KD}) and control cells. Molecular weights are represented on both side of the Figure.

5

Figure 17A and Figure 17B: Knocked down cells have conserved a transformed phenotype. Figure 17A, *in vitro* analysis of the transformed

phenotype using soft agar assay. Left panel, ERV^{KD} (right plates) and control B16 (left plates) cells (2×10^3 or 2×10^4) were plated onto a semi-solid

10

layer for 4 weeks, and then colonies were numbered (right panel). Figure

17B, assay for the transformed phenotype *in vivo* using immuno-incompetent mice. ERV^{KD} and control B16 cells (2×10^5) were injected subcutaneously into the flank of either X-irradiated (5 Gy) C57Bl/6 (left panel) or SCID mice (right panel) (2-5 independent experiments with 5 mice

15

per group) and tumor growth was determined by measuring tumor area (vertical axis, mm²) as a function of time (horizontal axis, days post injection).

Figure 18A, Figure 18B and Figure 18C: Inhibition of tumor cell growth and increased mouse survival upon ERV knockdown. Figure 18A,

20

tumor cell growth of control (black dots) and ERV^{KD} B16 cells (white dots) engrafted into immunocompetent C57Bl/6 mice (22 mice per group; same experimental conditions as in Figure 17B). Tumor area (vertical axis, mm²) is measured as a function of time (horizontal axis, days post injection).

25

Figure 18B, percentage of survivors (vertical axis) among the control (black dots) and ERV^{KD} B16 cells (white dots) engrafted mice (10 mice per group) as a function of time (horizontal axis, days post injection). Figure 18C,

percentage of survivors (vertical axis) (10 mice per group) among MelARV *env* - transduced ERV^{KD} B16 cells (grey dots) and ERV^{KD} B16 cells (white dots) engrafted mice as a function of time (horizontal axis, days post injection).

30

Figure 19: Immunostaining for ERV envelope protein detection.

Control, ERV^{KD}, and ERV^{KD}+env B16 cells were labelled with the 9B6 antibody (directed against the MelARV envelope protein; gift from E. Gorelik, Cancer Res 1988;48:4954-4958) revealed by a goat anti-mouse FITC antibody (Caltag, Burlingame, USA). Flow cytometry analysis was performed using a Facscalibur cytometer. The number of counts (vertical axis) is represented as a function of ERV envelope expression (horizontal axis).

Figure 20A and Figure 20B: *In vivo* systemic administration of siRNA

reduces tumor cell progression. Synthetic siRNA targeted to the 19 nt ERV (white dots) and control (gfp) (black dots) sequences referred to in Figure 16B were purchased from MWG Biotech. They were injected intraperitoneously (3 µg of siRNA in 50 µl of PBS), at day 12 after prior engraftment of 2x10⁵ B16 cells in the right flank of the mice. Figure 20A, the tumor area (vertical axis, mm²) is measured as a function of time (horizontal axis, days post tumour injection), siRNA injection is represented as an arrow. Figure 20B, the percentage of survivors (vertical axis) were monitored (5 mice per group in two independent experiments) as a function of time (horizontal axis, days post tumour injection).

DETAILED DESCRIPTION

The present invention provides a polypeptide having a sequence of 7 to 20 amino acid residues, which is capable of modulating the immunosuppressive properties of a viral protein or a fragment thereof against the host in which it is expressed when it substitutes the homologous sequence of said viral protein or fragment, said polypeptide comprising the minimum following consensus amino acid sequence:



wherein, X1 and X2 are selected to impact on said immunosuppressive properties, Y represents variable amino acid residues, and 3 and 1 represent the number of variable amino acid residues Y, respectively between X1 and C and between C and X2.

5 In all the sequences of the present invention, the amino acid one-letter code is used. X and Y are used to designate variable amino acid residues, X being determined to influence the immunosuppressive properties of a determined protein.

10 Y represents amino acid residues that can vary for different polypeptides and within one determined polypeptide. "(Y)₃" indicates that 3 amino acid residues are present between the X1 residues and the cysteine residue (C). The 3 amino acid residues can be different or identical and can be selected independently of each other. The indication of a particular amino acid residue in a sequence, like the cysteine in the sequence above,
15 means that this amino acid residue is invariant, *i.e.* it has a constant position in said sequence.

Optionally the consensus sequence can also be noted as follows:



20 wherein X₁ represents X1, X₂ represents X2, and Y₉ to Y₁₂ represent any amino acid. As intended herein amino acids Y₉ to Y₁₂ are identical or different.

In the present invention, the expressions "virus" or "viral" apply both exogenous or endogenous viruses or their compounds, unless
25 otherwise stated. Therefore, "viral protein" encompasses "viral-like proteins" which may also be referred to when describing the expression products of endogenous viruses, especially ERV, in particular HERV.

The above consensus sequence of the polypeptide according to the invention is called "immunosuppression-modulatory sequence"
30 meaning that, when it is present in the polypeptide having 7 to 20 amino acid residues, the polypeptides can be used to modulate

immunosuppressive properties of a protein which has been identified as harbouring such immunosuppressive properties or, as lacking such properties despite the fact that it comprises a peptidic motif having a sequence $X1-(Y)_3-C-(Y)_1-X2$.

5 More especially, X represents both amino acid residues (X1 and X2) directly involved, individually or together, in the modulation of the immunosuppressive properties of a protein comprising the above consensus sequence. They are respectively located at the N-terminal and C-terminal ends of the minimum polypeptide having 7 amino acid residues.

10 A protein is said to have immunosuppressive properties, when this protein, expressed in tumour cells engrafted in a host which would normally be rejected by said host, to the contrary allows these tumour cells to proliferate and to escape immune rejection by the host.

An *in vivo* procedure to assay the immunosuppressive activity of a protein is that used by Mangeney M. and Heidmann T., 1998 PNAS or by Blaise et al. 2001 represented in Figure 5. A wild-type or modified nucleic acid expressing the protein to be tested is transfected in tumour cell lines such as MCA 205 or Cl8.1 cell lines by known transfection methods. The tumour cells expressing the protein to be tested are then injected especially s.c. injection to a host, generally mice. Following said injection, the establishment of tumour or, to the contrary, its rejection, is determined and the tumour area is measured. *In vitro* assay could be carried out, using high doses of synthetic peptides but they are indirect and less convincing, since the expression "immunosuppressive" is relevant when applied to animals possessing a complete immune system and not to cell lines.

25 The expression "modified nucleic acid" as used herein refers to any genetic alteration such as nucleotide substitution, deletion or insertion that change the amino acid composition of the encoded polypeptide or protein. Thus, an amino acid sequence can substitute, *i.e.* replace a homologous sequence present in the original protein.

30

The terms "homologous sequence" in the protein which is tested for modulation of its immunosuppressive properties refer to a sequence having the same amino acid sequence as that replacing (i.e. substituting) it for the assay, i.e., $X_1-(Y)_3-C-(Y)_1-X_2$ except for the X_1 and X_2 residues; at least one of which and possibly both, are selected to be different from their corresponding amino acid residues in the original sequence. Thus, the Y amino acid residues are conserved between the homologous sequence of the protein to be modified and the sequence of the polypeptide having 7-20 amino acid residues as defined above.

Such homologous sequences are disclosed in Figure 9 for various viruses and are illustrated in the context of the TM subunit of various envelopes for several viruses in Benit L. et al. (J. Virol. Vol. 75, No. 23, December 2001, p. 11709-11719) in Figure 3.

The X_1 and X_2 amino acid residues are chosen to modulate the immunosuppressive properties of the original viral protein. The term "modulate" as used herein refers to an increase or decrease of the immunosuppressive activity of the modified protein with respect to the immunosuppressive activity of the original (i.e., non modified) protein, when tested in the same conditions.

The invention especially relates to an "immunosuppression-modulatory sequence" which allows a decrease in the immunosuppressive properties of the modified protein with respect to the originally immunosuppressive protein. The modulation is preferably significant meaning that the immune response of the host becomes detectable, and advantageously becomes sufficient to eliminate the pathogen agent or becomes sufficient to stop, stabilize or reverse the detrimental consequences of infection by said pathogen in a host or of the expression of endogenous viruses, especially of normally silent ERV, especially HERV, in a host.

In a particular embodiment, modulation results in decreasing the immunosuppressive properties of the original protein.

In a particular embodiment it corresponds to at least a two-fold decrease of the immunosuppressive properties of the original protein, in the modified, i.e., derived protein.

5 The above defined polypeptide of the invention having 7 to 20 amino acid residues and comprising sequence $X1-(Y)_3-C-(Y)_1-X2$ is such that $X1$ and/or $X2$ are selected to modulate the immunosuppressive properties of a protein and accordingly:

in a particular embodiment of the invention, $X1$ is an alkaline amino acid residue and $X2$ is an aromatic residue or *vice versa*.

10 As intended herein "alkaline" relates to basic amino acids.

In another particular embodiment of the invention, $X1$ is an alkaline residue or $X2$ is an aromatic residue or *vice versa*.

The inventors have observed that the modulation effect of $X1$ and $X2$ on immunosuppressive proteins is lower when only one of $X1$ or $X2$ residues is modified in an original immunosuppressive protein.

15 Therefore, modification of both $X1$ and $X2$ in an immunosuppression-modulatory sequence may be regarded as advantageous.

20 In another particular embodiment of the invention, residues $X1$ or $X2$ located in amino acid sequence represented as $X1-(Y)_3-C-(Y)_1-X2$ are selected as follows:

where $X1$ is chosen among R, H and K, $X2$ is chosen among F, W, Y and H or where $X1$ is chosen among F, W, Y and H, $X2$ is chosen among R, H and K.

25 In a further embodiment of the invention, $X1$ is R, H or K and $X2$ is F, or *vice versa*.

In a further embodiment of the invention, $X1$ is R and $X2$ is F, W, Y or H.

30 In another further embodiment of the invention $X1$ and $X2$ are selected from the group consisting of:

a. $X1$ is E, K or Q and $X2$ is A

- b. X1 is W and X2 is I or V
- c. X1 is R and X2 is F
- d. X1 is K and X2 is F.

5 The inventors have identified the effects of particular X1 and X2 residues, in a immunosuppression-modulatory sequence on modulation of the immunosuppressive properties of a viral envelope protein.

Their observations enable to consider that, when X1 is either glutamic acid (E) or glutamine (Q) and X2 can be alanine (A), the resulting viral envelope protein comprising the consensus sequence of the invention
10 harbours immunosuppressive properties. To the contrary, when X1 is arginine (R) and X2 is phenylalanine (F), the resulting viral envelope protein having the consensus sequence of the invention has low or has no immunosuppressive properties. Interestingly, whereas van der Waals interactions are suspected in the pair E/A, an electrostatic interaction may
15 occur in the pair R/F, between the positively charged side chain of Arginine and the pi-electrons (negative pole) of Phenylalanine.

Accordingly, in a particular embodiment of the invention, the polypeptide having 7 to 20 amino acid residues has an immunosuppression-modulatory sequence $X1-(Y)_3-C-(Y)_1-X2$ suitable to
20 confer low or no immunosuppressive properties to a protein, wherein X1 is R and/or X2 is F.

In another embodiment, X1 is K and X2 is F to confer low or no immunosuppressive properties to a protein. In particular, such a protein has low immunosuppressive properties.

25 It is recalled that the immunosuppressive properties are assayed in a test as defined above and illustrated in the Examples.

The consensus sequence, $X1-(Y)_3-C-(Y)_1-X2$, can be identified in viral proteins and especially in viral envelope proteins. Particular envelope proteins are those of retroviruses that comprise two
30 subunits: the SU and TM subunits. Such consensus sequences have been found in MoMLV, Friend retrovirus, FeLV, HTLV-1, HTLV-2, STLV-1, GLV-

X, Pox viruses, MPMV or SSAV, or in Ebola or Marburg viruses or in endogenous retroviruses such as FRD, PyERV, PERV or HERV-T.

The Y amino acid residues thus identified in various proteins allow determining particular sequences of the invention such as E/Q-G-G-L/T/I-C-A/K/L/M/V/I-A or R-G-G-L/T/I-C-A/K/L/M/V/I-F. The "/" indicates that this sequence position accepts several types of amino acid residues according to the indications which are provided.

Therefore, the above-defined polypeptide of the invention comprises, in a particular embodiment, a minimum sequence which can be selected from the group consisting of:

QGGLCKA (SEQ ID NO: 17)
 QGGLCAA (SEQ ID NO: 18)
 QGGLCLA (SEQ ID NO: 19)
 QGGICLA (SEQ ID NO: 20)
 15 EGGLCAA (SEQ ID NO: 21)
 EGGLCVA (SEQ ID NO: 22), wherein these
 immunosuppression-modulatory sequences provide
 immunosuppressive properties to a protein comprising them, or
 20 RGGTCLF (SEQ ID NO: 23)
 KGGTCMF (SEQ ID NO: 24)
 KGRTCLF (SEQ ID NO: 25)
 KGGLCIF (SEQ ID NO: 26)
 RGGLCKF (SEQ ID NO: 27)
 RGGLCAF (SEQ ID NO: 28)
 25 RGGLCLF (SEQ ID NO: 29)
 RGGICLF (SEQ ID NO: 30)
 RGGLCVF (SEQ ID NO: 31)
 RGGTCVF (SEQ ID NO: 32), these immunosuppression-
 modulatory sequences providing low or no immunosuppressive properties
 30 to a protein comprising them.

More particularly, the above-defined polypeptide of the invention comprises, in another embodiment, a minimum sequence which can be selected from the group consisting of:

QGGLCKA (SEQ ID NO: 17)
 35 QGGLCAA (SEQ ID NO: 18)

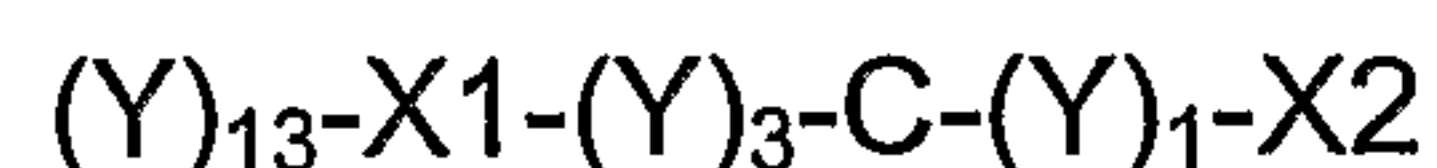
5 **QGGLCLA** (SEQ ID NO: 19)
 QGGICLA (SEQ ID NO: 20)
 EGGLCAA (SEQ ID NO: 21)
 EGGLCVA (SEQ ID NO: 22), wherein these
 immunosuppression-modulatory sequences provide
 immunosuppressive properties to a protein comprising them, or
 KGGTCMF (SEQ ID NO: 24)
 KGRTCLF (SEQ ID NO: 25)
 10 **KGGLCIF** (SEQ ID NO: 26), wherein these
 immunosuppression-modulatory sequences provide low
 immunosuppressive properties to a protein comprising them, or
 RGGTCLF (SEQ ID NO: 23)
 RGGLCKF (SEQ ID NO: 27)
 RGGLCAF (SEQ ID NO: 28)
 15 **RGGLCLF** (SEQ ID NO: 29)
 RGGICLF (SEQ ID NO: 30)
 RGGLCVF (SEQ ID NO: 31)
 RGGTCVF (SEQ ID NO: 32), these immunosuppression-
 modulatory sequences providing essentially no immunosuppressive
 20 properties to a protein comprising them.

As intended herein, "low immunosuppressive properties"
 relates to a polypeptide which provides lower immunosuppressive
 properties to a protein comprising it than polypeptides represented by SEQ
 ID NO: 17 to 22, but provides higher immunosuppressive properties to a
 25 protein comprising it than polypeptides represented by SEQ ID NO: 23 to
 and 27 to 32. In particular, a protein comprising a polypeptide which
 provides low immunosuppressive properties is less immunosuppressive
 than a HERV-W ENV R393Q F399A double mutant, such as represented
 by SEQ ID NO: 118. More particularly, the immunosuppressive index of a
 30 protein comprising a polypeptide which provides low immunosuppressive
 properties is positive but lower than the immunosuppressive index of said
 HERV-W ENV R393Q F399A double mutant, and preferably lower than
 50% the immunosuppressive index of said HERV-W ENV R393Q F399A
 double mutant.

All the polypeptides of the invention are encoded by nucleic acids that can be obtained by all known methods to enable expression of the polypeptides in host cells, especially in prokaryotic or eukaryotic cells. As example, nucleic acids can be isolated from samples expressing
5 viruses, using suitable probes and amplification technique. They can also be chemically synthesized or obtained by enzymatic digestion from existing plasmids or plasmids from the invention.

Furthermore, the polypeptides of the invention can also be chemically synthesized or semi-synthesized according to well-established
10 procedures.

A particular 20-amino acid polypeptide has the following consensus sequence:



As above explained, X1 and X2 are selected to impact on the
15 immunosuppressive properties of a tested i.e., original viral immunosuppressive protein in which the polypeptide is inserted, including by replacement of X1 and X2 residues in an homologous sequence as defined above, wherein Y represents variable amino acid residues, 3 and 1 represent the number of variable amino acid Y residues respectively
20 between X1 and C and between C and X2, and 13 represents the number of amino acid residues in the N-terminal part of the polypeptide. The Y residues can independently be identical or different in the sequence.

The identification of invariant amino acid residues in various protein sequences allows defining a particular sequence: $(Y)_2\text{-N-(Y)}_3\text{-L-(Y)}_2\text{-L-(Y)}_3\text{-X1-(Y)}_3\text{-C-(Y)}_1\text{-X2}$, i.e. from the N-terminal-end to C-terminal end:
25 two variable amino acid residues, an asparagine (N), three variable amino acid residues, a leucine (L), two variable amino acid residues, a leucine (L), three variable amino acid residues, the X1 residue, three variable amino acid residues, a cysteine (C), one variable amino acid residue and the X2
30 residue.

Optionally the above consensus sequence can be noted as follows:



wherein X_1 and X_2 are respectively identical to X_1 and X_2 , and Y_1 to Y_{14} represent any aminoacid. As intended herein amino acids Y_1 to Y_{14} can be identical or different.

Particular amino acid sequences presenting the capacity to modulate the immunosuppressive properties of a viral immunosuppressive protein in the above disclosed test, can be selected from the group consisting of:

AQNRRGLDLLFWE**Q**GGLCK**A** (SEQ ID NO: 33)
 LQNCRCCLDLLFLS**Q**GGLCA**A** (SEQ ID NO: 34)
 LQNRRGLDMLTAA**Q**GGLCL**A** (SEQ ID NO: 35)
 LQNRRGLDLLTAEB**Q**GGICL**A** (SEQ ID NO: 36)
 15 LQNRRGLDILFLQ**E**GGLCA**A** (SEQ ID NO: 37)
 LQNRRGLDLLFLK**E**GGLCA**A** (SEQ ID NO: 38)
 LQNRRGLDLLFLK**E**GGLCV**A** (SEQ ID NO: 39), wherein these immunosuppression-modulatory sequences provide immunosuppressive properties to a protein comprising them, or
 20 LQNRRALDLLTAER**R**GGTCL**F** (SEQ ID NO: 40)
 LQNWRALDLLTAK**R**GGTCL**F** (SEQ ID NO: 41)
 LQNWRALDLLIAK**R**GGTCV**F** (SEQ ID NO: 42)
 LQNRRGLDLLTAER**R**GGTCL**F** (SEQ ID NO: 43)
 LQNRRALDLLTAER**R**GGICL**F** (SEQ ID NO: 44)
 25 LQNRRGLDLLTAEK**G**GGLCI**F** (SEQ ID NO: 45)
 MQNRRALDLLTAD**K**GGTCM**F** (SEQ ID NO: 46)
 AQNRQALDLLMAEK**G**RTCL**F** (SEQ ID NO: 47)
 AQNRRGLDLLFWER**G**GGLCK**F** (SEQ ID NO: 48)
 LQNCRCCLDLLFLS**R**GGLCA**F** (SEQ ID NO: 49)
 30 LQNRRGLDMLTAAR**G**GGLCL**F** (SEQ ID NO: 50)
 LQNRRGLDLLTAER**R**GGICL**F** (SEQ ID NO: 51)
 LQNRRGLDILFLQ**R**GGLCA**F** (SEQ ID NO: 52)
 LQNRRGLDLLFLK**R**GGLCA**F** (SEQ ID NO: 53)
 LQNRRGLDLLFLK**R**GGLCV**F** (SEQ ID NO: 54), these
 35 immunosuppression-modulatory sequences providing low or no immunosuppressive properties to a protein comprising them.

According to a preferred embodiment, particular amino acid sequences presenting the capacity to modulate the immunosuppressive properties of a viral immunosuppressive protein in the above disclosed test, can be selected from the group consisting of:

- 5 AQNRRGLDLLFWE**Q**GGLCK**A** (SEQ ID NO: 33)
 LQNCRCCLDLLFLS**Q**GGLCA**A** (SEQ ID NO: 34)
 LQNRRGLDMLTAA**Q**GGLCL**A** (SEQ ID NO: 35)
 LQNRRGLDLLTAE**Q**GGICL**A** (SEQ ID NO: 36)
 LQNRRGLDILFLQ**E**GGLCA**A** (SEQ ID NO: 37)
 10 LQNRRGLDLLFLK**E**GGLCA**A** (SEQ ID NO: 38)
 LQNRRGLDLLFLK**E**GGLCV**A** (SEQ ID NO: 39), wherein
 these immunosuppression-modulatory sequences provide
 immunosuppressive properties to a protein comprising them, or
 LQNRRGLDLLTAE**K**GGLCI**F** (SEQ ID NO: 45)
 15 MQNRRALDLLTAD**K**GGTCM**F** (SEQ ID NO: 46)
 AQNRQALDLLMAE**K**GRTCL**F** (SEQ ID NO: 47), wherein
 these immunosuppression-modulatory sequences provide low
 immunosuppressive properties to a protein comprising them, or
 LQNRRALDLLTAER**G**GGTCL**F** (SEQ ID NO: 40)
 20 LQNWRALDLLTAK**R**GGTCL**F** (SEQ ID NO: 41)
 LQNWRALDLLIAK**R**GGTCV**F** (SEQ ID NO: 42)
 LQNRRGLDLLTAER**G**GGTCL**F** (SEQ ID NO: 43)
 LQNRRALDLLTAER**G**GGICL**F** (SEQ ID NO: 44)
 AQNRRGLDLLFWER**G**GGLCK**F** (SEQ ID NO: 48)
 25 LQNCRCCLDLLFLS**R**GGLCA**F** (SEQ ID NO: 49)
 LQNRRGLDMLTAAR**G**GGLCL**F** (SEQ ID NO: 50)
 LQNRRGLDLLTAER**G**GGICL**F** (SEQ ID NO: 51)
 LQNRRGLDILFLQ**R**GGLCA**F** (SEQ ID NO: 52)
 LQNRRGLDLLFLK**R**GGLCA**F** (SEQ ID NO: 53)
 30 LQNRRGLDLLFLK**R**GGLCV**F** (SEQ ID NO: 54), these
 immunosuppression-modulatory sequences providing essentially no
 immunosuppressive properties to a protein comprising them.

The present invention also relates to the use of a first mutation of a first amino acid and optionally of a second mutation of a second amino acid
 35 in a wild type viral envelope (ENV) protein essentially comprising the
 following sequence:



wherein the first amino acid to be mutated is X_1 and the second amino acid to be mutated is X_2 , and Y_1 to Y_{12} represent any amino acid, for manufacturing a mutated ENV protein having a modified immunosuppressive activity with respect to said wild type ENV protein.

5 The expression "wild type viral envelope protein" relates to an envelope protein in which amino acid X_1 has not been mutated. In particular, it is not excluded that other mutations or modifications have been brought to the envelope protein.

10 The expression "essentially comprising" means that at least two of the four constant amino acids of the above sequence (represented in bold) are present in said wild type viral envelope. Two amino acids are sufficient to unambiguously determine the position of X_1 and X_2 in the envelope sequence. Advantageously, the above sequence is usually localized in the transmembrane (TM) subunit, more particularly in the ectodomain of the TM subunit.

15 As intended herein, amino acids Y_1 to Y_{12} , independently of each other are different or identical.

As intended herein the mutated ENV protein essentially carries the following sequence:

20 $\text{NY}_1\text{Y}_2\text{Y}_3\text{LY}_4\text{Y}_5\text{LY}_6\text{Y}_7\text{Y}_8\text{X}'_1\text{Y}_9\text{Y}_{10}\text{Y}_{11}\text{CY}_{12}\text{X}'_2$

wherein X'_1 corresponds to the mutated X_1 and X'_2 corresponds to the mutated X_2 .

25 The expression "modified immunosuppressive activity" means that the mutated ENV protein has either increased or decreased immunosuppressive activity with respect to the corresponding wild-type ENV protein. In particular, the mutated ENV protein can be essentially deprived of any residual immunosuppressive activity. In another instance, the mutated ENV protein can have immunosuppressive activity whereas the corresponding wild-type ENV protein is essentially deprived of immunosuppressive activity. The immunosuppressive activity can be

30

measured as described above and in the Examples, for instance by using the immunosuppressive index method.

Advantageously, mutated ENV proteins having a modified immunosuppressive activity have many applications, in particular therapeutic applications, which will be discussed hereafter.

In a preferred embodiment of the above-defined use, structures responsible for the antigenicity of the mutated ENV protein are essentially preserved.

As intended herein, the expression "structures responsible for antigenicity" relates to structures of the protein which are liable to interact with components of the immune system such as antibodies or membrane receptors of immune cells, in particular T cells.

According to the invention, at least one or more of these structures presents the same conformation in the mutated ENV protein with respect to the corresponding wild type ENV protein. Advantageously, this means that an immune reaction elicited against a mutated ENV protein will also be directed against the corresponding wild type ENV protein.

According to a preferred embodiment, the invention also relate to the above -defined use of a first mutation of a first amino acid and optionally of a second mutation of a second amino acid in a wild type viral envelope (ENV) protein essentially comprising the following sequence:



wherein the first amino acid to be mutated is X_1 and the second amino acid to be mutated is X_2 , and Y_1 to Y_{12} represent any amino acid, for manufacturing a mutated ENV protein having a decreased immunosuppressive activity with respect to said wild type ENV protein.

In a most preferred embodiment, the decrease in immunosuppressive activity is such that almost no residual activity is seen in the mutated ENV protein.

According to a preferred embodiment, the invention also relates to the above-defined use of a first mutation of a first amino acid and a second

mutation of a second amino acid in a wild type viral envelope (ENV) protein essentially comprising the following sequence:



wherein the first amino acid to be mutated is X_1 and the second amino acid to be mutated is X_2 , and Y_1 to Y_{12} represent any amino acid,
 5 for manufacturing a mutated ENV protein having a decreased immunosuppressive activity with respect to said wild type ENV protein.

The mutation of X_1 alone is sufficient to modify the immunosuppressive activity of the mutated ENV protein with respect to the
 10 corresponding wild type ENV. However, it is advantageous that X_2 be also mutated because it ensures that the structure of the mutated ENV protein is essentially conserved with respect to the corresponding wild type ENV protein.

In a preferred embodiment of the above-defined use, the mutation is
 15 a substitution.

In another preferred embodiment of the above-defined use, X_1 is substituted by R or H.

In another preferred embodiment of the above-defined use, X_2 is substituted by F, M, Y or W.

20 In a further preferred embodiment of the above-defined use, X_1 is E, K, or Q and is substituted by R or H.

In a preferred embodiment of the above defined use, the ENV protein is HERV-H ENV and X_1 is K.

25 In a further preferred embodiment of the above-defined use, X_2 is A, V, L, I, or K and is substituted by F, M, Y, or W.

In a particularly preferred embodiment of the above defined use, the ENV protein is a HERV ENV, in particular selected from:
 30 HERV-FRD ENV (SEQ ID NO: 82), wherein X_1 is Q427 and X_2 is A433, or
 HERV-T ENV (SEQ ID NO: 84), wherein X_1 is Q516 and X_2 is A522, or
 HERV-R ENV (SEQ ID NO: 86), wherein X_1 is E561 and X_2 is K567, or
 HERV-V ENV (SEQ ID NO: 88), wherein X_1 is Q381 and X_2 is V387, or

HERV-R(b) ENV (SEQ ID NO: 90), wherein X_1 is E391 and X_2 is L397.

HERV relates to Human Endogenous RetroVirus, which have been described previously. HERV ENV proteins have been found to be expressed in cancer cells. The HERV ENV listed above present an immunosuppressive activity and can help cancer cells carrying them escape immune response. These HERV are well known to the man skilled in the art and are in particular discussed in Benit et al. J. Virol. 2001, 75:11709-11719. As will be apparent later HERV ENV proteins having decreased immunosuppressive activity are advantageous to prepare vaccines inhibiting the activity of wild type ENV proteins expressed by cancer cells.

In an advantageous embodiment of the above-defined use, the ENV protein is HERV-FRD ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 120,

SEQ ID NO: 122.

SEQ ID NO: 120 carries the mutation Q427R.

SEQ ID NO: 122 carries the mutation Q427R + A433F.

The mutated HERV-FRD ENV represented by SEQ ID NO: 120 or 122 presents a decreased immunosuppressive activity with respect to the corresponding wild-type HERV-FRD ENV.

In another advantageous embodiment of the above-defined use, the ENV protein is HERV-V ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 124,

SEQ ID NO: 126.

SEQ ID NO: 124 carries the mutation Q381R.

SEQ ID NO: 126 carries the mutation Q381R + V387F.

The mutated HERV-V ENV represented by SEQ ID NO: 124 or 126 presents a decreased immunosuppressive activity with respect to the corresponding wild-type HERV-V ENV.

In another advantageous embodiment of the above-defined use, the ENV protein is HERV-T ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 128,

5 SEQ ID NO: 130.

SEQ ID NO: 128 carries the mutation Q516R.

SEQ ID NO: 130 carries the mutation Q516R + A522F.

10 The mutated HERV-T ENV represented by SEQ ID NO: 128 or 130 presents a decreased immunosuppressive activity with respect to the corresponding wild-type HERV-T ENV.

In another advantageous embodiment of the above-defined use, the ENV protein is HERV-R ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 146,

15 SEQ ID NO: 148.

SEQ ID NO: 146 carries the mutation E561R.

SEQ ID NO: 148 carries the mutation E561R + K567F.

20 The mutated HERV-R ENV represented by SEQ ID NO: 128 or 130 presents a decreased immunosuppressive activity with respect to the corresponding wild-type HERV-R ENV.

In another particularly preferred embodiment of the above defined use, the ENV protein is selected from:

HTLV-1 ENV (SEQ ID NO: 92), wherein X_1 is Q389 and X_2 is A395, or

HTLV-2 ENV (SEQ ID NO: 94) wherein X_1 is Q385 and X_2 is A391, or

25 FeLV ENV (SEQ ID NO: 96), wherein X_1 is E527 and X_2 is A533, or

PERV ENV (SEQ ID NO: 98), wherein X_1 is E545 and X_2 is A551, or

STLV-1 ENV (SEQ ID NO: 100), wherein X_1 is Q389 and X_2 is A395, or

MoMLV ENV (SEQ ID NO: 70), wherein X_1 is E551 and X_2 is A557, or

MPMV ENV (SEQ ID NO: 72), wherein X_1 is Q471 and X_2 is A477, or

30 FV ENV (SEQ ID NO: 102), wherein X_1 is E561 and X_2 is A567.

HTLV-1 and 2 relate to Human T-cell Leukemia Virus type 1 and 2.

FeLV relates to Feline Leukemia Virus.

PERV relates to Porcine Endogenous RetroVirus.

STLV-1 relates to Simina T-cell Leukemia Virus type 1.

MoMLV relates to Moloney Murine Leukemia Virus.

5 MPMV relates to Mason-Pfizer Monkey Virus.

FV relates to the mouse Friend Virus.

10 These virus are well known to the man skilled in the art and are notably described in Benit et al. J. Virol. 2001, 75:11709-11719. The propagation of these viruses is notably favoured by the presence of an immunosuppressive ENV protein, which helps viruses escape the immune response. As will be apparent later viral ENV proteins having decreased immunosuppressive activity are advantageous to inhibit the activity of wild type ENV proteins expressed by viruses.

15 In an advantageous embodiment of the above-defined use, the ENV protein is FeLV ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 104,

SEQ ID NO: 106.

SEQ ID NO: 104 carries the mutation E527R.

20 SEQ ID NO: 106 carries the mutation E527R + A533F.

The mutated FeLV ENV represented by SEQ ID NO: 104 or 106 presents a decreased immunosuppressive activity with respect to the corresponding wild-type FeLV ENV.

25 In another advantageous embodiment of the above-defined use, the ENV protein is HTLV-1 ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 108,

SEQ ID NO: 110.

SEQ ID NO: 108 carries the mutation Q389R.

30 SEQ ID NO: 110 carries the mutation Q389R + A395F.

The mutated HTLV-1 ENV represented by SEQ ID NO: 108 or 110 presents a decreased immunosuppressive activity with respect to the corresponding wild-type HTLV-1 ENV.

In another advantageous embodiment of the above-defined use, the ENV protein is HTLV-2 ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 112,

SEQ ID NO: 114.

SEQ ID NO: 112 carries the mutation Q385R.

SEQ ID NO: 114 carries the mutation Q385R + A391F.

The mutated HTLV-2 ENV represented by SEQ ID NO: 112 or 114 presents a decreased immunosuppressive activity with respect to the corresponding wild-type HTLV-2 ENV.

In another advantageous embodiment of the above-defined use, the ENV protein is PERV ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 150,

SEQ ID NO: 152.

SEQ ID NO: 150 carries the mutation E545R.

SEQ ID NO: 152 carries the mutation E545R + A551F.

The mutated PERV ENV represented by SEQ ID NO: 150 or 152 presents a decreased immunosuppressive activity with respect to the corresponding wild-type PERV.

The present invention also relates to the above use of a first mutation of a first amino acid and optionally of a second mutation of a second amino acid in a wild type viral envelope (ENV) protein essentially comprising the following sequence:

NY₁Y₂Y₃LY₄Y₅LY₆Y₇Y₈X₁Y₉Y₁₀Y₁₁CY₁₂X₂

wherein the first amino acid to be mutated is X₁ and the second amino acid to be mutated is X₂, and Y₁ to Y₁₂ represent any amino acid,

for manufacturing a mutated ENV protein having an increased immunosuppressive activity with respect to said wild type ENV protein.

5 The mutation of X_1 alone is sufficient to increase the immunosuppressive activity of the mutated ENV protein with respect to the corresponding wild type ENV. However, it is advantageous that X_2 be also mutated because it ensures that the structure of the mutated ENV protein is essentially conserved with respect to the corresponding wild type ENV protein.

10 Advantageously, it is possible according to the invention to obtain a mutated ENV protein with immunosuppressive activity whereas the corresponding wild-type ENV protein is essentially deprived of such an activity. Such mutated ENV proteins with increased immunosuppressive activity are useful to inhibit the immune system, for instance in graft rejections or autoimmune diseases.

15 In a preferred embodiment of the above mentioned use for manufacturing a mutated ENV protein having an increased immunosuppressive activity, the mutation is a substitution.

20 In another preferred embodiment of the above mentioned use for manufacturing a mutated ENV protein having an increased immunosuppressive activity, X_1 is substituted by E, *K* or Q and X_2 is substituted by A.

25 In another preferred embodiment of the above mentioned use for manufacturing a mutated ENV protein having an increased immunosuppressive activity, the ENV protein is HERV-W ENV, such as represented by SEQ ID NO: 74, and the sequence of the mutated HERV-W ENV is preferably selected from

SEQ ID NO: 116,

SEQ ID NO: 118.

SEQ ID NO: 116 carries the mutation R393E/Q.

30 SEQ ID NO: 118 carries the mutation R393E/Q + F399A.

The mutated HERV-W ENV represented by SEQ ID NO: 116 or 118 presents an increased immunosuppressive activity with respect to the corresponding wild-type HERV-W which is essentially deprived of such an activity.

5 The present invention also provides a polypeptide derived from a determined antigenic and immunosuppressive protein, said polypeptide comprising an amino acid sequence (so-called "immunosuppression-modulatory sequence") represented by $X1-(Y)_3-C-(Y)_1-X2$ wherein in said polypeptide Y represents variable amino acid
10 residues, 3 and 1 represent the number of variable amino acid Y residues, respectively between X1 and C and between C and X2, and X1 and X2 are chosen to confer to said polypeptide, altered immunosuppressive properties with respect to the immunosuppressive properties of said determined protein.

15 The term "derived" as used herein indicates that the amino acid sequence, and especially the immunosuppression-modulatory sequence, in the polypeptide, is modified with respect to the sequence of the determined protein. Said "determined" protein is hence the original protein whose modification is required to modulate its immunosuppressive
20 properties. A polypeptide according to the invention can be derived, biologically or chemically, from a determined protein by substitution, deletion, addition, recombination or insertion of one or several amino acid residues or sequences, provided the consensus sequence of the invention is such that X1 and X2 are selected to modulate the immunosuppressive
25 properties of the starting determined protein, and therefore provided X1 and/or X2 are mutated by substitution with respect to their original corresponding residues in said determined immunosuppressive protein. In case of sequence insertion, the immunosuppression-modulatory sequence can replace a homologous sequence present in the determined protein, or
30 can replace a sequence known or likely to be involved in the same function of modulation of the immunosuppressive properties as the inserted

sequence, or can be inserted within the starting amino acid sequence. In all cases, the open reading frame of the amino acid sequence following the site of insertion (at the C-terminal part of the polypeptide) is conserved.

5 Obviously, the invention can be carried out with or without actually starting from said determined protein to derive the polypeptide of the invention. Hence, said determined protein is a reference for the design of the derived polypeptide rather than a necessary starting material from a biological or chemical point of view.

10 In a particular embodiment of the invention, the derived polypeptide has lower immunosuppressive properties than said determined starting polypeptide and advantageously has substantially lost said immunosuppressive properties, e.g. has no immunosuppressive properties.

15 The expressions "polypeptide" and "protein" throughout the present invention define molecules, whatever their length (except otherwise stated in the present description) comprising an amino acid sequence.

In a particular embodiment, the polypeptide or protein is multimeric, especially trimeric.

20 "Determined" as used herein refers to a starting protein from which the polypeptide of the invention is designed, i.e., derived to have modulated immunosuppressive properties. This protein can be a wild-type protein (for example isolated from a viral, especially retroviral, strain) or a protein previously modified (for example expressed from a vector in a host). Such protein is chosen among those having antigenic and immunosuppressive properties.

25 The determined protein has immunosuppressive properties as defined above: when this determined protein is expressed in tumour cells normally rejected by an engrafted host, it allows these tumour cells to proliferate and to escape immune rejection.

30 Second, it is an antigenic protein, i.e. it is capable of being recognized by antibodies formed in a host to whom it is administered. Advantageously it is capable of inducing an immune response, in the host

to whom it is administered in appropriate known conditions, and accordingly said antigenic protein is advantageously an immunogenic protein. This involves that said host produces antibodies against epitopes of the protein.

In view of these desired property of the protein to be antigenic, especially immunogenic, and in view of the required property for the derived polypeptide to substantially retain these antigenic, especially immunogenic properties, the determined protein used to derive the polypeptide of the invention encompasses native or naturally occurring proteins or antigenic, especially immunogenic, fragments thereof, provided said fragments further have immunosuppressive properties. It also encompasses modified proteins with respect to the native or naturally occurring protein, provided the modified proteins have antigenic and immunosuppressive properties.

The determined protein used as reference to derive the polypeptide of the invention can be a viral protein, *i.e.* coded by nucleic acids of infectious agents like viruses, or a protein coded by nucleic acid of viral origin, such as endogenous retroviruses, especially HERV. A particular protein is a protein originating from a subclass of viruses: retroviruses. In a particular embodiment, the determined protein is an envelope protein, *i.e.*, the expression product of the env gene.

"Nucleic acid" as used herein refers to viral nucleic acids in DNA or RNA forms, including cellular nucleic acids such as genomic DNA, complementary DNA, coding sequences. All the nucleic acid quoted in the present application can be single or double-stranded.

The X1 and X2 amino acid residues of the X1-(Y)₃-C-(Y)₁-X2 motif are chosen as described above.

The above defined polypeptide of the invention derived from an antigenic and immunosuppressive protein and comprising sequence X1-(Y)₃-C-(Y)₁-X2 can be defined as follows:

in a particular embodiment of the invention, X1 is an alkaline amino acid residue and X2 is an aromatic residue or *vice versa*.

In another particular embodiment of the invention, X1 is an alkaline residue or X2 is an aromatic residue or *vice versa*.

5 The inventors have observed that the modulation effect of X1 and X2 on immunosuppressive properties of proteins is usually lower when only one of X1 or X2 residues is modified in an original immunosuppressive protein.

Therefore, modification of both X1 and X2 is an immunosuppression-modulatory sequence may be regarded as advantageous.

10 In another particular embodiment of the invention, residues X1 or X2 located in amino acid sequence represented as X1-(Y)₃-C-(Y)₁-X2 are selected as follows:

where X1 is chosen among R, H and K, X2 is chosen among F, W, Y and H or where X1 is chosen among F, W, Y and H, X2 is chosen
15 among R, H and K.

In a further embodiment of the invention, X1 is R, H or K and X2 is F, or *vice versa*.

In a further embodiment of the invention, X1 is R and X2 is F, W, Y or H.

20 The inventors have especially identified that a polypeptide, derived from an antigenic and immunosuppressive protein, has altered immunosuppressive properties compared to the immunosuppressive properties of the protein from which is derived when particular interesting X1 and X2 residues are respectively R and F or K and F.

25 The determined protein can advantageously be a viral protein and particularly a retroviral protein or a protein of viral origin like one of an HERV, having antigenic and immunosuppressive properties.

Known naturally occurring low or non-immunosuppressive envelope proteins of HERV-W, H1, F(c)1 or F(c)2 are not, as such, the
30 object of the present invention.

In a particular embodiment of the present invention, the polypeptide derived from an antigenic protein has altered immunosuppressive properties and especially reduced immunosuppressive properties, while retaining its antigenic properties.

5 In another particular embodiment, these proteins have, further to antigenic and immunosuppressive properties, infectious and/or fusion properties.

When the determined starting protein further has fusion and infectious properties, such as those identified for viral envelope proteins, one of these or both properties can be retained, but not necessary, in the derived polypeptide.

10 The evaluation or measurement of fusion and/or infectious properties to determine whether these properties of the original determined protein are maintained in the derived polypeptide of the invention can provide useful indications as to whether the derived polypeptide has substantially retained the structure, especially the antigenic structure, e.g., immunogenic determinants, of the original determined protein.

15 A protein is said to have fusion properties when cells transfected with nucleic acids encoding said protein are able to form syncytia (multi-nucleated cells) with other cells probably not expressing the same protein. Indeed, it is suspected that a strong expression of a protein with fusion properties blocks the expression of the receptors of said protein involved in the fusion event. Therefore, the capacity of fusion can be defined by the formation of syncytia between cells expressing said protein with fusion properties and cells expressing its receptor. Cells can be transfected having recourse to various known methods such as calcium phosphate precipitation or with liposomes, such as Lipofectamine™.

25 A protein is said to have infectious properties when pseudotypes coated with this protein are able to infect cells. "Pseudotypes" as used herein refers to viral particles in which an ENV protein from a different strain is incorporated. MLV core particles are currently used.

Pseudotypes are produced in cell lines (such as 293T cells) in which a vector encoding the infectious protein is co-transfected with one or several vector(s) encoding the GAG and POL proteins of another viral strain.

Particular polypeptides having the properties described are derived from viral envelope protein (ENV) and especially retroviral envelope proteins. Such retroviral ENV can be selected from the group of retroviruses consisting of: MoMLV, Friend retrovirus, FeLV, HTLV-1, STLV-1 and MPMV. Other interesting polypeptides are those encoded by nucleic acids of viral origin such as HERV. As far as viruses are concerned, Ebola and Marburg viruses have ENV proteins from which the polypeptides of the invention can be derived.

Said envelope protein can be all or part of the native or naturally occurring protein or from an antigenic, especially immunogenic variant thereof, including a fragment thereof, i.e., an analogue of a naturally occurring viral envelope protein as far as antigenic, especially immunogenic properties, and immunosuppressive properties are concerned.

Within the amino acid sequence of determined proteins described above, inventors have identified particular residues that are involved in the regulation of immunosuppression. Such a sequence, called immunosuppression-modulatory sequence which confers immunosuppressive properties to a protein is the following: E/Q-G-G-L/T/I-C-A/K/L/M/V/I-A, wherein "/" indicates that this sequence position accepts several types of amino acid residues. Thus, proteins comprising an immunosuppression-modulatory sequence selected from the group consisting of

QGGLCKA (SEQ ID NO: 17)

QGGLCAA (SEQ ID NO: 18)

QGGLCLA (SEQ ID NO: 19)

QGGICLA (SEQ ID NO: 20)

EGGLCAA (SEQ ID NO: 21)

EGGLCVA (SEQ ID NO: 22)

are particular determined proteins having immunosuppressive properties, from which the polypeptides of the invention can be derived by modifying the terminal E/Q and or A residues figuring X1 and X2 positions of the consensus sequence of the invention.

5 As described above, the term "derived" as used herein indicates that the amino acid sequence, and especially the immunosuppression-modulatory sequence, of the polypeptide is modified with respect to the sequence of the determined protein in order to impact on immunosuppressive properties, especially to decrease said properties.
10 These altered immunosuppressive properties can be the consequence of substitution of the X1 and X2 residues according to the amino acid characteristics described above.

 These altered immunosuppressive properties can also be the consequence of the insertion of the polypeptide comprising X1-(Y)₃-C-(Y)₁-
15 X2 sequence wherein X1 and X2 are selected to alter the immunosuppressive properties, in a permissive site of the chosen protein.

 "Permissive site" as used herein refers to a site which does not substantially alter the antigenic properties of a protein.

 The insertion can replace a homologous sequence or a
20 sequence involved in immunosuppression. The polypeptide of 7 to 20 amino acid residues according to the invention can also be inserted without deletion of amino acid residues from the determined protein.

 A polypeptide derived from a determined protein as described above, and having altered immunosuppressive properties comprises a
25 sequence having the following sequence R-G-G-L/T/I-C-A/K/L/M/V/I-F, and particularly a sequence selected from the group consisting of:

 RGGLCKF (SEQ ID NO: 27)
 RGGLCAF (SEQ ID NO: 28)
 RGGLCLF (SEQ ID NO: 29)
30 RGGICLF (SEQ ID NO: 30)
 RGGLCVF (SEQ ID NO: 31)

The sequences given above have been derived by mutation of said X1 and X2 residues in identified naturally occurring retroviral ENV proteins.

5 The same strategy can be applied with viruses which express proteins presenting a sequence similar to $X1-(Y)_3-C-(Y)_1-X2$. In particular, the Y residues can be different amino acid residues from those described above (Benit et al. 2001).

10 Moreover, the structure, e.g. their 3-dimensional structure of the determined ENV proteins of the present application have been shown to share similar structural features with that of other viruses and especially with other retroviruses, despite amino acid sequence diversity. Thus, a highly conserved organization of the TM structure has been found in proteins of Ebola or Marburg viruses, most probably relevant to a common mechanism for triggering the fusion process and viral entry. Consequently,
15 a same approach can be applied to identify particular sequences, involved in the modulation of the immunosuppression in such viruses.

The present invention also relates to a mutated ENV protein resulting from the mutation of a wild type ENV protein essentially carrying the following sequence:

20 $NY_1Y_2Y_3LY_4Y_5LY_6Y_7Y_8X_1Y_9Y_{10}Y_{11}CY_{12}X_2$

wherein amino acid X_1 and optionally amino acid X_2 are mutated, and Y_1 to Y_{12} represent any amino acid, said mutated ENV protein having a modified immunosuppressive activity with respect to the wild type ENV protein, or a fragment thereof, provided that said fragment carries the mutated
25 amino acid X_1 and optionally X_2 , that it has an immunosuppressive activity similar to that of the mutated ENV protein, and that optionally its antigenic structure is essentially similar to the structure it adopts in the context of the mutated ENV protein,
or a protein derived from the mutated ENV protein, or fragments thereof, by
30 insertion, deletion or substitution of at least one amino acid, provided that said derived protein carries the mutated amino acid X_1 and X_2 , that it has an

immunosuppressive activity similar to that of the mutated ENV protein, and that, optionally, its antigenic structure is essentially similar to that of the mutated ENV protein, or fragment thereof.

As intended herein the mutated ENV protein essentially carries the following sequence:

NY₁Y₂Y₃LY₄Y₅LY₆Y₇Y₈X'₁Y₉Y₁₀Y₁₁CY₁₂X'₂

Wherein X'₁ corresponds to the mutated X₁ and X'₂ corresponds to the mutated X₂.

As intended herein fragments of the mutated ENV protein according to the invention are in particular at least 7 amino acids long and comprise the mutated amino acid X₁. Optionally, fragments are at least 7 amino acids long and comprise both X₁ and X₂. Preferred fragments of the mutated ENV protein according to the invention are notably constituted of the TM subunit or of the ectodomain of the TM subunit.

In a preferred embodiment of the invention the above mentioned protein derived from the mutated ENV protein presents at least 80% sequence identity with said mutated ENV protein, in particular at least 90% sequence identity.

In a preferred embodiment of the above-defined mutated ENV protein, or fragment thereof, the structures responsible for the antigenicity of said mutated ENV protein, or fragment thereof, are essentially preserved with respect to the wild type ENV protein.

According to a preferred embodiment, the present invention relates to an above-defined mutated ENV protein resulting from the mutation of a wild type ENV protein essentially comprising the following sequence:

NY₁Y₂Y₃LY₄Y₅LY₆Y₇Y₈X₁Y₉Y₁₀Y₁₁CY₁₂X₂,

wherein amino acid X₁ and optionally amino acid X₂ are mutated, and Y₁ to Y₁₂ represent any amino acid, said mutated ENV protein having a decreased immunosuppressive activity with respect to the wild type ENV protein,

or a fragment thereof, provided that said fragment carries the mutated amino acid X_1 and optionally X_2 , that it has an immunosuppressive activity similar to that of the mutated ENV protein, and that optionally its antigenic structure is essentially similar to the structure it adopts in the context of the mutated ENV protein,

or a protein derived from the mutated ENV protein, or fragments thereof, by insertion, deletion or substitution of at least one amino acid, provided that said derived protein carries the mutated amino acid X_1 and X_2 , that it has an immunosuppressive activity similar to that of the mutated ENV protein, and that, optionally, its antigenic structure is essentially similar to that of the mutated ENV protein, or fragment thereof.

According to a preferred embodiment, the present invention relates to an above-defined mutated ENV protein resulting from the mutation of a wild type ENV protein essentially comprising the following sequence:

NY₁Y₂Y₃LY₄Y₅LY₆Y₇Y₈X₁Y₉Y₁₀Y₁₁CY₁₂X₂,

wherein amino acid X_1 and amino acid X_2 are mutated, and Y_1 to Y_{12} represent any amino acid, said mutated ENV protein having a decreased immunosuppressive activity with respect to the wild type ENV protein,

or a fragment thereof, provided that said fragment carries the mutated amino acid X_1 and X_2 , that it has an immunosuppressive activity similar to that of the mutated ENV protein, and that optionally its antigenic structure is essentially similar to the structure it adopts in the context of the mutated ENV protein,

or a protein derived from the mutated ENV protein, or fragments thereof, by insertion, deletion or substitution of at least one amino acid, provided that said derived protein carries the mutated amino acid X_1 and X_2 , that it has an immunosuppressive activity similar to that of the mutated ENV protein, and that, optionally, its antigenic structure is essentially similar to that of the mutated ENV protein, or fragment thereof.

In a preferred embodiment of the above-defined mutated ENV protein, or fragment thereof, the mutation is a substitution.

In another preferred embodiment of the above-defined mutated ENV protein, or fragment thereof, X_1 is substituted by R or H.

In another preferred embodiment of the above-defined mutated ENV protein, or fragment thereof, X_2 is substituted by F, M, Y or W.

5 In another preferred embodiment of the above-defined mutated ENV protein, or fragment thereof X_1 is E, K, or Q and is substituted by R or H.

In a preferred embodiment, the above defined mutated ENV protein, or fragment thereof, is HERV-H ENV wherein X_1 is K.

10 In another preferred embodiment of the above-defined mutated ENV protein, or fragment thereof, X_2 is A, V, L, I, or K and is substituted by F, M, Y, or W.

In a particularly preferred embodiment of the above-defined mutated ENV protein, or fragment thereof, the ENV protein is a HERV ENV, in particular selected from:

15 HERV-FRD ENV (SEQ ID NO: 82), wherein X_1 is Q427 and X_2 is A433, or
HERV-T ENV (SEQ ID NO: 84), wherein X_1 is Q516 and X_2 is A522, or
HERV-R ENV (SEQ ID NO: 86), wherein X_1 is E561 and X_2 is K567, or
HERV-V ENV (SEQ ID NO: 88), wherein X_1 is Q381 and X_2 is V387, or
HERV-R(b) ENV (SEQ ID NO: 90), wherein X_1 is E391 and X_2 is L397.

20 In an advantageous embodiment of the above-defined mutated ENV protein, or fragment thereof, the ENV protein is HERV-FRD ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 120

SEQ ID NO: 122

25 In an advantageous embodiment of the above-defined mutated ENV protein, or fragment thereof, the ENV protein is HERV-V ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 124

SEQ ID NO: 126

In an advantageous embodiment of the above-defined mutated ENV protein, or fragment thereof, the ENV protein is HERV-T ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 128

5 SEQ ID NO: 130

In an advantageous embodiment of the above-defined mutated ENV protein, or fragment thereof, the ENV protein is HERV-R ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 146,

10 SEQ ID NO: 148.

In a particularly preferred embodiment of the above-defined mutated ENV protein, or fragment thereof, the ENV protein is selected from:

HTLV-1 ENV (SEQ ID NO: 92), wherein X_1 is Q389 and X_2 is A395, or

HTLV-2 ENV (SEQ ID NO: 94) wherein X_1 is Q385 and X_2 is A391, or

15 FeLV ENV (SEQ ID NO: 96), wherein X_1 is E527 and X_2 is A533, or

PERV ENV (SEQ ID NO: 98), wherein X_1 is E545 and X_2 is A551, or

STLV-1 ENV (SEQ ID NO: 100), wherein X_1 is Q389 and X_2 is A395, or

MoMLV ENV (SEQ ID NO: 70), wherein X_1 is E551 and X_2 is A557, or

MPMV ENV (SEQ ID NO: 72), wherein X_1 is Q471 and X_2 is A477, or

20 FV ENV (SEQ ID NO: 102), wherein X_1 is E561 and X_2 is A567.

In an advantageous embodiment of the above-defined mutated ENV protein, or fragment thereof, the ENV protein is FeLV ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 104

25 SEQ ID NO: 106

In an advantageous embodiment of the above-defined mutated ENV protein, or fragment thereof, the ENV protein is HTLV-1 ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 108

30 SEQ ID NO: 110

In an advantageous embodiment of the above-defined mutated ENV protein, or fragment thereof, the ENV protein is HTLV-2 ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 112

5 SEQ ID NO: 114

In an advantageous embodiment of the above-defined mutated ENV protein, or fragment thereof, the ENV protein is PERV ENV and the sequence of the mutated ENV protein is selected from:

SEQ ID NO: 150,

10 SEQ ID NO: 152.

According to a preferred embodiment, the present invention relates a mutated ENV protein as defined above resulting from the mutation of a wild type ENV protein essentially comprising the following sequence:

NY₁Y₂Y₃LY₄Y₅LY₆Y₇Y₈X₁Y₉Y₁₀Y₁₁CY₁₂X₂ according to claim 27 or 28,

15 wherein amino acid X₁ and optionally amino acid X₂ are mutated, and Y₁ to Y₁₂ represent any amino acid, said mutated ENV protein having an increased immunosuppressive activity with respect to the wild type ENV protein,

or a fragment thereof, provided that said fragment carries the mutated amino acid X₁ and X₂, that it has an immunosuppressive activity similar to that of the mutated ENV protein, and that optionally its antigenic structure is essentially similar to the structure it adopts in the context of the mutated ENV protein,

20 or a protein derived from the mutated ENV protein, or fragments thereof, by insertion, deletion or substitution of at least one amino acid, provided that said derived protein carries the mutated amino acid X₁ and X₂, that it has an immunosuppressive activity similar to that of the mutated ENV protein, and that, optionally, its antigenic structure is essentially similar to that of the mutated ENV protein, or fragment thereof.

25

In a preferred embodiment of the above-defined mutated ENV protein having increased immunosuppressive activity, or fragment thereof, the mutation is a substitution.

5 In a preferred embodiment of the above-defined mutated ENV protein having increased immunosuppressive activity, or fragment thereof, X_1 is substituted by E, K, or Q and X_2 is substituted by A.

In a preferred embodiment of the above-defined mutated ENV protein having increased immunosuppressive activity, or fragment thereof, the ENV protein is HERV-W ENV, such as represented by SEQ ID NO: 74, and the sequence of the mutated HERV-W ENV is selected from:

10 SEQ ID NO: 116

SEQ ID NO: 118

The present invention also relates to a protein, characterized in that it comprises at least one polypeptide as defined above, or at least one mutated ENV protein, or a fragment thereof, as defined above, provided that when said polypeptide originates from a wild type ENV protein then said protein comprising said polypeptide is different from said wild type ENV protein.

20 The present invention also relates to nucleic acids, and especially polynucleotides, encoding polypeptides of the invention. In a particular embodiment, these nucleic acids are inserted in a vector. The recombinant vector can be a plasmid, a phage for bacterium introduction or a YAC able to transform yeast, or any expression vector.

25 In addition, the recombinant vector comprises transcription regulation regions (including promoter) allowing either inducible expression or conditional expression of the nucleic acid under control or if appropriate, constitutive expression. A tissue specific transcription region can also be used. Moreover, the recombinant vector comprises an origin of replication and/or marker genes.

30 In a particular embodiment of the invention, the vector comprises also nucleic acid encoding viral GAG and/or POL proteins or

sufficient fragments thereof to express functional viral proteins. Optionally, the vector can comprises nucleic acids encoding viral accessory proteins, like NEF, TAT or fragments thereof.

Alternatively, GAG and POL coding sequences can be inserted in separate vectors, including in vector(s) different from the ENV expressing vector.

In a particular embodiment of the invention, a provirus genome is modified with a nucleic acid encoding a polypeptide of the invention having antigenic properties but altered immunosuppressive properties with respect to a determined protein or a nucleic acid encoding a polypeptide of the invention having infectious, fusion and antigenic properties, but altered immunosuppressive properties with respect to a determined protein.

The present invention also relates to cells comprising nucleic acids encoding polypeptides of the invention.

In a particular embodiment, a cell is transformed with a polynucleotide of the invention, in a way that the polynucleotide is integrated in the cell genome either by a recombination with the homologous cellular sequence or by insertion in the cellular genome. The cell can also be transfected with a vector of the invention, by methods well known to the man skilled in the art. The transfection or infection can occurred *ex vivo*, *i.e.* in an artificial environment outside the living organism.

In another embodiment, a vector containing a nucleic acid encoding a polypeptide according to the invention cells is complemented with the introduction of other nucleic acids, contained in additional vectors, especially encoding viral GAG protein and/or POL protein.

These cell lines are useful to the production of recombinant viral particles. In a particular embodiment, the GAG and POL polypeptides originate from the same virus strain as the ENV protein. In another embodiment, the GAG and POL polypeptides originate from a different strain from the ENV protein.

The recombinant viral particles produced comprise a nucleic acid encoding a functional POL protein, a nucleic acid encoding a functional GAG protein and a nucleic acid encoding the polypeptide of the invention.

Moreover, the ENV protein can be chosen among viral
5 amphotropic ENV protein according to the host, *i.e.* able to infect cells of a species from which the virus is not originated, or viral ecotropic ENV proteins according to the host, *i.e.* able to replicate only in the cells of the species from which the virus is originated.

To ensure that the recombinant viral particles be infectious
10 and replicative, the vector comprises various nucleic sequences chosen among transcription, expression and encapsidation signals, such as LTRs, cPPT, PPT3', CTS, SA, SD, psi sequence and RRE. However, such elements can be deleted to produce non-replicative viral particles. Moreover, the proviral genome comprises nucleic acids encoding
15 accessory proteins.

Optionally the particles can be prepared to express additional compounds useful for medical application in a host.

The present invention also relates to a nucleic acid coding for a polypeptide as defined above, for a mutated ENV protein according as
20 defined above or for a protein as defined above.

In a preferred embodiment the above-defined nucleic acid is characterized in that it is represented by a sequence selected from the list comprising:

SEQ ID NO: 103,
25 SEQ ID NO: 105,
SEQ ID NO: 107,
SEQ ID NO: 109,
SEQ ID NO: 111,
SEQ ID NO: 113,
30 SEQ ID NO: 115,
SEQ ID NO: 117.

SEQ ID NO: 119,
SEQ ID NO: 121,
SEQ ID NO: 123,
SEQ ID NO: 125,
5 SEQ ID NO: 127,
SEQ ID NO: 129,
SEQ ID NO: 145,
SEQ ID NO: 147,
SEQ ID NO: 149, and
10 SEQ ID NO: 151.

The above mentioned SEQ ID NO: 103 to 129 and SEQ ID NO: 147 to 151 (odd numbers) respectively encode SEQ ID NO: 104 to 130 and SEQ ID NO: 146 to 152 (even numbers).

15 The present invention also relates to an eukaryotic or prokaryotic expression vector, characterized in that it comprises a nucleic acid as defined above as well as the elements necessary for the expression of said nucleic acid.

20 In a preferred embodiment, the above-defined eukaryotic or prokaryotic expression vector is a viral vector, in particular a pox vector, such as a fowlpox, a canarypox, or a MVA (modified vaccinia virus Ankara) vector, an adenoviral vector, a measles vector, or a CMV (cytomegalovirus) vector.

25 In a further preferred embodiment, the above-defined eukaryotic or prokaryotic expression vector is a viral vector, in particular a canarypox vector, comprising a nucleic acid sequence coding for an as above defined mutated ENV protein, or a fragment thereof, in particular a mutated FeLV ENV, such as represented by SEQ ID NO: 103 or SEQ ID NO: 105, as well as optionally a nucleic acid coding for a GAG protein originating from the same virus as said mutated ENV.

The present invention also relates to a recombinant cell, characterized in that it comprises a nucleic acid as defined above, or an eukaryotic or prokaryotic expression vector as defined above.

5 The present invention also relates to a composition comprising a polypeptide of the invention having altered immunosuppressive properties with respect to a determined protein and particularly a polypeptide substantially retaining antigenic properties, especially immunogenic properties of the protein from which they derive.

10 A particular composition of the invention has lower immunosuppressive properties with respect to the starting determined protein, or even has substantially no immunosuppressive properties.

15 Other compositions comprise polynucleotides or vectors comprising nucleic acid encoding polypeptides of the invention. In this case, tissue specific promoters can be chosen depending upon the organ in which the composition is administered, for example injected and depending upon the expression intensity required.

20 Other compositions of the invention comprise recombinant viral particles or viruses harbouring the polypeptides of the invention and optionally expressing further compounds having a medical interest in a host.

25 The polypeptides and compositions of the invention are useful for the design of active principle for drugs and have accordingly interesting properties for the prophylaxis and or treatment of infections especially viral infections or for the treatment of detrimental consequences, especially malignant states, including tumors, resulting from the viral infection or also for the prophylaxis and/or for the treatment of detrimental consequences, in particular malignant states, including tumors associated with the expression of endogenous viruses, especially HERV, which are normally silent in a host. The expression "treatment" encompasses the curative effect achieved
30 with the polypeptides and compositions of the invention and also the

alleviation of the symptoms observed in a patient or the improvement of the patient's condition.

5 In a particular embodiment, the composition of the invention further comprises additional active compounds useful for the prophylaxis or the treatment of infections, especially viral infections, in particular retroviral infections, including cytokines or useful for the treatment of consequences resulting from the expression of normally silent ERV.

10 When used for administration either for systemic or local administration, especially by injection, the composition further comprises a pharmaceutically suitable excipient or carrier and/or vehicle.

Several types of compositions can be used to elicit an immune response against an antigenic polypeptide of the invention.

15 First, a composition comprising a nucleic acid is administered to a host, for instance injected (known as DNA vaccination) and said nucleic acid expresses *in vivo* a polypeptide according to the invention. DNA vaccines usually consist of plasmid vectors comprising eukaryotic promoter, cloning site, a polyadenylation sequence, a selectable marker and a bacterial origin of replication. All these elements are well known to the man skilled in the art. The delivery of naked DNA has shown to be
20 poorly efficient, and some carriers are needed to improve the delivery of DNA into cells. Two types of carriers have been developed: viral carriers (adenoviruses, lentiviruses) or non-viral carriers such as polymers (and especially cationic polymers), encapsulated-DNA (liposomes) or DNA linked to gold microparticles.

25 Another type of composition comprises a polypeptide of the invention having altered immunosuppressive properties with respect to a determined protein and having antigenic properties. Such a composition may be immunogenic, *i.e.* it is capable of elicit an immune response in a host in which it is administered. However, since proteins are sometimes
30 non-immunogenic or poorly immunogenic, an adjuvant can be administered with the polypeptide, to elicit or improve the immune response. An adjuvant

is defined as any substance that enhances the immunogenicity of an antigen mixed with said adjuvant. Some adjuvants convert soluble antigens into small particles, such as aluminium hydroxide gel, oil in water emulsion or immune stimulatory complexes (ISCOMs). Another class of adjuvants comprises sterile constituents of bacteria such as cell wall or polysaccharides, Freund adjuvant.

Therefore, a composition comprising a polypeptide having antigenic properties but altered immunosuppressive properties with respect to a determined protein is interesting in the elicitation of an immune response in a host in which it is administered and in the production of a humoral and/or cell-mediated immune response.

Indeed, the administration, e.g., the injection, of a polypeptide having non-immunosuppressive properties provides a more efficient immune reaction than the administration of the determined protein (having immunosuppressive properties), because the immune system of the host is fully functional.

In a particular embodiment, a polypeptide according to the invention has antigenic, fusion and infectious properties but has altered immunosuppressive properties with respect to a determined immunosuppressive protein.

Altered immunosuppressive properties according to the invention advantageously correspond to decreased immunosuppressive properties with respect to the original starting protein.

Viral particles coated with a polypeptide having said properties described above can be constructed in recombinant cell lines transfected with gag-pol vectors and vector comprising a nucleic acid encoding said polypeptide.

Optionally, these viral particles also express other compounds of therapeutic or prophylactic interest.

Interestingly, such viral particles are able to infect and to fuse with the cells of a host, and incorporate a non-immunosuppressive

envelope protein. A composition comprising such viral particles elicits an efficient immune reaction, better than viral particle incorporating the determined protein having immunosuppressive properties. Indeed, the envelope protein is not able to immunosuppress its host, resulting in an optimal immune reaction. Another consequence is that viral particles that would have the capacity to replicate, due to recombination events which do not involve the ENV gene, would have their propagation in the host limited, since recombinant viral particle cannot evade the immune response.

A composition comprising viral particles coated with an antigenic envelope protein with fusion and infectious properties appears to be an efficient and safe vaccine.

Interestingly, such viral particles can be either replicative (functional) or non-replicative. This can have consequences on the time of residence of the particles once administered in the host and on the quality of the immune response.

All compositions quoted above can be injected in a host via different routes: subcutaneous (s.c.), intradermal (i.d.), intramuscular (i.m.) or intravenous (i.v.) injection, oral administration and intranasal administration or inhalation.

The present invention also relates to a pharmaceutical or a vaccine composition comprising as active substance:

at least one polypeptide as defined above, or

at least one mutated ENV protein, or fragments thereof, as defined above, or

at least one nucleic acid as defined above, or

at least one prokaryotic or eukaryotic expression vector as defined above, or

at least one recombinant cell as defined above,

in association with a pharmaceutically acceptable carrier.

As will be described hereafter these pharmaceutical compositions are particularly useful for treating cancers, immune disorders or viral diseases.

5 The present invention also relates to the use of at least one protein comprising or constituted of a mutated ENV protein, or fragments thereof, having decreased immunosuppressive activity as defined above, or of a nucleic acid coding for said protein, for the manufacture of a medicament or a vaccine intended for the prevention and/or the treatment of viral diseases, such as HTLV or FeLV infections.

10 The administration to an individual of mutated ENV protein having decreased immunosuppressive activity is liable to protect said individual from infection by the corresponding virus. Indeed, the immune response elicited against the mutated ENV protein is also directed against the corresponding wild type ENV protein. As demonstrated herein, this immune
15 response effectively blocks the immunosuppressive activity of the wild type ENV protein and prevents the immune escape of the virus.

Furthermore, the mutated ENV protein is also liable to act as a molecular decoy which competes with the viral wild-type ENV for binding to its natural receptor, thus inhibiting the activity of said wild-type ENV.

20 The present invention also relates to the use of at least one protein comprising or constituted of a mutated HERV ENV protein, or fragments thereof, as defined above, or of a nucleic acid coding for said protein, for the manufacture of a medicament or a vaccine intended for the prevention and/or the treatment of cancer.

25 As demonstrated herein, blocking the activity of HERV ENV proteins expressed by cancer cells prevents immune escape of these cells. As such, the immune response effectively elicited against mutated HERV ENV proteins having decreased immunosuppressive activity would also be directed against wild-type HERV ENV expressed by cancer cells and thus
30 prevent them from enabling immune escape of these cancer cells.

Furthermore, the mutated ENV protein is also liable to act as a molecular decoy which competes with the wild-type ENV expressed by cancer cells for binding to its natural receptor, thus inhibiting the activity of said wild-type ENV.

5 The present invention also relates to the use of at least one protein comprising or constituted of a mutated ENV protein having increased immunosuppressive activity, or fragments thereof, as defined above, or of a nucleic acid coding for said protein, for the manufacture of a medicament or
10 a vaccine intended for the prevention and/or the treatment of pathologies requiring an inhibition of the immune system, such as autoimmune diseases, allergies or graft rejections.

As intended herein graft rejections also encompass Graft Versus Host Disease (GVHD).

15 The present invention also relates to the use of at least one polypeptide as defined above, or of a protein comprising said polypeptide as defined above, or of a nucleic acid coding for said polypeptide or said protein, for the manufacture of a medicament intended for the prevention and/or the treatment of cancer, of viral diseases, or of pathologies requiring an inhibition of the immune system, such as autoimmune diseases,
20 allergies or graft rejections.

Polypeptides as defined above, and proteins comprising them, can have several applications. When originating from wild type immunosuppressive ENV protein they can be used directly to inhibit the immune system. Otherwise, whether originating from an
25 immunosuppressive or non-immunosuppressive ENV protein they can be used as decoys intended to bind to the natural receptors of the corresponding wild type ENV proteins expressed by cancer cells or viruses, which prevents the activity of said wild type ENV proteins.

30 The present invention also relates to the use of at least one protein or of a nucleic acid coding for said protein, said protein comprising or being constituted of:

- an immunosuppressive ENV protein essentially comprising the following sequence:

$$NY_1Y_2Y_3LY_4Y_5LY_6Y_7Y_8X_1Y_9Y_{10}Y_{11}CY_{12}X_2,$$

wherein amino acids Y_1 to Y_{12} represent any amino acid, amino acid X_1 represents E, K or Q, and optionally amino acid X_2 represents A,

- or a fragment thereof, provided that said fragment carries amino acid X_1 and optionally X_2 , and that it has an immunosuppressive activity similar to that of said ENV protein,

- or a protein derived from said ENV protein, or fragments thereof, by insertion, deletion or substitution of at least one amino acid, provided that said derived protein carries amino acid X_1 and optionally X_2 , and that it has an immunosuppressive activity similar to that of the mutated ENV protein, for the manufacture of a medicament or a vaccine intended for the prevention and/or the treatment of cancers, of viral diseases, or of pathologies requiring an inhibition of the immune system, such as autoimmune diseases, allergies or graft rejections.

In a preferred embodiment of the above-defined use at least one protein comprising or constituted of an immunosuppressive ENV protein essentially comprising the following sequence:

$$NY_1Y_2Y_3LY_4Y_5LY_6Y_7Y_8X_1Y_9Y_{10}Y_{11}CY_{12}X_2,$$

for the manufacture of a medicament or a vaccine intended for the prevention and/or the treatment of cancers, of viral diseases, or of pathologies requiring an inhibition of the immune system, such as autoimmune diseases, allergies or graft rejections, the ENV protein is selected from:

HERV-T ENV, such as represented by SEQ ID NO: 84, or

HERV-R ENV, such as represented by SEQ ID NO: 86, or

HERV-V ENV, such as represented by SEQ ID NO: 88, or

HERV-R(b) ENV, such as represented by SEQ ID NO: 90, or

HTLV-1 ENV, such as represented by SEQ ID NO: 92, or

HTLV-2 ENV, such as represented by SEQ ID NO: 94, or

FeLV ENV, such as represented by SEQ ID NO: 96, or
PERV ENV, such as represented by SEQ ID NO: 98, or
STLV-1 ENV, such as represented by SEQ ID NO: 100, or
FV ENV, such as represented by SEQ ID NO: 102.

5 As for the above-mentioned polypeptides, these proteins, and fragments thereof, can have several application. They can be used either directly to inhibit the immune system or as decoys intended to bind to the natural receptors of the corresponding wild type ENV proteins expressed by cancer cells or viruses.

10 The invention also relates to a method for producing antibodies comprising:

- 15 a. modifying the nucleotide immunosuppression-modulatory sequence in a way to modulate the immunosuppression effect, but to retain the fusion, infectious and immunosuppressive properties,
- b. expressing the modified gene,
- c. purifying the modified polypeptide,
- d. injecting the modified polypeptide in an animal to induce a immune response,
- 20 e. purifying the produced antibodies reacting against the modified polypeptide.

The invention also provides a method to modulate the immunosuppressive properties of a antigenic and immunosuppressive protein while retaining its antigenic properties comprising:

- 25 a. identifying the nucleic acid sequence encoding an immunosuppression-modulatory sequence encoding a consensus amino acid sequence as defined above in a nucleic acid sequence encoding said antigenic and immunosuppressive properties,

- b. identifying the codons encoding amino acids X1 and X2 impacting on the immunosuppressive properties in sequence X1-(Y)₃-C(Y)₁-X2 as defined above,
- c. modifying the codons encoding said both amino acids in such a way that the resulting protein retains its antigenic properties but has modified immunosuppressive properties,
- d. expressing the obtained modified nucleic acid sequence encoding said antigenic protein having modified immunosuppressive properties.

A particular method to modulate the immunosuppressive properties of an antigenic and immunosuppressive protein having further infectious and fusion properties while retaining its fusion, infectious and antigenic properties comprises:

- a. identifying the immunosuppression-modulatory sequence of an env gene encoding an amino acid sequence similar to that defined above,
- b. modifying the codons coding amino acids impacting on the immunosuppressive properties in such a way that the resulting protein retains its fusion, infectious and antigenic properties but has modified its immunosuppressive properties.

The invention also provides a method to prepare attenuated virus comprising:

- a. modifying the gene coding for an antigenic and immunosuppressive protein of a virus in a way to modulate its immunosuppressive properties, but to retain its antigenic properties,
- b. expressing the modified gene in a recombinant cell lines, to produce attenuated recombinant viral particles integrating a modified proviral genome.

The invention also concerns a method to prepare attenuated virus comprising:

- a. modifying the gene coding for an antigenic and immunosuppressive ENV protein of a virus having further fusion and infectious properties in a way to modulate its immunosuppressive properties but to retain its fusion, infectious and antigenic properties,
- b. expressing the modified gene in a recombinant cell lines, to produce attenuated recombinant viral particles integrating a modified proviral genome.

The invention also more generally relates to the use non-immunosuppressive or low-immunosuppressive polypeptides for the preparation of an immunogenic composition suitable for prophylaxis, or treatment of a viral disease or of a malignant state, or a tumor disease.

Naturally occurring proteins which have no immunosuppressive or low-immunosuppressive properties can be used accordingly; they encompass HERV-W or HERV-H.

The present invention relates to the use of a polypeptide as defined above, or of a mutated protein or a protein as defined above, for the preparation of ligands of ENV proteins selected from:

- polyclonal or monoclonal antibodies, or fragments thereof, such as Fab or F(ab)'₂ fragments,
- scFv polypeptides,
- aptamers,
- binding peptides.

Such ligands and methods for preparing them are well known to man skilled in the art.

The present invention also relates to antibodies or fragments thereof, scFv polypeptides, aptamers, or binding peptides, directed against mutated ENV proteins as defined above, or proteins or polypeptides comprising

them as defined above, provided that said antibodies or fragments thereof, scFv polypeptides, aptamers, or binding peptides do not bind to the corresponding wild type ENV proteins.

5 The present invention also relates to the use of polypeptides as defined above, or of proteins as defined above, for screening compounds liable to modulate the immunosuppressive activity of viruses or tumor cells.

10 The present invention also relates to the use of antibodies or fragments thereof, scFv polypeptides, aptamers, or binding peptides as defined above, for screening compounds liable to modulate the immunosuppressive activity of viruses or tumor cells.

15 In a preferred embodiment of the above defined uses of polypeptides as defined above, of proteins as defined above, or of antibodies or fragments thereof, scFv polypeptides, aptamers, or binding peptides as defined above, the compounds to screen are peptides, in particular peptides comprising from 5 to 30 amino acids, such as peptides originating from combinatorial peptide libraries.

EXAMPLES

EXAMPLE 1

METHODS:

5

Mice and Cell Lines.

The cell lines used in these tests were:

- 293T, embryonal kidney cells (ATCC CRL11268),
- HeLa, human epithelioid carcinoma cells (ATCC CCL2)
- 10 - MCA205, methylcholanthrene-induced murine fibrosarcoma cells
(Shu and Rosenberg, 1985)
- NIH 3T3, mouse fibroblasts

Cells were cultured in DMEM supplemented with 10% fetal calf serum, streptomycin (100 µg/ml) and penicillin (100 units/ml).

15

In order to test the immunosuppressive effect of the modified protein, C57BL/6 and BALB/c mice, 8- to 12-wk-old, obtained from Janvier (Laval, France), were used.

20

Constructions.

The vectors expressing the envelope of HERV-W and HERV-T (phCMV-envW and phCMV-envT) have been previously described (Blaise et al., 2003). In brief, they comprise a promoter (human cytomegalovirus early promoter), the rabbit β -globin intron and polyadenylation sequences. The
25 cDNA of HERV-W env was inserted between the EcoRI sites of the vector (Figure 3A).

The envelope gene of MPMV was retrieved from the pTMO vector (Brody et al., 1994) by PCR using the following primers:

Atacatctcgagaccggtccaactagaacccatgaacttcaattatcatttcacatctgga (SEQ ID NO: 55) and

5 Atacatacgcgctctatgttaagggtcaaataatgagccacc (SEQ ID NO: 56) digested with XhoI and MluI (underlined), and cloned into phCMV-envT digested with the same enzymes. The phCMV-envMPMV expression vector containing and expressing the envelope gene of MPMV was obtained (Figure 2A). These vectors are used in the cell-cell fusion assay and for the production of
10 pseudotypes.

Amino-acid positions * in the following description of the constructions were numbered according to the model structure of the TM subunit of HERV-W generated with the Swiss-Model software (Figure 8) (<http://swissmodel.expasy.org/>) and the structure of Moloney murine
15 leukaemia virus TM subunit as a template (Protein Data Bank ID: 1MOF(1), <http://www.resb.org/pdf/>). The positions 44 and 50 according to this numbering scheme represent therefore the following positions when identified in the SU-TM precursors of the corresponding envelopes disclosed as NCBI sequence accession number:

20

Envelope	Position*36	Position*44	Position*47	Position*50	NCBI sequence accession number
HERV-W	A385	R393	T396	F399	AF072503 ⁽²⁾
MPMV	G463	Q471	I474	A477	AF033815 ⁽³⁾
MoMLV	G543	E551	L554	A557	AF033811 ⁽³⁾

NCBI URL: <http://www.ncbi.nlm.nih.gov:80/entrez/>

(1) Fass D, Harrison SC, Kim PS. Nat Struct Biol. 1996 May; 3(5): 465-9.

25 (2) Blond, J.L., Beseme, F., Duret, L., Bouton, O., Bedin, F., Perron, H., Mandrand, B. and Mallet, F.J. Virol. 73(2), 1175-1185 (1999)

(3) Petropoulos, C.J. Appendix 2: Retroviral taxonomy, protein structure, sequences and genetic maps, in RETROVIRUSES: 757, Coffin, J.M. (Ed.); Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, NY, USA (1997)

5

Site-directed mutagenesis of phCMV-envW was performed as described previously (Kunkel et al., 1987), using single-stranded uracilated DNA as template and mutagenic oligonucleotides (mutation in bold face), which also introduced silently a restriction site (underlined) for easier screening:

10

A36G: tagtccttcaaatacgccg**cggt**tttagacttgctaa (SEQ ID NO: 57),

R44Q: **aca**agggggtac**ct**gtttattttaggggaaga (SEQ ID NO: 58),

T47I: ccgctgaaagagggggg**catat**gtttattttagggga (SEQ ID NO: 59),

F50A: aaccgctgaaagagggggtac**ct**gtttag**gct**tttaggggaaga (SEQ ID NO: 60),

R44Q/F50A: aaccgctga**aca**agggggtac**ct**gtttag**gct**tttaggggaaga (SEQ ID NO:

15

61).

Site-directed mutagenesis of phCMV-envMPMV was performed by the same method except that PCR fragments linking a silently XhoI-introducing antisense primer (cttcggcgtctctc**gag**agacgccgaag) (SEQ ID NO: 62) to the mutagenic primers Silent: caaaacagaagaggattagatctacttacagc (SEQ ID NO: 63),

20

Q44R: tacttacagcagag**ag**aggaggtatctgcttag (SEQ ID NO: 64),

A50F: gggaggtatctgcttat**tttt**tacaggaaaaatgtt (SEQ ID NO: 65),

Q44R/A50F: acttacagcagag**ag**aggaggtatctgcttat**tttt**tacaggaaaaatg) (SEQ ID

25

NO: 66) were used instead of synthetic oligonucleotides.

Mutant derivatives of pDFG-envW were constructed by triple ligation of the BstBI-BsrGI and BsrGI-BstEII fragments of pDFG-envW with the BstEII-BstBI fragment of phCMV-envW.

30

pDFG-envMPMV and its mutant derivatives (Figure 2B) were constructed by ligation of the AgeI-MluI fragments of phCMV-envMPMV into the pDFG-MoTMTag vector digested with the same enzyme. The pDFG plasmid is an envelope expressing vector containing LTRs, splice sites (SD and SA) a psi sequence and an IRES (internal ribosome entry site) element, as well as a selection gene (antibiotic resistant gene). These vectors (Figures 1B, 2B and 3B) are used in the Envelope-Expressing Tumor Cells and *in Vivo* Assay.

Fusion property: the Cell–Cell Fusion Assay.

HeLa cells were transfected using Lipofectamine (Invitrogen, 2 µg of DNA for 5×10^5 cells). Fusion activity of envelope glycoproteins was measured 24 h after transfection with the corresponding expression vectors (Figures 1A, 2A and 3A). To visualize syncytia, cells were fixed in methanol and stained by adding May–Grünwald and Giemsa solutions (Sigma) according to the manufacturer's instructions. The fusion index, which represents the percentage of fusion events in a cell population is defined as $[(N - S)/T] \times 100$, where N is the number of nuclei in the syncytia, S is the number of syncytia, and T is the total number of nuclei counted (Figure 4). A phCMV vector not expressing envelope protein was used as a negative control.

Infectious property: the Infectivity Assay.

7.5×10^5 293T cells were cotransfected with 1.75 µg of CMV-gag-pol-MoMLV, 1.75 µg MFG-nls-lacZ and 0.55 µg phCMV vector (Figures 1A, 2A and 3A) expressing the envelope glycoproteins (wild-type or mutated) using the phosphate calcium method. MFG-nls-lacZ vector comprises the MoMLV LTRs, the psi sequence, a NLS (nuclear localisation signal) and the LacZ gene. Supernatants containing the pseudotypes (viral body of MoMLV with envelope protein from another virus strain) were recovered 2 days later, filtered, serially diluted in culture medium and used for infection of 4×10^3 HeLa cells in 96-well culture plates in the presence of 4 µg/mL polybrene.

Plates were fixed 2 days later, X-gal coloured for 1 hour, and foci of β -galactosidase-expressing infected cells were counted to determine pseudotype titers (number of infectious particles by ml of supernatant). A phCMV vector not expressing envelope protein was used as a negative control.

Immunosuppressive properties: the Establishment of Envelope-Expressing Tumor Cells and *in Vivo* Assay.

pDFG retroviral expression vectors (1.75 μ g) were packaged by transient cotransfection into 7.5×10^5 293T cells with 1.75 μ g of CMV-gag-pol-MoMLV and 0.55 μ g CMV-envAmpho, using the calcium phosphate method. Supernatants were recovered 2 days later, filtered and used for infection of 5×10^5 MCA205 tumor cells in the presence of 4 μ g/mL polybrene, as described in Mangeney & Heidmann, 1998. Cells were maintained in selective medium (400 units/mL hygromycin) for 2 weeks. For *in vivo* assays, tumor cells were trypsinized, centrifuged and resuspended in PBS at a concentration of 1×10^7 cells/mL. 100 μ L of each suspension were injected s.c. in the shaved right flank of 3 C57/BL6 and 8 to 10 BALB/c mice. Tumor establishment was determined by palpation and tumor area (mm^2) was determined by measuring perpendicular tumor diameters (Figure 5). Immunosuppression index is defined as $i = (S_{\text{env}} - S_{\text{none}}) / S_{\text{none}}$, wherein S_{env} is the maximum area reached by a tumour expressing an envelop protein and S_{none} is the maximum area reached by a tumour not expressing envelop protein (negative control).

RESULTS

1- Determination of the infectious properties of various wild-type envelope proteins

5 The infectiosity of envelope proteins was tested in NIH 3T3 cells (MoMLV) or HeLa cells (HERV-W and MPMV). Figure 6 shows that the three wild-type envelope proteins (lines 1, 5 and 9) were able to sustain an infection.

2- Determination of the immunosuppressive effects of various wild-type envelope proteins

10 The immunosuppressive effect of MPMV retrovirus and HERV-W was tested in MCA205 cells, injected in allogenic balb/c or syngenic C57Bl/6 mice. Figure 7 shows that tumour expressing MPMV (black bars) were large comparing to tumours expressing HERV-W (white bars). Whereas
15 inventors confirmed the immunosuppressive effect of MPMV envelope, they showed that HERV-W was not able to immunosuppress an allogenic host.

In conclusion, the envelope proteins of MPMV and HERV-W have the same properties in term of fusogenicity and infectiosity, but differ for their
20 immunosuppressive properties.

3- Strategy for the identification of envelope protein with altered immunosuppressive properties

Based on the different properties of HERV-W and MPMV, inventors
25 attempted to identify domains in the amino acid sequence, which could be involved in the modulation of immunosuppression.

A putative 17 amino acid immunosuppressive domain (ISU) was previously characterized in several publications between amino acid 30 and amino
30 acid 47 of the cristallized subdomain, the TM domain, respectively two leucines (L) in the MoMLV (Blaise et al. 2001 J Virol. 82, 1597-1600).

A two-step strategy was applied; the first step was to modify an envelope protein that in such a way that the derived protein (i.e., the modified protein) retains the fusion and infectious properties of the corresponding none modified protein. Once such a modified envelope protein has been identified, its immunosuppressive effect was tested and compared to that of the none modified protein.

4- Study of modified HERV-W

One difficulty lays in the fact that previous attempts to modify the amino acid composition of the TM subunit have lead to the loss of association of SU-TM and have altered the infectivity. A deletion from Leucine 30 to Threonine 40 of the MPMV immunosuppressive domain for instance completely abrogates the infectivity of the envelope proteins (Brody et al. 1992 J Virol 66, 3466-3475; Brody et al. 1994 Virology 202, 673-683).

Despite these unsuccessful attempts, the inventors studied the amino acid composition of the ISU domain, and their possible impact on the structure of the domain and achieve a novel definition of said ISU domain involved in immunosuppressive properties observed *in vivo*. They further determined that some positions in the amino acid sequence of proteins together with the nature of the amino acid residues at these positions were critical for the immuno suppressive effect.

The inventors especially designed some modifications in the amino acid sequence of a non-immunosuppressive envelope protein, i.e., HERV-WEnv protein, to render it immunosuppressive, using for instance substitution of determined residues by the corresponding residues of MPMV.

a. Infectious properties

The A36G and T47I substitutions of the HERV-W envelope do not modify the infectiosity, the fusogenicity and the immunosuppressive effect of the envelope protein (Table 1). These two amino acids appear not to be determinant for these functions. To the contrary, the R44Q or F50A substitutions strongly altered both the infectious and fusion properties of the envelope protein (Table 1, and Figure 6, lines 2 and 3).

A double mutant comprising both the R44Q and F50A substitutions was constructed. Surprisingly, the double mutant retained fusion and infectious properties similar to those of the wild type polypeptide (Table 1 and Figure 6, line 4).

This result and the design of this modified envelope protein using some homologous positions found in the envelope of MoMLV (Figure 8) suggest that these two amino acids could interact together because of both their respective location in the structure of the TM unit of the envelope protein, and their nature. This possible interaction may explain the compensatory behaviour of this pair of mutations. This was unexpected, because of the previous attempts that fail to identify such amino acids.

b. Immunosuppressive properties

Another result, as surprising as the above-mentioned, arises from the study of the immunosuppressive effect. Indeed, whereas the wild-type HERV-W envelope protein was not immunosuppressive in view of the size of the tumours, the HERV-W double mutant was more immunosuppressive than the wild-type MPMV envelope proteins (Table 1 and Figure 7, white bars).

Moreover, inventors identified two amino acids positions in the sequence, one of which was previously not reported as forming part of the ISU domain (position 50), which, taken together, revealed to be involved in the

modulation of the immunosuppressive effect of the HERV-W envelope proteins.

Mutant	Fusion	Infection	Immunosuppression
Wild Type	55.0 ± 3.7 %	800 ± 200	-0.30 ± 0.06
R44Q	32.5 ± 1.3 %	< 10	-0.12 ± 0.30
F50A	5.6 ± 3.0 %	< 10	-0.16 ± 0.14
R44Q+F50A	53.0 ± 2.8 %	947 ± 542	0.61 ± 0.10
A36G	54.5 ± 4.5 %	3950 ± 2250	-0.02 ± 0.01
T47I	50.5 ± 1.2 %	300 ± 80	-0.25 ± 0.04
Negative control	3.2 ± 1.2 %	< 10	0.00 ± 0.00

5 Table 1: Results obtained for fusion, infectious and immunosuppression properties of HERV-W modified envelope proteins.

5- Study of modified retrovirus envelope proteins

10 To confirm the fact that these amino acids residues belong to a determinant of immunosuppression, other retroviruses comprising similar amino acid at positions 44 (E or Q) and 50 (F) were screened. Several of these retroviruses have been identified and are disclosed in Figure 9: Moloney Murine Leukaemia virus (MoMLV), Friend virus, Feline Leukaemia virus (FeLV), Human T-cell lymphotropic virus type-1 (HTLV-1) and simian T-cell lymphotropic virus type-1 (STLV-1).

15 In two of them, MPMV and MoMLV viruses, amino acid residues 44 and 50 were substituted by the corresponding amino acids found in HERV-W. The following constructs were made: E44R, A50F and E44R/A50F (MoMLV) and, Q44R, A50F and Q44R/A50F (MPMV).

a. Infectious property

25 Interestingly, in MoMLV, the simple mutant loses its infectivity properties (Table 2 and Figure 6, lines 6 and 7), whereas the double mutant has the same properties as the wild-type protein (Table 2 and Figure 6, line 8).

In MPMV, slight differences were observed between mutants and wild-type, but only the double mutant presents properties strictly identical to the wild-type proteins (Table 3 and Figure 6, lines 10 to 12).

b. Immunosuppressive properties

5

In MoMLV, both a protein with the E44R substitution or a double mutant (E44R+A50F) have their immunosuppressive properties reduced *in vivo* (Table 2).

10

In MPMV, both a protein with the Q44R substitution or a double mutant (Q44R+A50F) have their immunosuppressive properties reduced *in vivo* (Table 3).

Mutant	Infection	Immunosuppression
wt	$4.59 \pm 1.97.10^5$	0.60 ± 0.20
E44R	$6.97 \pm 3.98.10^4$	0.03 ± 0.01
A50F	$< 10^1$	n/d
E44R+A50F	$4.34 \pm 2.11.10^5$	0.00 ± 0.01
Negative control	$<10^1$	-0.00 ± 0.00

15

Table 2: Results obtained for infectious and immunosuppression properties of MoMLV modified envelope proteins (MoMLV is not fusigenic).
n/d: not determined

Mutant	Fusion	Infection	Immunosuppression
wt	$47.8 \pm 3.0 \%$	$3.3 \pm 0.4 10^4$	0.45 ± 0.09
Q44R	$29.8 \pm 6.4 \%$	$3.6 \pm 0.5 10^3$	-0.32 ± 0.12
A50F	$37.2 \pm 5.9 \%$	$8.9 \pm 2.7 10^3$	0.01 ± 0.01
Q44R+A50F	$52.6 \pm 3.4 \%$	$2.8 \pm 1.0 10^4$	-0.27 ± 0.06
Negative control	$5.1 \pm 2.2 \%$	$< 10^1$	0.00 ± 0.00

20

Table 3: Results obtained for fusion, infectious and immunosuppression properties of MPMV modified envelope proteins.

Taken together, all these results allow to draw the following conclusions:

Firstly, a single mutation seems sufficient to modify the immunosuppressive properties of a retroviral immunosuppressive envelope protein. Indeed, the substitution of the glutamine or glutamic acid in position 44 with an arginine reduced the immunosuppressive behaviour of the mutants. However, the fusion and infectious properties, even if not abolished, are strongly reduced (MPMV).

Secondly, double mutants (at positions 44 and 50) have reduced immunosuppressive properties when compared to the corresponding wild-type envelope protein. Interestingly, MPMV double mutants have fusion properties as efficient as those of wild-type protein, and high infectious properties. The interest of such a protein in the production of viral particles and live vaccine is promising.

EXAMPLE 2

METHODS

Mice and cell lines: Swiss mice (FV permissive), 10 weeks old, were obtained from Janvier (Laval, France). The cell lines 293T (ATCC CRL11268), HeLa (ATCC CCL2), NIH/3T3 (ATCC CRL-1658) and MCA205 (REF) were cultured in DMEM supplemented with 10% fetal calf serum, streptomycin (100 µg/ml) and penicillin (100 units/ml).

Constructions: Plasmids p57 (Oliff et al. *J Virol* 33, 475-86 (1980)) and pET28(+)(b) (Novagen) were used.

phCMV-envFV was constructed as phCMV-envMPMV (Example 1), using p57 as PCR template and primers 16 and 17. Mutant derivatives were constructed by inserting into the ClaI/AvrII opened vector two PCR products, the first digested with ClaI, the second with AvrII. These fragments were generated with phosphorylated primer pairs 1-2 and 3-4

for E14R mutation (which corresponds to the E561R mutation of the full length ENV), 1-5 and 3-6 for A20F mutation (which corresponds to the A567F mutation of the full length ENV), and 1-2 and 4-6 for E14R+A20F mutation. pDFG-envFV and its mutant derivative were constructed by inserting the AgeI/MluI fragments of phCMV-envFV into pDFG-MoTMTag digested with the same enzymes. The double mutant p57 was constructed by inserting the BstZ11I/BsmI fragment of the double mutant phCMV-envFV into p57 digested with the same enzymes.

The bacterial expression vector for the SU subunit of the FV envelope protein was constructed by inserting a PCR fragment generated with phCMV-envFV as a template and primer pair 7-8, and digested with NcoI and XhoI, into pET28(+)-b digested with the same enzymes.

The bacterial expression vectors for the SU and the TM subunits of the FV envelope protein were constructed by inserting a PCR fragment generated with wild-type or double-mutant phCMV-envFV as a template and primer pair 7-8 or 9-10, and digested with NcoI and XhoI, into pET28(+)-b digested with the same enzymes.

SEQUENCE	SEQ ID
1 CAACCTTACCAACCCTGATAAACTCAAGA	SEQ ID NO: 131
2 CAGTCCTCCTCTTTTATAGGAACAACAGGTCTAGGC	SEQ ID NO: 132
3 TGTGCTGCCCTAAAAGAAGAATGTTGTT	SEQ ID NO: 133
4 GGACTAAAGCCTGGACTACTGAGATCCTG	SEQ ID NO: 134
5 CAGTCCTCCTCTTTTATAGGAACAACAGGT	SEQ ID NO: 135
6 TGTGCTTTCCTAAAAGAAGAATGTTGTTTCTAT	SEQ ID NO: 136
7 ATACATCCATGGCGTGTTCACGCTCCCAAATCCCCTA	SEQ ID NO: 137
8 ATACATCTCGAGTTCTCTTTTATGTCTATAGGATTTTCAAAC	SEQ ID NO: 138
9 ATACATCCATGGCTGCCGTACAAGATGATCTCA	SEQ ID NO: 140
10 ATACATCTCGAGATCTCTTACTAGGCCTGTATGGTCAGC	SEQ ID NO: 141

Virus production, quantitation and inactivation: 7.5×10^5 293T cells were transfected with 4 μg of p57 DNA using a calcium phosphate transfection kit (Invitrogen). 48h later, cell supernatants were used to infect 5×10^5 NIH/3T3 cells in the presence of 4 $\mu\text{g/mL}$ polybrene and infected cells were cultured for 4 additional days. Viral particles were collected from cell supernatants, concentrated by ultracentrifugation, resuspended in PBS, and frozen. Inactivation was performed by exposing a viral suspension in PBS to UV light at 0.5 mW/cm^2 during 30 minutes.

Immunosuppression assay: MCA205 cells were transduced with either an envelope gene expression vector or an empty vector, and engrafted into allogenic mice where they established transient tumors, as described in example 1. The immunosuppression index was calculated as $(A_{\text{env}} - A_{\text{none}})/A_{\text{none}}$, where A_{env} and A_{none} are the mean tumor areas obtained with cells expressing the envelope gene and the empty cassette, respectively.

Cell-cell fusion and infectivity assays were performed as described in Example 1, with pHCMV-envFV and their mutant derivatives as envelope expression vectors.

Viral load assay: RNA from 2 μl of concentrated virus or 20 μl of cell supernatant or serum was extracted using the RNeasy microkit (QIAGEN), reverse-transcribed using the MoMuLV reverse transcription kit (Applied) and random hexamers as primers, and cDNA was quantitated by real-time PCR using the Platinum SYBR Green qPCR kit (Invitrogen) and primers CTCAGGGAGCAGCGGGA (SEQ ID NO: 142) and TAGCTTAAGTCTGTTCCAGGCAGTG (SEQ ID NO: 143).

Recombinant proteins: Recombinant proteins were produced in BL21(DE3) *E.coli* cells (Stratagene) using pET28(+)-b (Novagen) as an expression vector. The SU subunit was produced as inclusion bodies, and

the wild-type and mutant TM subunits as soluble material. They were purified on HiTrap Chelating HP columns (Amersham) according to the manufacturer's instructions. The TM subunits were further purified on a Superdex 75 HR10/30 column (Amersham) to isolate the major trimeric form, their LPS contents were quantitated using the LAL QCL-1000 kit (Cambrex) and adjusted to 5 µg/mg of protein by addition of *E.coli* LPS (strain 0111:B4, Sigma).

Mice immunization: Mice were injected thrice at one week interval with either 100 µg of recombinant TM subunits or 1.5×10^{10} RNA copies of an intact or UV-inactivated FV viral particles. 100 µg of CpG (phosphorothioate oligonucleotide TCCATGACGTTCTGACGTT (SEQ ID NO: 144)) was systematically added as an adjuvant. Sera were collected 4 days after the last immunization. Inactivated viral particles-immunized mice were challenged with 10^6 RNA copies of the wild-type FV, and post-challenge sera were collected 5 days later.

Immunological FV detection: Recombinant SU subunit was produced as inclusion bodies in BL21(DE3) *E.coli* cells (Stratagene) using pET28(+)-b (Novagen) as an expression vector, purified on a HiTrap Chelating HP column (Amersham) according to the manufacturer's instructions, and used to coat MaxiSorp microplates (Nunc) at a concentration of 2 µg/ml. IgG levels in serially diluted sera were quantitated using an anti-mouse IgG antibody conjugated to HRP (Amersham) and OPD as a chromogenic reagent (Sigma).

RESULTS

1. Loss of envelope protein-induced immunosuppression leads to complete immune rejection of an infectious retrovirus: The genetic, double-mutation-generated disjunction between immunosuppression and

infectivity evidenced in Example 1 opens the possibility to generate an entire retrovirus devoid of the immunosuppressive activity of its envelope protein, but still replicative and infectious.

5 The Friend Murine Leukemia Virus (FV) was chosen as a model, because the mouse genome does not contain a related endogenous retrovirus that could impair its *in vivo* detection.

10 The key residues of the FV envelope were replaced by those of Syncytin-1 (HERV-W ENV), and it was checked, as for the MPMV envelope, that the double mutation E14R + A20F (which corresponds to the E561R + A567F mutation of the full length ENV) reversed immunosuppression without altering infectivity (Figures 11A and 11B). The wild-type envelope gene was replaced by its non-immunosuppressive mutant in the FV molecular clone 57, and each type of retroviral particles was produced *in vitro*. The virus yields were similar as measured by a quantitative RT-PCR assay of the viral RNA in the cell supernatants.

15 As expected, both virus types display the same propagation kinetics in an *in vitro* infection assay in NIH/3T3 cells (Figure 11C), and similarly when injected *in vivo* in 5-Gray irradiated, immunocompromised mice (Figure 12A).

20 In normal mice, the wild-type FV first established high viremia in all mice during the primo-infection phase (at day 7 after virus injection, Figures 12A-12B). This phase was followed by the establishment of persistent infections, the mice being able to control viral replication to various extents, as expected with non-congenic, outbred mice. After 4 months, 80% of the infected mice disclosed an erythroleukemia syndrome, with a hematocrit level below 35%.

25 In contrast the mutated non-immunosuppressive FV was undetectable as early as 14 days after injection of even very high doses of viral copies (10^6 RNA copies, 10^2 ID₅₀) with no evidence for any pathology. Noteworthy, IgG directed against the FV envelope protein were detected persistently in mice infected with wild-type FV, but only transiently in mice infected with

30

the double-mutant FV (Figure 13), indicating complete clearance of the mutated virus.

In conclusion, the present experiments demonstrate that envelope-driven immunosuppression is essential for FV infection, as its absence leads to
5 thorough immune rejection of the incoming virus.

2. Increased immunogenicity of immunosuppression-negative recombinant envelope proteins and inactivated viral particles: As the key element for viral entry into the target cell, retroviral envelope proteins
10 are systematically included in every vaccinal formulation, either as recombinant proteins, as fragments thereof, or as genes carried by a defective viral vector. One could suspect that envelope protein-mediated immunosuppression could inhibit the response mounted against an immunogen containing the ISU, thus lowering its vaccinal efficiency.

To test this hypothesis, two kinds of ISU-containing immunogens were
15 generated: 1) recombinant proteins corresponding to the ectodomains of the TM subunit of the wild-type or mutant FV envelope protein, produced in *E.coli* as soluble - thus correctly folded - and trimeric forms displaying identical behavior upon purification; 2) wild-type and mutant FV particles
20 that were intact or inactivated by exposure to UV light, in order to preserve the native structure of their envelope proteins. These immunogens were injected thrice in Swiss mice to generate a strong secondary humoral response.

As illustrated in Figure 14A, only the mutant non-immunosuppressive
25 envelope protein raises such a response, with high IgG levels. In every cases, the signals obtained with plates coated with the wild-type or the mutant TM subunits were quantitatively the same, indicating that the anti-TM antibodies in the mice sera are not preferentially directed against the ISU itself but rather against other epitopes within the TM subunits.

Thus, the double mutation introduced in FV envelope protein does not
30 convert its ISU into a highly efficient epitope. In addition, IgM levels raised

by the wild-type envelope protein are much higher than those raised by its non-immunosuppressive mutant counterpart. These results suggest that the immunosuppressive domain of FV envelope protein directly inhibits the immune system, and that this effect does not require viral entry and replication in the target cell nor even any other viral component than the TM subunit alone.

Figure 14B confirms these results with MoMLV ENV and HERV-W ENV. Almost no IgG response is elicited against the wild type recombinant TM subunit of MoMLV ENV, whereas the non immunosuppressive double mutant (see Example 1) shows a strong IgG response. Furthermore, as expected, an IgG response is seen against the TM subunit HERV-W ENV, which is naturally deprived of immunosuppressive activity, whereas the immunosuppressive double mutant (see Example 1) elicits only a slight IgG response.

3. Loss of envelope protein-induced immunosuppression improves the vaccinal efficiency of inactivated viral particles: One could suspect that this antigenicity-inhibiting effect of the ISU might lower the efficiency of any vaccine formulation containing an immunosuppressive envelope protein, and thus, that the specific, double mutation-induced disruption of this effect might improve vaccinal efficiency.

To test this hypothesis, mice immunized with either wild type and double mutant inactivated viral particles or with intact double mutant viral particles were challenged with the intact wild-type FV. Serum viral loads were then assayed at peak viremia, five days after challenge (Figure 15).

The virus was detectable in all mice immunized with the wild-type inactivated FV, yet with a geometric mean viral load 50-fold lower than that of control mice immunized with the adjuvant only, indicating a significant but incomplete protection conferred by immunization with wild-type particles. In contrast, the viral loads of 6 of the 14 mice immunized with the non-

immunosuppressive inactivated double mutant FV were below the detection threshold of the assay, and the geometric mean viral load was reduced 7500-fold as compared to mice immunized with adjuvant only. Furthermore, the viral loads of 12 out of 14 mice immunized with the intact non-immunosuppressive double mutant FV were below the detection threshold and the geometrical mean viral load was also below the detection threshold.

These results show that disrupting immunosuppression by mutations that preserve the canonical function – thus the structure – of an envelope protein improves the efficiency of vaccinal formulation based on such proteins.

EXAMPLE 3

METHODS

Mice and cell lines: C57BL/6 and SCID mice, 8-12 weeks old, were obtained from Janvier (France). B16 (murine melanoma cell line of C57BL/6 origin, EACC 94042254) and 293T (human embryonic kidney cells, ATCC CRL11268) were maintained in DMEM supplemented with 10% heat-inactivated foetal calf serum and antibiotics.

Constructions: a plncxH1 expression vectors derived from the plncx (Miller and Rosman Biotechniques 1989;7: 989-90) and the pSUPER (Brummelkamp et al. Science 2002;296: 550-3) vectors was constructed to generate short transcripts directed against MelARV (targeted to the genomic transcript within the gag sequence; nt positions 1220-1238 from the start codon), or against the green fluorescent protein transcript (nt position 215-233 from the start codon) as a control. They were obtained by

first inserting annealed 64-mer oligonucleotides (sequences in Figure 1B) into pSUPER opened at the *Bgl*II and *Hind*III sites, followed by introduction of the *Bam*HI-*Hind*III fragment from these constructs into pIncx opened at the corresponding sites. The expression vector for the MelARV envelope (pDFG MelARVenv) and the control (pDFG none) were constructed by introducing (or not) a RT-PCR product, generated from the MelARV viral RNA using an *Age*I-containing primer at the envelope 5'-end and a *Xho*I-containing primer at the envelope 3'-end, into a hygromycin-containing pDFG vector (Mangeney and Heidmann Proc Natl Acad Sci USA 1998;95: 14920-14925) opened at the same sites.

Establishment of ERV^{KD} B16 tumor cells: 7.5×10^5 293T cells were cotransfected with the pIncxH1 vector (1.75 μ g) and expression vectors for the MLV proteins (0.55 μ g for the amphotropic MLV envelope vector and 1.75 μ g for the MLV gag and pol vector, see Blaise et al. J Virol 2004;78: 1050-1054). Thirty six hours post-transfection, viral supernatants were collected for infection of the B16 tumor cells (2.5 ml of supernatant for 5×10^5 cells, with 8 μ g/ml polybrene). Cells were maintained in selective medium (1 mg/ml neomycin) for three weeks. In some experiments, the pDFG MelARVenv expression vector (or control pDFG none) was additionally introduced into the cells using the same protocol and infected cells were selected with 300 units/ml hygromycin.

Expression of MelARV proteins: Analysis of MelARV expression was performed by Western blot analyses. The supernatants of 10^7 cells were collected, centrifuged for 10 min at 100xg, filtered and concentrated by ultra-centrifugation in a SW41 Beckman rotor (150,000xg, 1 hour, 4°C). Pellets were resuspended in lysis buffer, submitted to SDS-PAGE, blotted and revealed with an anti-Env mAb (Ciancolo et al. J Exp Med 1984; 159:964-969) and an anti-Gag goat serum (Viromed Biosafety Labs).

***In vitro* transformation assay:** Both control- and ERV^{KD}- B16 cells were plated in soft agar to determine the efficiency of anchorage-independent growth. Cells (2×10^3 or 2×10^4) were plated in 5 ml of 0.33% agar in DMEM with 10% foetal bovine serum overlaid onto a solid layer of 0.5% agar in DMEM supplemented with 10% foetal bovine serum. The culture was maintained for 4 weeks, the colonies were stained with INT solution (Sigma-Aldrich) and then counted.

Tumor progression *in vivo*: For *in vivo* assays, tumor cells were washed three times with PBS, scrapped without trypsination, and subcutaneously inoculated in the shaved area of the right flank of the mice. Tumor establishment was determined by palpation and tumor area was determined by measuring perpendicular tumor diameters.

CD4⁺CD25⁺ T cell purification and adoptive transfer in syngenic C57BL/6 mice: CD4⁺CD25⁺ cells were freshly isolated from spleens of C57BL/6 mice engrafted with 2×10^5 B16 cells 17 days before. Cells were purified by a two step procedure of negative and positive selections, using MACS magnetic beads (mouse regulatory T cell isolation kit, Miltenyi Biotec), according to the manufacturer's instructions. Fifty thousands purified lymphocytes were transferred intravenously into naive C57BL/6 mice. Recipient mice were challenged the same day with 2×10^5 control- or ERV^{KD}- B16 cells in the right flank.

RESULTS

1. Knocking down ERV does not modify the transformed phenotype of B16 melanoma cells.

An RNA interference approach was used based on stable vectors producing short double-stranded RNA (dsRNA) directed against the viral genome of the MelARV element and the irrelevant *gfp* gene as a control. The rationale of the

procedure and the structure of the plasmids used are illustrated in Figures 16A-16B. Figure 16C clearly shows that the ERV-specific dsRNA vector almost completely abolished ERV expression in the transduced B16 cells (ERV^{KD} B16 cells), with a >10-fold reduction in the amount of both the Env and Gag viral proteins as compared to the control transduced cells (control B16 cells). As a next step, the transformed phenotype of the ERV^{KD} and control B16 cells was assayed both *in vitro* and *in vivo*. *In vitro*, the anchorage-independent growth rate was measured after plating in semi-solid media (soft agar assay). As illustrated in Figure 17A, the ERV^{KD} B16 cell line gave rise to a similar number of colonies as the control B16 cells. *In vivo*, the growth rate of the two cell populations was analyzed after engrafting into X-irradiated or SCID mice. As illustrated in Figure 17B, both cell populations have a transformed phenotype, with similar growth rates. Altogether, these results show that knocking down the MelARV endogenous retrovirus has no effect on the transformed state of the melanoma cells.

2. Knocking down ERV inhibits B16 tumor cell growth *in vivo* and increases survival of immunocompetent hosts.

To investigate whether tumor cells may overwhelm the antitumor response *in vivo* through an ERV-dependent mechanism, the Inventors explored the impact of the knocking down of MelARV on tumor progression by injecting C57BL/6 immunocompetent mice with the control and the ERV^{KD} B16 cells. As illustrated in Figure 18A, growth of control B16 cells, as expected, led to large tumors in most of the animals, whereas the ERV^{KD} B16 cells yielded tumors of a limited size and in only a small number of engrafted mice. The difference in tumor cell growth is also clearly substantiated by the extent of animal survival (Figure 18B): as soon as day 70, 90% of the mice engrafted with the control B16 cells had been killed by their tumor, whereas 80% of mice engrafted with ERV^{KD} B16 cells were alive and tumor-free (and still so at day 130). In an attempt to identify the MelARV genes involved in the observed effects, an expression vector (lacking the dsRNA-targeted sequence) for the sole MelARV

env gene was introduced back into the ERV^{KD} B16 cells. The resulting double-transduced ERV^{KD}+*env* (or control) B16 cells were then engrafted into C57BL/6 mice. As illustrated in Figure 18C, this resulted in partial reversion of the knockdown effect, with already 50% of the mice engrafted with the Env-expressing cells dead by day 70. This reversion indicates that the *env* gene is - at least in part - responsible for tumor immune escape. The partial effect of the reversion is most likely explained by the lower expression (Figure 19) of the Env protein when expressed by the exogenous vector.

Along this line, it is of interest that a first series of experiments using synthetic siRNA targeted to MelARV, and injected intraperitoneously 12 days after engraftment of B16 cells into immunocompetent mice, actually resulted in a 1/3 inhibition of tumor growth as compared to mice injected with control siRNA (Figure 20A) and, as illustrated in the supplementary Figure 20B, in a reproducible increase in survival delay.

The present data demonstrate that tumors are able to overwhelm the immune system by expressing the envelope of an ERV and that blocking ERV expression resulted in enhanced tumor rejection.

It is noteworthy that in humans the expression of ERV *env* genes, mainly restricted to placenta and testis in normal tissues, can be observed in several tumor types such as seminomas and melanomas. Such HERV ENV proteins have been shown to be immunosuppressive. Therefore, inhibiting the expression or the activity of these ENV proteins is a promising approach to enhance immune response against ENV-expressing tumors. Such an inhibition of the activity of the tumoral ENV proteins could be performed, for instance, by an immune response elicited by a prophylactic or a therapeutic vaccination with mutated ENV proteins depleted of their immunosuppressive activity according to the invention or by compounds directly binding to tumoral ENV proteins.

CLAIMS

1. Polypeptide having a sequence of 7 to 20 amino acid residues, encoded by a nucleic acid derived from a viral *env* gene,
 which is capable of decreasing the immunosuppressive properties of a viral ENV protein or a fragment thereof, against the host in which it is expressed when it substitutes the homologous sequence of said viral protein or fragment,
 said polypeptide corresponding to a region of the transmembrane subunit of said ENV protein and comprising the minimum following consensus amino acid sequence:



wherein X_1 and X_2 are selected to impact on said immunosuppressive properties,
 and wherein

- i. X_1 is R, H or K, and X_2 is such that it ensures that the structure of the viral protein is conserved; or
- ii. X_1 is R, H or K and X_2 is F, W, Y or H,

and wherein Y_9 to Y_{12} represent variable amino acid residues.

2. Polypeptide according to claim 1, encoded by a nucleic acid derived from a retroviral *env* gene, which is capable of decreasing the immunosuppressive properties of a retroviral ENV protein or a fragment thereof, against the host in which it is expressed when it substitutes the homologous sequence of said retroviral protein or fragment.

3. Polypeptide according to claim 1 or 2, wherein:

- X_1 is R and X_2 is F; or
- X_1 is K, and X_2 is F.

4. Polypeptide according to claim 2 or 3, said retroviral *env* gene being the *env* gene of a virus, the virus being an endogenous retrovirus, a human endogenous retrovirus, a MoMLV, a Friend retrovirus (FV), FeLV, HTLV-1, HTLV-2, STLV-1 or MPMV.

5. Polypeptide according to claim 4, said endogenous retrovirus being HERV-FRD, HERV-T, HERV-R, HERV-V, HERV-R(b) or PERV.

6. Polypeptide according to any one of claims 1 to 5, comprising the following sequence R-G-G-L/T/I-C-A/K/L/M/V/I-F.

7. Polypeptide according to any one of claims 1 to 5, comprising the sequence:

KGGTCMF (SEQ ID NO: 24);
KGRTCLF (SEQ ID NO: 25);
KGGLCIF (SEQ ID NO: 26);
RGGTCLF (SEQ ID NO: 23);
RGGLCKF (SEQ ID NO: 27);
RGGLCAF (SEQ ID NO: 28);
RGGLCLF (SEQ ID NO: 29);
RGGICLF (SEQ ID NO: 30);
RGGLCVF (SEQ ID NO: 31); or
RGGTCVF (SEQ ID NO: 32).

8. Polypeptide according to any one of claims 1 to 7, having the consensus sequence:

$$Y_{13}Y_{14}NY_1Y_2Y_3LY_4Y_5LY_6Y_7Y_8X_1Y_9Y_{10}Y_{11}CY_{12}X_2,$$

wherein X_1 and X_2 are as defined in claim 1 and Y_1 to Y_{14} represent any amino acid.

9. Polypeptide according to any one of claims 1 to 8, having the sequence:

LQNRRLDLLTAEKGGLCIF (SEQ ID NO: 45);
MQNRRALDLLTADKGGTCMF (SEQ ID NO: 46);
AQNRQALDLLMAEKGRTCLF (SEQ ID NO: 47);
LQNRRALDLLTAERGRTCLF (SEQ ID NO: 40);
LQNRALDLLTAKRGGTCLF (SEQ ID NO: 41);
LQNRALDLLIAKRGGTCVF (SEQ ID NO: 42);
LQNRRLDLLTAERGRTCLF (SEQ ID NO: 43);
LQNRALDLLTAERGICLF (SEQ ID NO: 44);
AQNRRLDLLFWERGGLCKF (SEQ ID NO: 48);
LQNCRLDLLFLSRGGLCAF (SEQ ID NO: 49);
LQNRRLDMLTAARGGLCLF (SEQ ID NO: 50);
LQNRRLDLLTAERGICLF (SEQ ID NO: 51);
LQNRRLDILFLQRGGLCAF (SEQ ID NO: 52);
LQNRRLDLLFLKRGGLCF (SEQ ID NO: 53); and
LQNRRLDLLFLKRGGLCVF (SEQ ID NO: 54).

10. A mutated ENV protein resulting from the mutation of a wild type viral ENV protein, said wild-type viral ENV protein comprising in its transmembrane subunit the sequence $NY_1Y_2Y_3LY_4Y_5LY_6Y_7Y_8X_1Y_9Y_{10}Y_{11}CY_{12}X_2$,

wherein X_1 is E, K or Q and X_2 is A, V, L, I, or K and Y_1 to Y_{12} represent any amino acid,

wherein in the mutated ENV protein, X_1 is substituted by R or H, and wherein said mutated ENV protein has a decreased immunosuppressive activity with respect to the wild type ENV protein;

or

a fragment of the mutated ENV protein that is at least 7 amino acids long, provided that (a) said fragment carries X_1 as defined for the mutated ENV protein and X_2 as defined above, that (b) it has an immunosuppressive activity similar to that of the mutated ENV protein, and that (c) its antigenic structure is similar to the structure it adopts in the context of the mutated ENV protein;

or

a protein presenting at least 80% sequence identity to the mutated ENV protein, provided that (a) said protein carries X_1 as defined for the mutated ENV protein and X_2 as defined above, that (b) it has an immunosuppressive activity similar to that of the mutated ENV protein, and that (c) its antigenic structure is similar to that of the mutated ENV protein, or that of the fragment of the mutated ENV protein.

11. The mutated ENV protein according to claim 10, resulting from the mutation of a wild type viral ENV protein, said wild type viral protein being a wild type retroviral ENV protein.

12. The mutated ENV protein according to claim 10 or 11, resulting from the mutation of a wild type viral ENV protein, said wild type viral protein being a wild type ENV protein of a virus, the virus being an endogenous retrovirus, a human endogenous retrovirus, a MoMLV, a Friend retrovirus (FV), FeLV, HTLV-1, HTLV-2, STLV-1 or MPMV.

13. The mutated ENV protein according to claim 12, said endogenous retrovirus being HERV-FRD, HERV-T, HERV-R, HERV-V, HERV-R(b) or PERV.

14. The mutated ENV protein or fragment according to any one of claims 10 to 13, wherein amino acid X_2 is substituted by F, M, Y or W.

15. The mutated ENV protein or fragment thereof, according to any one of claims 10 to 14, wherein the ENV protein is:

HERV-FRD ENV (SEQ ID NO: 82), wherein X_1 is Q427 and X_2 is A433;

HERV-T ENV (SEQ ID NO: 84), wherein X_1 is Q516 and X_2 is A522;

HERV-R ENV (SEQ ID NO: 86), wherein X_1 is E561 and X_2 is K567;
HERV-V ENV (SEQ ID NO: 88), wherein X_1 is Q381 and X_2 is V387; or
HERV-R(b) ENV (SEQ ID NO: 90), wherein X_1 is E391 and X_2 is L397.

16. The mutated ENV protein or fragment according to claim 15, wherein the ENV protein is HERV-FRD ENV and the sequence of the mutated ENV protein is SEQ ID NO: 120 or SEQ ID NO: 122.

17. The mutated ENV protein or fragment according to claim 15, wherein the ENV protein is HERV-V ENV and the sequence of the mutated ENV protein is SEQ ID NO: 124 or SEQ ID NO: 126.

18. The mutated ENV protein or fragment according to claim 15, wherein the ENV protein is HERV-T ENV and the sequence of the mutated ENV protein is SEQ ID NO: 128 or SEQ ID NO: 130.

19. The mutated ENV protein or fragment according to claim 15, wherein the ENV protein is HERV-R ENV and the sequence of the mutated ENV protein is SEQ ID NO: 146 or SEQ ID NO: 148.

20. The mutated ENV protein or fragment according to any one of claims 10 to 14, wherein the ENV protein is:

HTLV-1 ENV (SEQ ID NO: 92), wherein X_1 is Q389 and X_2 is A395;
HTLV-2 ENV (SEQ ID NO: 94) wherein X_1 is Q385 and X_2 is A391;
FeLV ENV (SEQ ID NO: 96), wherein X_1 is E527 and X_2 is A533;
PERV ENV (SEQ ID NO: 98), wherein X_1 is E545 and X_2 is A551;
STLV-1 ENV (SEQ ID NO: 100), wherein X_1 is Q389 and X_2 is A395;
MoMLV ENV (SEQ ID NO: 70), wherein X_1 is E551 and X_2 is A557;
MPMV ENV (SEQ ID NO: 72), wherein X_1 is Q471 and X_2 is A477; or
FV ENV (SEQ ID NO: 102), wherein X_1 is E561 and X_2 is A567.

21. The mutated ENV protein or fragment according to claim 20, wherein the ENV protein is FeLV ENV and the sequence of the mutated ENV protein is SEQ ID NO: 104 or SEQ ID NO: 106.

22. The mutated ENV protein or fragment according to claim 20, wherein the ENV protein is HTLV-1 ENV and the sequence of the mutated ENV protein is SEQ ID NO: 108 or SEQ ID NO: 110.

23. The mutated ENV protein or fragment according to claim 20, wherein the ENV protein is HTLV-2 ENV and the sequence of the mutated ENV protein is SEQ ID NO: 112 or SEQ ID NO: 114.

24. The mutated ENV protein or fragment according to claim 20, wherein the ENV protein is PERV ENV and the sequence of the mutated ENV protein is SEQ ID NO: 150 or SEQ ID NO: 152.

25. A nucleic acid coding for a polypeptide as defined in any one of claims 1 to 9 or for a mutated ENV protein or fragment as defined in any one of claims 10 to 24.

26. The nucleic acid according to claim 25, characterized in that it is as set forth in SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 115, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 121, SEQ ID NO: 123, SEQ ID NO: 125, SEQ ID NO: 127, SEQ ID NO: 129, SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149, or SEQ ID NO: 151.

27. An eukaryotic or a prokaryotic expression vector, characterized in that it comprises a nucleic acid as defined in claim 25 or 26 as well as the elements necessary for the expression of said nucleic acid.

28. The eukaryotic or prokaryotic expression vector according to claim 27, wherein said vector is a viral vector.

29. The eukaryotic or prokaryotic expression vector according to claim 28, said viral vector being a pox vector, a modified vaccinia virus Ankara (MVA) vector, an adenoviral vector, a measles vector, or a cytomegalovirus (CMV) vector.

30. The eukaryotic or prokaryotic expression vector according to claim 29, said pox vector being a fowlpox or a canarypox.
31. The eukaryotic or prokaryotic expression vector according to any one of claims 27 to 30, comprising a mutated FeLV ENV as set forth in SEQ ID NO: 103 or SEQ ID NO: 105.
32. The eukaryotic or prokaryotic expression vector according to any one of claims 28 to 31, further comprising a nucleic acid coding for a GAG protein originating from the same virus as said mutated ENV.
33. The eukaryotic or prokaryotic expression vector according to claim 31 or 32, said vector being a pox vector.
34. The eukaryotic or prokaryotic expression vector according to claim 33, said vector comprising a nucleic acid coding for a GAG protein originating from FeLV.
35. A recombinant cell, characterized in that it comprises a nucleic acid as defined in claim 25 or 26, or an eukaryotic or prokaryotic expression vector as defined in any one of claims 27 to 34.
36. A pharmaceutical or a vaccine composition comprising:
- i) at least one polypeptide as defined in any one of claims 1 to 9;
 - ii) at least one mutated ENV protein or fragment as defined in any one of claims 10 to 24;
 - iii) at least one nucleic acid as defined in claim 25 or 26;
 - iv) at least one prokaryotic or eukaryotic expression vector as defined in any one of claims 27 to 34; or
 - v) at least one recombinant cell as defined in claim 35,
- in association with a pharmaceutically acceptable carrier.
37. Use of at least one protein comprising or consisting of a mutated ENV protein or fragment as defined in any one of claims 10 to 24, or of a nucleic acid coding for said protein or fragment, for the manufacture of a medicament or a vaccine for the prevention or the treatment of a viral disease.

38. The use according to claim 37, wherein said viral disease is a HTLV or FeLV infection.

39. Use of at least one protein comprising or consisting of a mutated ENV protein or fragment as defined in any one of claims 16 to 20, or of a nucleic acid coding for said protein or fragment, for the manufacture of a medicament or a vaccine for the prevention or the treatment of cancer.

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phCMV-envMoMLV -> Graphic Map

DNA sequence 6859 bp GCGGCCGCTCTA ... TCCACCGCGGTG circular

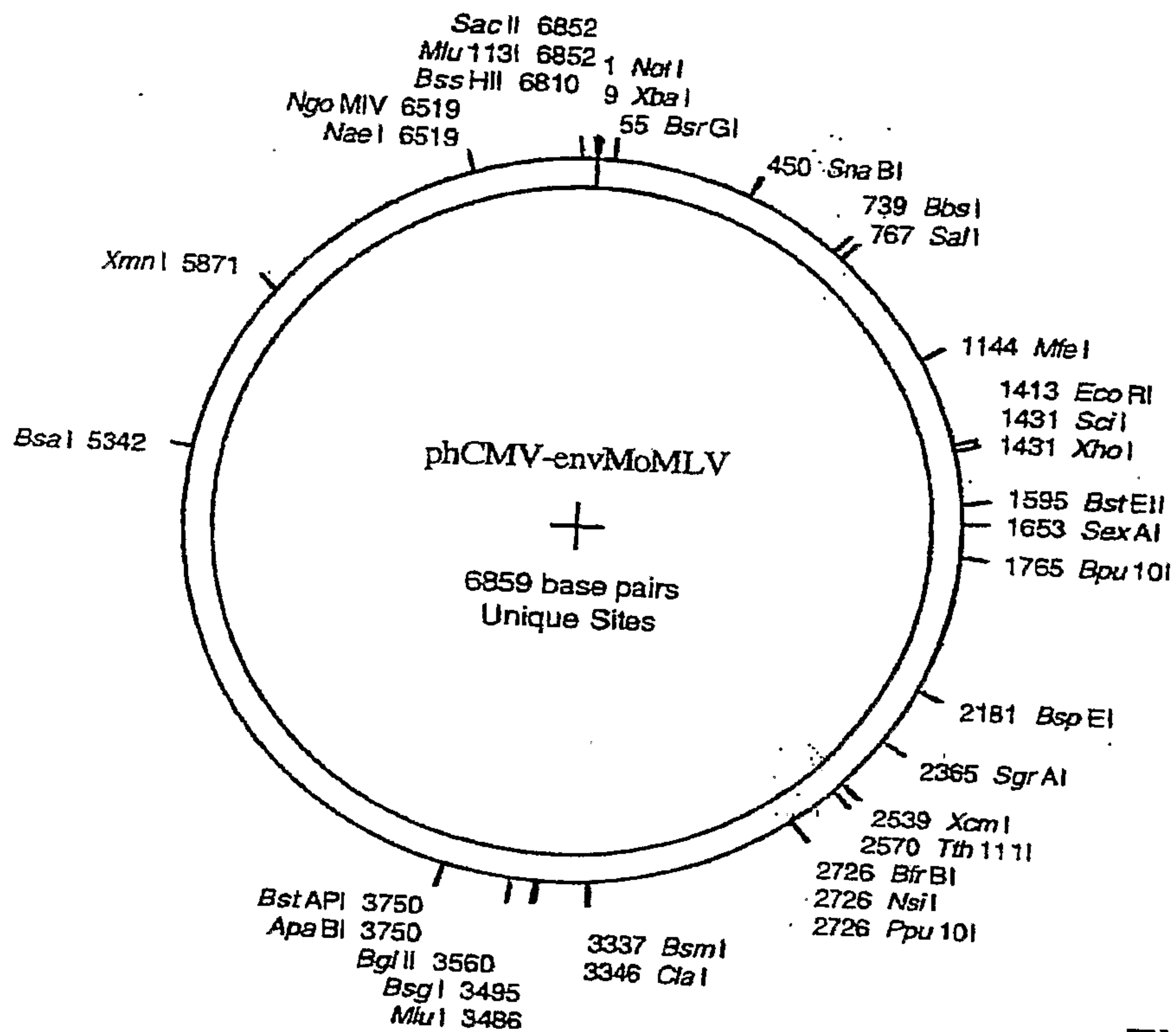


FIG 1A

pDFG-envMoMLV-iresHygro -> Graphic Map

DNA sequence 11787 bp GGATTAGTCCAA ... ACCGGATCCGCG circular

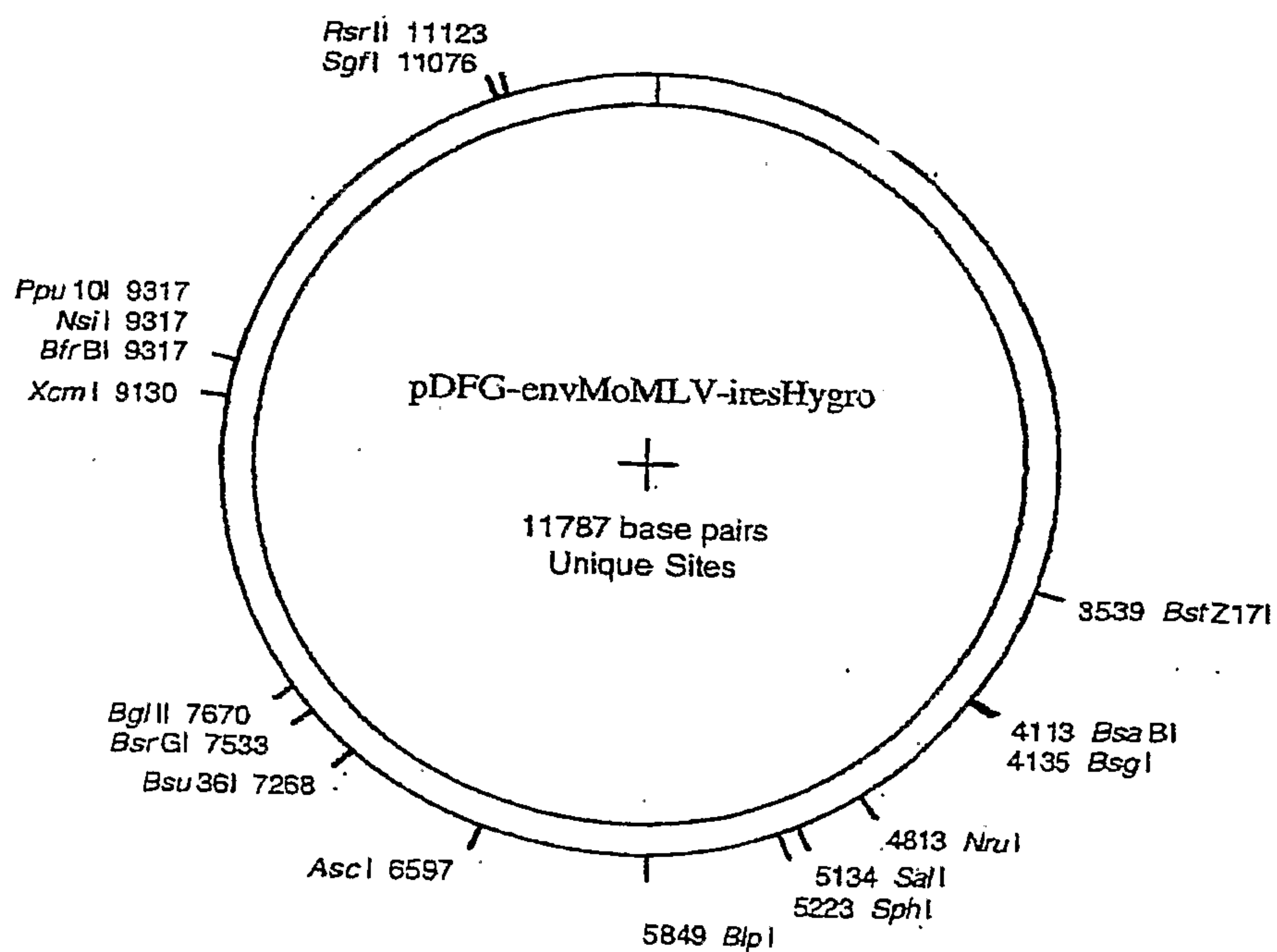
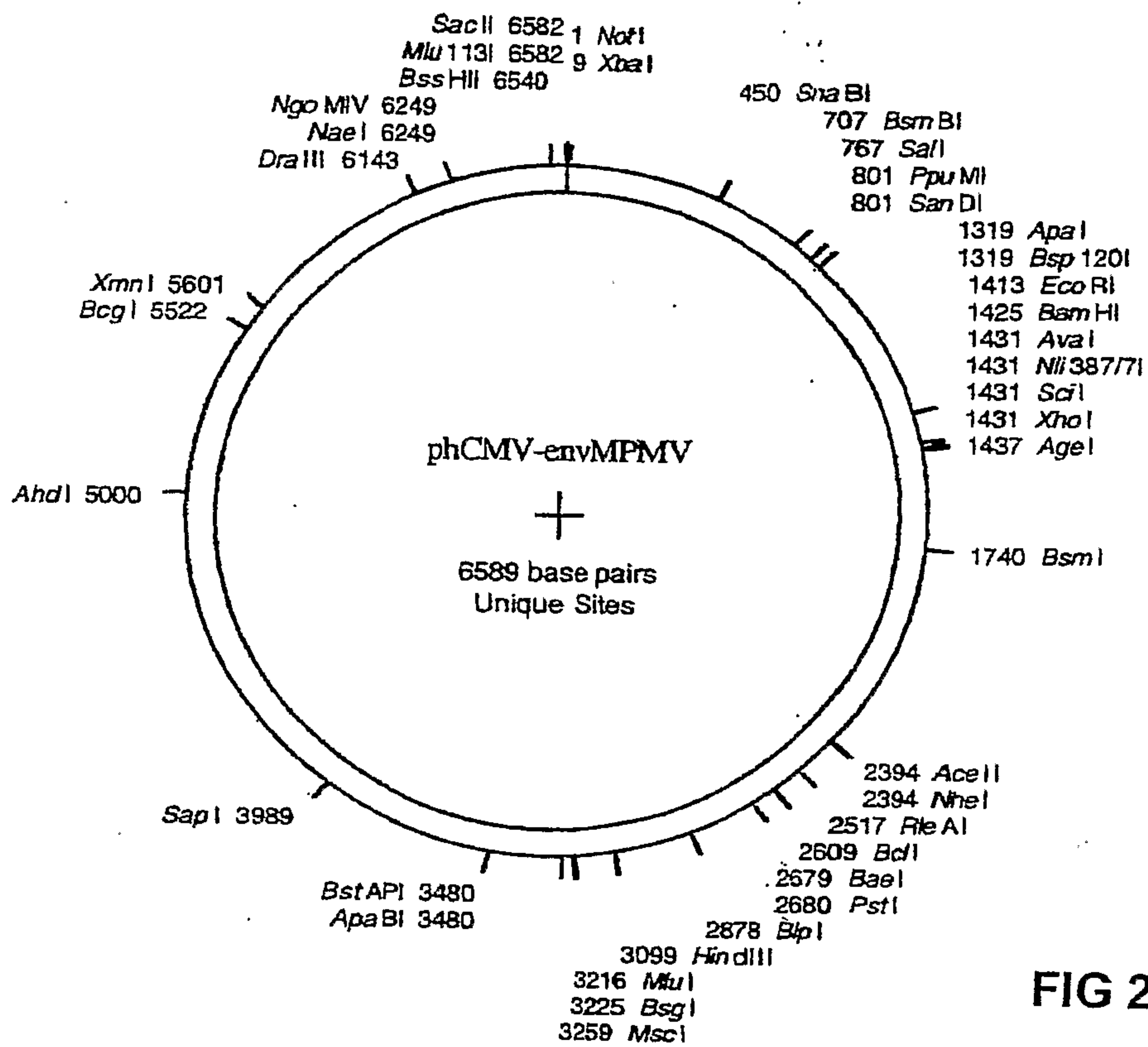


FIG 1B

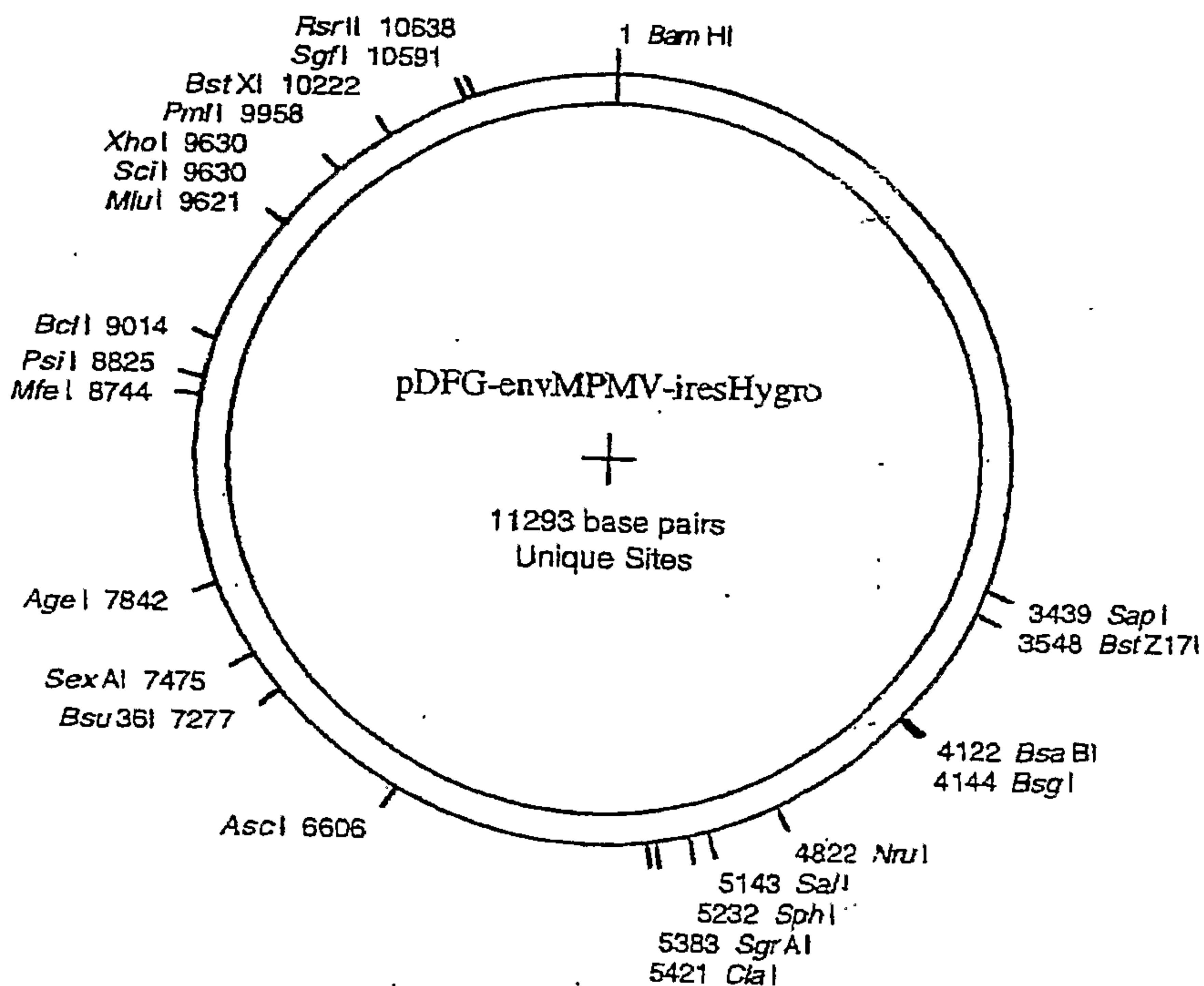
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phCMV-envMPMV -> Graphic Map

DNA sequence 6589 bp GCGGCCGCTCTA ... TCCACCGGGTC circular

**FIG 2A****pDFG-envMPMV-iresHygro -> Graphic Map**

DNA sequence 11293 bp GGATCCCGGGGA ... GGACACAATACC circular

**FIG 2B**

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phCMV-EnvW -> Graphic Map

DNA sequence 6570 bp GCGGCCGCTCTA ... TCCACCCGCGTG circular

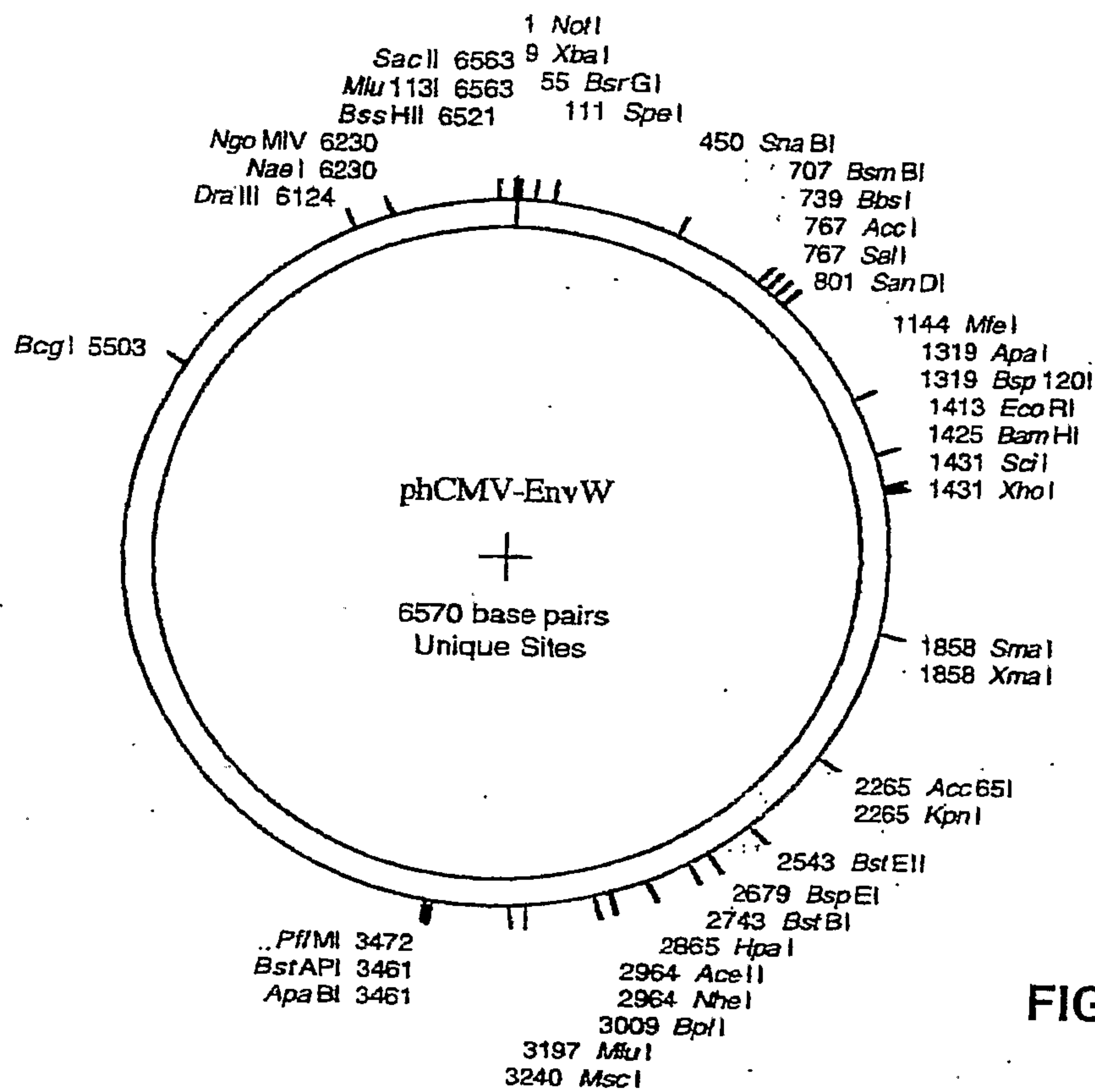


FIG 3A

pDFG-envW-iresHygro -> Graphic Map

DNA sequence 11383 bp GGATCCGCGGCA ... GGAGACAATACC circular

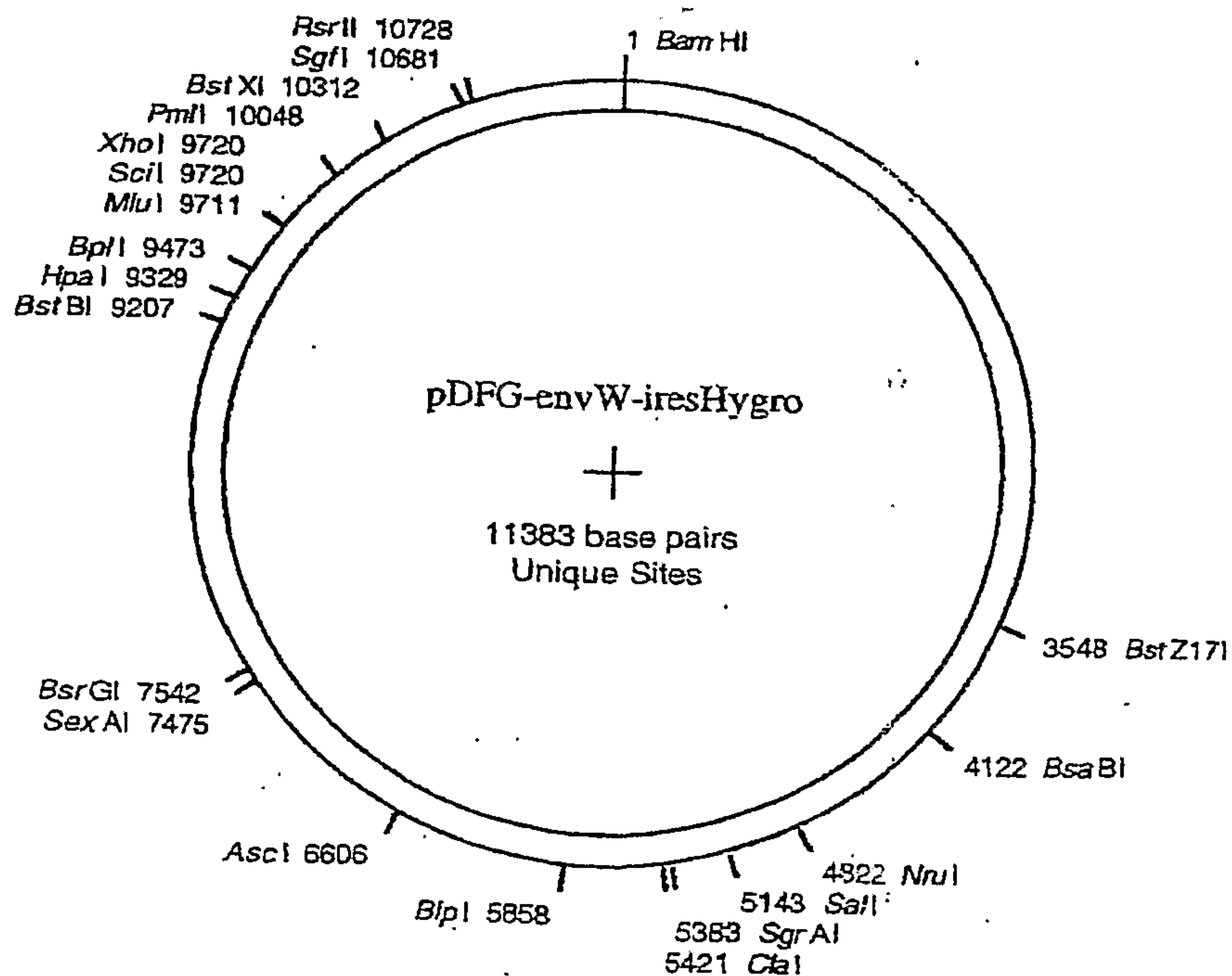


FIG 3B

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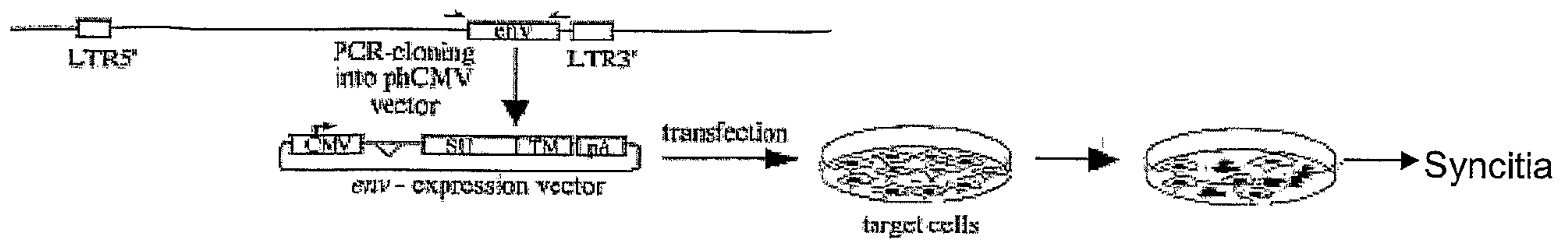


Figure 4

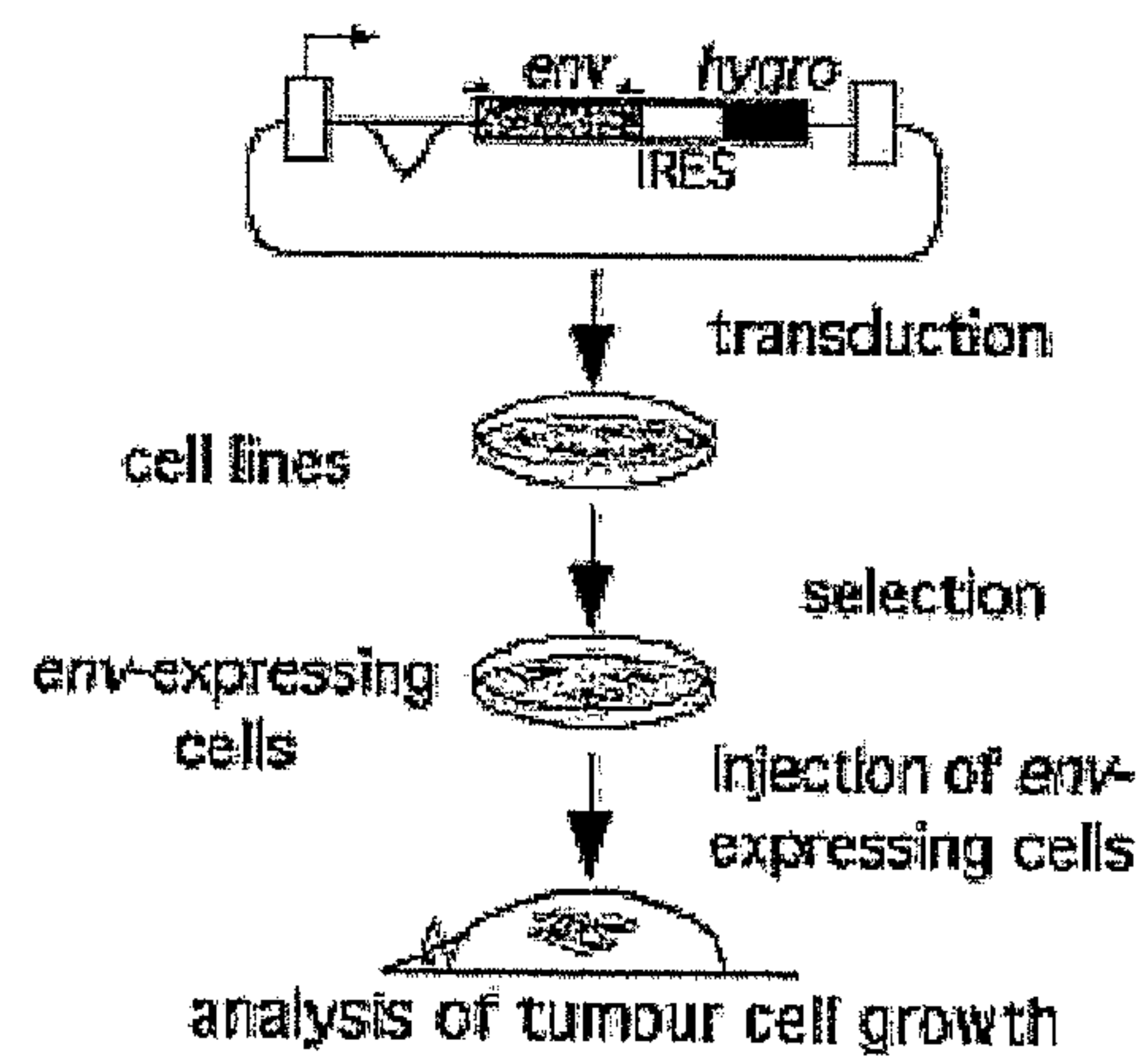


Figure 5

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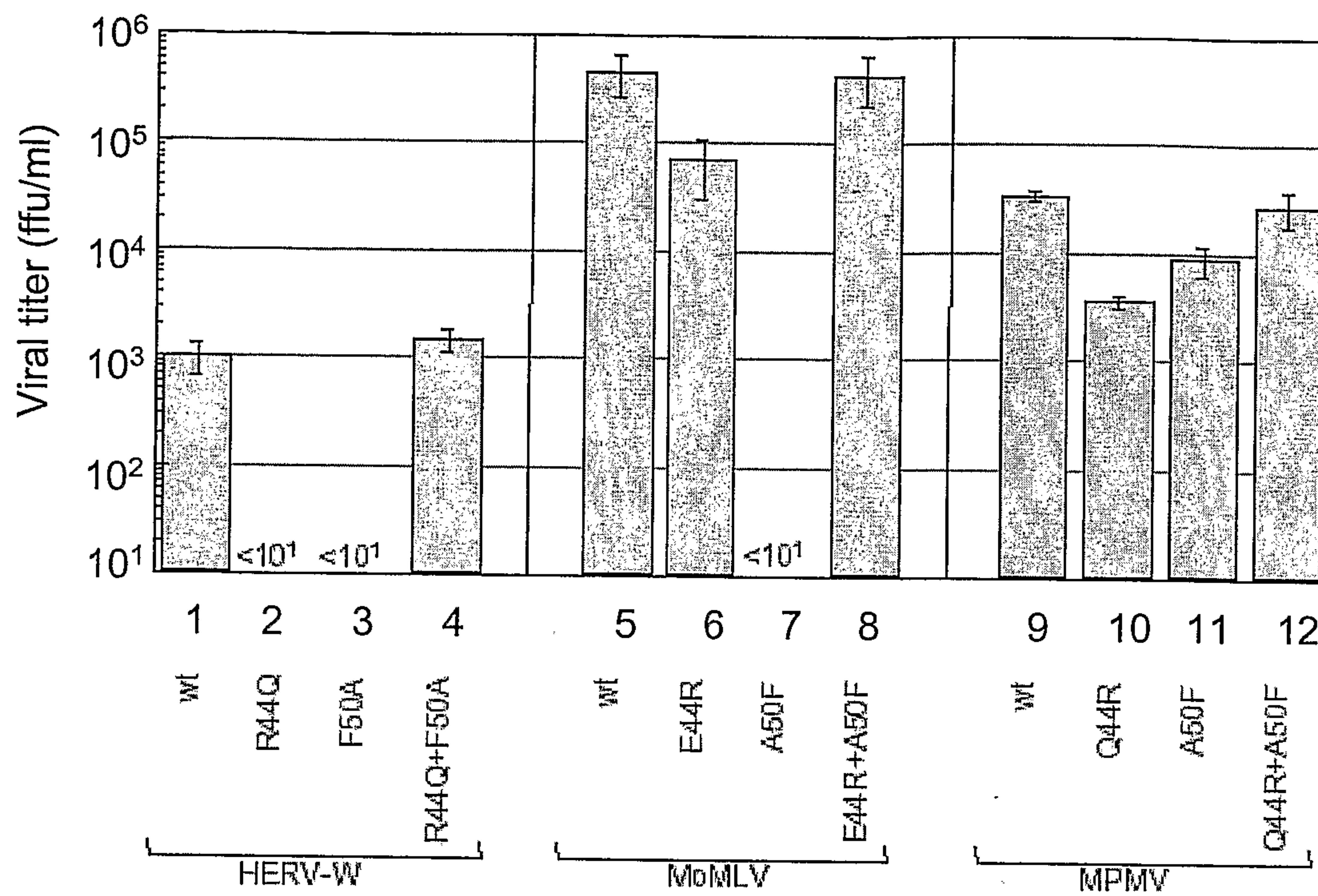


Figure 6

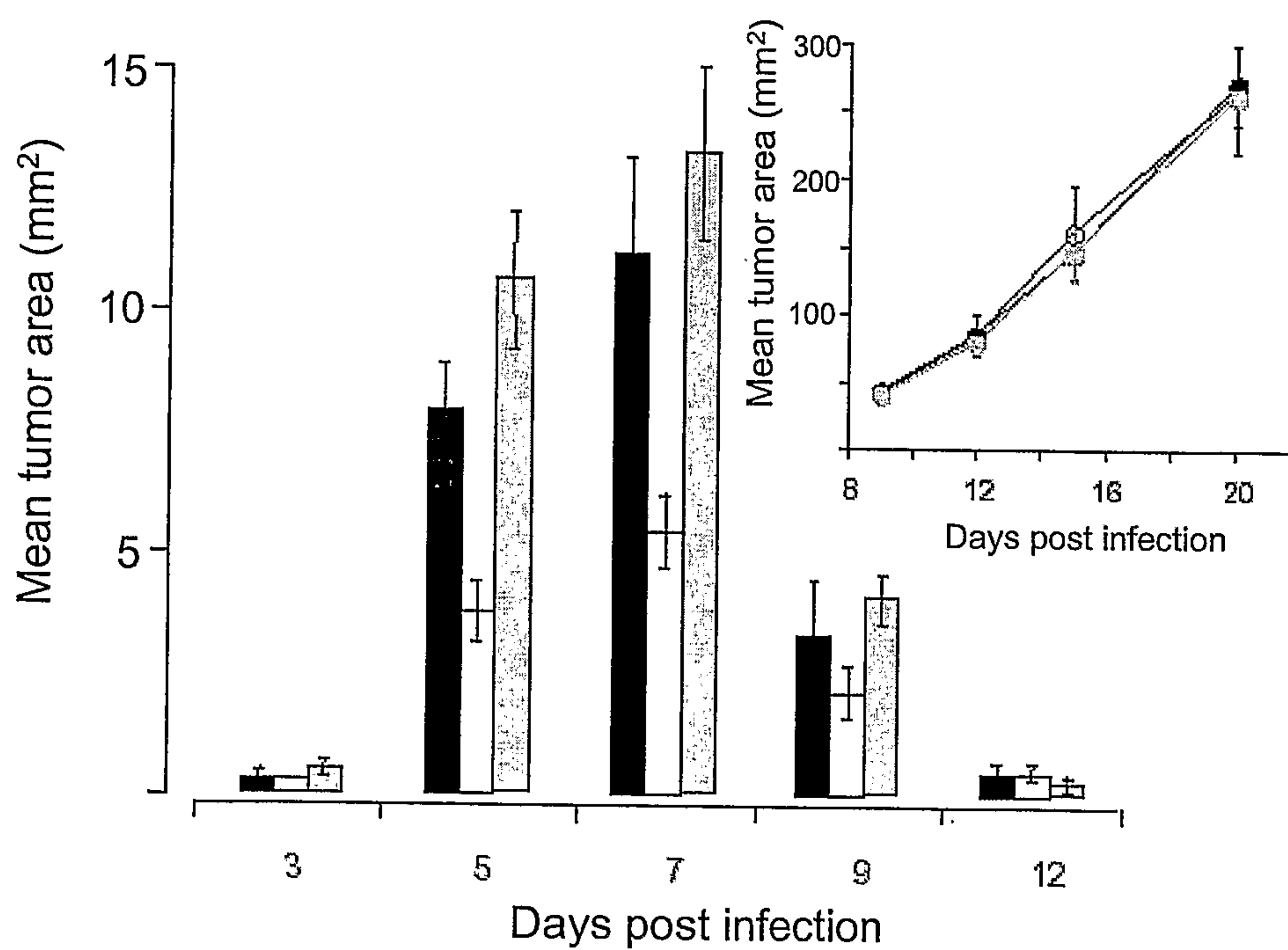


Figure 7

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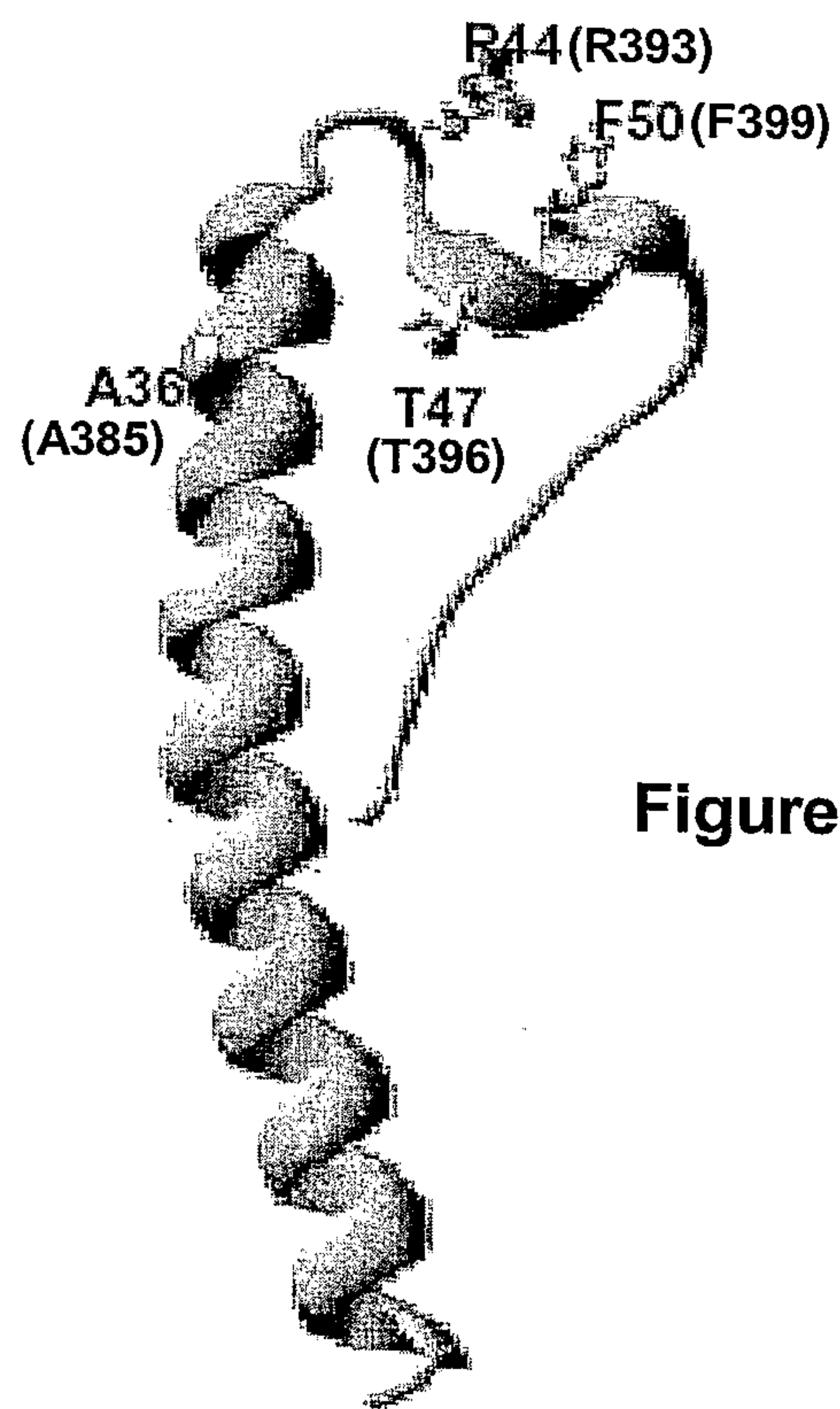


Figure 8

Name	Origin	Nucleotide sequence	Accession Number
HERV-T	Human endogenous	LQNCRCLDLLFLSQGGGLCAA	AC 078 899
HTLV-1	Human retrovirus	AQNRRGLDLLFWEQGGGLCKA	AY 324 800
STLV-1	Simian retrovirus	AQNRRGLDLLFWEQGGGLCKA	AY 324 800
HERV-FRD	Human endogenous	LQNRRGLDMLTAAQGGGLCLA	AL 136 139
MPMV	Human retrovirus	LQNRRGLDLLTAEQGGICLA	AF 033 815
Fowlpox	Poxvirus	LQNRRGLDLLTAEQGGICLA	NC 002 188
MoMLV	Human retrovirus	LQNRRGLDLLFLKEGGGLCAA	AF 033 811
Friend	Human retrovirus	LQNRRGLDLLFLKEGGGLCAA	M 90 673
PyERV	python endogenous	LQNRRGLDLLFLKEGGGLCVA	AF 500 297
PERV	porcine endogenous	LQNRRGLDLLFLKEGGGLCVA	AY 364 236
FeLV	feline leukaemia virus	LQNRRGLDILFLQEGGLCAA	M 18248
SSAV	Simian Sarcoma-associated virus	LQNRRGLDLLFLKEGGGLCAA	J 02396
GLV-X	Gibbon leukemia virus X	LQNRRGLDLLFLKEGGGLCAA	U 60065
EBOLA	Filovirus	ILNRKAIDFLLQRWGGTCHI	EVU 31033
Marburg	Filovirus	LINRHAIDFLLTRWGGTCKV	MVVIRPR
HERV-W	Human endogenous	LQNRRALDLLTAERGGTCLF	AF 072 503
HERV-W	Human endogenous	LQNWRALDLLTAKRGGTCLF	
HERV-W	Human endogenous	LQNWRALDLLIAKRGGTCVF	
HERV-H1	Human endogenous	LQNRRGLDLLTAEKGGLCIF	AJ 289 709
HERV-F(c)1	Human endogenous	MQNRRALDLLTADKGGTCMF	AL 354 685
HERV-F(c)2	Human endogenous	AQNRRQALDLLMAEKGRTCLE	AC 016 222

Figure 9

Figure 10**A. Nucleotide sequence encoding envMoMLV**

ATGGCGCGTTCAACGCTCTCAAAACCCCTTAAAAATAAGGTTAACCCGCG
AGGCCCCCTAATCCCCTTAATTCTTCTGATGCTCAGAGGGGTCAGTACTG
CTTCGCCCCGGCTCCAGTCCTCATCAAGTCTATAATATCACCTGGGAGGTA
ACCAATGGAGATCGGGAGACGGTATGGGCAACTTCTGGCAACCACCCTCT
GTGGACCTGGTGGCCTGACCTTACCCAGATTTATGTATGTTAGCCCACC
ATGGACCATCTTATTGGGGGCTAGAATATCAATCCCCTTTTTCTTCTCCC
CCGGGGCCCCCTTGTTGCTCAGGGGGCAGCAGCCCAGGCTGTTCCAGAGA
CTGCGAAGAACCTTTAACCTCCCTCACCCCTCGGTGCAACACTGCCTGGA
ACAGACTCAAGCTAGACCAGACAACCTCATAAATCAAATGAGGGATTTTAT
GTTTGCCCCGGGCCCCACCGCCCCCGAGAATCCAAGTCATGTGGGGGTCC
AGACTCCTTCTACTGTGCCTATTGGGGCTGTGAGACAACCGGTAGAGCTT
ACTGGAAGCCCTCCTCATCATGCGGATTTTCATCACAGTAAACAACAATCTC
ACCTCTGACCAGGCTGTCCAGGTATGCAAAGATAATAAGTGGTGCAACCC
CTTAGTTATTTCGGTTTACAGACGCCGGGAGACGGGTACTTCTTGGACCA
CAGGACATTACTGGGGCTTACGTTTGTATGTCTCCGGACAAGATCCAGGG
CTTACATTTGGGATCCGACTCAGATACCAAAATCTAGGACCCCGCGTCCC
AATAGGGCCAAACCCCGTTCTGGCAGACCAACAGCCACTCTCCAAGCCCA
AACCTGTTAAGTCGCCTTCAGTCACCAAACCACCCAGTGGGACTCCTCTC
TCCCCTACCCAACCTTCCACCGGCGGGAACGGAAAATAGGCTGCTAAACTT
AGTAGACGGAGCCTACCAAGCCCTCAACCTCACCAGTCCTGACAAAACCC
AAGAGTGCTGGTTGTGTCTAGTAGCGGGACCCCCCTACTACGAAGGGGTT
GCCGTCTTGGGTACCTACTCCAACCATACTCTGCTCCAGCCAACCTGCTC
CGTGGCCTCCCAACACAAGTTGACCCTGTCCGAAGTGACCGGACAGGGAC
TCTGCATAGGAGCAGTTCCCAAAACACATCAGGCCCTATGTAATACCACC
CAGACAAGCAGTCGAGGGTCTTATTATCTAGTTGCCCCCTACAGGTACCAT
GTGGGCTTGTAGTACCGGGCTTACTCCATGCATCTCCACCACCATACTGA
ACCTTACCACTGATTATTGTGTTCTTGTGCAACTCTGGCCAAGAGTCACC
TATCATTTCCCCCAGCTATGTTTACGGCCTGTTTGAGAGATCCAACCGACA
CAAAAGAGAACCGGTGTCGTTAACCCCTGGCCCTATTATTGGGTGGACTAA
CCATGGGGGGGAATTGCCGCTGGAATAGGAACAGGGACTACTGCTCTAATG
GCCACTCAGCAATTCCAGCAGCTCCAAGCCGCAGTACAGGATGATCTCAG
GGAGGTTGAAAAATCAATCTCTAACCTAGAAAAGTCTCTCACTTCCCTGT
CTGAAGTTGTCCTACAGAATCGAAGGGGCCTAGACTTGTTATTTCTAAAA
GAAGGAGGGCTGTGTGCTGCTCTAAAAGAAGAATGTTGCTTCTATGCGGA
CCACACAGGACTAGTGAGAGACAGCATGGCCAAATTGAGAGAGAGGCTTA
ATCAGAGACAGAACTGTTTGTAGTCAACTCAAGGATGGTTTGTAGGGACTG
TTTAACAGATCCCCTTGGTTTACCACCTTGATATCTACCATTTATGGGACC
CCTCATTTGTACTCCTAATGATTTTGCTCTTCGGACCCTGCATTCTTAATC
GATTAGTCCAATTTGTTAAAGACAGGATATCAGTGGTCCAGGCTCTAGTT
TTGACTCAACAATATCACCCAGCTGAAGCCTATAGAGTACGAGCCATAG

B. Protein sequence of envMoMLV

MARSTLSKPLKNKVNPRGPLIPLILLMLRGVSTASPGSSPHQVYNITWEV
TNGDRETVWATSGNHPLWTWWPDLTPDLCMLAHHGPSYWGLEYQSPFSSP
PGPPCCSGGSSPGCSRDC EEPLTSLTPRCNTAWNRLKLDQTTHKSNEGFY

VCPGPHRPRESKSCGGPDSFYCAYWGCETTGRAYWKPSSSWDFITVNNNL
TSDQAVQVCKDNKWCNPLVIRFTDAGRRTSWTTGHWGLRLYVSGQDPG
LTFGIRLRYQNLGPRVPIGPNPVLADQQPLSKPKPVKSPSVTKPPSGTPL
SPTQLPPAGTENRLLNLVDGAYQALNLTSPDKTQECWLCLVAGPPYYEGV
AVLGTYSNHTSAPANCSVASQHKLTLESEVTGQGLCIGAVPKTHQALCNTT
QTSSRGSYYLVAPTGTMWACSTGLTPCISTTILNLTTDYCVLVELWPRVT
YHSPSYVYGLFERSNRHKREPVSLTLALLLGGLTMGGIAAGIGTGTTALM
ATQQFQQLQAAVQDDLREVEKSI SNLEKSLTSLSEVVLQNRRLDLLFLK
EGGLCAALKEECCFYADHTGLVRDSMAKLRERLNQRQKLFESTQGWFEGL
FNRSPWFTTLISTIMGPLIVLLMILLFGPCILNRLVQFVKDRISVVQALV
LTQQYHQLKPIEYEP*

C. Nucleotide sequence encoding envMPMV

ATGAACTTCAATTATCATTTTCATCTGGAGCTTAGTGATACTATCTCAAAT
ATCTCAAGTTCAAGCCGGTTTTGGAGATCCGCGTGAAGCCCTGGCAGAAA
TACAACAAAAACATGGTAAACCTTGTGACTGTGCTGGAGGATATGTTTCC
TCCCCACCGATTAACTCTCTTACAACCTGTTTCTTGCTCTACTCATACTGC
TTATTCAGTGACAACTCCCTAAAATGGCAGTGTGTGTCAACTCCCACTA
CCCCTAGCAATACACATATAGGAAGTTGTCCCGGTGAATGCAACACGATC
TCATATGATTCTGTACATGCCTCTTGCTATAACCACTATCAACAATGTAA
CATTGGTAATAAAACATATCTCACTGCCACTATAACTGGAGATAGAACTC
CTGCCATTGGTGACGGGAATGTCCCTACAGTACTAGGGACTAGTCACAAC
CTCATTACAGCAGGCTGTCCCAATGGTAAAAAGGGCCAAGTGGTCTGTTG
GAATAGCCGACCTTCTGTTCAATATCTGATGGAGGAGGGCCTCAAGATA
AGGCCCGCGACATTATAGTAAATAAAAAGTTTGAGGAATTGCACAGGTCG
CTGTTCCCGAAGCTTTCTTACCATCCTCTGGCCTTGCCCGAAGCCCGTGG
TAAAGAAAAAATTGACGCACACACTCTTGATCTCCTTGCCACTGTACATA
GTTTACTCAATGCTTCCCAACCCAGTTTAGCCGAAGATTGCTGGCTGTGC
TTACAGTCAGGAGATCCCGTTCCTCTTGCCCTGCCCCTATAATGATACACT
CTGCTCTAACTTTGCCTGTTTATCTAATCACTCCTGCCCTTTAACCCECCC
CTTTTTTTAGTACAGCCCTTTAACTTCACTGATTCCAATTGCCTTTACGCT
CATTATCAAAACAACCTCATTTGACATAGATGTAGGTCTAGCTAGCTTTAC
TAATTGCTCTAGCTATTATAACGTTTCTACAGCCTCCAAACCCTCTAATT
CCCTATGCGCCCCAAACAGCTCGGTTTTTGTATGCGGTAACAATAAGGCA
TACACTTATCTACCCACAAATTGGACGGGAAGTTGTGTACTTGCTACTCT
TTTGCCCGATATAGACATCATTCAGGTAGTGAGCCTGTCCCCATTCCAG
CTATTGATCATTTTTTTAGGCAAGGCCAAAAGAGCAATCCAACCTTATCCCC
CTGTTCTGATAGGGTTAGGTATAACTACTGCAGTATCTACTGGGGCTGCTGG
TCTAGGGGTTTCCATCACTCAATATACAAAATTATCTCATCAACTAATAT
CAGATGTTCAAGCTATTTCTAGCACTATAACAAGATCTCCAAGATCAGGTA
GACTCTCTAGCAGAAGTAGTACTGCAAAACAGAAGAGGATTAGATCTACT
TACAGCAGAGCAGGGAGGTATCTGCTTAGCCTTACAGGAAAAATGTTGTT
TCTACGCCAATAAATCTGGAATCGTCAGAGACAAGATTAAAAACCTACAA
GACGACTTAGAAAGACGCCGAAGACAACCTGATCGACAACCCATTTTGGAC
CAGTTTTTCATGGATTCTCCCTTATGTTATGCCCTATTAGGCCCTTTGC
TTTGCTTATTGCTTGTGTTATCTTTCGGTCCAATTATTTTCAACAAGCTT
ATGACCTTTATTAAACATCAAATTGAGAGCATCCAGGCCAAACCTATACA
AGTCCATTATCATCGCCTTGAACAAGAAGACAGTGGTGGCTCATATTTGA
CCTTAACATAG

D. Protein sequence of envMPMV

MNFNYHFIWSLVILSQISQVQAGFGDPREALAEIQQKHGKPCDCAGGYVS
SPPINSLTTVSCSTHTAYSVTNSLKWQCVSTPTTPSNTHIGSCPGECONTI
SYDSVHASCYNHYYQQCNIGNKTYLTATITGDRTPAIGDGNVPTVLGTSHN
LITAGCPNGKKGQVVCWNSRPSVHISDGGGPQDKARDIIVNKKFEELHRS
LFPELSYHPLALPEARKEKIDAHTLDLLATVHSLLNASQPSLAEDCWLC
LQSGDPVPLALPYNDTLCNFACLSNHSCPLTPPFLVQPFNFTDSNCLYA
HYQNNSTFDIDVGLASFNCSSYYNVSTASKPSNSLCAPNSSVFVCGNNKA
YTYLPTNWTGSCVLATLLPDIDIIPGSEVPPIPAIDHFLGKAKRAIQLIIP
LFVGLGITTAVSTGAAGLGVSIQYTKLSHQLISDVQAISSTIQDLQDQV
DSLAEVVLQNRRLDLLTAEQGGICLALQEKCCFYANKSGIVRDKIKNLQ
DDLERRRRQLIDNPFWTSFHGFLPYVMPLLGPLLCLLLVLSFGPIIFNKL
MTFIKHQIESIQAKPIQVHYHRLEQEDSGGSYLTLT*

E. Nucleotide sequence encoding envHERV-W (envW)

ATGGCCCTCCCTTATCATATTTTTCTCTTTACTGTTCTTTTACCCTCTTT
CACTCTCACTGCACCCCCTCCATGCCGCTGTATGACCAGTAGCTCCCCTT
ACCAAGAGTTTCTATGGAGAATGCAGCGTCCCGGAAATATTGATGCCCCA
TCGTATAGGAGTCTTTCTAAGGGAACCCCCACCTTCACTGCCACACCCA
TATGCCCCGCAACTGCTATCACTCTGCCACTCTTTGCATGCATGCAAATA
CTCATTATTGGACAGGAAAAATGATTAATCCTAGTTGTCCTGGAGGACTT
GGAGTCACTGTCTGTTGGACTTACTTCACCCAAACTGGTATGTCTGATGG
GGGTGGAGTTCAAGATCAGGCAAGAGAAAAACATGTAAAAGAAGTAATCT
CCCAACTCACCCGGGTACATGGCACCTCTAGCCCCCTACAAAGGACTAGAT
CTCTCAAACTACATGAAACCTCCGTACCCATACTCGCCTGGTAAGCCT
ATTTAATACCACCCTCACTGGGCTCCATGAGGTCTCGGCCCAAACCCCTA
CTAACTGTTGGATATGCCTCCCCCTGAACTTCAGGCCATATGTTTCAATC
CCTGTACCTGAACAATGGAACAACCTCAGCACAGAAATAAACACCACTTC
CGTTTTAGTAGGACCTCTTGTTTCCAATCTGGAAATAACCCATACCTCAA
ACCTCACCTGTGTAAAATTTAGCAATACTACATACACAACCAACTCCCAA
TGCATCAGGTGGGTAACTCCTCCACACAAATAGTCTGCCTACCCTCAGG
AATATTTTTTGTCTGTGGTACCTCAGCCTATCGTTGTTTGAATGGCTCTT
CAGAATCTATGTGCTTCCTCTCATTTCTTAGTGCCCCCTATGACCATCTAC
ACTGAACAAGATTTATACAGTTATGTCATATCTAAGCCCCGCAACAAAAG
AGTACCCATTCTTCCTTTTGTATAGGAGCAGGAGTGCTAGGTGCACTAG
GTACTGGCATTGGCGGTATCACAACCTCTACTCAGTTCTACTACAAACTA
TCTCAAGAACTAAATGGGGACATGGAACGGGTGCGCGACTCCCTGGTCAC
CTTGCAAGATCAACTTAACTCCCTAGCAGCAGTAGTCCTTCAAAATCGAA
GAGCTTTAGACTTGCTAACCGCTGAAAGAGGGGGAACCTGTTTATTTTAA
GGGGAAGAATGCTGTTATATGTTAATCAATCCGGAATCGTCACTGAGAA
AGTTAAAGAAATTCGAGATCGAATACAACGTAGAGCAGAGGAGCTTCGAA
ACACTGGACCCTGGGGCCTCCTCAGCCAATGGATGCCCTGGATTCTCCCC
TTCTTAGGACCTCTAGCAGCTATAATATTGCTACTCCTCTTTGGACCCTG
TATCTTTAACCTCCTTGTTAACTTTGTCTCTTCCAGAATCGAAGCTGTAA
AACTACAAATGGAGCCCAAGATGCAGTCCAAGACTAAGATCTACCGCAGA
CCCCTGGACCGGCCTGCTAGCCACGATCTGATGTTAATGACATCAAAGG
CACCCCTCCTGAGGAAATCTCAGCTGCACAACCTCTACTACGCCCCAATT

CAGCAGGAAGCAGTTAG

F. Protein sequence of envHERV-W (envW)

MALPYHIFLFTVLLPSFTLTAPPPCRCMTSSSPYQEFLWRMQRPGNIDAP
SYRSLSKGTPTFTAHTHMPRNCYHSATLCMHANTHYWTGKMINPSCPGGL
GVTVCWTYFTQTGMSDGGGVQDQAREKHVKEVISQLTRVHGTSSPYKGLD
LSKLHETLRTHTRLVSLFNTTTLTGLHEVSAQNPTNCWICLPLNFRPYVSI
PVPEQWNNFSTEINTTSVLVGPLVSNLEITHTSNLTCVKFSNTTYTTNSQ
CIRWVTPPTQIVCLPSGIFVCGTSAYRCLNGSSESMCFLSFLVPPMTIY
TEQDLYSYVISKPRNKRVPILPFVIGAGVLGALGTGIGGITTSTQFYYKL
SQELNGDMERVADSLVTLQDQLNSLA AVLQNRALDLLTAERGGTCLFL
GEECCYYVNQSGIVTEKVKEIRDRIQRRAEELRNTGPWGLLSQWMPWILP
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PLDRPASPRSDVNDIKGTPPEEISAAQPLLRPNSAGSS*

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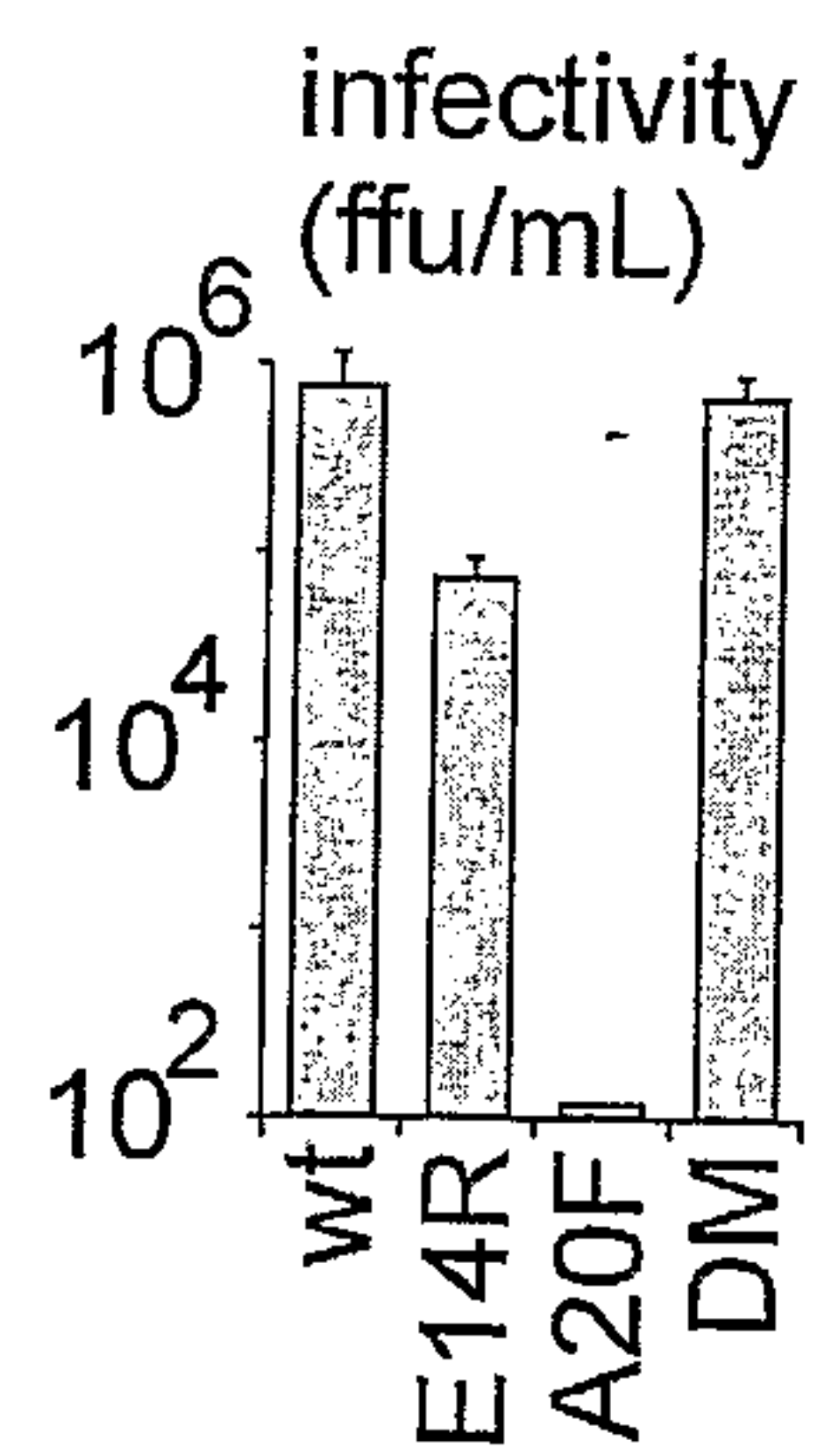


Figure 11A

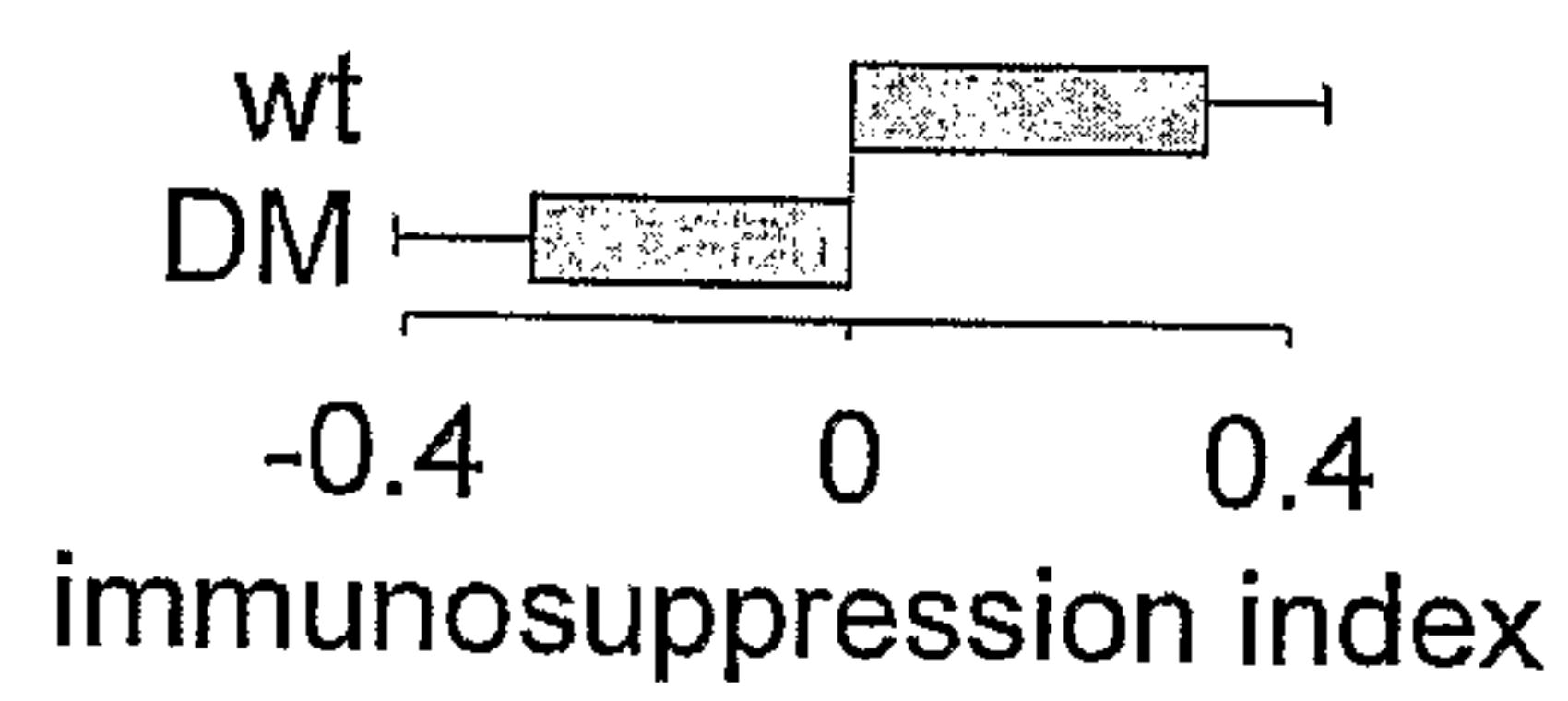


Figure 11B

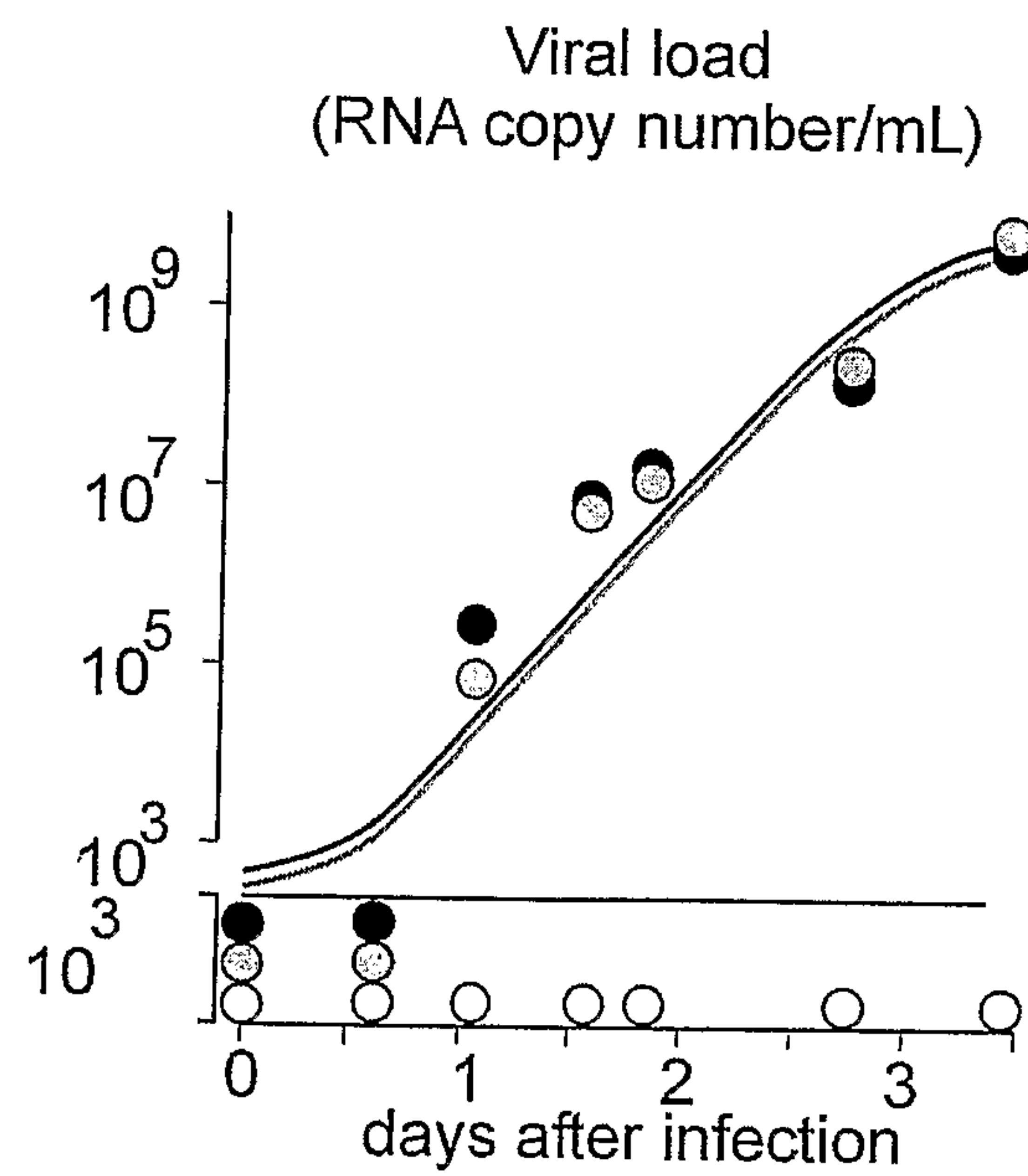


Figure 11C

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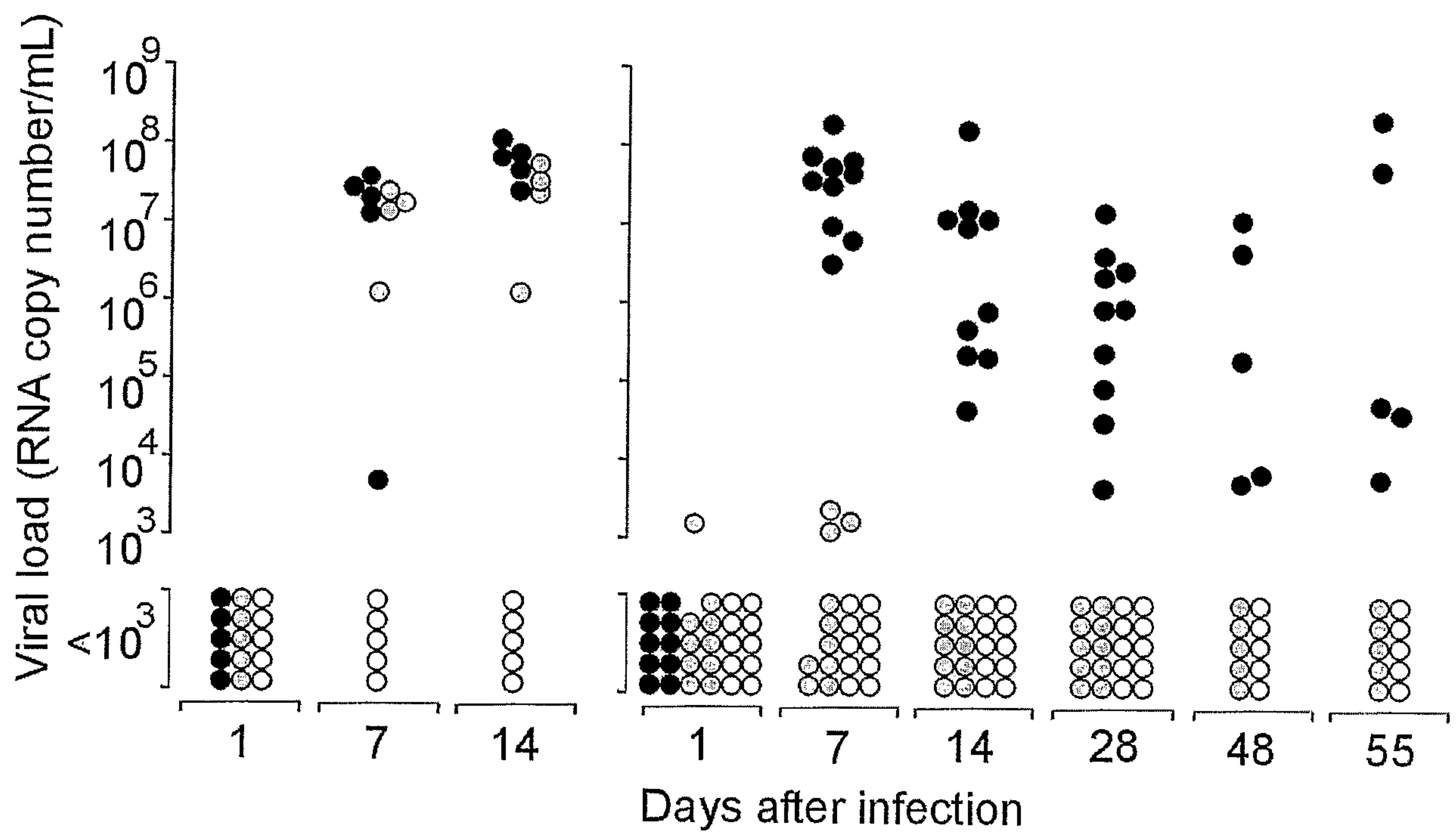


Figure 12A

Figure 12B

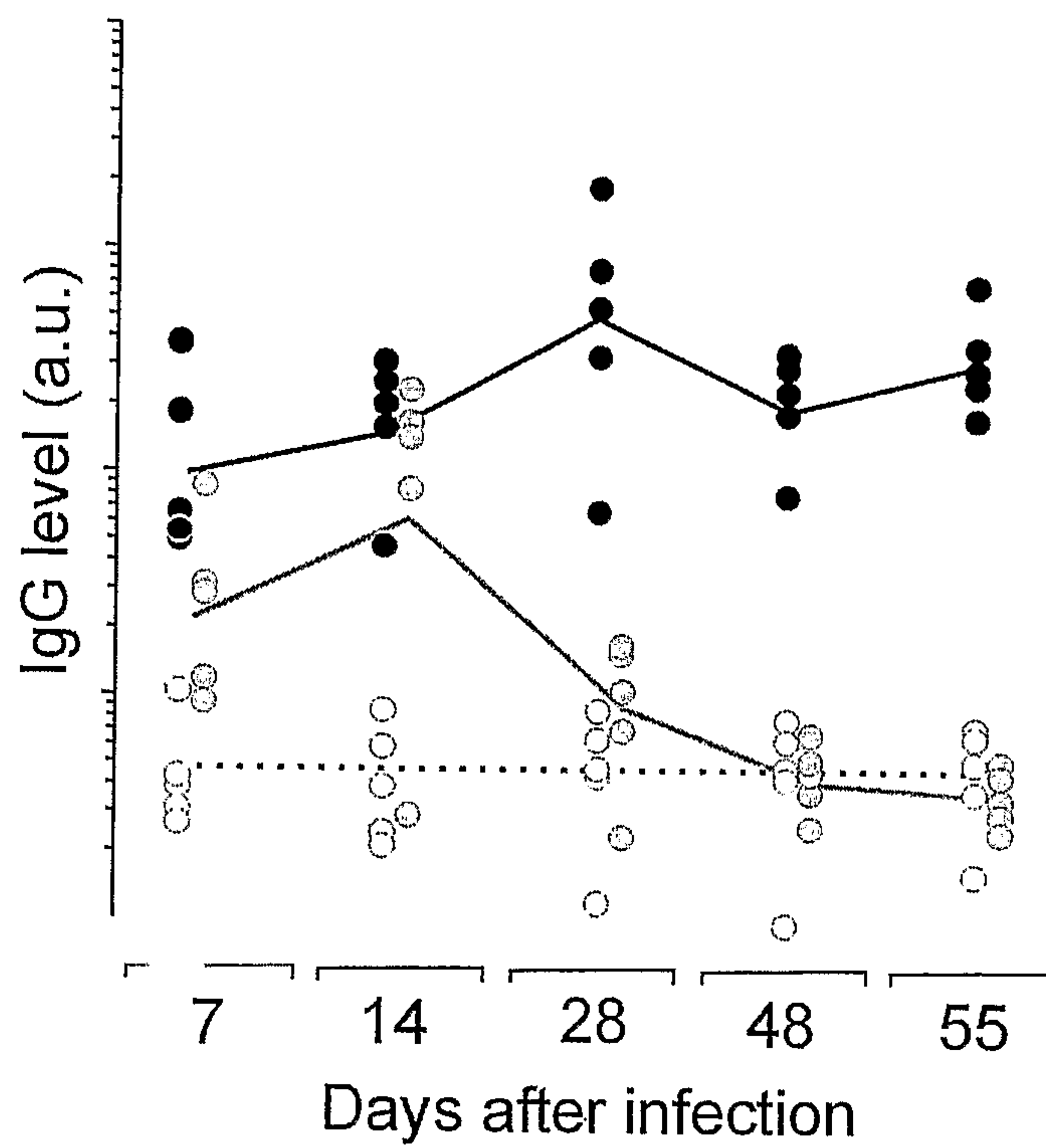


Figure 13

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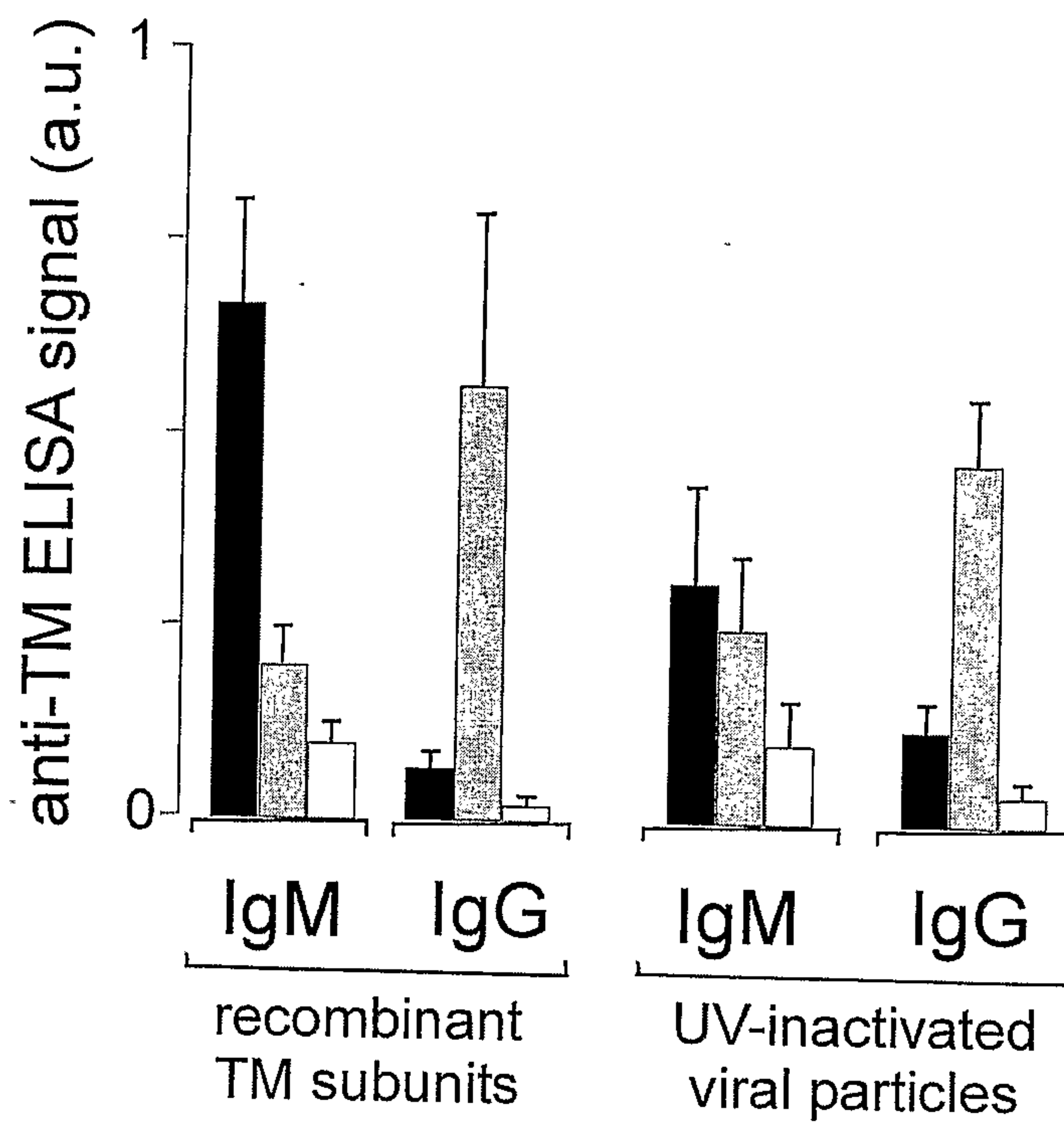


Figure 14A

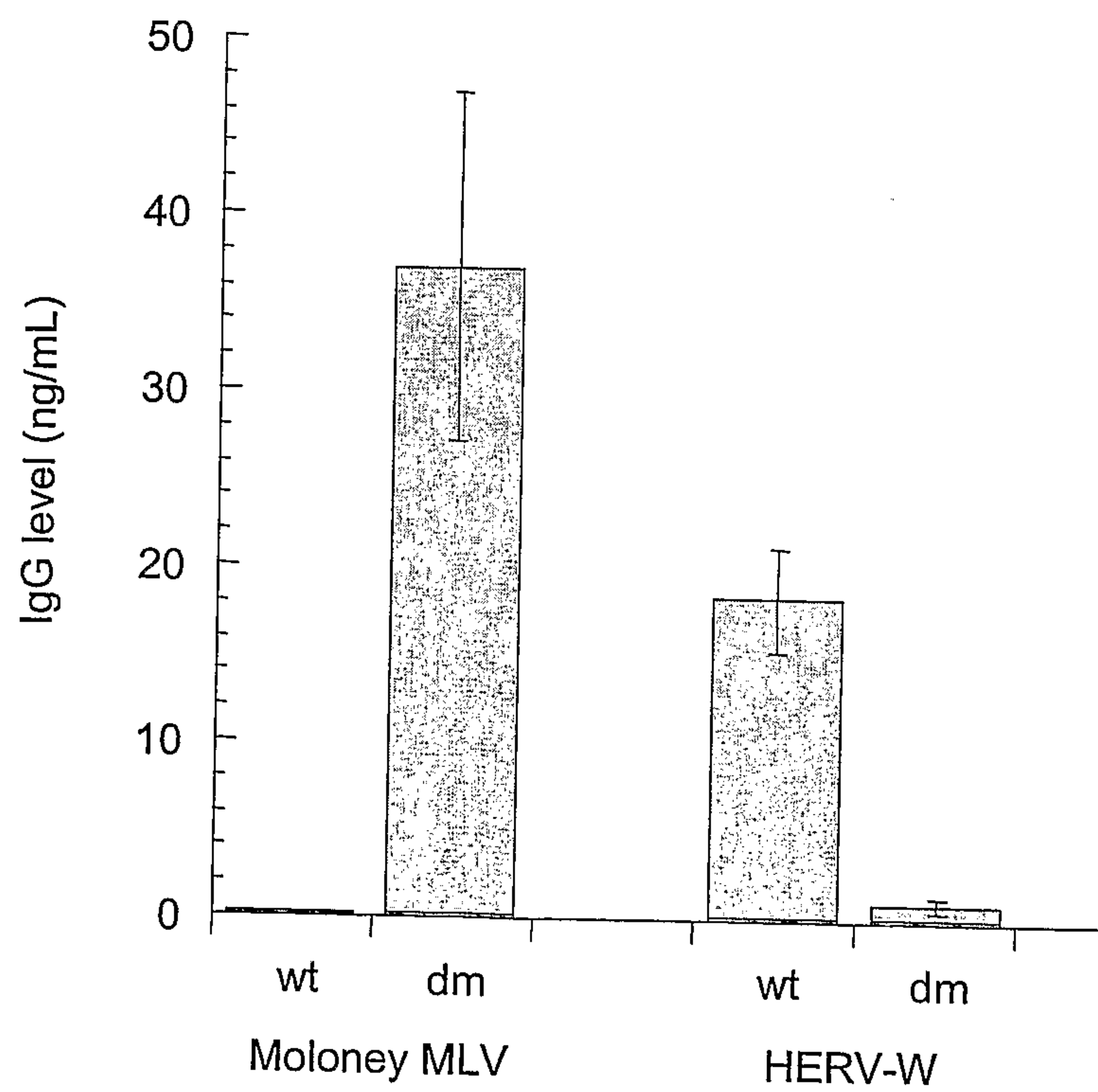


Figure 14B

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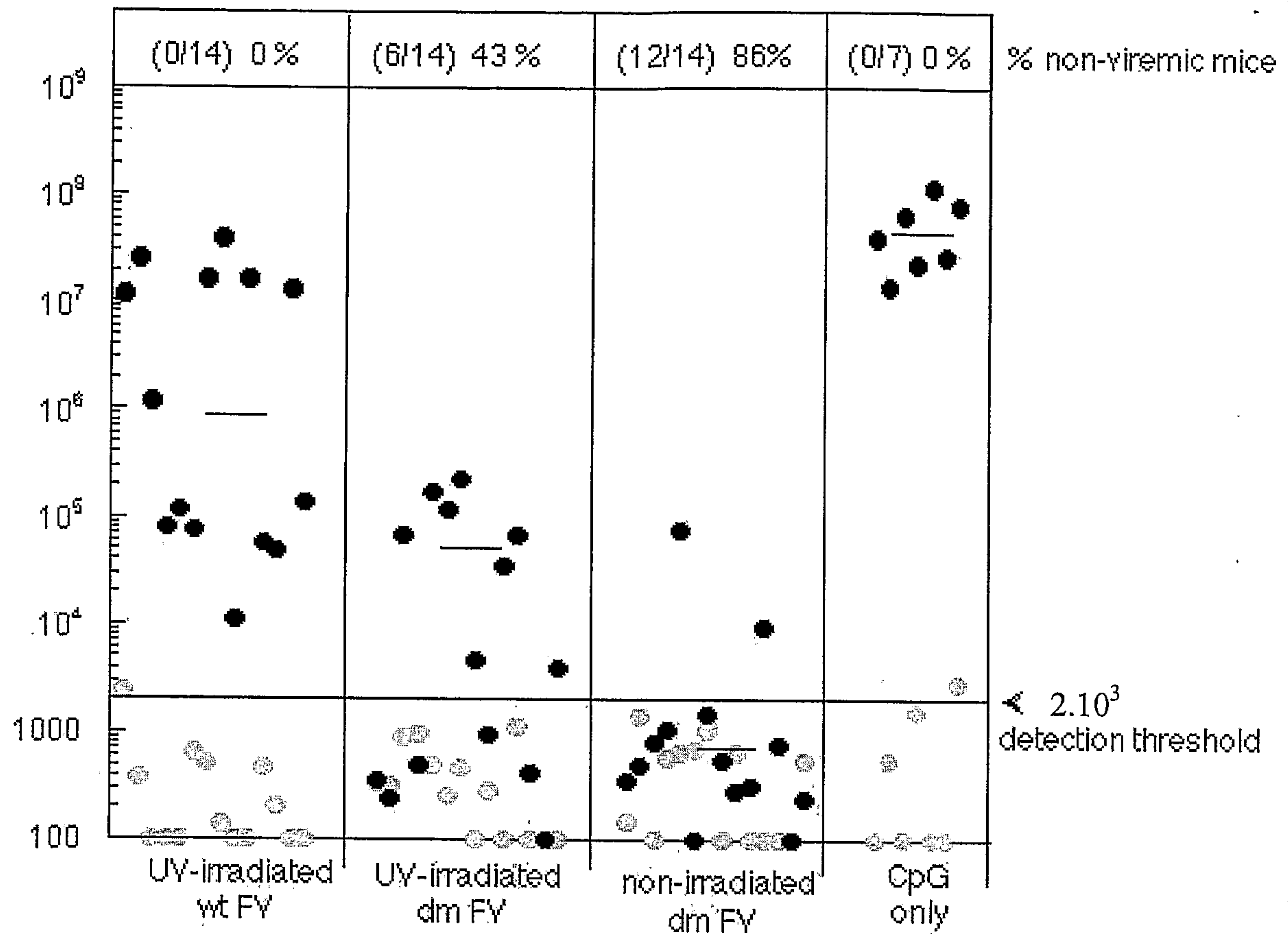


Figure 15

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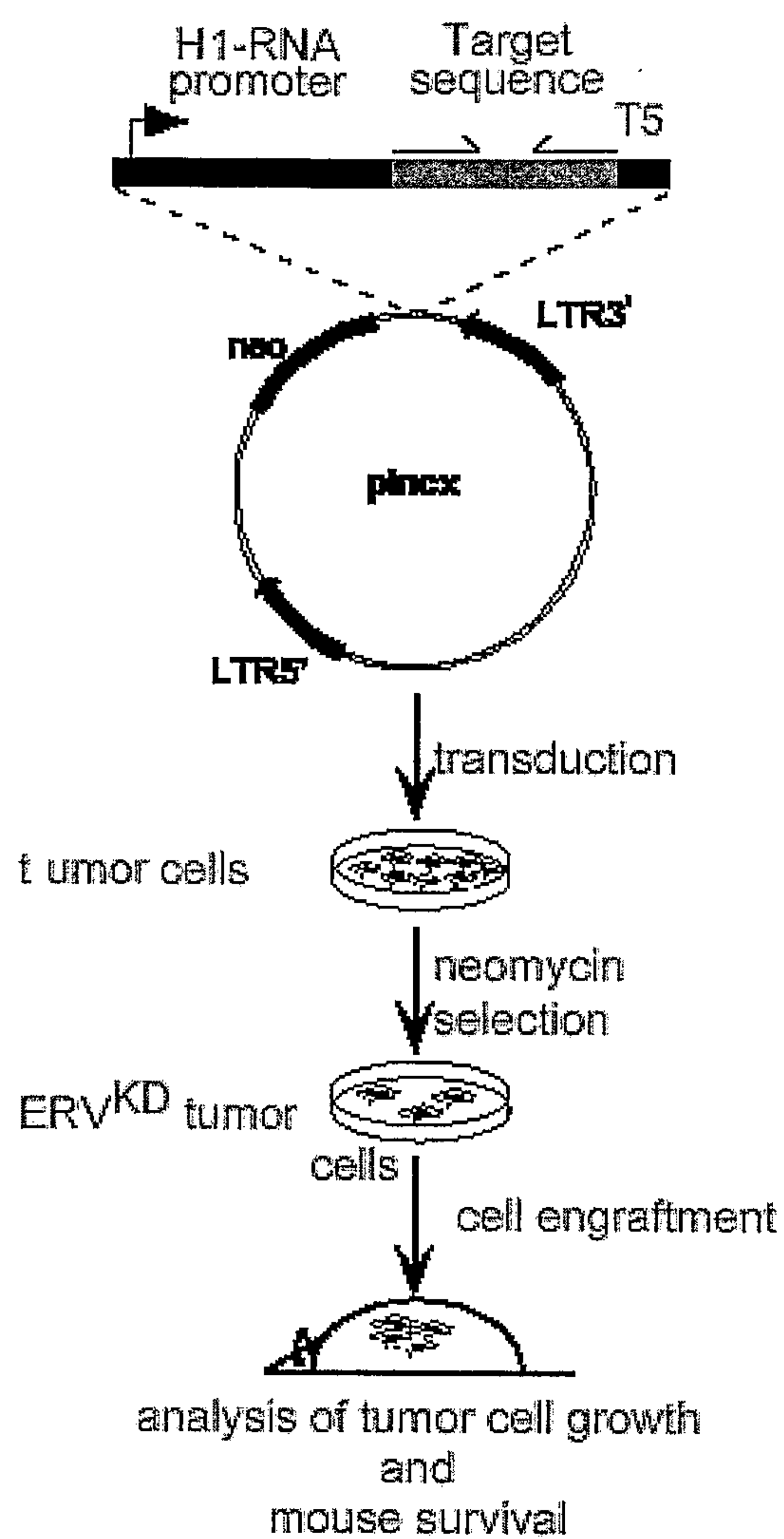


Figure 16A

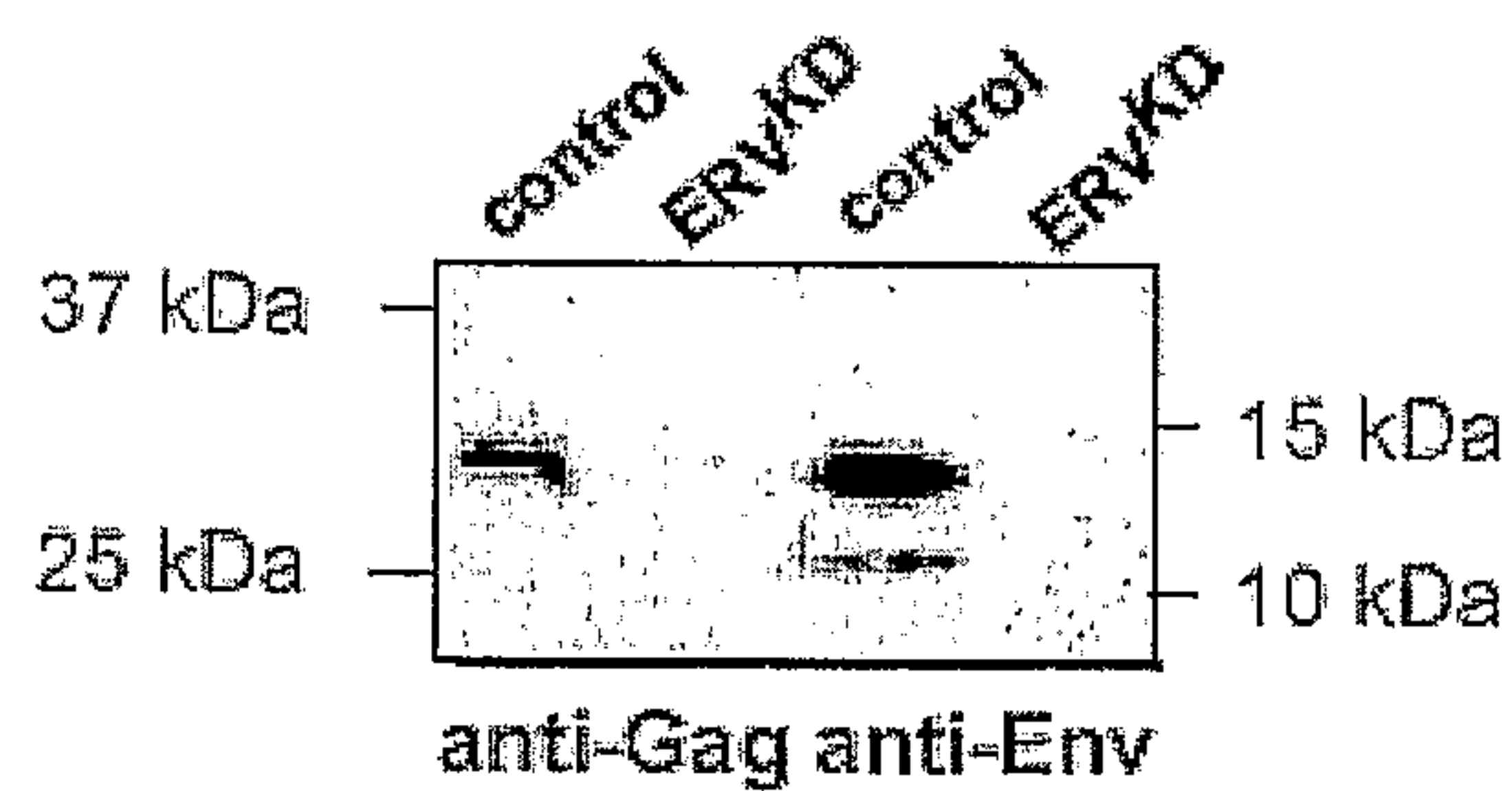
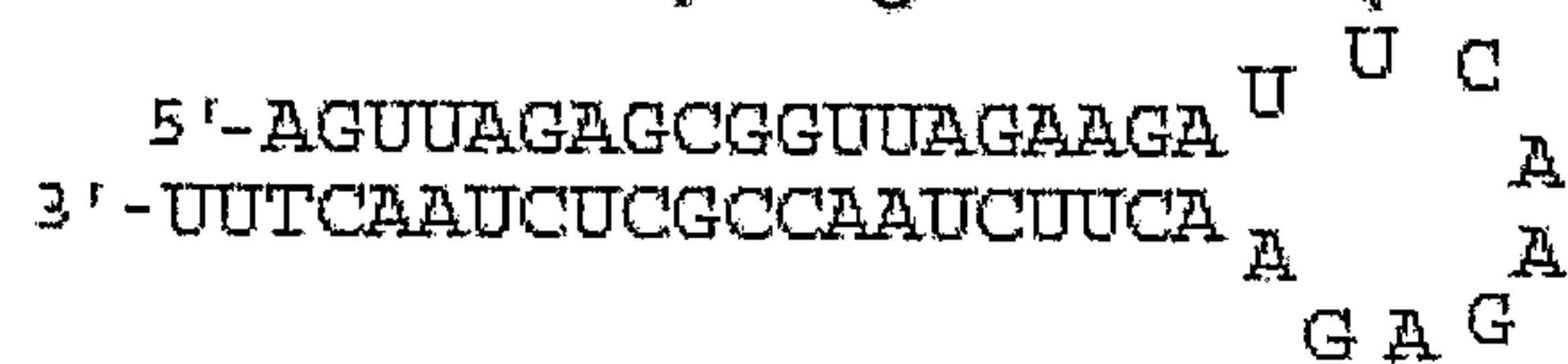


Figure 16C

predicted transcripts against ERV (1220-1238)



predicted control transcripts against GFP (215-233)

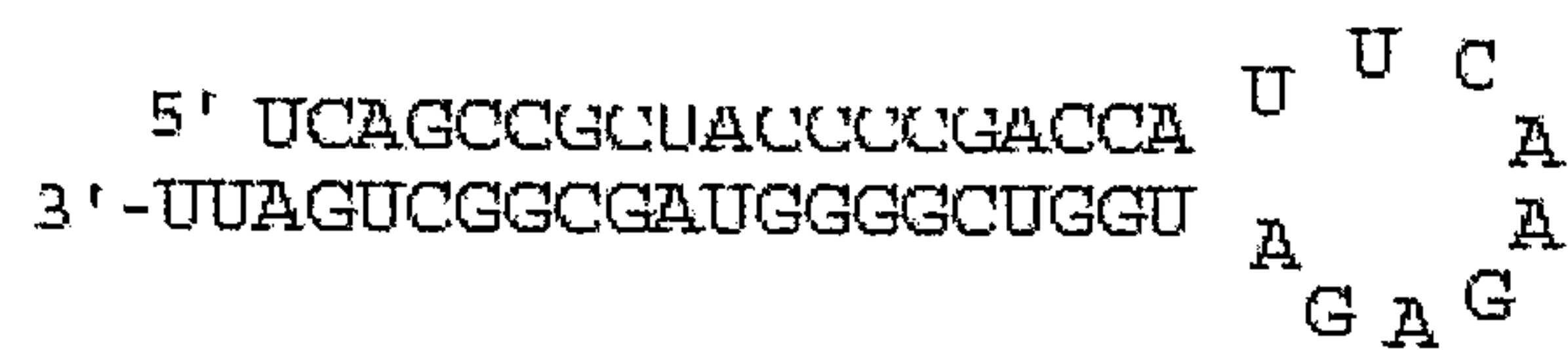


Figure 16B

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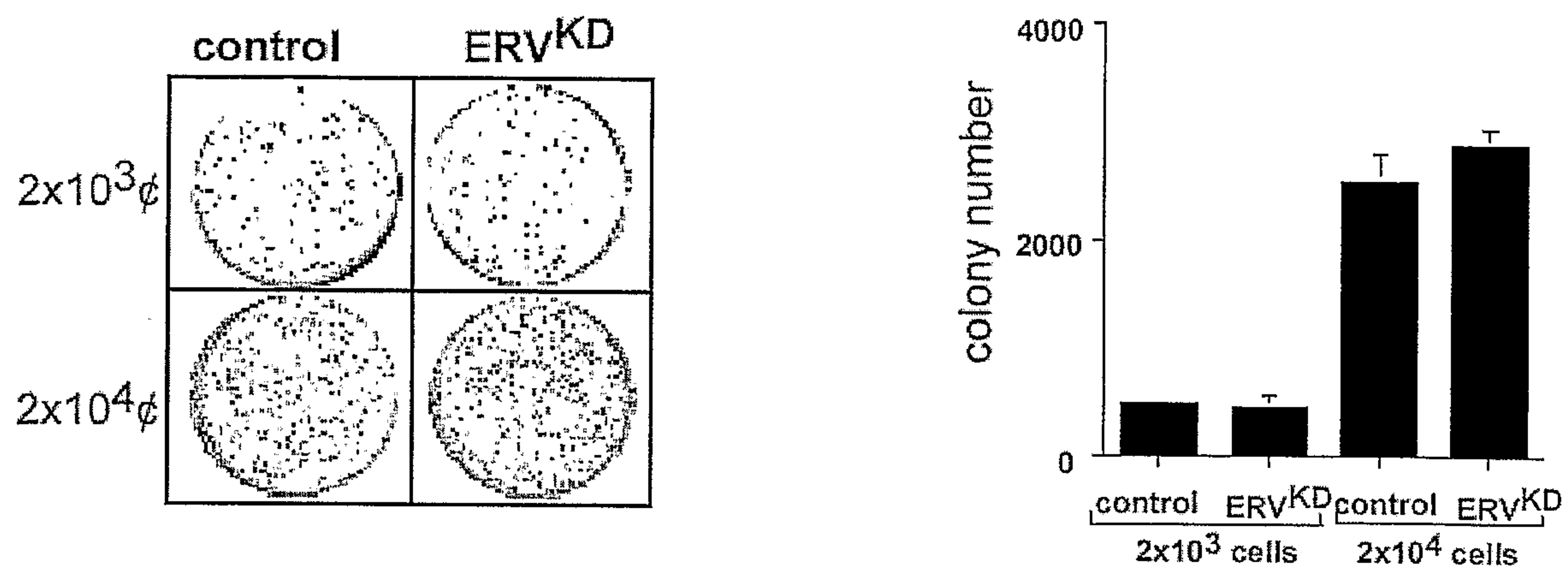


Figure 17A

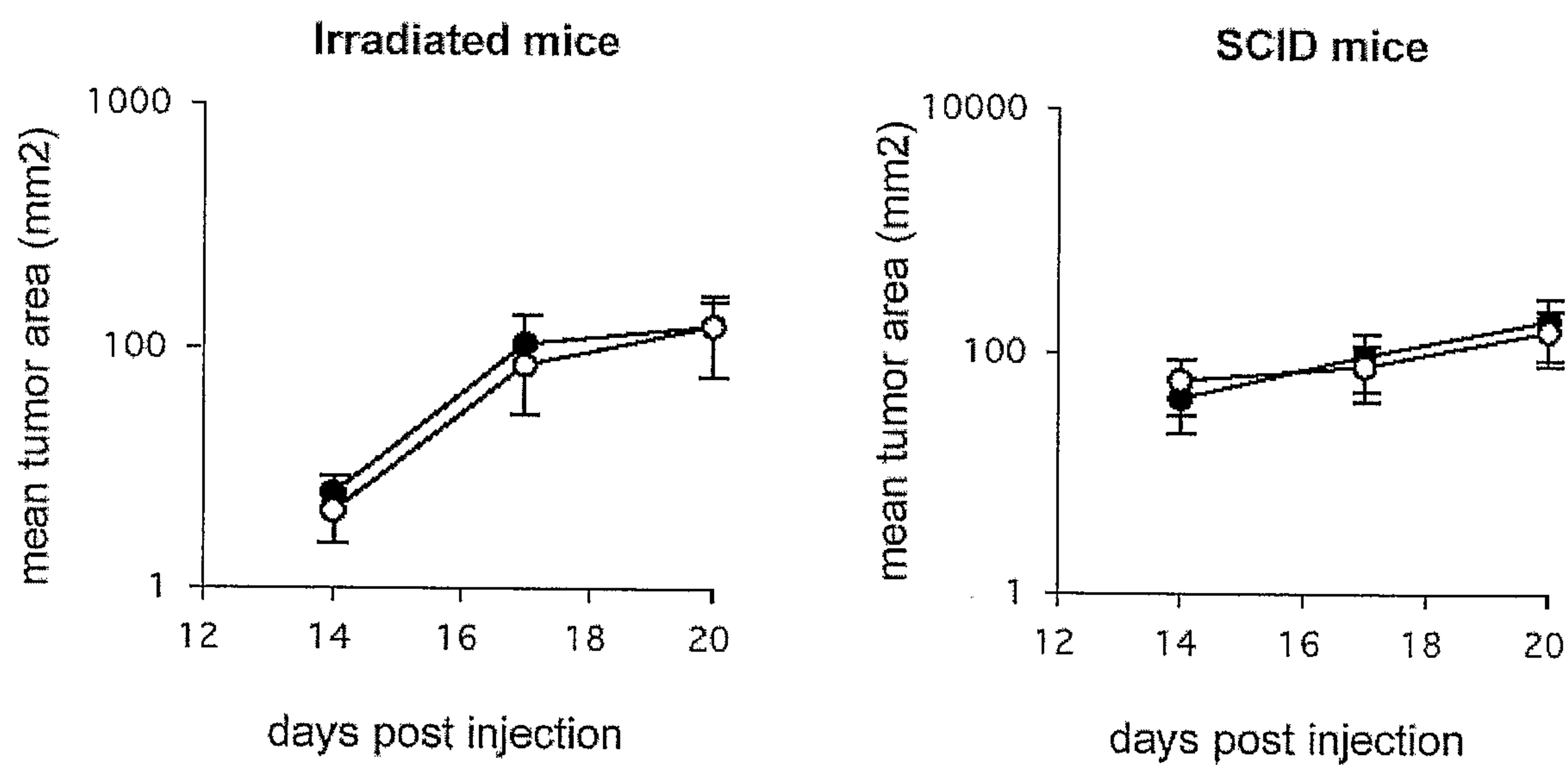


Figure 17B

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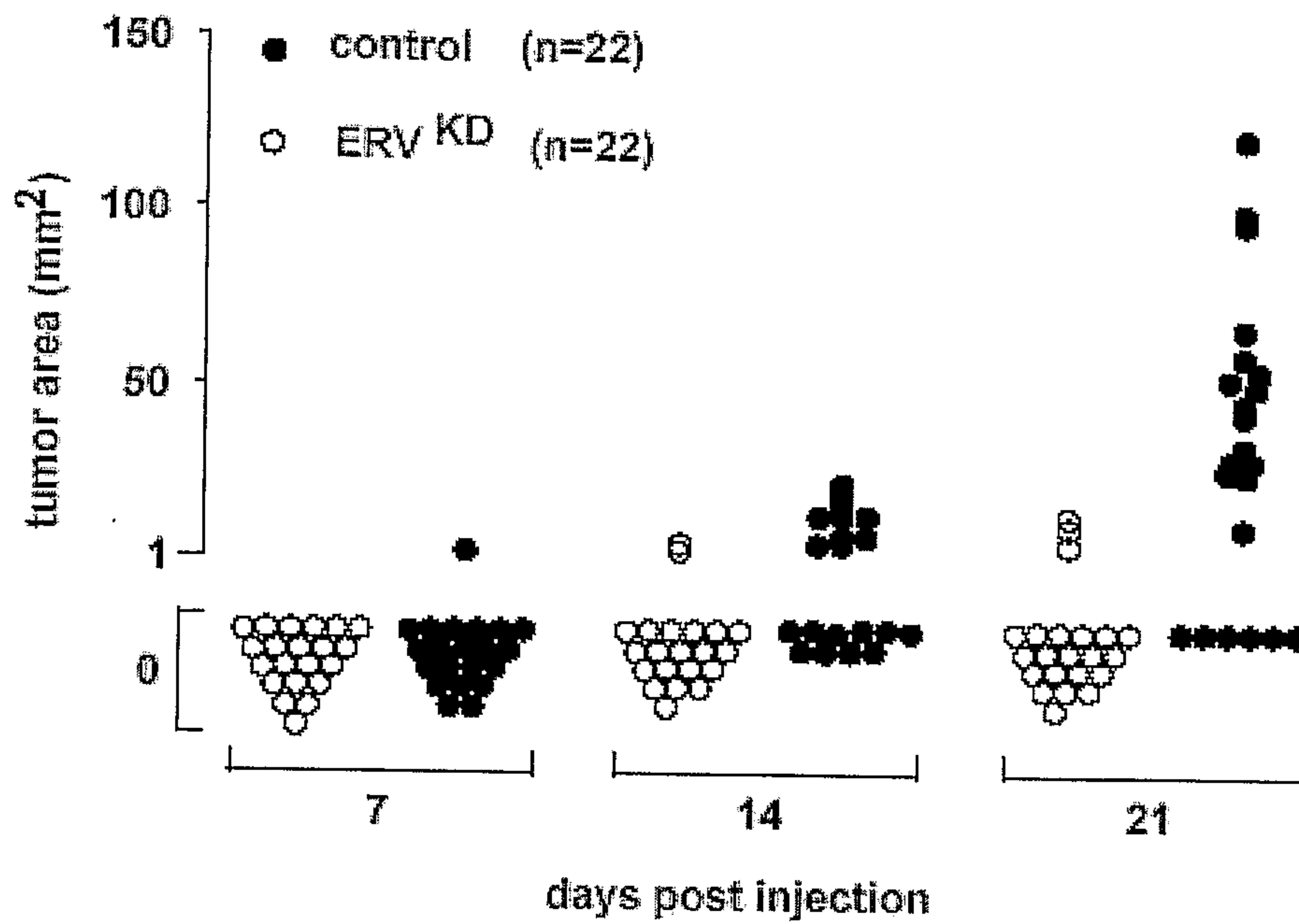


Figure 18A

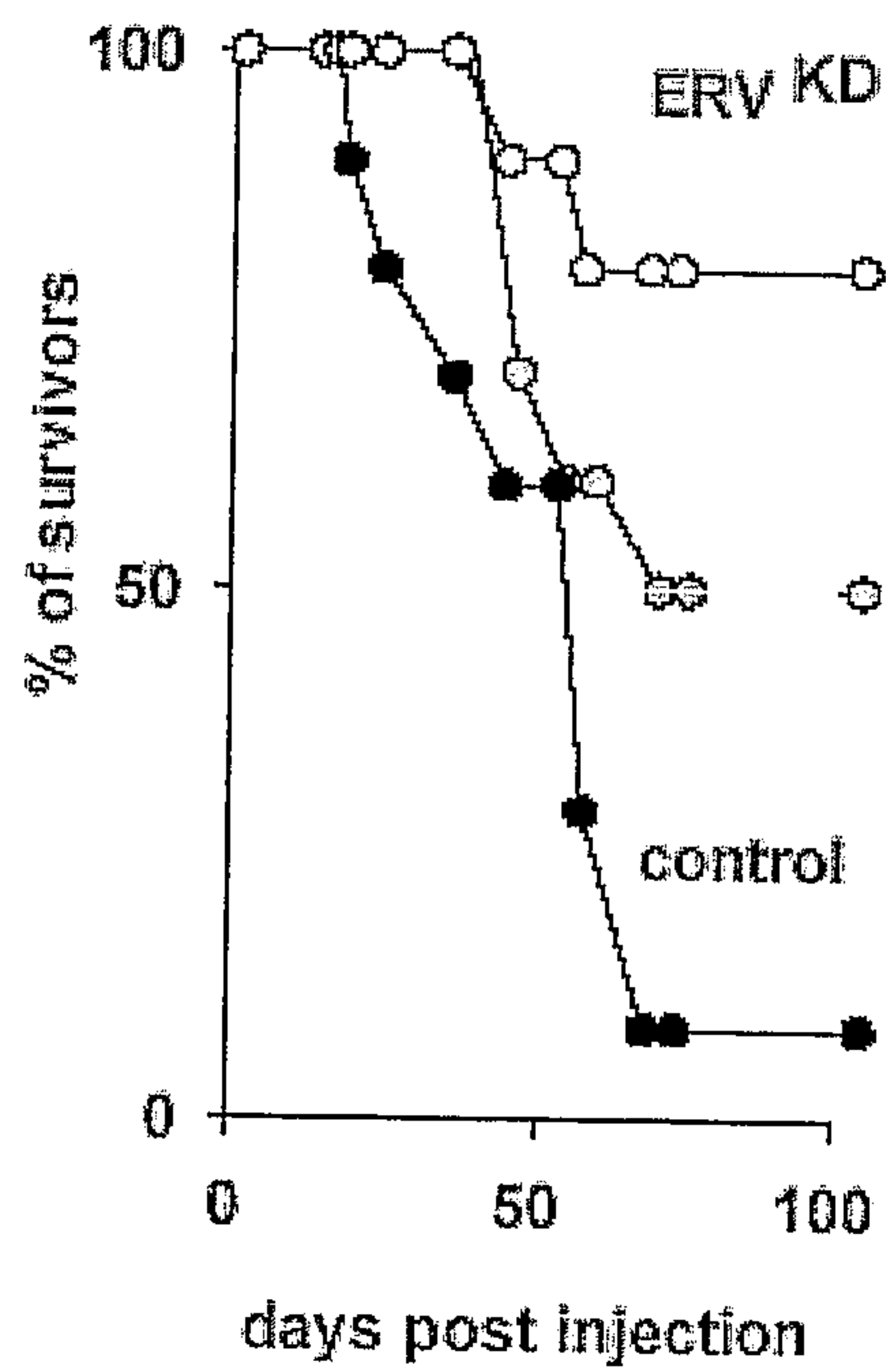


Figure 18B

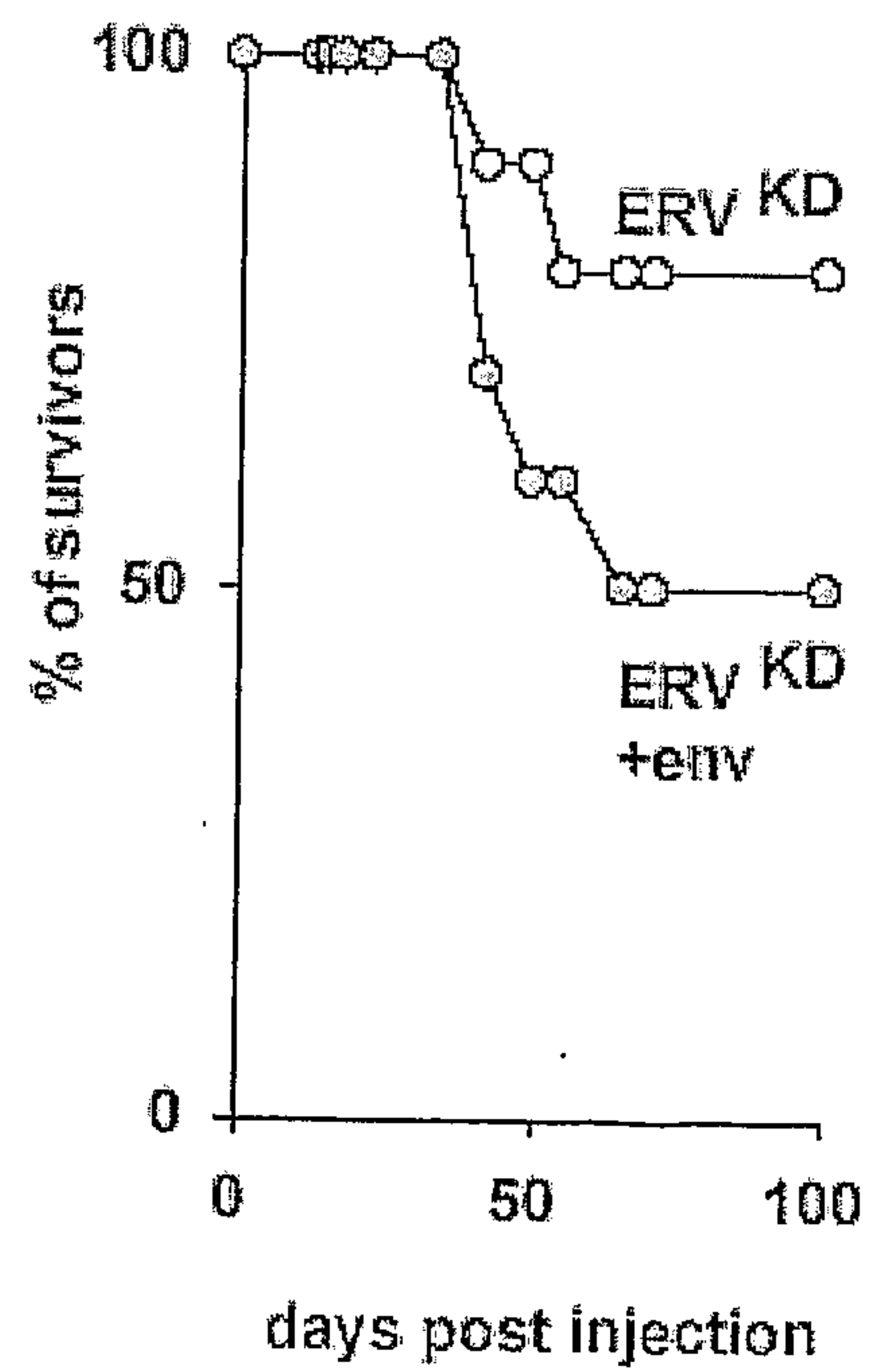


Figure 18C

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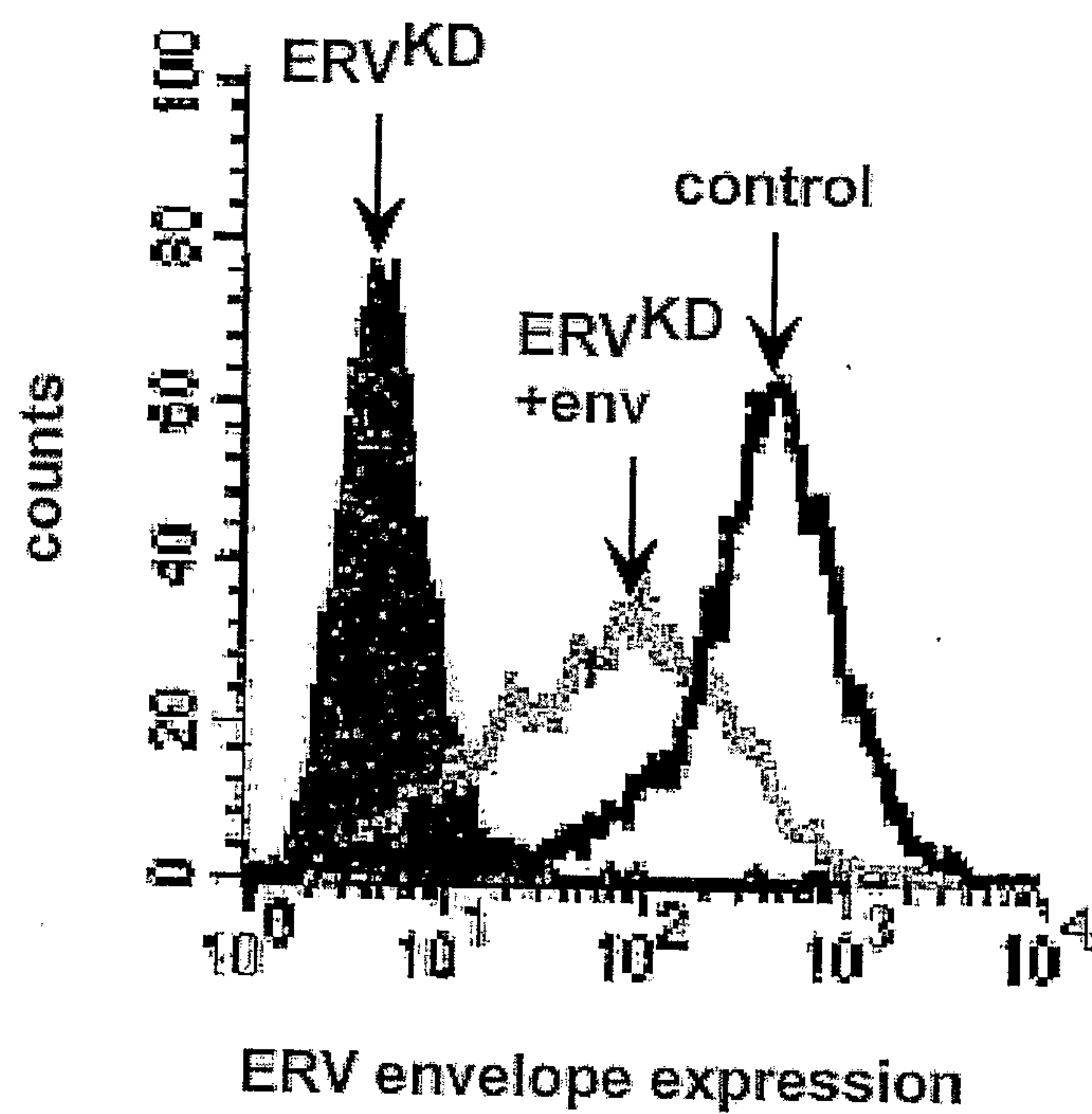


Figure 19

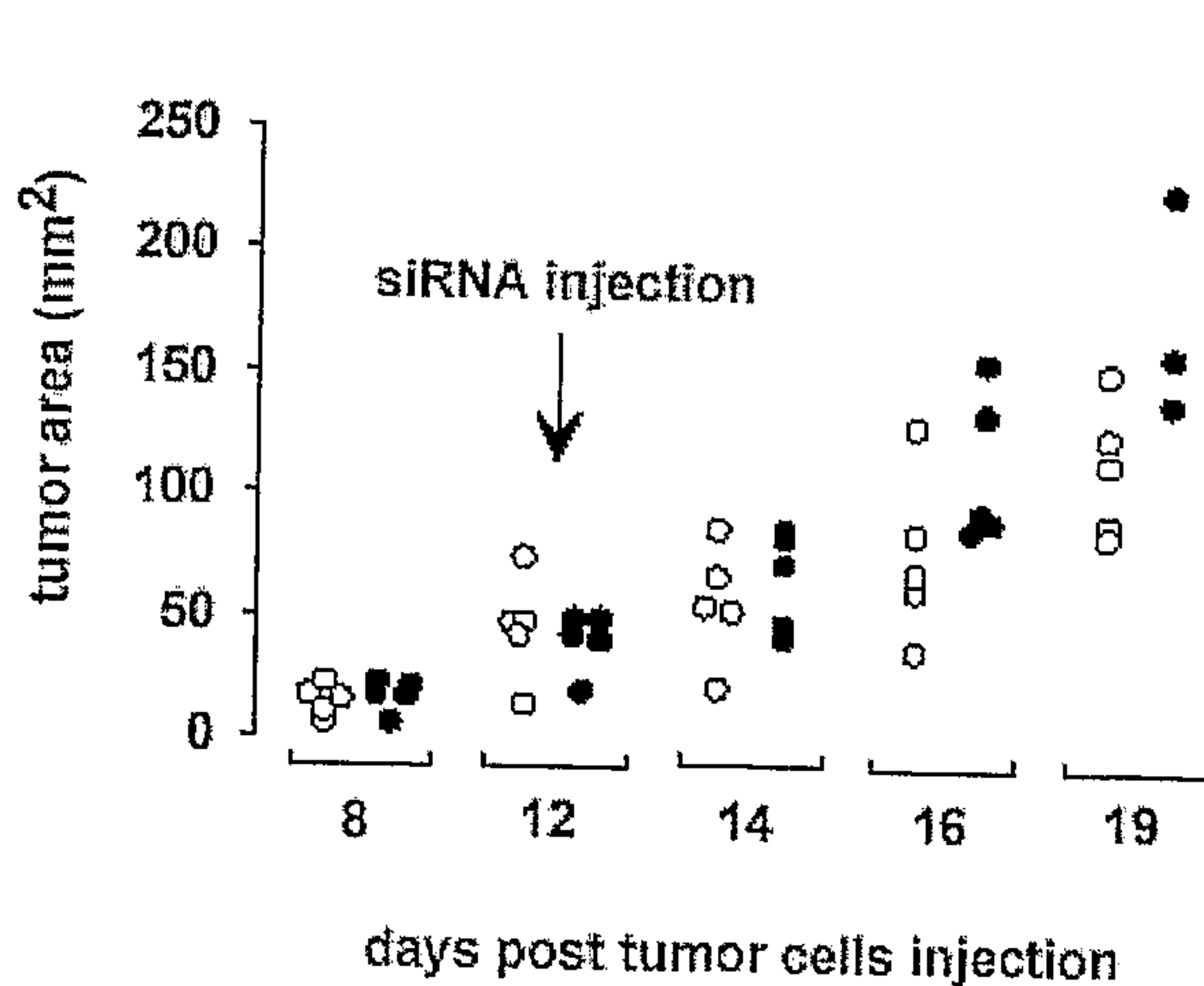


Figure 20A

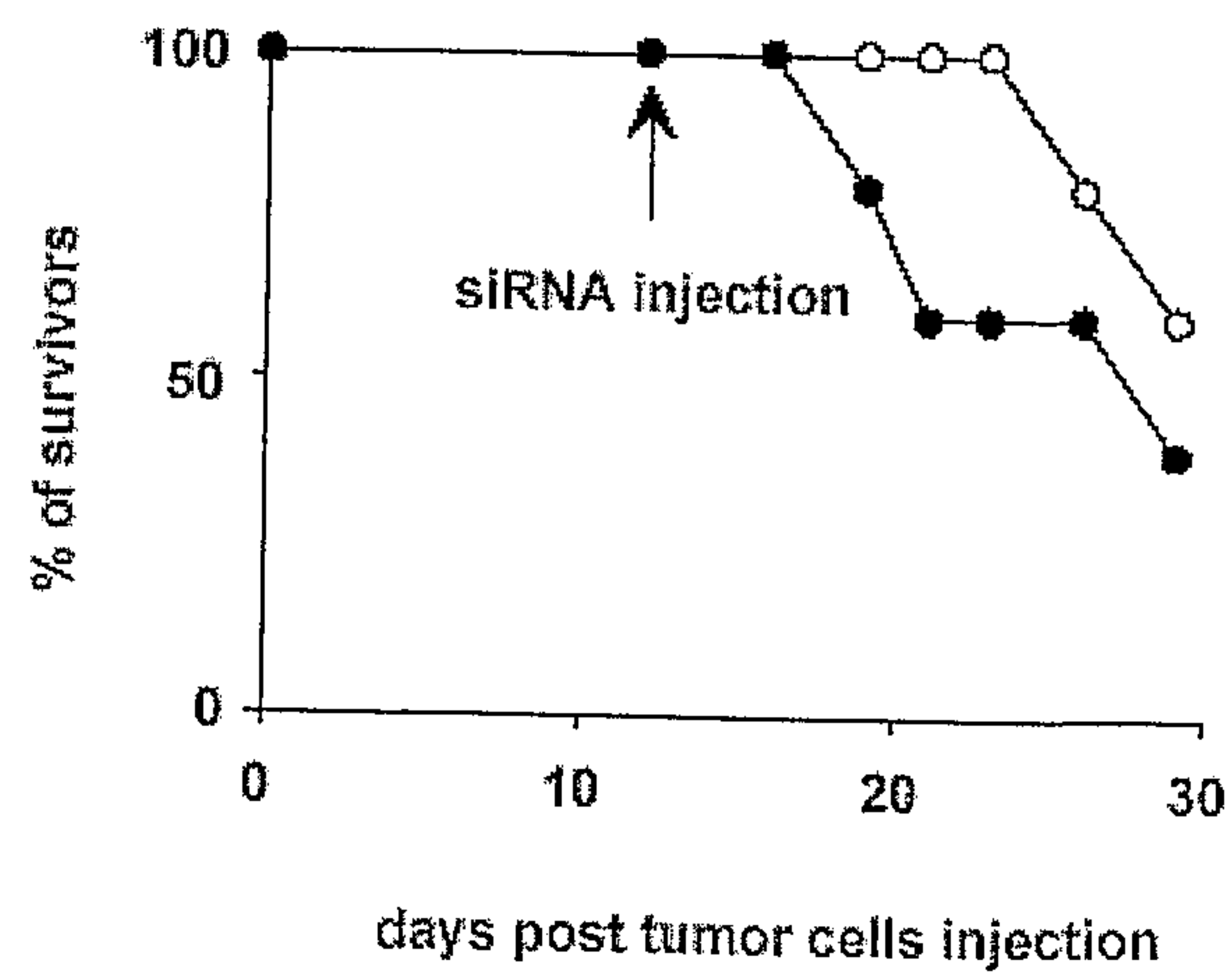


Figure 20B