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(54) Title: COMPOSITIONS AND METHODS FOR TREATING RETROVIRUS INFECTIONS

(57) **Abrégé/Abstract:**

The present invention relates to at least one nucleic acid (i) comprising or consisting of, or (ii) encoding a nucleic acid comprising or consisting of, a sequence selected from the group consisting of : 1) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4, and 2) a sequence derived from SEQ ID NO: 1 to 4 by substitution deletion or insertion of at least one nucleotide, provided that a nucleic acid consisting of the sequence derived from SEQ ID NO: 1 to 4 is liable to induce HIV-1 expression in latent HIV-1-infected cells, for use as a medicament, in particular for treating retrovirus infections.

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(54) Title: COMPOSITIONS AND METHODS FOR TREATING RETROVIRUS INFECTIONS

(57) Abstract: The present invention relates to at least one nucleic acid (i) comprising or consisting of, or (ii) encoding a nucleic acid comprising or consisting of, a sequence selected from the group consisting of: 1) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4, and 2) a sequence derived from SEQ ID NO: 1 to 4 by substitution deletion or insertion of at least one nucleotide, provided that a nucleic acid consisting of the sequence derived from SEQ ID NO: 1 to 4 is liable to induce HIV-1 expression in latent HIV-1 -infected cells, for use as a medicament, in particular for treating retrovirus infections.



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## Compositions and methods for treating retrovirus infections

The present invention relates to compositions and methods for treating retrovirus infections, in particular in asymptomatic individuals in whom the retrovirus  
5 is in a latent state.

Retroviruses, such as Human Immunodeficiency Virus (HIV), are able to stay in infected cells in a latent state. The mechanisms which are responsible for latency and reactivation of the virus are poorly understood. It seems that the replication of the virus in CD4+ T lymphocyte cells is dependent in part upon the cell cycle of the  
10 host cell. HIV entry into activated CD4+ lymphocytes generally leads to a productive infection whereas no infective production is generally obtained after entry into non-activated CD4+ lymphocytes.

Some patients referred as to Elite HIV-1 Controllers are infected individuals who are able to maintain their virus at undetectable levels for many years in absence  
15 of treatment (Goudsmit *et al.* (2002) *AIDS* **16**:791-793). This capacity has today no clear explanation and concerns have been expressed regarding the ability of these individuals to manage long-term control of the infection.

Besides, HIV latency is a major problem for the current HIV antiviral therapies. In fact, these therapies do not eradicate the infection because of the latent, resistant  
20 reservoir of viruses. For example, Highly Active Anti-Retroviral Therapy (HAART), in which a cocktail of anti-retroviral drugs is administered to HIV-1 infected patients, fails to eradicate definitively HIV-1 infection because of this HAART refractory latent viral reservoirs (Marcello (2006) *Retrovirology* **3**:7). Accordingly, the risk is always present, in such patients, that the infection reactivates, for instance upon a decrease  
25 of the efficiency of the administered drugs.

Accordingly, there is a need to fully eradicate the latent retrovirus reservoir in these patients.

One of the therapeutic strategies which has been suggested for achieving such a goal consists in reactivating latent retroviruses in infected cells, thereby  
30 inducing retroviral particles production and restoring sensitivity to medication. Such a strategy could thus lead to a complete recovery of infected patients.

Some molecules promoting reactivation of retroviruses are known. Prostratin, for instance, was shown to be able to up-regulate HIV expression in the CD8+ T lymphocytes of an infected patient undergoing HAART. Prostatin was thus, proposed

to be a good candidate for the elimination of the persistent viral repertoire. However, the results obtained with prostratin are somewhat heterogeneous and a need for other molecules still exists (Kulkosky (2001) *Blood* **98**:3006-3015, Korin *et al.* (2002) *Virology* **76**:8118-8123).

5        MicroRNAs (miRNAs) are a newly discovered class of RNAs generally 20-25 nucleotides in length. They are involved in gene expression regulation at the post-transcriptional level by degrading or blocking translation of specific messenger RNAs mRNAs. The miRNA pathway, from synthesis to action, has been well described in terms of components of the pathway, which notably comprise, among others, the  
10        proteins known as Drosha, DGCR8, Dicer, RCK/p54, LSm-1, GW182, and XRN1 (Bartel (2004) *Cell* **116**:281-297). It has been recently shown that 2'-O-methyl-oligoribonucleotide antisense inhibitors of five miRNA, namely mir-28, mir-125b, mir-150, mir-223 and mir-382, could induce HIV-1 infectious particles production from CD4+ T cells obtained HIV-1 infected individuals under HAART (Huang *et al.* (2007)  
15        *Nat. Med.* **13**:1241-1247). It was thus proposed to such anti-miRNA inhibitors to reverse HIV-1 latency *in vivo*. However, concerns were raised regarding the potential toxicity of these inhibitors (Zhang (2008) *Int J Biochem Cell*).

Accordingly, it is an object of the present invention to provide alternative compounds and methods useful for reactivating latent retroviral reservoirs in infected  
20        individuals.

### Summary of the invention

In this regard, the present invention arises from the unexpected finding by the inventors that contacting latent cells infected by HIV-1 with particular miRNAs,  
25        namely miR-34a, miR-122, miR-206 and miR-210 (respectively represented by SEQ ID NO: 1 to 4), induced HIV-1 expression by these cells. Unexpectedly also, the same inventors have found that inhibiting the expression of components of the miRNA pathway, such as Drosha, DGCR8, Dicer, RCK/p54, LSm-1, GW182, and XRN1, induced HIV-1 expression in latent infected cells.

30        The present invention thus relates to at least one nucleic acid

- (i) comprising or consisting of, or
  - (ii) encoding a nucleic acid comprising or consisting of,
- a sequence selected from the group consisting of:

- 1) SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4, and

- 2) a sequence derived from SEQ ID NO: 1 to 4 by substitution, deletion or insertion of at least one nucleotide, provided that a nucleic acid consisting of the sequence derived from SEQ ID NO: 1 to 4 is liable to induce HIV-1 expression in latent HIV-1-infected cells,
- 5 for use as a medicament, in particular for treating retrovirus infections.

The present invention also relates to at least one compound inhibiting the activity of at least one component of the miRNA pathway for use in the treatment of retrovirus infections.

The present invention also relates to a method for treating retrovirus infections  
10 in an individual comprising administering said individual with a therapeutically effective amount of at least one nucleic acid as defined above or at least one compound as defined above.

In an embodiment of the above-defined nucleic acid, compound or method, the nucleic acid or compound is used in combination with at least one other anti-  
15 retroviral compound.

The present invention also relates to the *in vitro* use of a nucleic acid as defined above or of a compound inhibiting a component of the miRNA pathway selected from the group consisting of DGCR8, RCK/p54, LSm-1, GW182, and XRN1, for the production of retroviral particles from cells harbouring a retroviral vector.

20 The present invention also relates to an *in vitro* method for the production of retroviral particles, comprising:

- contacting cells harbouring a retroviral vector with a nucleic acid as defined above or of a compound inhibiting a component of the miRNA pathway selected from the group consisting of DGCR8, RCK/p54, LSm-1, GW182, and XRN1;
  - 25 - letting the cells express the retroviral vector;
- whereby retroviral particles are produced from the cells.

In an embodiment of the invention, the above-defined *in vitro* use and *in vitro* method involve no step of culturing the cells with T cells.

The inventors have also identified 51 genes which are targeted by miR-34a, miR-206, miR-210 and miR-122, and which inhibition of the expression by siRNAs or shRNAs activates viral replication of HIV-1.  
30

The present invention thus also relates to a modulator of the activity of a gene selected from the group consisting of DGUOK, MIR16, PPP1R11, ARHGAP1, TEDDM1, QDPR, C14orf32, C1orf19, ATP1B3, FLJ10241, ANP32E, TAGLN2,

ARF3, PTMA, PPIB, PRCP, PTPRK, OBSL1, SLC44A1, PPIAL4, SERP1, EBPL, CBX6, ZBED3, NP, PRSS21, PPIA, C5orf13, E2F2, CACYBP, TROAP, APOBEC3A, C7orf44, ORC6L, WNT10B, VIM, CDC6, MCERS1, NAG18, PPP1CC, DULLARD, ASF1B, PLP2, MTHFD2, PIGS, KIF2C, NRM, PEG10, C22orf9, COL4A2, and  
 5 SNX26,

for use as a medicament, in particular for treating retrovirus infections.

The present invention also relates to a method for treating retrovirus infections in an individual, comprising administering the individual with at least one modulator of the activity of a gene selected from the group consisting of DGUOK, MIR16,  
 10 PPP1R11, ARHGAP1, TEDDM1, QDPR, C14orf32, C1orf19, ATP1B3, FLJ10241, ANP32E, TAGLN2, ARF3, PTMA, PPIB, PRCP, PTPRK, OBSL1, SLC44A1, PPIAL4, SERP1, EBPL, CBX6, ZBED3, NP, PRSS21, PPIA, C5orf13, E2F2, CACYBP, TROAP, APOBEC3A, C7orf44, ORC6L, WNT10B, VIM, CDC6, MCERS1, NAG18, PPP1CC, DULLARD, ASF1B, PLP2, MTHFD2, PIGS, KIF2C, NRM, PEG10,  
 15 C22orf9, COL4A2, and SNX26.

In an embodiment of the above defined modulator for use as a medicament or method of treatment involving the modulator, modulators of the expression of each one of DGUOK, MIR16, PPP1R11, ARHGAP1, TEDDM1, QDPR, C14orf32, C1orf19, ATP1B3, FLJ10241, ANP32E, TAGLN2, ARF3, PTMA, PPIB, PRCP, PTPRK,  
 20 OBSL1, SLC44A1, PPIAL4, SERP1, EBPL, CBX6, ZBED3, NP, PRSS21, PPIA, C5orf13, E2F2, CACYBP, TROAP, APOBEC3A, C7orf44, ORC6L, WNT10B, VIM, CDC6, MCERS1, NAG18, PPP1CC, DULLARD, ASF1B, PLP2, MTHFD2, PIGS, KIF2C, NRM, PEG10, C22orf9, COL4A2, and SNX26, are used.

In an embodiment of the above-defined modulator for use a medicament or  
 25 method of treatment involving the modulator, the modulator is used in combination with at least one other anti-retroviral compound.

## Detailed description of the invention

### *Nucleic acid*

30 As intended herein the nucleic acid of the invention can be of any type, it can notably be natural or synthetic, DNA or RNA, single or double stranded. In particular, where the nucleic acid is synthetic, it can comprise non-natural modifications of the bases or bonds, in particular for increasing the resistance to degradation of the nucleic acid. Where the nucleic acid is RNA the modifications notably encompass

capping its ends or modifying the 2' position of the ribose backbone so as to decrease the reactivity of the hydroxyl moiety, for instance by suppressing the hydroxyl moiety (to yield a 2'-deoxyribose or a 2'-deoxyribose-2'-fluororibose), or substituting the hydroxyl moiety with an alkyl group, such as a methyl group (to yield a 2'-O-methyl-ribose).

SEQ ID NO: 1, 2, 3 and 4 respectively represent the sequences of miRNAs miR-34a, miR-122, miR-206 and miR-210. These miRNAs are notably described in Griffiths-Jones (2004) *Nucleic Acids Res* **32**:D109-D111; Griffiths-Jones *et al.* (2008) *Nucleic Acids Res* **36**:D154-D158.

Where the nucleic acid of the invention comprises or consists of SEQ ID NO: 1, 2, 3 or 4, or of the sequences derived therefrom, the nucleic acid of the invention is intended to directly exert its effect on its cellular targets. In this case, the nucleic acid is preferably a RNA molecule.

Where the nucleic acid of the invention encodes a nucleic acid comprising or consisting of SEQ ID NO: 1, 2, 3 or 4, or of the sequences derived therefrom, the nucleic acid of the invention is intended to be expressed within cells where the nucleic acid it encodes, in particular a RNA molecule, will exert its effect on its cellular targets. In this case the nucleic acid of the invention is preferably a DNA molecule, more preferably a double stranded DNA molecule. Besides, as will be clear to one of skill in the art, the nucleic acid according to the invention preferably also comprises genetic elements ensuring expression of the encoded nucleic acid, in particular a promoter sequence of RNA polymerase II or III.

Methods for delivering nucleic acids into cells *in vitro* or *in vivo* are well known to one of skill in the art and are notably described in Nguyen *et al.* (2008) *Curr Opin Mol Ther* **10**:158-67 and Dykxhoorn *et al.* (2006) *Gene Therapy* **13**:541-552.

Preferably, where the nucleic acid of the invention comprises SEQ ID NO: 1, 2, 3, or 4, or a sequence derived therefrom, it is less than 1000 nucleotides long, more preferably less than 100 nucleotides long, and most preferably less than 50 nucleotides long.

Preferably, the sequence derived from SEQ ID NO: 1 to 4 by substitution deletion or insertion of at least one nucleotide presents at least 85%, more preferably at least 90%, and most preferably at least 95% identity with the sequence from which

it is derived. As intended herein, the percentage of identity between two sequences is obtained by aligning the two sequences so as to maximize the number of positions of each sequence for which the nucleotides are identical and dividing the number of positions of each sequence for which the nucleotides are identical by the number of  
5 nucleotides of the longer of the two sequences.

As intended herein "latent HIV-1-infected cells" are cells in which HIV-1 sequences can be found integrated in one of their chromosomes and which do not express HIV-1 encoded RNAs or proteins. Such cells, in particular peripheral blood multinuclear cells, more particularly T cells, even more particularly CD4+ T cells, can  
10 notably be obtained from asymptomatic patients infected by HIV-1, such as HAART treated patients or elite HIV-1 controller patients. Determining whether HIV-1 sequences can be found integrated in one of the chromosomes of said cells can be carried out by numerous methods well known to one of skill in the art, such as Polymerase Chain Reaction (PCR) experiments conducted with HIV-1 specific  
15 primers. Determining whether said cells are latent can be carried out by measuring the expression of a HIV-1 encoded RNA or protein (*e.g.* the p24 antigen), by said cells, in particular using respectively quantitative Reverse-Transcriptase Polymerase Chain Reaction (qRT-PCR) or immunological methods, such as Enzyme-Linked Immunosorbent Assays (ELISA) as is notably described in the Examples. Latent cells  
20 express essentially no HIV-1-encoded RNAs and proteins, which can be defined as a level of expression which is undetectable (*e.g.* lower than 40 HIV-1 RNA copies/ml when using quantitative RT-PCR) or which is not significantly different from that of control cells, for instance non-HIV-1 infected cells.

As intended herein, establishing whether a nucleic acid is liable to induce HIV-  
25 1 expression in latent HIV-1-infected cells can be determined by comparing the expression level of a HIV-1 encoded protein, such as the p24 antigen, in cells contacted with a nucleic acid according to the invention with identical control cells which have not been contacted with the nucleic acid of the invention. If the contacted cells present a significantly altered level of expression of the HIV-1-encoded protein  
30 with respect to the control cells, the nucleic acid will be said liable to induce HIV-1 expression in latent HIV-1-infected cells.

In a preferable embodiment of the invention, a RNA molecule consisting of SEQ ID NO: 1, a RNA molecule consisting of SEQ ID NO: 2, a RNA molecule consisting of SEQ ID NO: 3, and a RNA molecule consisting of SEQ ID NO: 4 are

administered to a patient in need thereof in combination or are present together in a same medicament or pharmaceutical composition.

### *Compound*

5           As intended herein a “component of the miRNA pathway” relates to any one of the cellular proteins involved in the synthesis, the maturation, and the action of microRNAs (miRNAs). The components of the miRNA pathway are well known by one of skill in the art and are notably described in Bartel (2004) *Cell* **116**:281-97 and Beckham and Parker (2008) *Cell Host Microbe* **3**:206-12. In particular, the  
10 components of the miRNA pathway are selected from Drosha, DGCR8 (which are involved in the maturation pre-miRNAs upon their synthesis by RNA polymerases II or III), Dicer (which is involved in the maturation of pre-miRNAs to miRNAs), RCK/p54, LSm-1, GW182, and XRN1 (which are involved in the degradation of targeted mRNAs). More preferably, the components of the miRNA pathway are  
15 selected from the group consisting of DGCR8, RCK/p54, LSm-1, GW182, and XRN1.

More preferably, the components of the miRNA pathway are selected from the group consisting of DGCR8, RCK/p54, LSm-1, GW182, and XRN1.

By way of example Drosha is represented by SEQ ID NO: 6, DGCR8 is represented by SEQ ID NO: 8, Dicer is represented by SEQ ID NO: 10, RCK/p54 is  
20 represented by SEQ ID NO: 12, LSm-1 is represented by SEQ ID NO: 14, GW182 is represented by SEQ ID NO: 16, and XRN1 is represented by SEQ ID NO: 18.

As intended herein, the compound of the invention can be of any type. In particular, the compound of the invention may have the ability to directly interfere with the activity of a component of the miRNA pathway. The compound can also interfere  
25 with the expression of the component of the miRNA pathway at the transcriptional or the translational level. Where the compound interferes with the expression of the component of the miRNA pathway at the translational level, it can notably be an effector nucleic acid targeting a mRNA encoding a component of the miRNA pathway or a nucleic acid encoding said effector nucleic acid, such as a viral vector. In  
30 particular, the effector nucleic acid can be a DNA or RNA antisense oligonucleotide or a small interfering RNA (siRNA).

The effector nucleic acid of the invention can comprise non-natural modifications of the bases or bonds, in particular for increasing their resistance to

degradation. Where the nucleic acid is RNA, Modifications notably encompass capping its ends or modifying the 2' position of the ribose backbone so as to decrease the reactivity of the hydroxyl moiety, for instance by suppressing the hydroxyl moiety (to yield a 2'-deoxyribose or a 2'-deoxyribose-2'-fluororibose), or substituting the hydroxyl moiety with an alkyl group, such as a methyl group (to yield a 2'-O-methyl-ribose).

Preferably, effector nucleic acids according to the invention are less than 50 nucleotides long, more preferably less than 40 nucleotides long, and most preferably less than 30 nucleotides long. Preferably also, effector nucleic acids according to the invention are at least 10 nucleotides long, more preferably at least 15 nucleotides long, and most preferably at least 20 nucleotides long.

The siRNAs of the invention are preferably double-stranded.

As intended herein the term "siRNA" encompasses "small hairpin RNA (shRNA)". shRNAs are formed of a self-hybridizing single stranded RNA molecule liable to yield a double-stranded siRNA upon processing of the single-stranded part of the shRNA linking the hybridized parts of the shRNA. As is well known to one of skill in the art, shRNAs transcribed from a nucleic acid which has been delivered into a target cell are the precursors of choice for siRNAs where the production of the siRNAs is to occur within a cell. As will be clear to one of skill in the art, the preferred length given above for the effector nucleic acids apply to shRNAs considered in their hybridized conformation and should be doubled if the shRNAs are considered in their unhybridized conformation.

It is well within the reach of one of skill in the art to devise a siRNA intended to target a specific mRNA where the sequence of the mRNA is known either partially or in totality and to deliver siRNAs, or nucleic acids encoding siRNAs and shRNAs into cells *in vitro* or *in vivo*, as is notably reported by Dykxhoorn *et al.* (*op. cit.*) and Nguyen *et al.* (*op. cit.*)

By way of example, siRNAs targeting Drosha, DGCR8, Dicer, RCK/p54, LSm-1, GW182, and XRN1 are respectively represented by SEQ ID NO: 19, 20, 21, 22, 23, 24 and 25.

### *Modulator*

As intended herein, the modulator of the invention can be of any type. Besides, as will be clear to one of skill in the art, the modulator of the invention may

either activate or inhibit (*i.e.* interfere with) the activity of a gene selected from the group consisting of DGUOK, MIR16, PPP1R11, ARHGAP1, TEDDM1, QDPR, C14orf32, C1orf19, ATP1B3, FLJ10241, ANP32E, TAGLN2, ARF3, PTMA, PPIB, PRCP, PTPRK, OBSL1, SLC44A1, PPIAL4, SERP1, EBPL, CBX6, ZBED3, NP, 5 PRSS21, PPIA, C5orf13, E2F2, CACYBP, TROAP, APOBEC3A, C7orf44, ORC6L, WNT10B, VIM, CDC6, MCERS1, NAG18, PPP1CC, DULLARD, ASF1B, PLP2, MTHFD2, PIGS, KIF2C, NRM, PEG10, C22orf9, COL4A2, and SNX26.

Advantageously, a modulator of the invention inhibiting the activity of one of the above genes is useful to activate the viral replication of a retrovirus, thereby 10 enabling to eradicate a latent retrovirus reservoir in an individual.

Equally advantageously, a modulator of the invention activating the activity of one of the above genes is useful to inhibit the viral replication of a retrovirus.

In particular, the modulator of the invention may have the ability to directly activate or inhibit the activity of the genes selected from the group consisting of 15 DGUOK, MIR16, PPP1R11, ARHGAP1, TEDDM1, QDPR, C14orf32, C1orf19, ATP1B3, FLJ10241, ANP32E, TAGLN2, ARF3, PTMA, PPIB, PRCP, PTPRK, OBSL1, SLC44A1, PPIAL4, SERP1, EBPL, CBX6, ZBED3, NP, PRSS21, PPIA, C5orf13, E2F2, CACYBP, TROAP, APOBEC3A, C7orf44, ORC6L, WNT10B, VIM, CDC6, MCERS1, NAG18, PPP1CC, DULLARD, ASF1B, PLP2, MTHFD2, PIGS, 20 KIF2C, NRM, PEG10, C22orf9, COL4A2, and SNX26. The modulator can also activate or inhibit the expression of these genes at the transcriptional or the translational level.

Where the modulator interferes with the expression of the genes selected from the group consisting of DGUOK, MIR16, PPP1R11, ARHGAP1, TEDDM1, QDPR, 25 C14orf32, C1orf19, ATP1B3, FLJ10241, ANP32E, TAGLN2, ARF3, PTMA, PPIB, PRCP, PTPRK, OBSL1, SLC44A1, PPIAL4, SERP1, EBPL, CBX6, ZBED3, NP, PRSS21, PPIA, C5orf13, E2F2, CACYBP, TROAP, APOBEC3A, C7orf44, ORC6L, WNT10B, VIM, CDC6, MCERS1, NAG18, PPP1CC, DULLARD, ASF1B, PLP2, MTHFD2, PIGS, KIF2C, NRM, PEG10, C22orf9, COL4A2, and SNX26, at the 30 translational level, it can notably be an effector nucleic acid targeting a mRNA encoding one of these genes or a nucleic acid encoding said effector nucleic acid, such as a viral vector. In particular, the effector nucleic acid can be a DNA or RNA antisense oligonucleotide or a small interfering RNA (siRNA).

The effector nucleic acid of the invention can comprise non-natural modifications of the bases or bonds, in particular for increasing their resistance to degradation. Where the nucleic acid is RNA, Modifications notably encompass capping its ends or modifying the 2' position of the ribose backbone so as to decrease the reactivity of the hydroxyl moiety, for instance by suppressing the hydroxyl moiety (to yield a 2'-deoxyribose or a 2'-deoxyribose-2'-fluororibose), or substituting the hydroxyl moiety with an alkyl group, such as a methyl group (to yield a 2'-O-methyl-ribose).

Preferably, effector nucleic acids according to the invention are less than 50 nucleotides long, more preferably less than 40 nucleotides long, and most preferably less than 30 nucleotides long. Preferably also, effector nucleic acids according to the invention are at least 10 nucleotides long, more preferably at least 15 nucleotides long, and most preferably at least 20 nucleotides long.

The siRNAs of the invention are preferably double-stranded.

As intended herein the term "siRNA" encompasses "small hairpin RNA (shRNA)". shRNAs are formed of a self-hybridizing single stranded RNA molecule liable to yield a double-stranded siRNA upon processing of the single-stranded part of the shRNA linking the hybridized parts of the shRNA. As is well known to one of skill in the art, shRNAs transcribed from a nucleic acid which has been delivered into a target cell are the precursors of choice for siRNAs where the production of the siRNAs is to occur within a cell. As will be clear to one of skill in the art, the preferred length given above for the effector nucleic acids apply to shRNAs considered in their hybridized conformation and should be doubled if the shRNAs are considered in their unhybridized conformation.

It is well within the reach of one of skill in the art to devise a siRNA intended to target a specific mRNA where the sequence of the mRNA is known either partially or in totality and to deliver siRNAs, or nucleic acids encoding siRNAs and shRNAs into cells *in vitro* or *in vivo*, as is notably reported by Dykxhoorn *et al.* (*op. cit.*) and Nguyen *et al.* (*op. cit.*)

Where the modulator activates the activity of the genes selected from the group consisting of DGUOK, MIR16, PPP1R11, ARHGAP1, TEDDM1, QDPR, C14orf32, C1orf19, ATP1B3, FLJ10241, ANP32E, TAGLN2, ARF3, PTMA, PPIB, PRCP, PTPRK, OBSL1, SLC44A1, PPIAL4, SERP1, EBPL, CBX6, ZBED3, NP, PRSS21, PPIA, C5orf13, E2F2, CACYBP, TROAP, APOBEC3A, C7orf44, ORC6L,

WNT10B, VIM, CDC6, MCRS1, NAG18, PPP1CC, DULLARD, ASF1B, PLP2, MTHFD2, PIGS, KIF2C, NRM, PEG10, C22orf9, COL4A2, and SNX26, it can notably a nucleic acid expressing one of these genes, such as an expression vector, in particular a viral vector, harbouring a sequence of one of these genes.

- 5 The above genes are well known to one of skill in the art and are notably represented by the NCBI accession numbers or the SEQ ID NOs listed in the following table. The NCBI accession numbers and the SEQ ID NOs refer to the sequences of the mRNAs or to the coding sequences (CDS) of the listed genes.

| Gene Symbol | Gene Name                                                                | Accession number (NCBI) | SEQ ID NO : |
|-------------|--------------------------------------------------------------------------|-------------------------|-------------|
| DGUOK       | deoxyguanosine kinase                                                    | NM_080916               | 26          |
| MIR16       | membrane interacting protein of RGS16                                    | AY463154                | 27          |
| PPP1R11     | protein phosphatase 1, regulatory (inhibitor) subunit 11                 | NM_021959               | 28          |
| ARHGAP1     | Rho GTPase activating protein 1                                          | NM_004308               | 29          |
| TEDDM1      | transmembrane epididymal protein 1                                       | NM_172000               | 30          |
| QDPR        | quinoid dihydropteridine reductase                                       | NM_000320               | 31          |
| C14orf32    | mitogen-activated protein kinase 1 interacting protein 1-like            | NM_144578               | 32          |
| C1orf19     | homolog of S. cerevisiae tRNA splicing endonuclease 15                   | NM_052965               | 33          |
| ATP1B3      | ATPase, Na <sup>+</sup> /K <sup>+</sup> transporting, beta 3 polypeptide | NM_001679               | 34          |
| FLJ10241    | ATP5S-like                                                               | NM_018035               | 35          |
| ANP32E      | acidic (leucine-rich) nuclear phosphoprotein 32 family, member E         | NM_030920               | 36          |
| TAGLN2      | transgelin 2                                                             | NM_003564               | 37          |
| ARF3        | ADP-ribosylation factor 3                                                | NM_001659               | 38          |
| PTMA        | prothymosin alpha                                                        | NM_002823               | 39          |
| PPIB        | peptidylprolyl isomerase B (cyclophilin B)                               | NM_000942               | 40          |
| PRCP        | prolylcarboxypeptidase (angiotensinase C)                                | NM_005040               | 41          |
| PTPRK       | protein tyrosine phosphatase, receptor type, K                           | NM_002844               | 42          |
| OBSL1       | obscurin-like 1                                                          | NM_015311               | 43          |
| SLC44A1     | solute carrier family 44, member 1                                       | NM_080546               | 44          |
| PPIAL4      | peptidylprolyl isomerase A (cyclophilin A)-like 4A                       | NM_178230               | 45          |
| SERP1       | stress-associated endoplasmic reticulum protein 1                        | NM_014445               | 46          |
| EBPL        | emopamil binding protein-like                                            | NM_032565               | 47          |
| CBX6        | chromobox homolog 6                                                      | NM_014292               | 48          |
| ZBED3       | zinc finger, BED-type containing 3                                       | NM_032367               | 49          |
| NP          | nucleoside phosphorylase                                                 | NM_000270               | 50          |
| PRSS21      | protease, serine, 21 (testisin)                                          | NM_144956               | 51          |
| PPIA        | peptidylprolyl isomerase A (cyclophilin A)                               | NM_021130               | 52          |
| C5orf13     | chromosome 5 open reading frame 13                                       | NM_004772               | 53          |
| E2F2        | E2F transcription factor 2                                               | NM_004091               | 54          |
| CACYBP      | calcyclin binding protein                                                | NM_014412               | 55          |
| TROAP       | trophinin associated protein (tastin)                                    | NM_005480               | 56          |

|          |                                                                                                      |           |    |
|----------|------------------------------------------------------------------------------------------------------|-----------|----|
| APOBEC3A | apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A                                  | NM_145699 | 57 |
| C7orf44  | chromosome 7 open reading frame 44                                                                   | NM_018224 | 58 |
| ORC6L    | origin recognition complex, subunit 6 like (yeast)                                                   | NM_014321 | 59 |
| WNT10B   | wingless-type MMTV integration site family, member 10B                                               | NM_003394 | 60 |
| VIM      | vimentin                                                                                             | EF445046  | 61 |
| CDC6     | homolog of <i>S. cerevisiae</i> cell division cycle 6                                                | NM_001254 | 62 |
| MCRS1    | microspherule protein 1                                                                              | NM_006337 | 63 |
| NAG18    | NAG18                                                                                                | AF210651  | 64 |
| PPP1CC   | protein phosphatase 1, catalytic subunit, gamma isoform                                              | NM_002710 | 65 |
| DULLARD  | homolog of <i>Xenopus laevis</i> dullard                                                             | NM_015343 | 66 |
| ASF1B    | homolog B of <i>S. cerevisiae</i> ASF1 anti-silencing function 1                                     | NM_018154 | 67 |
| PLP2     | proteolipid protein 2 (colonic epithelium-enriched)                                                  | NM_002668 | 68 |
| MTHFD2   | methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase | NM_006636 | 69 |
| PIGS     | phosphatidylinositol glycan anchor biosynthesis, class S                                             | NM_033198 | 70 |
| KIF2C    | kinesin family member 2C                                                                             | NM_006845 | 71 |
| NRM      | nurim (nuclear envelope membrane protein)                                                            | NM_007243 | 72 |
| PEG10    | paternally expressed 10                                                                              | NM_015068 | 73 |
| C22orf9  | chromosome 22 open reading frame 9                                                                   | NM_015264 | 74 |
| COL4A2   | collagen, type IV, alpha 2                                                                           | NM_001846 | 75 |
| SNX26    | sorting nexin 26                                                                                     | NM_052948 | 76 |

### Administration

Where a therapeutic use of: the nucleic acid of the invention, the compound of the invention, or the modulator of the invention, or a medicament or a pharmaceutical composition comprising the nucleic acid of the invention, the compound of the invention, or the modulator of the invention, is contemplated, the nucleic acid, the compound, and the modulator can be associated to one or more pharmaceutically acceptable carriers. In particular, it is preferred that the pharmaceutically acceptable carrier be suitable for delivering nucleic acid into cells. Carriers suitable for delivering nucleic acid into cells are well known to one of skill in the art and notably comprise cationic lipids or peptides, nanoparticles and liposomes, optionally linked to moieties, such as antibodies or antibody fragments, having a specificity towards a specific receptor of the target cells, notably T cells.

Either local or systemic routes can be used for administering the nucleic acid of the invention, the compound of the invention or the modulator of the invention. Examples of administration procedures for nucleic acids are notably described in Nguyen *et al.* (*op. cit.*) and Dykxhoorn *et al.* (*op. cit.*)

### *Anti-Retroviral compound*

Preferably, the other anti-retroviral compound as defined above is selected from the group consisting of a reverse-transcriptase inhibitor and a protease inhibitor, such as described in Hammer *et al.* (2008) *JAMA* **300**:555-70.

Reverse-transcriptase inhibitors are a well-known class of anti-retroviral compounds targeting the retroviral enzyme which catalyses reverse-transcription of the RNA genome of the retrovirus to DNA. Reverse-transcriptase inhibitors notably comprise:

- 10       - Nucleoside analogs reverse transcriptase inhibitors (NRTIs), such as Zidovudine (*i.e.* AZT), Didanosine, Zalcitabine, Stavudine, Lamivudine, Abacavir, and Emtricitabine;
- Nucleotide analogs reverse transcriptase inhibitors (NtRTIs, such as Tenofovir and Adefovir;
- 15       - Non-nucleoside reverse transcriptase inhibitors (NNRTIs), such as Efavirenz, Nevirapine, Delavirdine, Etravirine.

Protease inhibitors are a well-known class of anti-retroviral compounds targeting the retroviral enzyme which catalyses cleavage of polyproteins expressed by retroviral genomes. Protease inhibitors notably comprise: Saquinavir, Ritonavir, Indinavir, Nelfinavir, Amprenavir, Lopinavir, Atazanavir, Fosamprenavir, Tipranavir, and Darunavir.

Anti-retroviral compounds are often used in combinations. For instance, in the frame of Highly Active Antiretroviral Therapy (HAART), two or more different anti-retroviral compounds are used in combination, for instance 2 NRTIs and a protease inhibitor or 2 NRTIs and a NNTI. The definition of combinations suited for a particular retrovirus-infected patient are within the ordinary skills of one skilled in the art.

### *Retrovirus infection*

As intended herein the terms "retrovirus" or "retroviral" relate to viruses of the *Retroviridae* family, more particularly of the *Lentivirus* genus. The retroviruses of the invention notably encompass the Human Immunodeficiency Virus (HIV), in particular HIV-1 and HIV-2, the Simian Immunodeficiency Virus (SIV), and the Feline Immunodeficiency Virus (FIV).

Preferably, the nucleic acid of the invention and the compound of the invention, or medicaments or pharmaceutical compositions comprising the nucleic acid of the invention or the compound of the invention, are intended to treat asymptomatic patients infected by a retrovirus.

5 As intended herein, the expression “asymptomatic patients infected by a retrovirus” refers to individuals harbouring cells in which retroviral sequences can be found integrated in one of their chromosomes but who do not express the retroviral sequences. Identification of such individuals is well within the common skills of one of skill in the art and notably involves measuring blood, serum or plasma levels of  
10 retrovirus RNAs or antigens (e.g. the p24 antigen of HIV-1) using respectively qRT-PCR and immunological techniques for instance. In particular, patients are said to be asymptomatic if the retroviral sequences expression products (i.e. RNAs and proteins) are undetectable.

Where the asymptomatic patients are infected by HIV-1, they can notably be  
15 under Highly Active Antiretroviral Therapy (HAART) or elite HIV-1 controllers.

#### *Production of retroviral particles*

As intended herein a “retroviral particle” or a “retroviral vector” relate to particles or vectors derived from viruses the *Retroviridae* family, more particularly of  
20 the *Lentivirus* genus, which notably encompass the Human Immunodeficiency Virus (HIV), in particular HIV-1 and HIV-2, the Simian Immunodeficiency Virus (SIV), and the Feline Immunodeficiency Virus (FIV).

The retroviral particle or vector of the invention can respectively comprise and encode elements (e.g. nucleic acids and proteins) which are not of a retroviral origin.  
25 The retroviral particles can notably harbour envelope proteins intended to target it to specific cells and tissues, in particular to deliver transgenes. Such particles and vectors are well known in the art, as reported by Cronin *et al.* (2005) *Curr. Gene Ther.* 5:387-398, and are generally referred to as pseudotyped retroviral particles and vectors.

30 Methods for producing retroviral particles from retroviral vectors are well known to one of skill in the art and the method of the invention can be easily implemented, in particular in view of the following examples.

Advantageously, the above-defined *in vitro* use and *in vitro* method can be carried out without culturing the cells with T cells.

## Sequence Description

| Sequence description        | SEQ ID<br>NO : |
|-----------------------------|----------------|
| miR-34a                     | 1              |
| miR-122                     | 2              |
| miR-206                     | 3              |
| miR-210                     | 4              |
| Drosha nucleotide sequence  | 5              |
| Drosha amino acid sequence  | 6              |
| DGCR8 nucleotide sequence   | 7              |
| DGCR8 amino acid sequence   | 8              |
| Dicer nucleotide sequence   | 9              |
| Dicer amino acid sequence   | 10             |
| RCK/p54 nucleotide sequence | 11             |
| RCK/p54 amino acid sequence | 12             |
| LSm-1 nucleotide sequence   | 13             |
| LSm-1 amino acid sequence   | 14             |
| GW182 nucleotide sequence   | 15             |
| GW182 amino acid sequence   | 16             |
| XRN1 nucleotide sequence    | 17             |
| XRN1 amino acid sequence    | 18             |
| siRNA targeting Drosha      | 19             |
| siRNA targeting DGCR8       | 20             |
| siRNA targeting Dicer       | 21             |
| siRNA targeting RCK/p54     | 22             |
| siRNA targeting LSm-1       | 23             |
| siRNA targeting GW182       | 24             |
| siRNA targeting XRN1        | 25             |
| DGUOK                       | 26             |
| MIR16                       | 27             |
| PPP1R11                     | 28             |
| ARHGAP1                     | 29             |
| TEDDM1                      | 30             |
| QDPR                        | 31             |
| C14orf32                    | 32             |
| C1orf19                     | 33             |
| ATP1B3                      | 34             |
| FLJ10241                    | 35             |
| ANP32E                      | 36             |
| TAGLN2                      | 37             |
| ARF3                        | 38             |
| PTMA                        | 39             |
| PPIB                        | 40             |
| PRCP                        | 41             |
| PTPRK                       | 42             |

|          |    |
|----------|----|
| OBSL1    | 43 |
| SLC44A1  | 44 |
| PPIAL4   | 45 |
| SERP1    | 46 |
| EBPL     | 47 |
| CBX6     | 48 |
| ZBED3    | 49 |
| NP       | 50 |
| PRSS21   | 51 |
| PPIA     | 52 |
| C5orf13  | 53 |
| E2F2     | 54 |
| CACYBP   | 55 |
| TROAP    | 56 |
| APOBEC3A | 57 |
| C7orf44  | 58 |
| ORC6L    | 59 |
| WNT10B   | 60 |
| VIM      | 61 |
| CDC6     | 62 |
| MCRS1    | 63 |
| NAG18    | 64 |
| PPP1CC   | 65 |
| DULLARD  | 66 |
| ASF1B    | 67 |
| PLP2     | 68 |
| MTHFD2   | 69 |
| PIGS     | 70 |
| KIF2C    | 71 |
| NRM      | 72 |
| PEG10    | 73 |
| C22orf9  | 74 |
| COL4A2   | 75 |
| SNX26    | 76 |

### Brief description of the drawings

- 5 **FIG. 1** depicts the effect of knockdown of RCK/p54, LSm-1, GW182, XRN1, DGCR8, Drosha or control protein CDK9 by transfection with specific siRNA (horizontal axes ), on virus production (vertical axes), compared to virus production after transfection with scramble siRNA (sc).

**FIG. 2** shows virus production estimated by the quantity of p24 antigen (vertical axes) present in PBMCs isolated from healthy donor in contact with PBMCs isolated from HAART-treated HIV-1 from three patients (1, 2, 3) transfected with scramble siRNA (sc) or with Drosha, DGCR8 or RCK/p54 specific siRNA (horizontal axes).

5

**FIG. 3** shows the effects of different miRNA or of control miRNA (ctrl) (horizontal axes) on the LTR activity (vertical axes), in HeLa cells containing an integrated LTR-luciferase construct with an empty vector (white bars) or with a Tat expressing vector (hatched bars).

10

**FIG. 4** shows the HIV production (vertical axes) in HeLa cells transfected with siRNA specific for different miRNA, with scramble siRNA (sc), or with DGCR8 specific siRNA (horizontal axes) and infected with HIV-1 harbouring the luciferase gene.

15 **FIG. 5** shows HIV reactivation (vertical axes), in PBMCs isolated from a first patient Elite HIV-1 Controller with undetectable viremia, after transfection with control miRNA (diamonds) or with a mix of miRNA: miR-34a, miR-206, miR-122 and miR-210 (squares) in function of time (in days, horizontal axes).

20 **FIG. 6** shows HIV reactivation (vertical axes), in PBMCs isolated from a second patient Elite HIV-1 Controller with undetectable viremia, after transfection with control miRNA (diamonds) or with a mix of miRNA: miR-34a, miR-206, miR-122 and miR-210 (squares) in function of time (in days, horizontal axes).

25 **FIG. 7** shows HIV reactivation (vertical axes), in PBMCs isolated from a third patient Elite HIV-1 Controller with undetectable viremia, after transfection with control miRNA (diamonds) or with a mix of miRNA: miR-34a, miR-206, miR-122 and miR-210 (squares) in function of time (in days, horizontal axes).

30 **FIG. 8** shows HIV reactivation (vertical axes), in PBMCs isolated from a fourth patient Elite HIV-1 Controller with undetectable viremia, after transfection with control miRNA (diamonds) or with a mix of miRNA: miR-34a, miR-206, miR-122 and miR-210 (squares) in function of time (in days, horizontal axes).

**FIG. 9** shows HIV reactivation (vertical axes), in PBMCs isolated from a fifth patient Elite HIV-1 Controller with undetectable viremia, after transfection with control miRNA (diamonds) or with a mix of miRNA: miR-34a, miR-206, miR-122 and miR-210 (squares) in function of time (in days, horizontal axes).

5

**FIG. 10** shows HIV reactivation (vertical axes), in PBMCs isolated from a first HAART-treated infected patient, with undetectable viremia, after transfection with control miRNA (diamonds) or with a mix of miRNA: miR-34a, miR-206, miR-122 and miR-210 (squares) in function of time (in days, horizontal axes).

10

**FIG. 11** shows HIV reactivation (vertical axes), in PBMCs isolated from a second HAART-treated infected patient, with undetectable viremia, after transfection with control miRNA (diamonds) or with a mix of miRNA: miR-34a, miR-206, miR-122 and miR-210 (squares) in function of time (in days, horizontal axes).

15

**FIG. 12** shows HIV reactivation (vertical axes), in PBMCs isolated from a third HAART-treated infected patient, with undetectable viremia, after transfection with control miRNA (diamonds) or with a mix of miRNA: miR-34a, miR-206, miR-122 and miR-210 (squares) in function of time (in days, horizontal axes).

20

**FIG. 13** shows HIV reactivation (vertical axes), in PBMCs isolated from a fourth HAART-treated infected patient, with undetectable viremia, after transfection with control miRNA (diamonds) or with a mix of miRNA: miR-34a, miR-206, miR-122 and miR-210 (squares) in function of time (in days, horizontal axes).

25

**FIG. 14** shows the expression of miR-210 by RT-PCR (vertical axes), in PBMCs isolated from Elite HIV-1 Controllers (squares), untreated HIV-1-infected patients (triangles) and HAART-treated HIV-1-infected patients (upside down triangles).

30

**FIG. 15** shows the expression of miR-34a by RT-PCR (vertical axes), in PBMCs isolated from Elite HIV-1 Controller (squares), untreated HIV-1-infected patients (triangles) and HAART-treated HIV-1-infected patients (upside down triangles).

**FIG. 16** shows the expression of miR-206 by RT-PCR (vertical axes), in PBMCs isolated from Elite HIV-1 Controllers (squares), untreated HIV-1-infected patients (triangles) and HAART-treated HIV-1-infected patients (upside down triangles).

- 5 **FIG. 17** shows the expression of miR-122 by RT-PCR (vertical axes), in PBMCs isolated from Elite HIV-1 Controllers (squares), untreated HIV-1-infected patients (triangles) and HAART-treated HIV-1-infected patients (upside down triangles).

## EXAMPLES

### Example 1: miRNA effectors are repressors of HIV-1 gene expression

- 5 To investigate whether RNAi effectors regulate HIV-1 replication, virus replication was analyzed in cells where expression of RNAi effectors was reduced using specific siRNA.

#### METHODS

- 10 HeLa cells were transfected with siRNA as indicated in Triboulet *et al.* (2007) *Science* **315** (5818):1579-82. 48 hours post transfection, cells were analyzed for RCK/p54, LSm-1, GW182, XRN1, DGCR8, DROSHA and CDK9 expression by western blot, or infected with a single round infectious virus (HIV-1-VSV-luc) and cell extracts were measured for luciferase activity 48 hrs after infection. RCK/p54 restricts
- 15 HIV-1 mRNA association with polysomes. Cytoplasmic extracts from HeLa cells that were transfected with the indicated siRNA and infected with HIV-1-VSVG-luc were run on glycerol gradient (7% to 47%). Fractions were collected and their RNA contents were monitored by measuring absorbance (OD) at 254 nm. HIV-1 mRNA and Hdm2 mRNA were quantified in all the fractions by Q-RT-PCR using specific
- 20 oligonucleotides.

#### RESULTS

- HeLa cells were transfected with siRNA specific to RCK/p54, LSm-1, GW182 XRN1 or DGCR8. As controls, HeLa cells were transfected with scrambled siRNA (Scr) or
- 25 CDK9 specific siRNA. Knockdown of RCK/p54, LSm-1, GW182 and XRN1 enhanced virus replication by up to 10 fold (**FIG. 1**). As previously shown, (Triboulet *et al.* (2007) *Science* **315** (5818):1579-82) knockdown of DROSHA and DGCR8 (**FIG. 1**), the two subunits of the microprocessor complex, increased virus production while knockdown of CDK9 subunit of the PTEFb complex that is known to be required for
- 30 viral gene expression, reduced it (**FIG. 1**). Interestingly, analysis of HIV-1 cytoplasmic mRNA distribution on glycerol gradient shows that knockdown of RCK/p54 shifted HIV-1 mRNA from the non-polysomal fraction to polysomes as compared to control siRNA transfected cells. Knockdown of RCK/p54 did not affect endogenous Hdm2 mRNA distribution.

These experiments show that GW182, RCK/p54, LSm-1 and XRN1 required for RNAi are repressors of HIV-1 gene expression by preventing HIV-1 mRNA translation.

5     **Example 2: HIV-1mRNA is physically associated with Argonaute2 and co-localizes with protein required for miRNA-mediated silencing**

The physical interaction between RNAi effectors and HIV-1 mRNA was investigated.

10    **METHODS**

HeLa cells were transfected with HIV-1 molecular clone pNL4-3, Myc-Ago2 or Myc-AgoPAZ9 as indicated. 48 hrs later cells were harvested and cytoplasmic extracts were prepared. Total RNA was purified from a fraction of harvested cells while the rest was subjected to immunoprecipitation using anti-Myc antibody. After washing, a  
15    fraction was used to analyze the amount of Myc-Ago2 and Myc-AgoPAZ9 immunoprecipitated by western blot and the rest of the Myc-IPs was used for RNA extraction. HIV-1 mRNAs (TAR and unspliced), Hdm2 and GAPDH mRNA were quantified from total RNA or from Myc immunoprecipitated mRNPs by RT-PCR using specific oligonucleotides. The experiment was also performed using <sup>32</sup>P-labelled  
20    nucleotides in the PCR reaction. PCR products were visualized by autoradiography.

**RESULTS**

HeLa cells were mock transfected or transfected with combinations of pNL4-3, Myc-Ago2, a central component of the RISC complex, or its RNA binding mutant Myc-Ago2PAZ9. First, the fact that Myc-Ago2 and Myc-Ago2DPAZ9 were equally  
25    expressed was verified. Second, cytoplasmic extracts were prepared and a fraction was used for total RNA extraction while the rest was subjected to immunoprecipitation using anti-Myc antibody to purify myc-Ago2 associated mRNP. Both total RNA and Myc-Ago2 associated RNA were reverse transcribed and  
30    subjected to PCR amplification using oligonucleotides specific for HIV-1 TAR RNA (a 5' structure associated with all HIV-1 mRNA) or HIV-1 unspliced mRNA, Hdm2 mRNA or GAPDH mRNA. PCR analysis of total RNA shows that equal amount of HIV-1, Hdm2 and GAPDH mRNAs were present in all samples. HIV-1 mRNAs (both TAR and unspliced) were associated with Myc-Ago2 but not with Myc-Ago2PAZ9

mutant. Hdm2 mRNA was absent in Myc-Ago2 mRNPs suggest that, under these conditions, Hdm2 is not regulated by RNAi. A similar experiment was performed to analyze the association of HIV-1 multispliced mRNA with Myc-Ago2 mRNPs. The RT-PCR reactions were performed in presence of ATP-32P and analyzed by  
5 autoradiography. HIV-1 multispliced mRNAs associate with Myc-Ago2 and weakly with Myc-Ago2PAZ9. Co-localisation of HIV-1 mRNA and effectors of RNAi such as Ago2, RCK/p54 and DCP1 within the P-Bodies was also observed by immunofluorescence using HIV-1 containing MS2 binding sites and MS2-GFP constructs.

10

The inventors show that HIV-1 mRNAs physically associate with Ago2, a central component of RISC, and co-localize with cellular proteins required for miRNA-mediated silencing such as RCK/p54 and DCP1/DCP2 in P-bodies. The fact that all HIV-1 mRNA species associate with the RISC suggest that cellular miRNA(s) target a  
15 sequence common to all of these mRNAs. Accordingly, Huang *et al.* (*op cit.*) identified 5 cellular miRNAs able to target the 3'UTR sequence present in all HIV-1 mRNAs. However, the fact that other cellular miRNA(s) able to target regions outside the 3'UTR may participate can not be ruled out.

20

### **Example 3: Accumulation of HIV-1 mRNA in P-bodies limits virus replication and is independent of A3G-mediated HIV-1 repression**

#### **METHODS**

25

HeLa CD4+ cells were transfected with siRNA as indicated. 48 hrs post transfection cells were analyzed for RCK/p54 and LSm-1 expression by western blot or infected with equal amount of HIV-1. Virus production was monitored 48 hrs post infection by measuring p24 antigen in culture supernatant. To analyze the infectivity of the virions produced from the different siRNA transfected HeLa cells, equal volumes of  
30 supernatant from siRNA transfected HeLa CD4+ were used to re-infect HeLa CD4+ cells. P24 antigen was measured in culture supernatant 48 hrs post infection. APOBEC3G and RNAi effectors-mediated HIV-1 inhibition involves different mechanisms. HeLa CD4+ cells were transfected with the indicated siRNA. 48 hrs later cells were analyzed for RCK/p54 and LSm-1 expression or co-transfected with

pNL4-3Dvif (lacking vif gene) and pCDNA or expression vectors for wild-type APOBEC3G or APOBEC3G double mutant lacking both deaminase and antiviral activity. HIV-1 production was measured 24 hrs post transfection in culture supernatant by quantifying p24 antigen. Infectivity assay was performed using equal  
5 amounts of p24 antigen to infect HeLa CD4+ cells. HIV-1 p24 antigen was measured 24 hrs post infection.

## RESULTS

Emerging evidence suggests physical and functional interactions between P-bodies  
10 and viral life cycles. Viral mRNA trafficking through P-bodies may represent a pool of translationally repressed viral transcripts for efficient packaging or formation of viral-replication complexes. Indeed, yeast retrotransposons Ty1 and Ty3 mRNA associate with P-bodies and this association is required for efficient retrotransposition. In case of BMV (Brome Mosaic Virus), formation of the virus replication complex occurs in P-  
15 bodies. In addition, P-bodies may also function in host defences against viruses and transposable elements. Indeed, the cellular factors APOBEC 3G and 3F, which are viral restriction factors, are found to accumulate in P-bodies. It has been suggested that 3G and 3F mediated HIV-1 restriction may involve viral mRNA targeting to P-bodies leading to their translational inhibition.

20 First, it was asked whether P-bodies are positive or negative regulators of HIV-1 replication. HeLa CD4+ cells were transfected with RCK/p54 or LSm-1 specific siRNA or control siRNA . Forty eight hours later cells were infected with equal amount of HIV-1. HIV-1 p24 antigen was measured in cell culture supernatant 48hrs post-infection. Knockdown of RCK/p54 and LSm-1 results in enhanced virus  
25 production as compared to infection of control siRNA transfected cells. To assess the infectivity of produced viruses, an equal volume of supernatant from Scr, RCK/p54 and LSm-1 siRNA transfected cells was used to infect HeLa CD4+ cells, and p24 in the culture supernatant was measured 48hrs later. Virus infectivity correlates with the amount of p24 produced showing that virions produced in RCK/p54 and LSm-1  
30 knocked down cells are fully competent for replication and have no defect such as RNA packaging. Since knockdown of RCK/p54 and LSm-1 were shown to result in P-bodies disruption, it was concluded from these experiments that accumulation of HIV-1 mRNA in P-bodies limits virus replication.

Second, it was asked whether APOBEC3G-mediated HIV-1 restriction requires effectors of miRNA-mediated mRNA translational inhibition associated and needed for P-bodies formation. Thus, APOBEC3G-mediated HIV-1 restriction in cells where RCK/p54 or LSm-1 expression is reduced was compared to control cells. HeLa cells were transfected with control siRNA or with siRNA specific for RCK/p54 or LSm-1. Forty eight hours later, cells were transfected with an HIV-1 molecular clone lacking the vif gene (pNL4-3Dvif) either alone or with wild-type A3G or A3G mutant lacking antiviral activity (A3Gdm). HIV-1 p24 antigen was measured in culture supernatant 48 hrs post-transfection. Interestingly, knock down of RCK/p54 or LSm-1 enhanced HIV-1 production regardless of A3G. Similarly, A3G but not A3Gdm reduced virus production regardless of RCK/p54 or LSm-1 expression. These results suggest that RCK/p54 or LSm-1 and A3G mediated HIV-1 repression involve different mechanisms. Next, the infectivity of HIV-1 produced from siRNA transfected cells was analyzed. Equal amount of p24 was used to infected HeLa CD4+ cells and HIV-1 p24 antigen was measured in culture supernatant 48 hrs post-infection. Virus produced in Scr siRNA transfected cells in presence of A3G show low infectivity than those produced in absence or in presence of A3Gdm. Similar HIV-1 restriction activity of A3G was observed when virus was produced in RCK/p54 or LSm-1 knocked down cells.

This experiment shows that accumulation of HIV-1 mRNA in P-bodies limits virus replication and that A3G-mediated HIV-1 restriction is independent of RNAi effectors RCK/p54 and LSm-1 and does not require P-bodies.

#### **Example 4: Endogenous levels of Drosha, DGCR8 and RCK/p54 contribute to HIV-1 latency in infected patients**

Taken together, these results show a physical repressive interaction between RNAi effectors and HIV-1 mRNA. Since cellular miRNAs were shown to play role in HIV-1 latency, it was asked whether RCK/p54, which is required for miRNA-mediated mRNA translational inhibition, contributes to HIV-1 silencing *in vivo*.

## METHODS

Implication of RNAi in HIV-1 latency. PBMCs were isolated from 3 patients undergoing active HAART. Isolated PBMCs were transfected with the indicated siRNA and either analyzed for RCK/p54, DGCR8 and DROSHA expression by western blot 48 hrs after transfection or co-cultured with activated PBMCs obtained from healthy donor. Virus replication was monitored every 3 to 4 days post co-culture by measuring p24 antigen in culture supernatant. Shown is the amount of p24 antigen at day 15 post co-culture. No virus was isolated from Sc transfected-PBMCs for up to 27 days

## RESULTS

PBMCs isolated from 3 HAART-treated HIV-1 infected patients with undetectable viremia were transfected with control siRNA or with siRNA specific for Drosha, DGCR8 or RCK/p54. Transfected cells were co-cultured with PHA/IL2-activated PBMCs isolated from healthy donors. Virus production was monitored every 3 days by measuring p24 antigen in the culture supernatant. Knockdown of Drosha results in virus reactivation in PBMCs isolated from HAART-treated HIV-1 infected patients (**FIG. 2**). HIV-1 reactivation is also seen when DGCR8, another component of the microprocessor complex, was knocked down using specific siRNA. Interestingly, knockdown of RCK/p54 lead to virus reactivation in naturally infected latent HIV-1 cells.

These results show that endogenous levels of Drosha, DGCR8 and RCK/p54 contribute to HIV-1 latency in infected patients.

### **Example 5: miR-34a, miR-206, miR-122 and miR-210 enhance virus expression in HeLa cells and in naturally isolated silent HIV-1 reservoirs.**

## METHODS

HIV-1 up-regulated cellular miRNA (Triboulet *et al.* (2007) *Science* **315** (5818):1579-82) were overexpressed in HeLa cells containing an integrated LTR-luciferase reporter construct. 24 hrs later, cells were transfected with empty or Tat-expressing vector. Luciferase activity was measured 24 hrs post-transfection (**FIG. 3**). HeLa CD4+ cells were transfected with the indicated miRNA or DGCR8 specific siRNA. 48 hrs post transfection cells were infected with HIV-1 expressing luciferase gene in nef

frame and luciferase activity was measured 24 hrs later (**FIG. 4**). PBMCs were isolated from 5 Elite HIV Controllers patients (**FIG. 5 to FIG. 9**) or 4 HIV-1-infected HAART-treated patients (**FIG. 10 to FIG. 13**). Isolated PBMCs were transfected with a mix of miR-34a, miR-206, miR-210 and miR-122 (miRmix) or with control miRNA (miR-32) as indicated. Transfected PBMCs were co-cultured with activated PBMCs obtained from healthy donors. Virus replication was monitored every 3 to 4 days post co-culture by measuring p24 antigen in culture supernatant. Small RNAs were purified from PBMCs isolated from healthy donors, HIV-1-infected HAART-treated, Elite HIV Controllers or HIV-1 infected untreated patients. miR-210, miR206, miR34a, miR-125 and U6 snoRNA were quantified by QRT-PCR using specific oligonucleotides. Results were normalized to U6 snoRNA.

## RESULTS

miRNA, through regulation of gene expression, play important role in the modulation of almost every cellular process investigated (cell differentiation, proliferation, apoptosis .....). In particular, miRNA were found to play an important role in immune system development and in the adaptive immune response. It is tempting to hypothesize that HIV-1 may use cellular miRNA to regulate genes important for its replication. Indeed, it was previously shown that infection of Jurkat cells with HIV-1 alters the miRNA expression profile with some miRNA being down-regulated while others were up-regulated (Triboulet *et al.* (2007) *Science* **315**:1579-1582). Two miRNAs (miR-17 and miR-20) of the down regulated miRNA cluster 17/92 target the histone acetyltransferase PCAF known to be required for Tat-mediated HIV-1 gene activation (Triboulet *et al. op. cit.*). In the present study, the function of HIV-1 up-regulated miRNA in virus replication was analyzed. *In silico* analysis show that none of HIV-1 induced miRNA can target viral mRNA suggesting that if HIV-1 induced miRNA play a role in virus replication, this effect will be mediated through targeting of HIV-1 repressive cellular genes. Among HIV-1 induced miRNAs, it was screened for those able to modulate HIV-1 promoter activity. HeLa cells containing integrated LTR-luciferase construct were transfected with the indicated miRNA either alone, to measure their effect on basal LTR activity, or cotransfected with Tat expression plasmid to analyse their effect on Tat-mediated transactivation of the LTR. While miR-34a and miR-206 enhanced basal LTR activity with no effect on Tat-mediated transactivation of the LTR, miR-210 and miR-122 had no effect on basal expression

level but enhanced Tat-mediated transcriptional activity toward the LTR (**FIG. 3**). miR-370 had no effect. As a control, miR-20a, which targets PCAF, reduced the ability of Tat to activate the LTR. This experiment suggests that miR-34a and miR-206 target a cellular gene involved in the repression of basal LTR activity while miR-122 and miR-210 target cellular (s) factor (s) that repress Tat transcriptional activity. Then, the effect of these miRNAs was analyzed on HIV-1 production using a single round infectious pNL4-3 molecular clone expressing luciferase inserted in nef open reading frame. Interestingly, miR-34a, miR-206, miR-122 and miR-210 enhanced virus expression in this assay with an impressive effect of miR-206 which enhanced virus production by 54 fold (**FIG. 4**). miR-370 had no significant effect on virus production in this assay.

In HIV-1 infected patients, there are two situations where HIV-1 is silenced at the gene expression level. First, HAART-treatment revealed the presence of silent HIV-1 reservoir which consists of memory CD4+ T cells containing integrated silent provirus. Second, HIV-infected individuals who are able to control their virus to undetectable levels for many years in the absence of treatment have been recently identified and referred to Elite HIV Controllers. The fact that miR-34a, miR-206, miR-122 and miR-210 enhanced viral LTR activity lead us to ask whether these miRNA may play role in HIV-1 silencing observed in infected patients. Thus, PBMCs isolated from 5 HIV-1 Elite Controllers and 4 HAART-treated HIV-1-infected patients with undetectable viremia were transfected with either control miRNA (miR-32) or a mix of miR-34a, miR-206, miR-122 and miR-210. Transfected PBMCs were co-cultured with PHA/IL2-activated PBMCs from healthy donors and p24 antigen in culture supernatant was measured every 3 to 4 days. Over expression of miRmix lead to virus reactivation in PBMCs from 5 Elite Controllers out of five tested (**FIG. 5 to FIG. 9**) and in 4 HAART-treated HIV-1-infected out of 4 tested (**FIG. 10 to FIG. 13**). miR control had no effect. These experiments show that miR-34a, miR-206, miR-122 and miR-210 are able to reactivate HIV-1 replication in naturally isolated silent HIV-1 reservoirs. Quantitative real time RT-PCR was then used to analyze the expression levels of these miRNAs in PBMCs from Elite Controllers, HAART-treated and HIV-1-infected untreated patients (**FIG. 14 to FIG.17**). Expression of miR-34a, miR-206 and miR-210 is low in PBMCs isolated from Elite HIV Controllers and HAART-treated HIV-1-infected patients compared to untreated HIV-1-infected patients. Expression of miR-122 was low in all the patients tested. Expression level of miR-125b, which is not

regulated by HIV-1, was similar in all PBMCs tested. Interestingly, as in Elite Controllers and HAART-treated patients, expression level of miR34a, miR-206 and miR-210 was low in healthy donors. These experiments suggest a correlation between the expression of miR34a, miR-206, miR-210 and HIV-1 replication.

5

#### **Example 4: identification of genes involved in the activation of HIV replication**

Among 135 putative target genes of miR-34a, miR-206, miR-210 and miR-122, the inventors have identified 51 genes (**Table 1**) which inhibition of the expression by siRNAs or shRNAs activates viral replication of HIV-1.

10

Briefly, a siRNA library specifically targeting the 135 putative target genes of miR-34a, miR-206, miR-210 and miR-122 has been generated. Each gene was thus specifically targeted by a pool of 4 siRNAs. The siRNAs were obtained from siGenome, Dharmacon.

HeLa cells were first transfected by siRNA pools with oligofectamine (Invitrogen). 48 h later, the cells were infected by a HIV virus pseudotyped a VSV-G envelope and expressing a luciferase report gene replacing the nef gene (HIV-VSVG-Luc). 48 h post-infection, cells were collected and the luciferase activity quantified (Luciferase assay kit, Promega). Luciferase activity was normalized with respect to the quantity of proteins in the cellular lysate measured by a Bradford assay.

20

51 genes could thus be identified which specific inhibition leads to an increase of viral replication in HeLa cells by a factor 5.

The above analysis was also carried out in other *in vitro* cell models closer to the physiological conditions of infection:

- 25 - HeLa CD4 cells, which express the CD4 receptor and the CCR5 and CXCR4 coreceptors; siRNAs were transfected according to the above procedure but the infected virus carried a HIV envelope (pNL4-3 –Luc);
- Jurkat T cells, peripheral blood mononuclear cells (PBMCs) from non-infected individuals as well as human macrophages; in these cases, genes are inhibited following transduction of shRNA-expressing lentiviral particles (TRC clones).

30

#### **Table 1:**

| Gene Symbol | Accession number (NCBI) | Gene ID |
|-------------|-------------------------|---------|
| DGUOK       | <u>NM_080916</u>        | 1716    |
| MIR16       | <u>AY463154</u>         | 53591   |
| PPP1R11     | <u>NM_021959</u>        | 6992    |
| ARHGAP1     | <u>NM_004308</u>        | 392     |
| TEDDM1      | <u>NM_172000</u>        | 127670  |
| QDPR        | <u>NM_000320</u>        | 5860    |
| C14orf32    | <u>NM_144578</u>        | 93487   |
| C1orf19     | <u>NM_052965</u>        | 116461  |
| ATP1B3      | <u>NM_001679</u>        | 483     |
| FLJ10241    | <u>NM_018035</u>        | 55101   |
| ANP32E      | <u>NM_030920</u>        | 81611   |
| TAGLN2      | <u>NM_003564</u>        | 8407    |
| ARF3        | <u>NM_001659</u>        | 377     |
| PTMA        | <u>NM_002823</u>        | 5757    |
| PPIB        | <u>NM_000942</u>        | 5479    |
| PRCP        | <u>NM_005040</u>        | 5547    |
| PTPRK       | <u>NM_002844</u>        | 6745    |
| OBSL1       | <u>NM_015311</u>        | 23363   |
| SLC44A1     | <u>NM_080546</u>        | 23446   |
| PPIAL4      | <u>NM_178230</u>        | 164022  |
| SERP1       | <u>NM_014445</u>        | 27230   |
| EBPL        | <u>NM_032565</u>        | 84650   |
| CBX6        | <u>NM_014292</u>        | 23466   |
| ZBED3       | <u>NM_032367</u>        | 84327   |
| NP          | <u>NM_000270</u>        | 4860    |
| PRSS21      | <u>NM_144956</u>        | 10942   |
| PPIA        | <u>NM_021130</u>        | 5478    |
| C5orf13     | <u>NM_004772</u>        | 9315    |
| E2F2        | <u>NM_004091</u>        | 1870    |
| CACYBP      | <u>NM_014412</u>        | 27101   |
| TROAP       | <u>NM_005480</u>        | 10024   |
| APOBEC3A    | <u>NM_145699</u>        | 200315  |
| C7orf44     | <u>NM_018224</u>        | 55744   |
| ORC6L       | <u>NM_014321</u>        | 23594   |
| WNT10B      | <u>NM_003394</u>        | 7480    |
| VIM         | <u>EF445046</u>         | 7431    |
| CDC6        | <u>NM_001254</u>        | 990     |
| MCRS1       | <u>NM_006337</u>        | 10445   |
| NAG18       | <u>AF210651</u>         | 57051   |
| PPP1CC      | <u>NM_002710</u>        | 5501    |
| DULLARD     | <u>NM_015343</u>        | 23399   |
| ASF1B       | <u>NM_018154</u>        | 55723   |
| PLP2        | <u>NM_002668</u>        | 5355    |
| MTHFD2      | <u>NM_006636</u>        | 10797   |
| PIGS        | <u>NM_033198</u>        | 94005   |
| KIF2C       | <u>NM_006845</u>        | 11004   |
| NRM         | <u>NM_007243</u>        | 11270   |
| PEG10       | <u>NM_015068</u>        | 23089   |
| C22orf9     | <u>NM_015264</u>        | 23313   |
| COL4A2      | <u>NM_001846</u>        | 1284    |
| SNX26       | <u>NM_052948</u>        | 115703  |

**CLAIMS**

1. At least one nucleic acid  
(i) comprising or consisting of, or  
5 (ii) encoding a nucleic acid comprising or consisting of,  
a sequence that is:  
    (i) SEQ ID NO: 1;  
    (ii) SEQ ID NO: 2;  
    (iii) SEQ ID NO: 3;  
10 (iv) SEQ ID NO: 4; or  
    (v) a sequence derived from any one of SEQ ID NOs: 1 to 4 by substitution,  
deletion or insertion of at least one nucleotide, and having at least 85% identity  
with any one of SEQ ID NOs: 1 to 4, provided that the nucleic acid consisting of  
the sequence derived from any one of SEQ ID NOs: 1 to 4 induces HIV-1  
15 expression in latent HIV-1-infected cells,  
for use as a medicament for treating an HIV-1 infection.
2. The at least one nucleic acid according to claim 1, consisting of a RNA molecule.
- 20 3. The at least one nucleic acid according to claim 1 or 2, wherein the medicament  
comprises a RNA molecule consisting of SEQ ID NO: 1, a RNA molecule consisting  
of SEQ ID NO: 2, a RNA molecule consisting of SEQ ID NO: 3, and a RNA molecule  
consisting of SEQ ID NO: 4.
- 25 4. The at least one nucleic acid according to any one of claims 1 to 3, in combination  
with at least one other anti-retroviral compound.
5. The at least one nucleic acid according to claim 4, wherein the at least one other  
anti-retroviral compound is a reverse-transcriptase inhibitor or a protease inhibitor.
- 30 6. The at least one nucleic acid according to any one of claims 1 to 5, for use for  
treating asymptomatic patients infected by HIV-1.

7. The at least one nucleic acid according to claim 6, wherein the patients are under Highly Active Antiretroviral Therapy (HAART).

8. The at least one nucleic acid according to claim 6, wherein the patients are elite  
5 HIV-1 controllers.

9. The *in vitro* use of a nucleic acid as defined in any one of claims 1 to 3, for the production of HIV-1 particles from cells harbouring an HIV-1 vector.

10 **10.** An *in vitro* method for the production of HIV-1 particles, comprising:  
- contacting cells harbouring an HIV-1 vector with a nucleic acid as defined in any one of claims 1 to 3; and  
- letting the cells express the HIV-1 vector,  
whereby HIV-1 particles are produced from the cells.

15 **11.** The *in vitro* method according to claim 10, involving no step of culturing the cells with T cells.

**12.** Use of at least one nucleic acid

20 (i) comprising or consisting of, or  
(ii) encoding a nucleic acid comprising or consisting of,  
a sequence that is:  
(i) SEQ ID NO: 1;  
(ii) SEQ ID NO: 2;  
25 (iii) SEQ ID NO: 3;  
(iv) SEQ ID NO: 4; or  
(v) a sequence derived from any one of SEQ ID NOs: 1 to 4 by substitution, deletion or insertion of at least one nucleotide, and having at least 85% identity with any one of SEQ ID NOs: 1 to 4, provided that the nucleic acid consisting of  
30 the sequence derived from any one of SEQ ID NOs: 1 to 4 induces HIV-1 expression in latent HIV-1-infected cells,  
as a medicament for the treatment of an HIV-1 infection.

- 13.** The use according to claim 12, wherein the at least one nucleic acid consists of a RNA molecule.
- 14.** The use according to claim 12 or 13, wherein the medicament comprises a RNA molecule consisting of SEQ ID NO: 1, a RNA molecule consisting of SEQ ID NO: 2, a RNA molecule consisting of SEQ ID NO: 3, and a RNA molecule consisting of SEQ ID NO: 4.
- 15.** The use according to any one of claims 12 to 14, for treating asymptomatic patients infected by HIV-1.
- 16.** The use according to claim 15, wherein the patients are under Highly Active Antiretroviral Therapy (HAART).
- 17.** The use according to claim 15, wherein the patients are elite HIV-1 controllers.
- 18.** Use of at least one nucleic acid
- (i) comprising or consisting of, or
  - (ii) encoding a nucleic acid comprising or consisting of,
- a sequence that is:
- (i) SEQ ID NO: 1;
  - (ii) SEQ ID NO: 2;
  - (iii) SEQ ID NO: 3;
  - (iv) SEQ ID NO: 4; or
  - (v) a sequence derived from any one of SEQ ID NOs: 1 to 4 by substitution, deletion or insertion of at least one nucleotide, and having at least 85% identity with any one of SEQ ID NOs: 1 to 4, provided that the nucleic acid consisting of the sequence derived from any one of SEQ ID NOs: 1 to 4 induces HIV-1 expression in latent HIV-1-infected cells,
- in the manufacture of a medicament for the treatment of an HIV-1 infection.
- 19.** The use according to claim 18, wherein the at least one nucleic acid consists of a RNA molecule.

**20.** The use according to claim 18 or 19, wherein the medicament comprises a RNA molecule consisting of SEQ ID NO: 1, a RNA molecule consisting of SEQ ID NO: 2, a RNA molecule consisting of SEQ ID NO: 3, and a RNA molecule consisting of SEQ ID NO: 4.

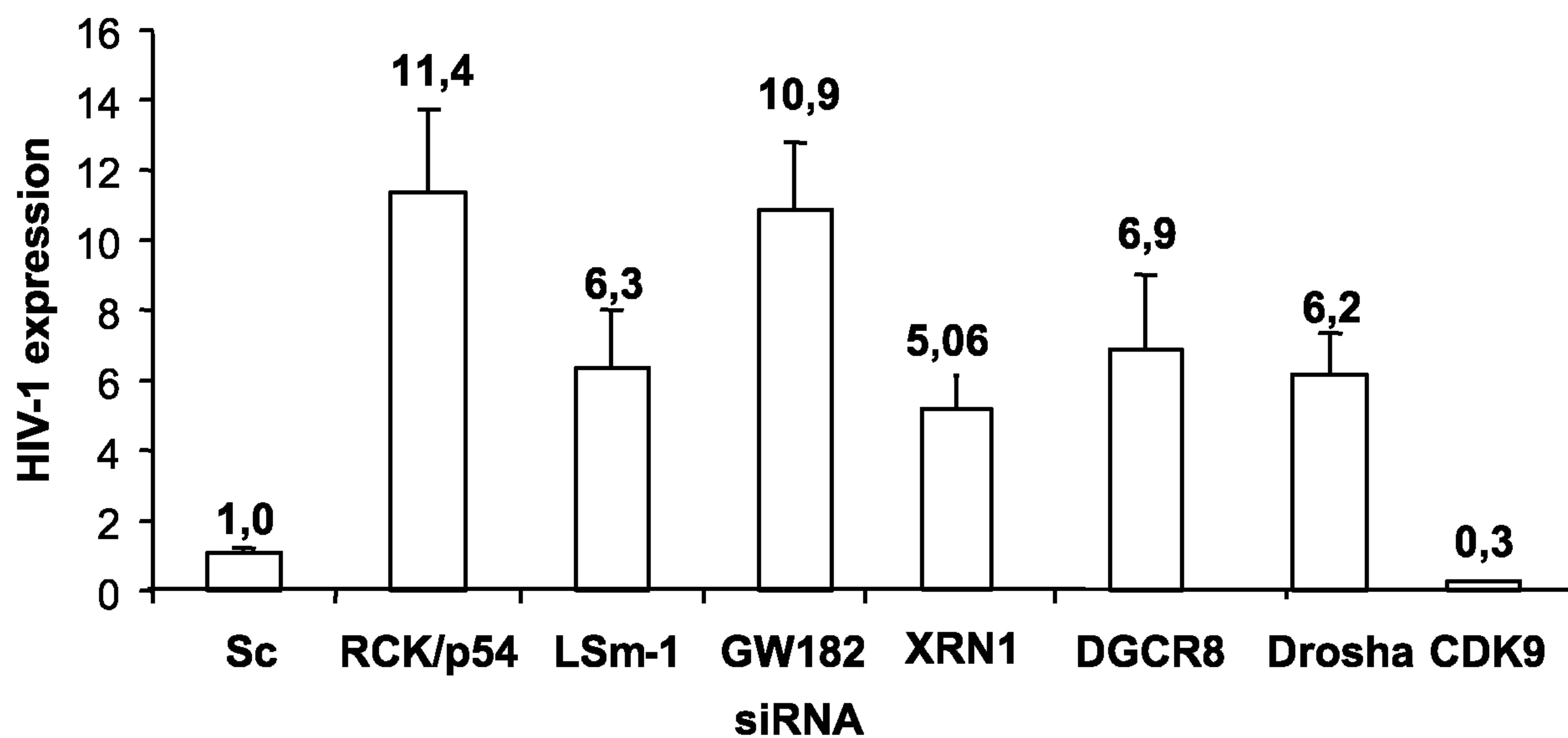
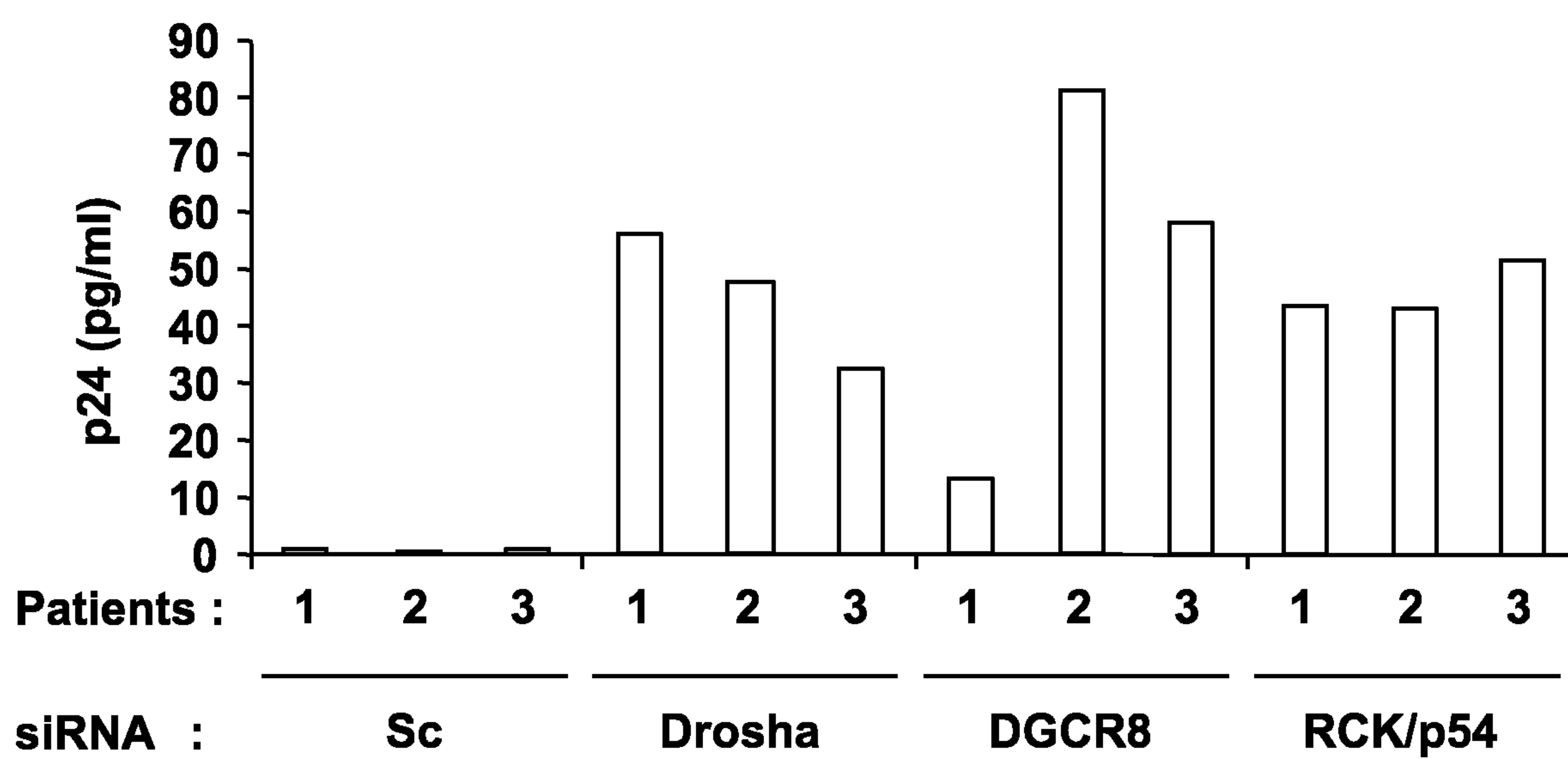
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**21.** The use according to any one of claims 18 to 20, wherein the medicament is for treating asymptomatic patients infected by HIV-1.

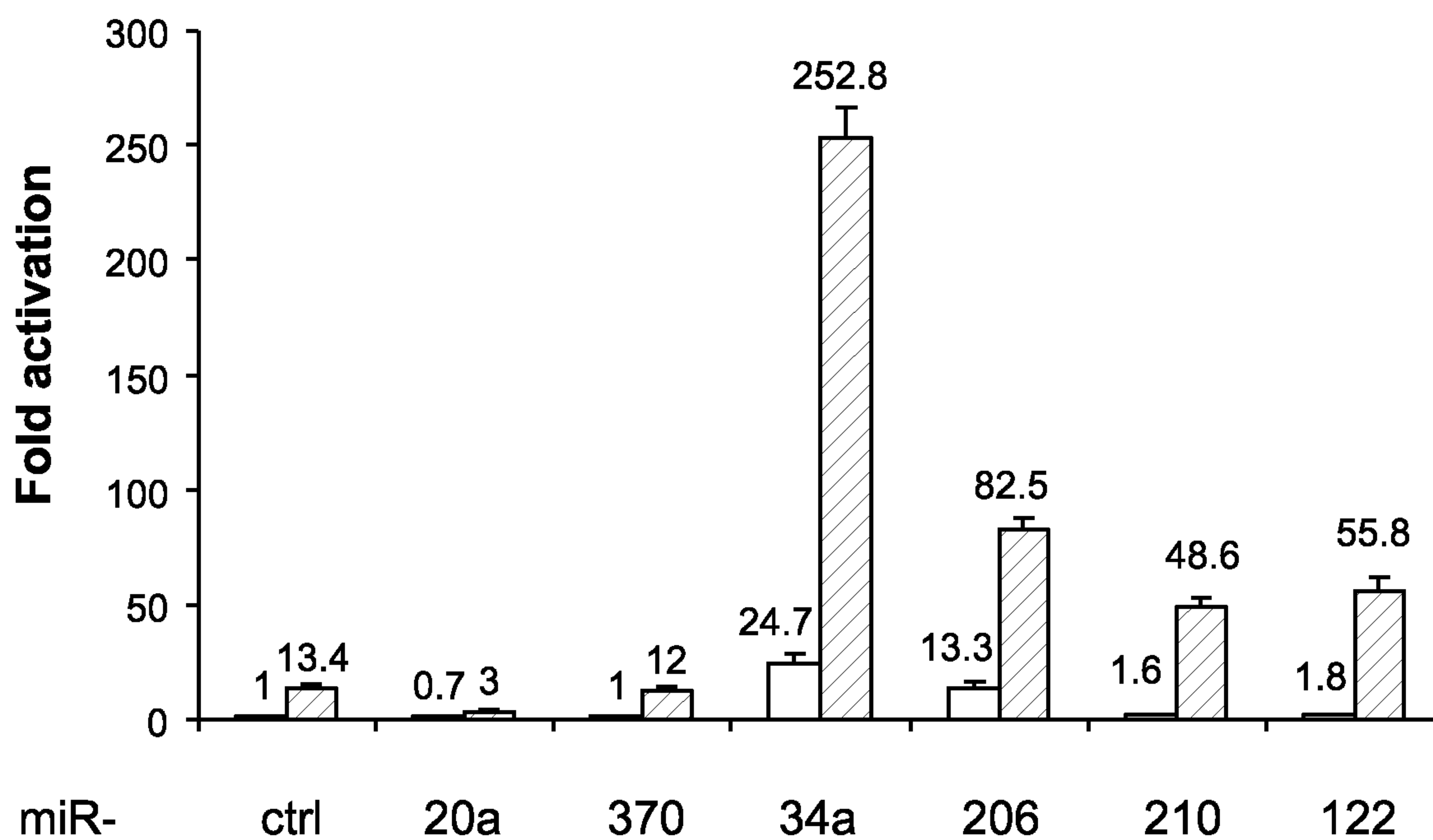
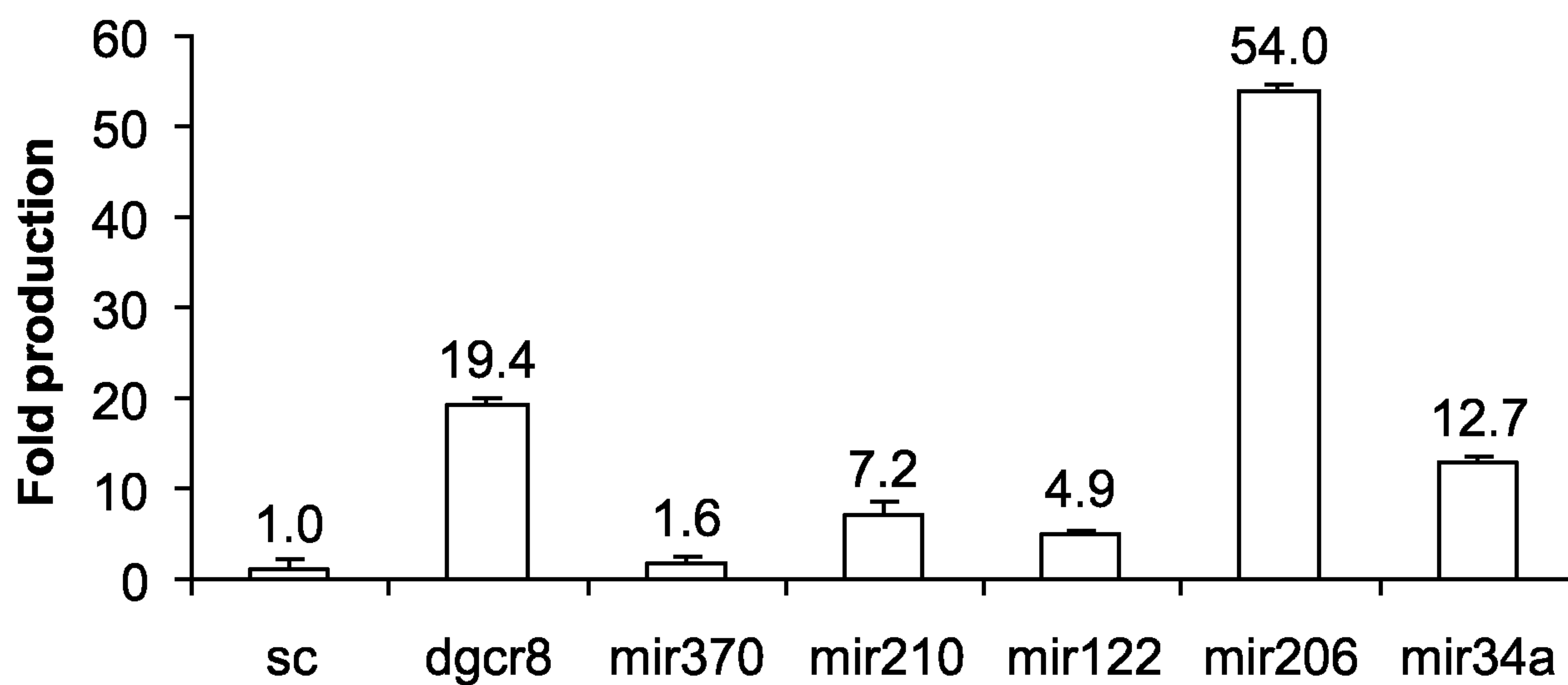
**22.** The use according to claim 21, wherein the patients are under Highly Active  
10 Antiretroviral Therapy (HAART).

**23.** The use according to claim 21, wherein the patients are elite HIV-1 controllers.

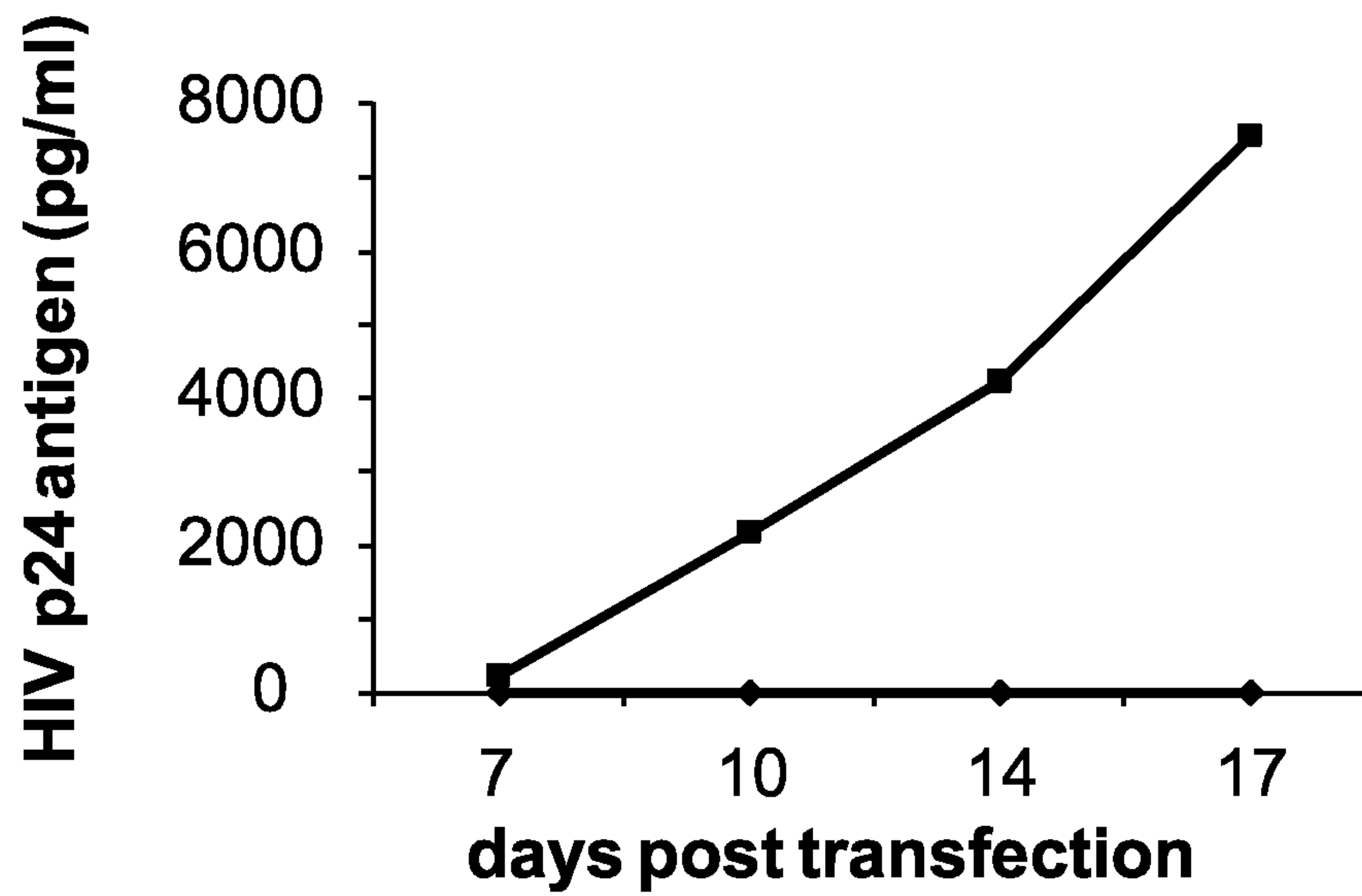
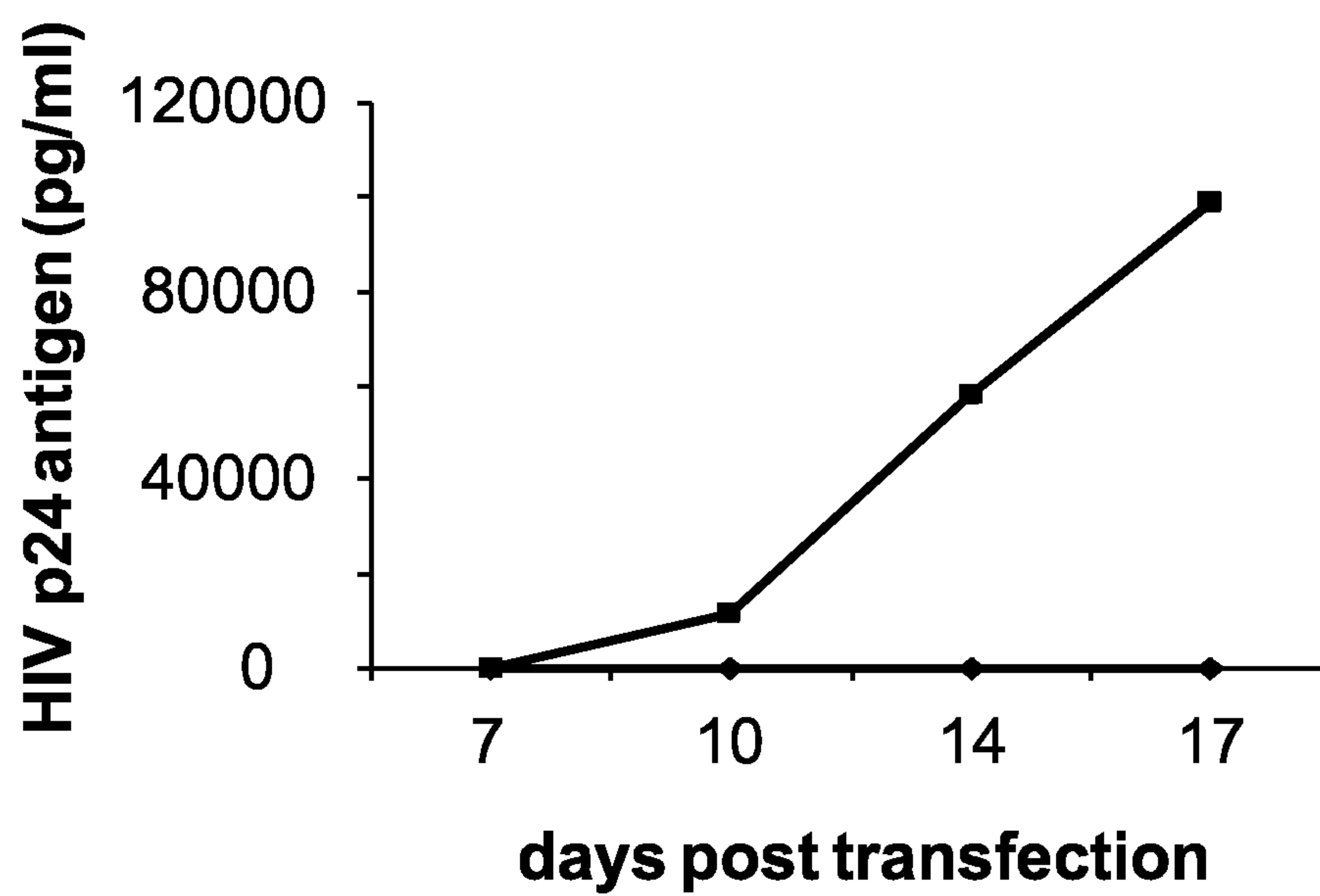
1/9

FIG.1FIG.2

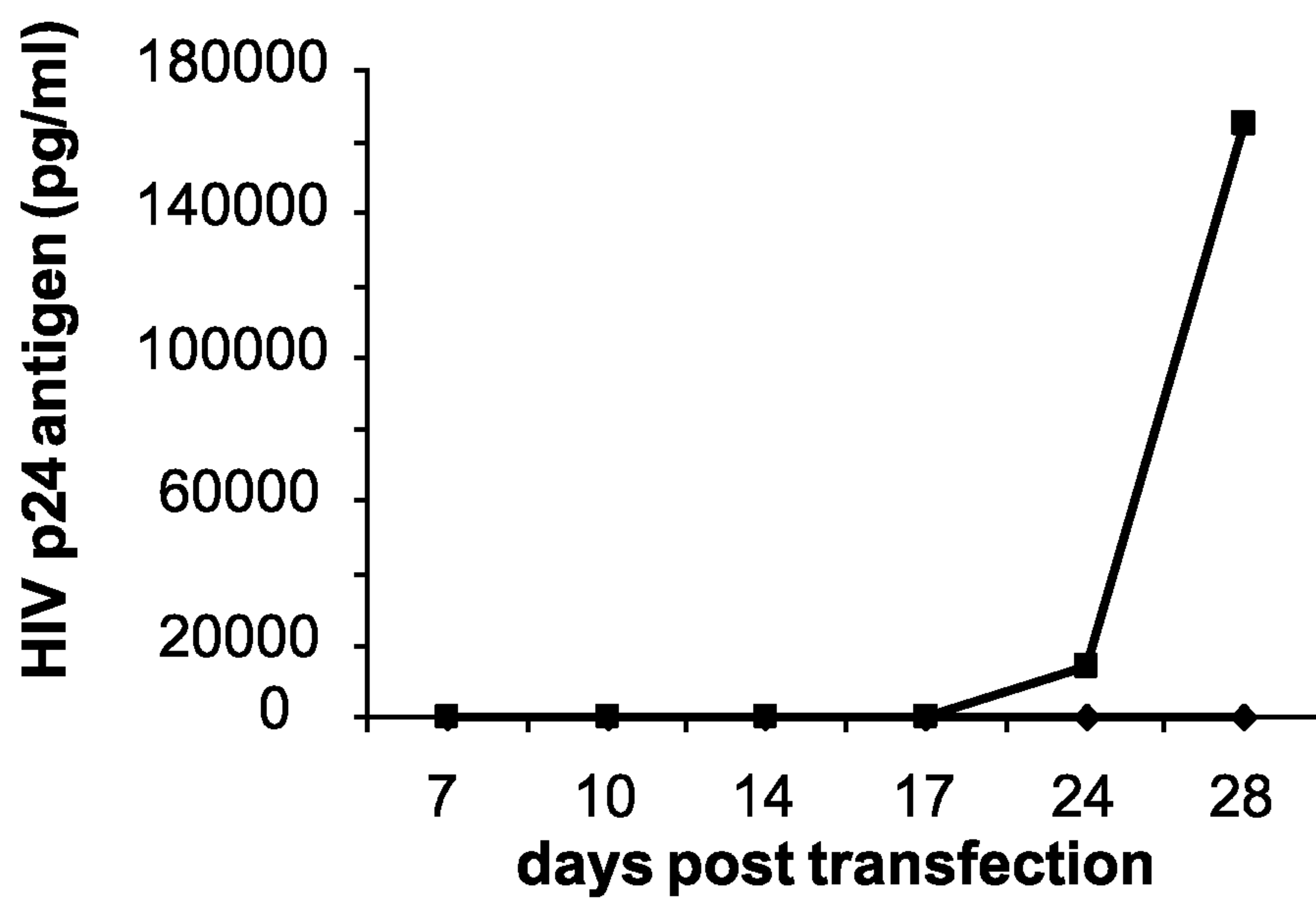
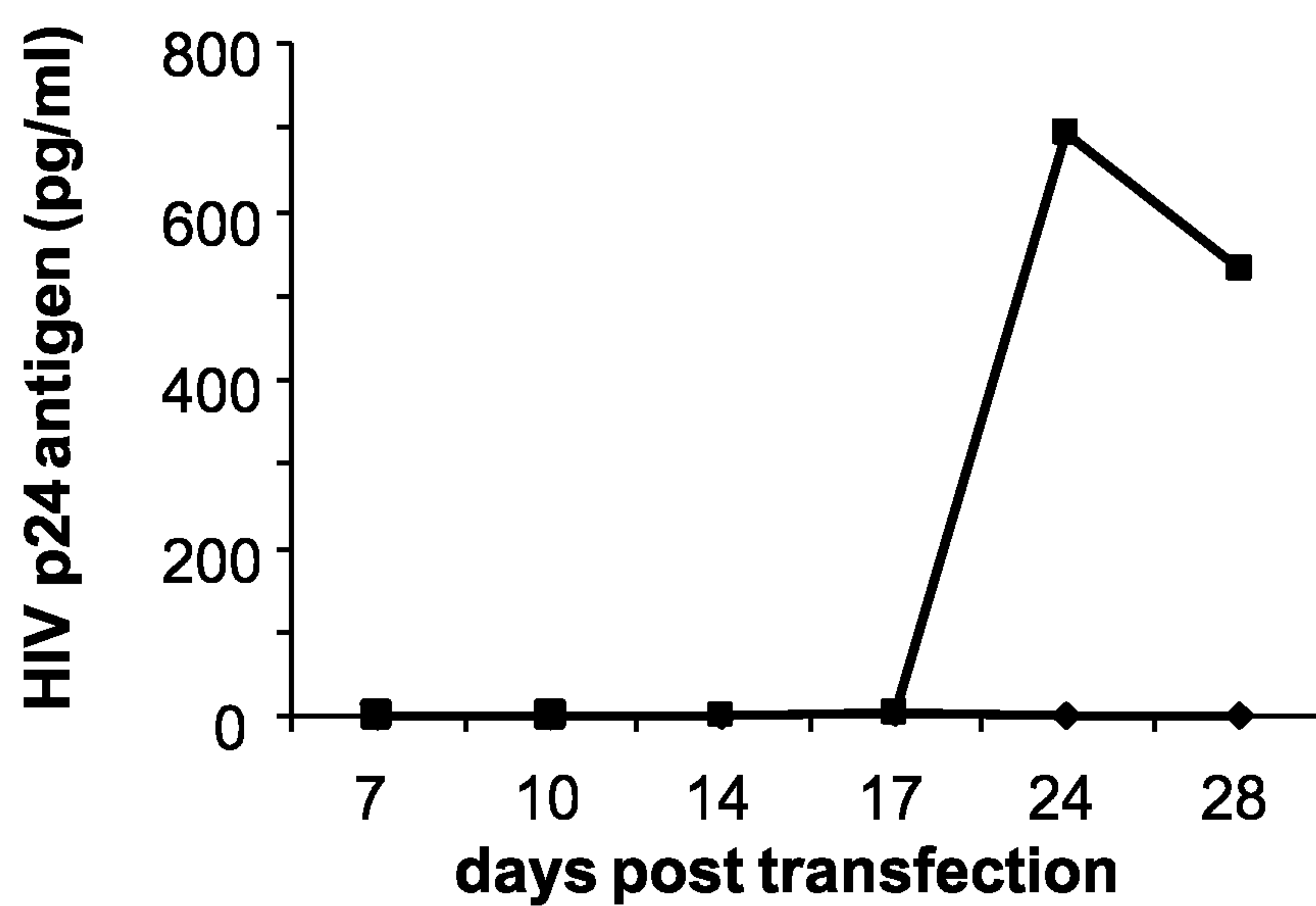
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FIG.3FIG.4

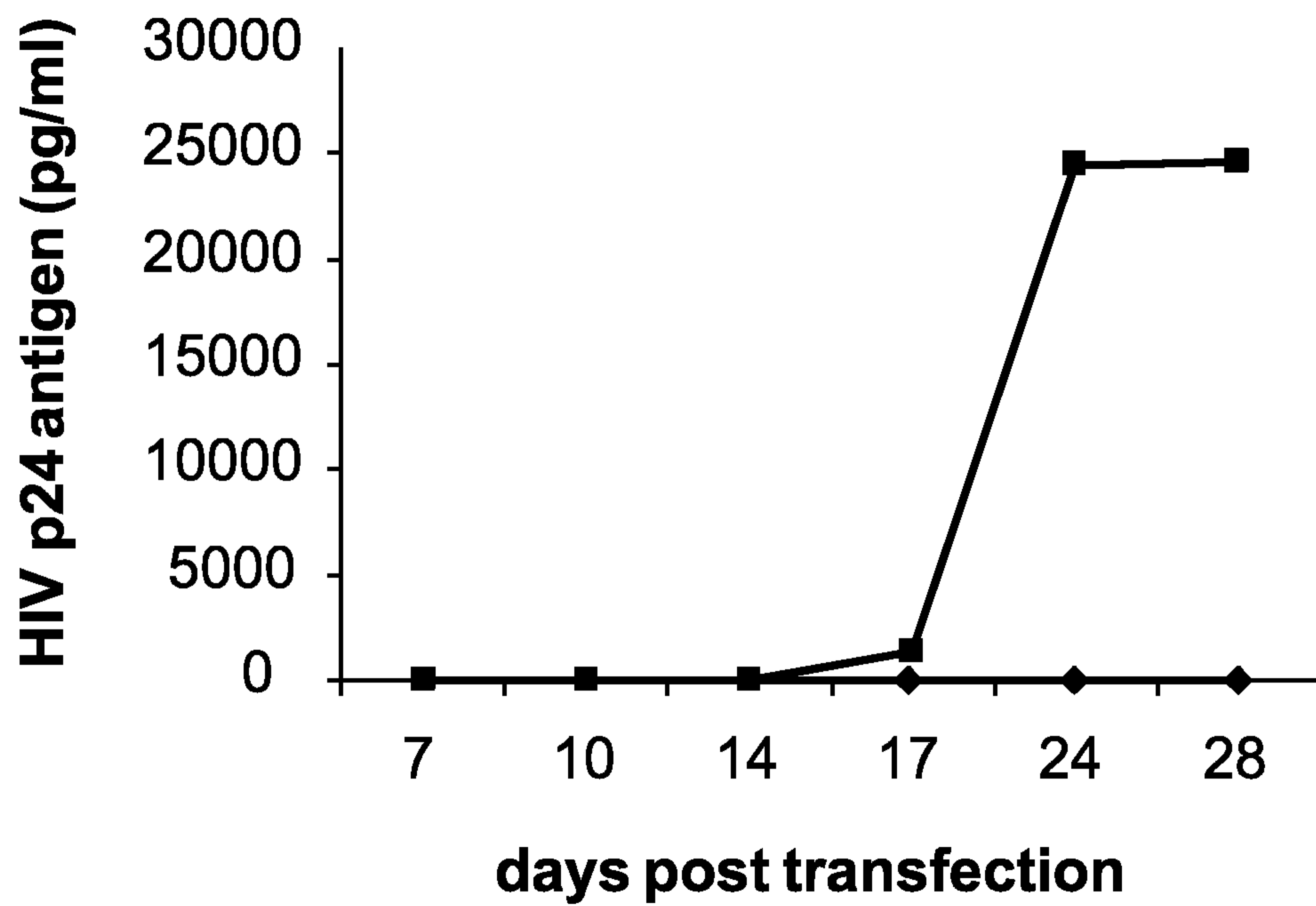
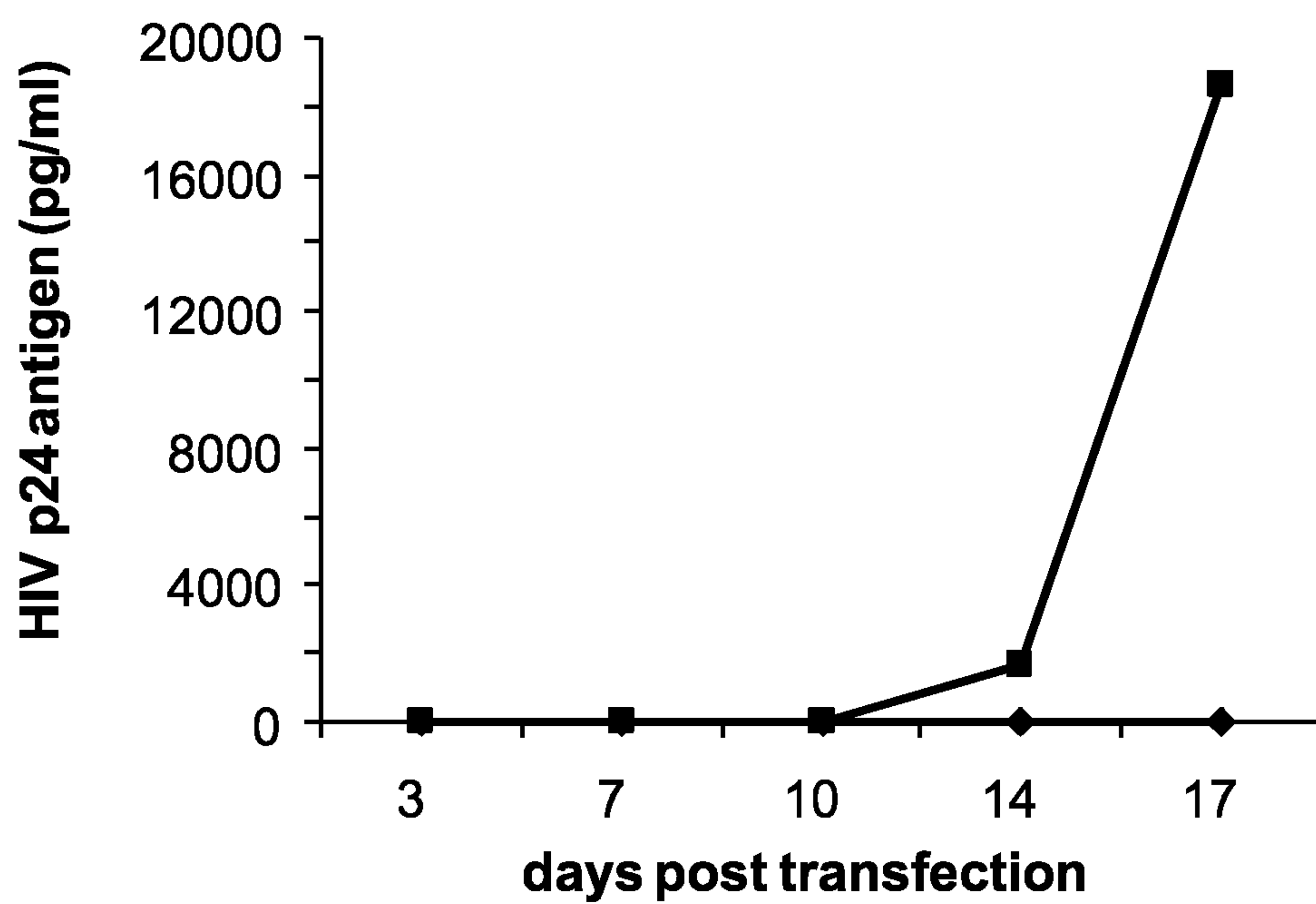
3/9

FIG.5FIG.6

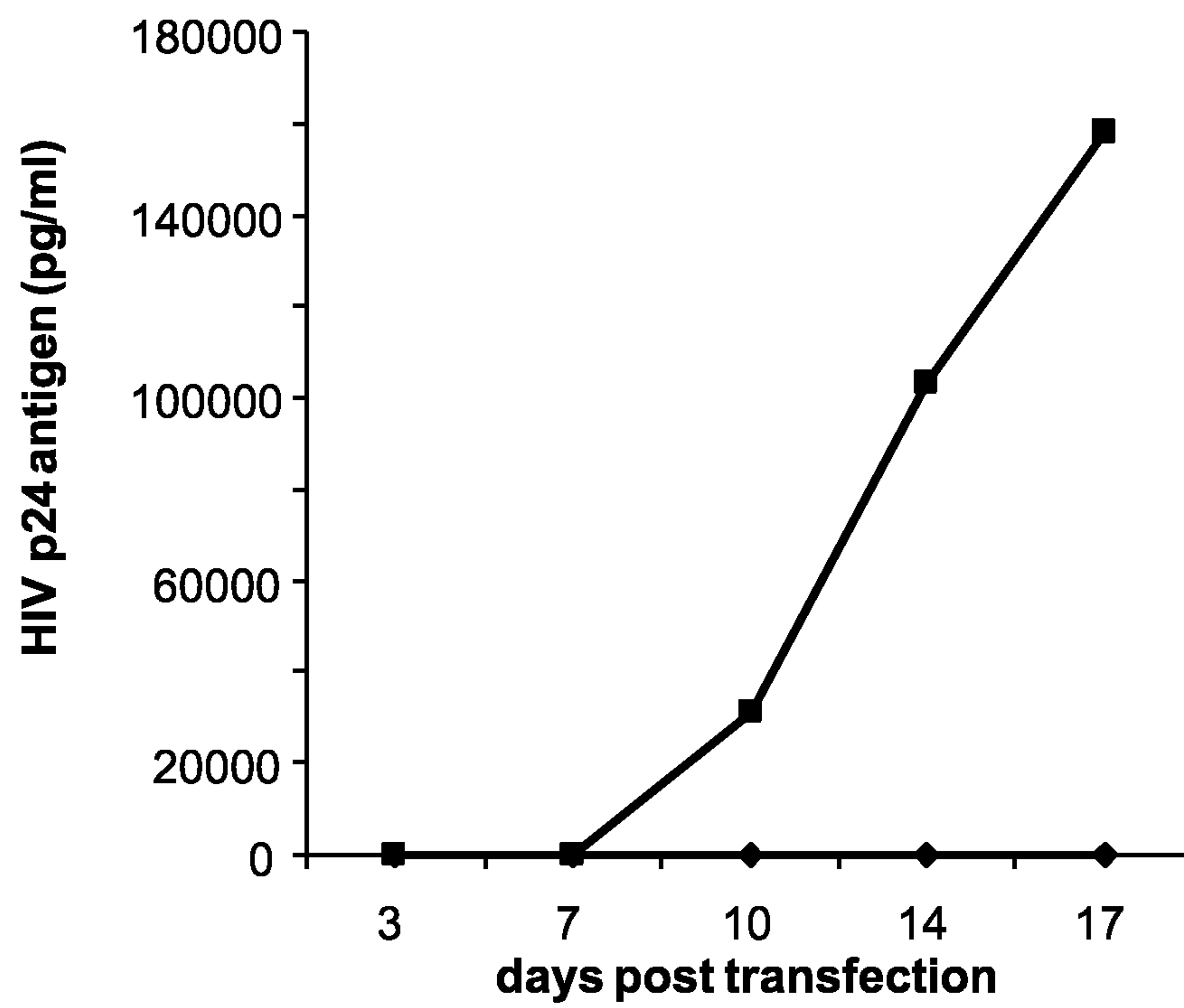
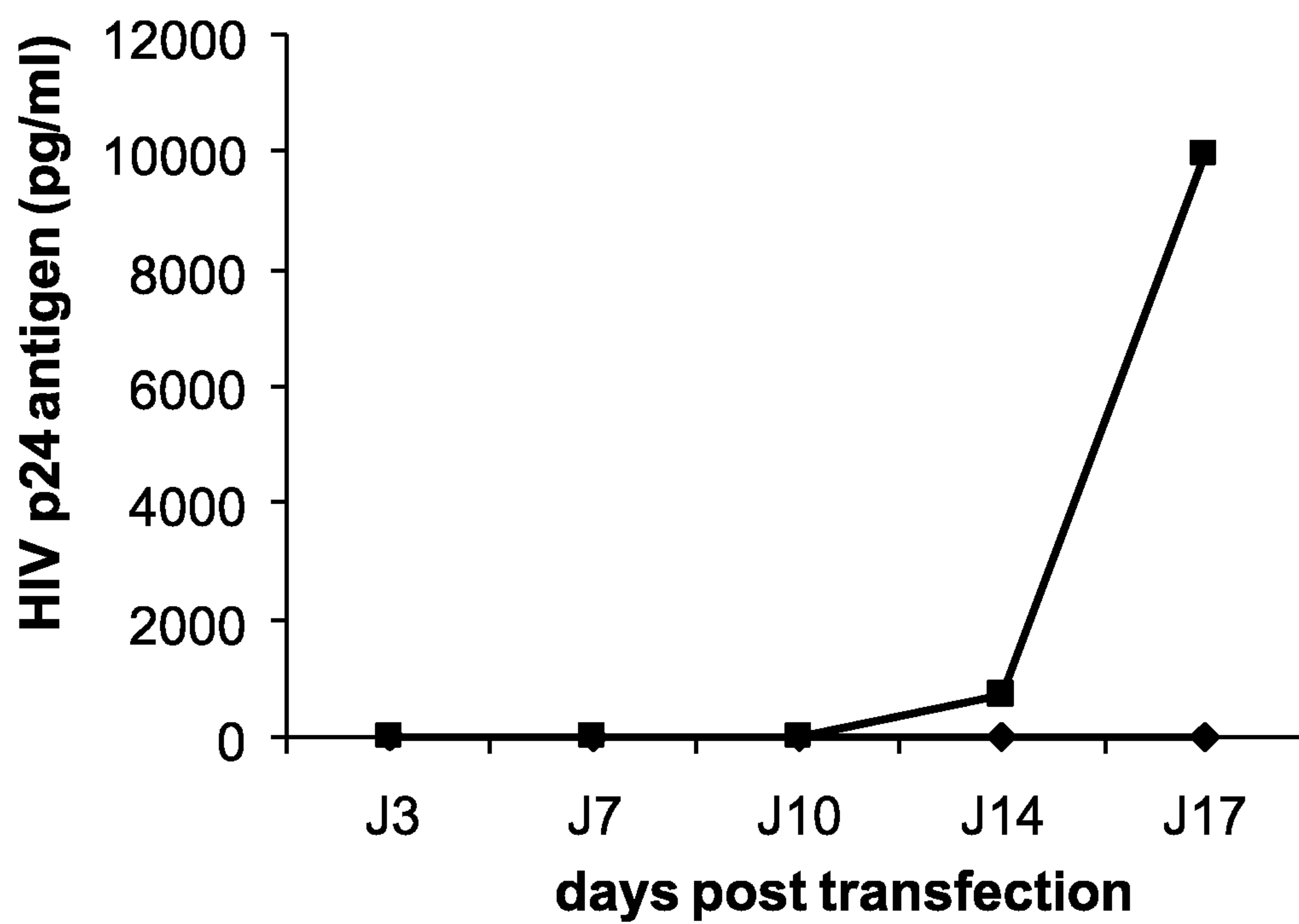
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FIG.7FIG.8

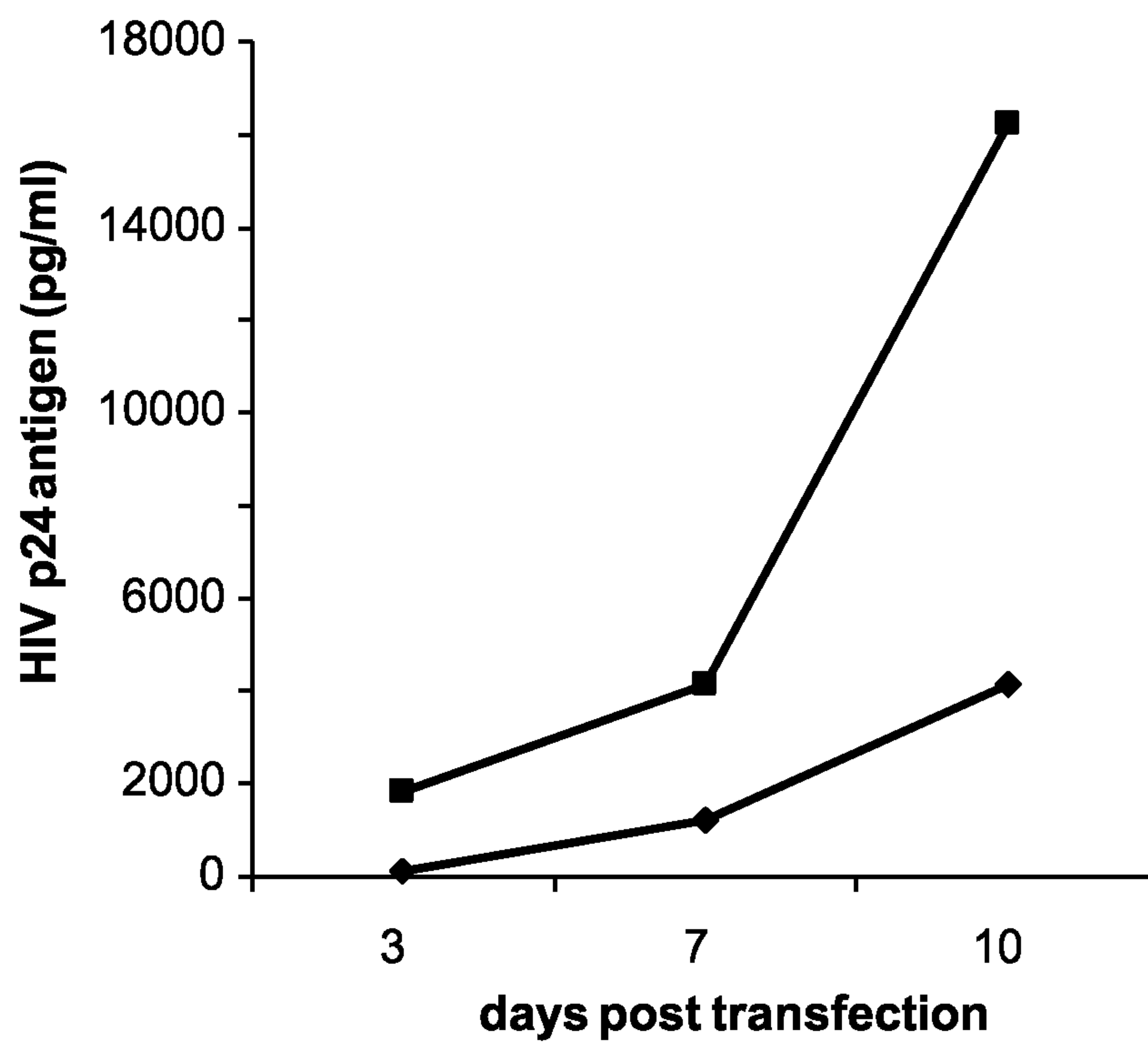
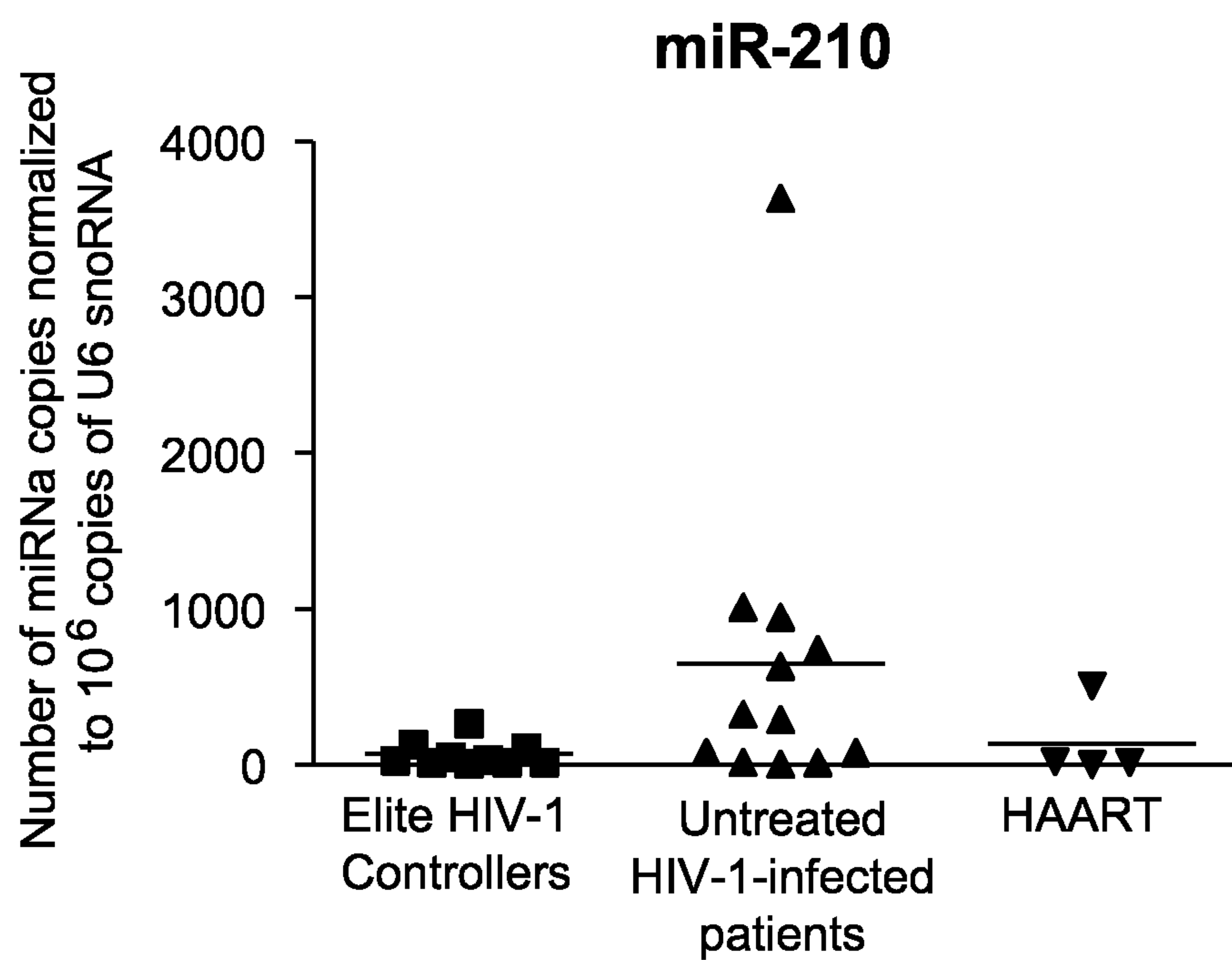
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FIG.9FIG.10

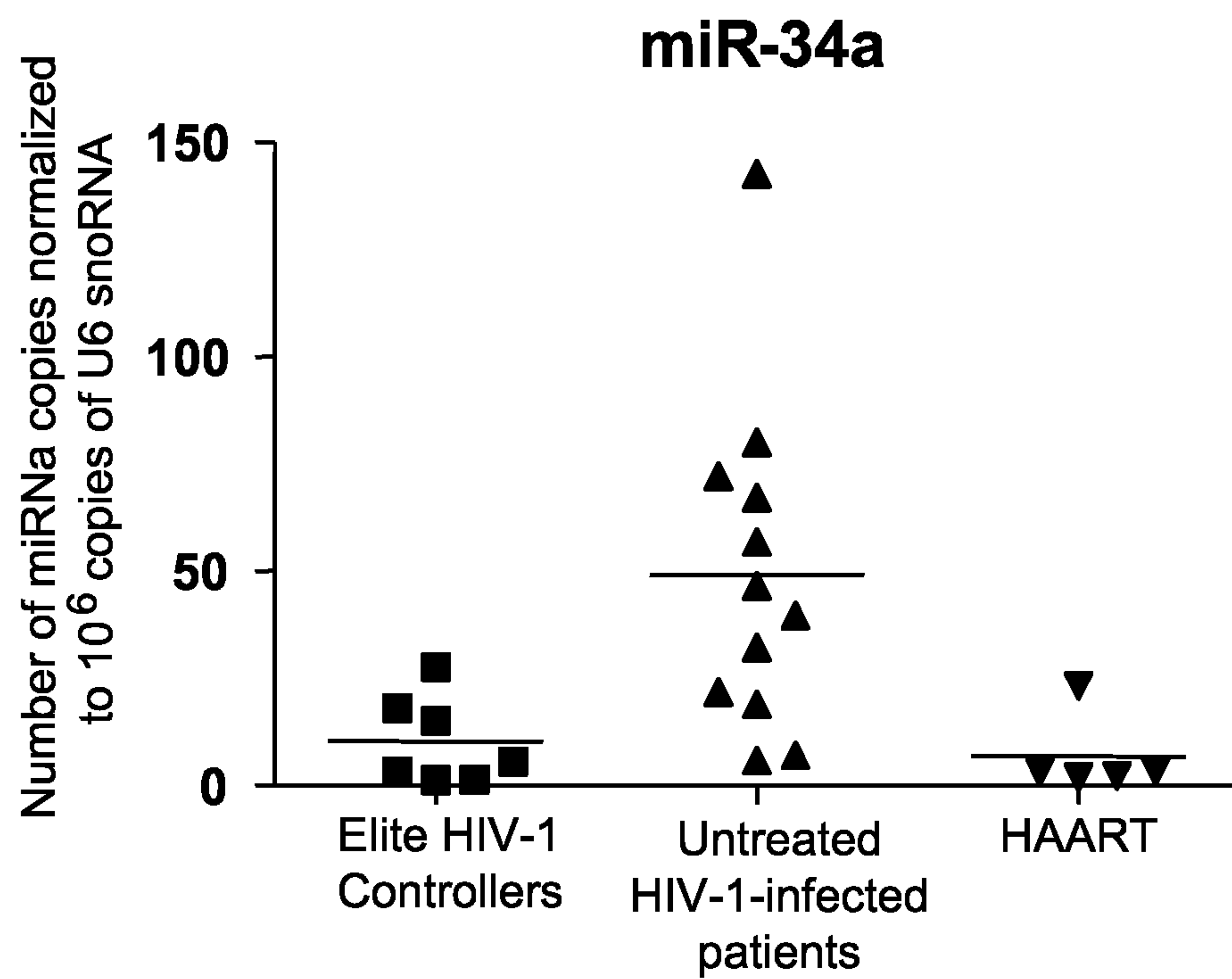
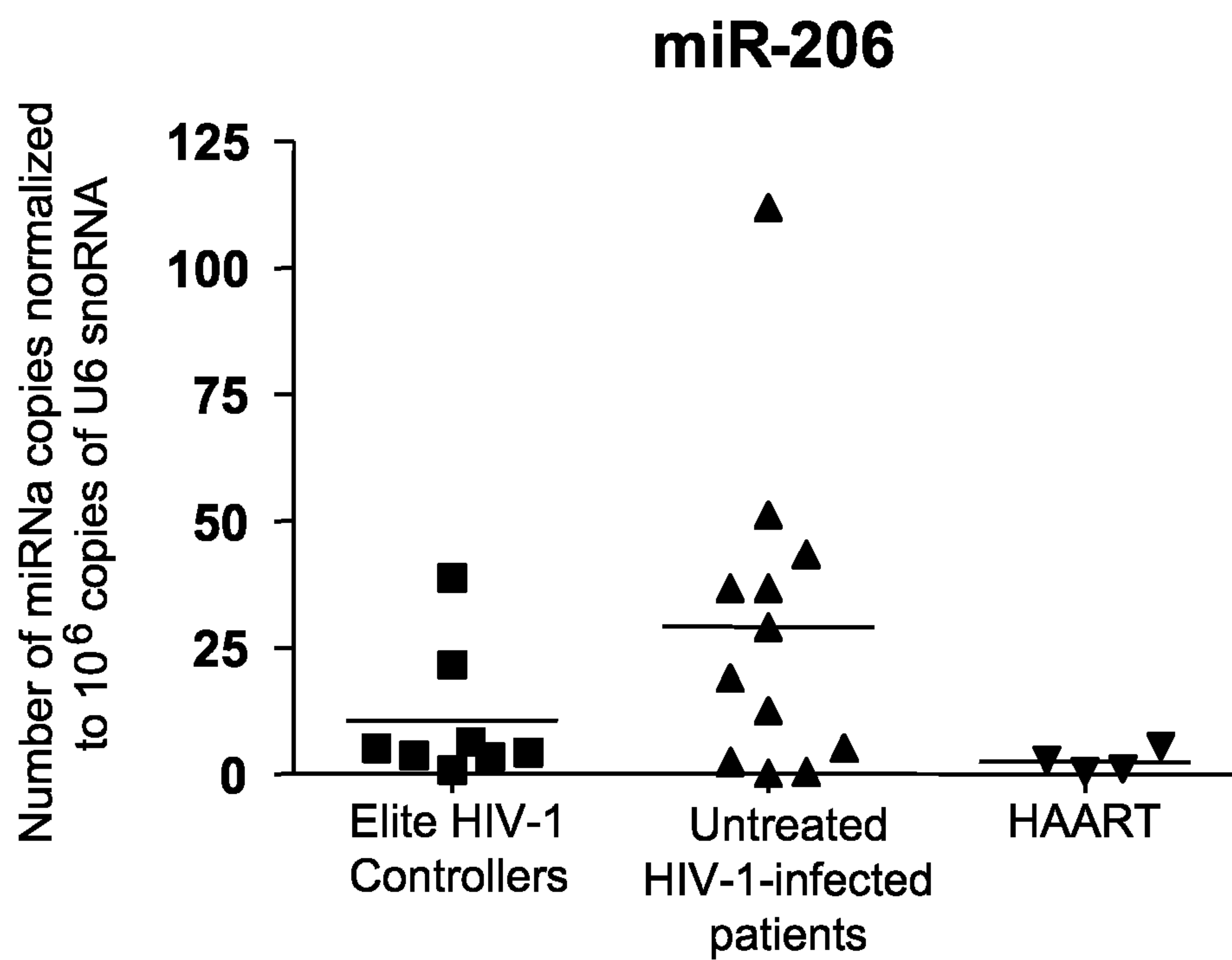
6/9

FIG.11FIG.12

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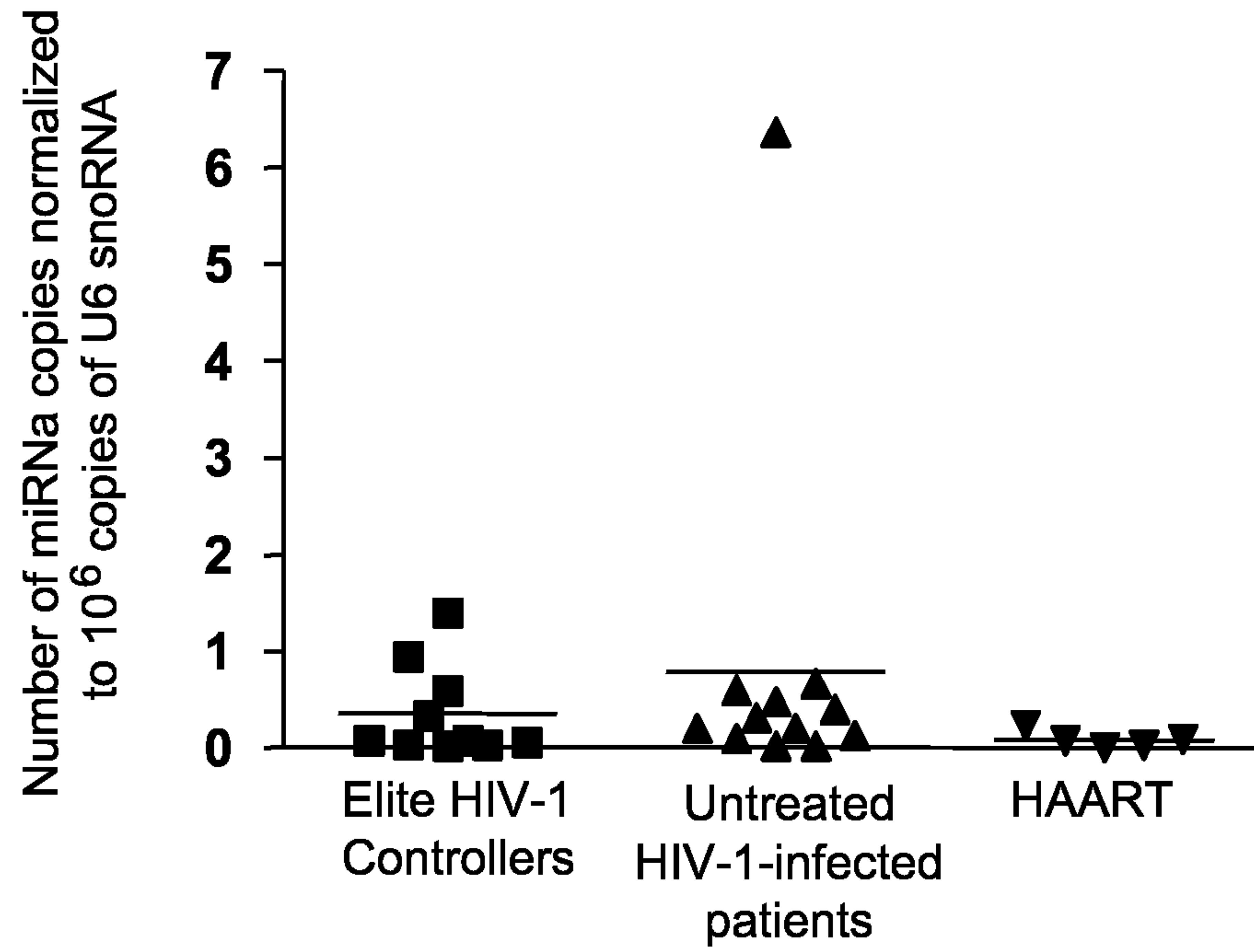
FIG.13FIG.14

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**FIG.15****FIG.16**

9/9

miR-122

FIG.17