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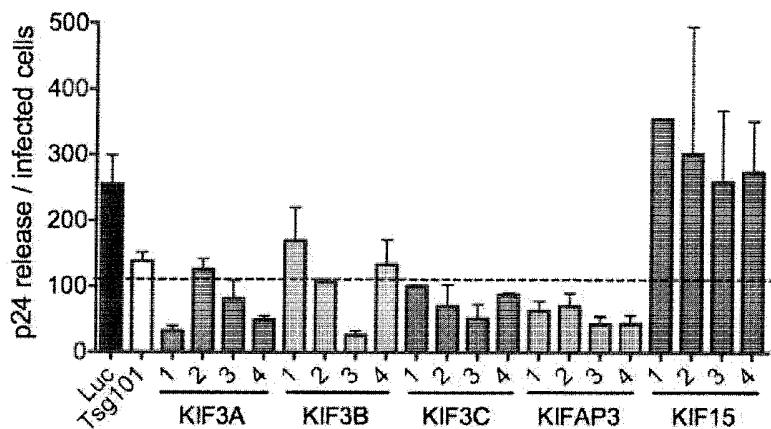
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(54) Title: USE OF KINESIN INHIBITORS IN HIV INFECTION TREATMENT AND A METHOD FOR SCREENING THEM

**Figure 9**



(57) Abstract: The present invention concerns new methods for treating HIV infection by using kinesin inhibitors and methods for identifying new molecules of interest for treating AIDS.

**Use of kinesin inhibitors in HIV infection treatment and a method for screening them.****Field of the invention**

The present invention relates to the field of medicine and provides new therapeutic agents  
5 for treating HIV infection. The present invention relates to a kinesin inhibitor which is effective in the treatment of HIV infection by targeting macrophages reservoirs and to methods of screening of molecules useful in the treatment of HIV infection, in particular using a tagged kinesin protein.

**10 Background of the invention**

Human immunodeficiency 1 (HIV-1), identified in 1983 as the cause of acquired immune deficiency syndrome (AIDS), remains a global health threat responsible for a world-wide pandemic. CD4+ T lymphocytes play a central role in the pathogenesis of human immunodeficiency virus (HIV) infection. In viremic patients, these cells are the major  
15 cellular target for the virus: HIV multiplies intensively in this sub population during the acute phase of the disease and continues to propagate in these same cells during the clinical latency phase. The problem of drug resistance in the treatment of HIV infection has become an increasingly significant barrier in the effectiveness of AIDS chemotherapy. Despite the initial success of monotherapy for HIV infection with agents such as AZT  
20 (Zidovudine), the benefits are short lived. A key factor in HIV chemotherapy failure is the rapid selection by HIV for drug-resistant strains. It is observed that resistance develops rapidly to reverse transcription inhibitors such as AZT and other drugs that reduce viral production. The resistance is due to the ability of the virus to mutate and circumvent the normal transcription pathway which the chemotherapy interrupts (Volberding et al; 1990).  
25 To limit viral escape, treatments involving more than one anti-retroviral drug have then been developed. In 1996, a major advance has been made in curing AIDS with the introduction of the highly active antiretroviral therapy (HAART), which usually includes a combination of reverse transcriptase and protease inhibitors. (Carpenter, C. et al. Antiretroviral therapy for HIV infection in 1997. JAMA 277, 1962, 1997). Two types of  
30 RT inhibitors exist, namely nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). Such HAART treatment has proved to be effective with a pronounced drop in viral load, increase in lymphocyte populations,

reduction in opportunistic infections and a doubling of time of progression to serious illness and/or death.

However, the viral infection is contained with such treatment but not eliminated. Indeed, multiple drug resistance and long-term persistence of drug-resistant viral reservoirs have  
5 been reported, making the emergence of secondary epidemics of multi-drug resistant HIV-1 a real long-term threat.

These observations suggest that even long term suppression of HIV-1 replication by HAART cannot totally eliminate HIV-1. The virus persists in cellular reservoirs because of viral latency, cryptic ongoing replication or poor drug penetration, and the residual virus  
10 may, after months or years of persistence and low-level replication, develop resistance to the drugs and reemerge. The continuing emergence of drug resistance further threaten the future therapy, thereby necessitates another treatment strategy. Many studies have been done to understand the viral production by cellular reservoirs.

It is known that the production of new infectious retroviral particles is an ordered process  
15 that includes many steps. The various components of the virions have to be transported to the assembly site to form new viral particles.

HIV-1 encodes only 15 proteins and thus must exploit multiple host cell proteins for successful infection. In particular, three major viral components, Gag, the envelope and genomic RNAs have to traffic inside the cell to reach their assembly site. It has long been  
20 established that Gag is the key component driving the assembly process. Gag is a polyprotein synthesized in the cytosol as a Pr55Gag precursor, which will be cleaved once in the viral particle to give rise to the following four main polypeptides: Matrix (p17), Capsid (p24), Nucleocapsid (p7) and the p6 domain. Pr55Gag probably coordinates the recruitment of different host proteins necessary for at least its transport to the assembly  
25 site, and the formation of the viral particles.

Targeting host factors required for the HIV cycle represents an alternative pharmacological strategy for treating HIV-infected individuals as drugs targeting such host factors are much less likely to escape mutation that interfere with the effectiveness of conventional antiretroviral drugs.

30 In attempts to identify those host proteins needed for HIV infection, several genome wide screens using various techniques have been carried out including 9 genome-wide 3 independent siRNA screens, (PLoS pathogens 5:e10000437 (2009), a genome-wide RNAi

screen coupled to interrogation of human interactome network databases (Köning et al. Cell, 135:49 (2008) and a genome-wide short hairpin RNA screening (Yeung et al., JBC 284:19463 (2009). Overall, although a large number of potential host proteins (up to 2410) linked to HIV replication were identified, very poor overlap in the results were obtained.

- 5 In addition, it is worth noting that most of these genome-wide studies were performed in non-myeloid cells and most of the time in cell lines such as COS, HeLa and 293T cells with very different characteristics from those of primary infected cells. Moreover, we are currently lacking functional experiments addressing the different roles of these proteins depending on cell type in contributing to the production of viral particles.
- 10 10 Resting memory CD4+ T cells and macrophages are the best characterized reservoir in the natural host which can be infected latently in the lymph nodes or in haematopoietic stem cells of the bone marrow and therefore contribute to the viral persistence by the formation of new virions (Alexaki A, Liu Y, Wigdahl B: Cellular reservoirs of HIV-1 and their role in viral persistence. Curr HIV Res 2008, 6:388-400).
- 15 15 Moreover, macrophages are relatively long-lived cells that are less prone to cell death as a result of HIV-1 infection than other cells and are therefore likely to be a major site of viral latency. With the half-lives of macrophages reported to be of the order of months to years, depending on the specific type of macrophage, and with reports of higher concentrations of antiviral drugs being required to inhibit HIV-1 replication in macrophages than in T cells,
- 20 20 infected macrophages pose a serious problem for viral eradication with current antiretroviral therapy. The problem of long-lived infected macrophages in the successful treatment of HIV-1 infection is highlighted by recently determined viral decay kinetics with current treatment regimens. The poor penetration of some antiretroviral drugs into the cerebrospinal fluid may result in the development of a sanctuary site, where macrophages,
- 25 25 in addition to microglial cells, can result in persistent viremia. If HIV-1 infection is to be cured, new strategies that will eradicate infected macrophages will be required. Very little data relating to host proteins involved in the HIV viral cycle in macrophage are available. Indeed, no genome-wide screen in such cells has been performed so far on such cell type. In addition, the potential role of the cellular cytoskeleton and cytoskeletal-related proteins
- 30 30 has not been investigated.

Accordingly, there is a significant need for alternative HIV treatments targeting host factors, especially host factors in viral reservoirs, in order to avoid immune viral escape.

Summary of the invention

The present invention provides new therapeutic agents for treating HIV infection. The inventors have shown that kinesins KIF3A, KIF3C and KIFAP3 play a key role in the 5 transport of Gag- containing viral compartments toward the cell periphery and that its silencing greatly reduces HIV virion release in macrophage.

Therefore in a first aspect, the present invention concerns kinesin inhibitors, preferably N-type kinesin inhibitors, more preferably kinesin 1 inhibitors or kinesin 2 inhibitors, even more preferably KIF3A, KIF3C and KIFAP3 inhibitors, for use in the treatment of HIV 10 infection.

In a second aspect, the present invention concerns a tagged kinesin comprising a kinesin motor domain, a stalk domain, a cargo domain and a tag allowing said tagged kinesin to be detected, and wherein said tag is located between said stalk domain and said cargo domain. In a third aspect, the present invention concerns a method for screening for compounds 15 useful for the treatment of HIV infection, said method comprising the steps of:

- a) providing or obtaining a candidate compound; and
- b) determining whether said candidate compound inhibits the activity and/or expression of a kinesin, preferably a N-type kinesin, more preferably a kinesin-1 or a kinesin-2, even more preferably kinesin KIF3A, KIF5B, KIF3C and/or KIFAP3, wherein 20 the ability of said candidate compound to inhibit the expression or activity of said kinesin indicates that said candidate compound is indicative of its usefulness for the treatment of HIV infection.

Brief description of the drawings

25 **Figure 1. The distribution of Virus-Containing Compartments is dependent on the microtubule network integrity**

(A) Ultrathin cryosections of macrophages infected with HIV-1 NLAD8 for 15 days were prepared and labelled with anti-Env antibodies and Protein A coupled to gold particles of 10 nm diameter (PAG10) and with anti-Pr55Gag antibodies and PAG15. Bar, 30 200 nm. (B) 3D reconstruction of macrophages infected with HIV-1 NLAD8 for 7 days and stained for Gag. (C) Confocal micrographs (one plane) of HIV-1-infected macrophages exposed to DMSO- or Nocodazole (10µM) for 1 hr. Cells were fixed and

stained with antibodies specific for the indicated proteins. Bar, 5 $\mu$ m. (D) Ultrathin cryosections of macrophages infected with HIV-1 NLAD8 for 15 days were double-immunogold labelled for Pr55Gag with PAG15 and for  $\alpha$ -tubulin with PAG10. Three representative profiles are presented. Tubulin staining was present at the limiting membrane of VCCs (see arrowheads).

5 **Figure 2: KIF3A is required for viral production by macrophages**

(A) Schematic representation of the experimental design. (B) Immunoblot analysis of KIF3A and Tsg101 expression in macrophages transfected with the indicated siRNA. All 10 siRNA specific for KIF3A reduced after 3 days its level of expression down to 20% in primary macrophages. (C) Macrophages were infected by HIV-1 and transfected with siRNA as described in (A). Cell viability was measured at day 4 p.i. using the CellTiter glo kit and normalized to the control (siLuc). (D) Primary macrophages were infected with HIV Gag-iGFP  $\Delta$ Env pseudotyped with VSV-G and transfected with siRNA as described 15 in (A). This virus has a single cycle in macrophages and does not induce syncitia formation. Percentages of GFP+ macrophages were estimated by flow cytometry at day 4 p.i.. (E) Infectivity of the virions produced by the macrophages subjected to the indicated siRNA was evaluated (see Experimental procedures). (F) KIF3A depletion does not affect the early steps of infection. The reporter cell line TZM-bl was transfected with the 20 indicated siRNA. Two days later, cells were infected with HIV-1. Cells were washed 8 hours later, reincubated for additional 16 hours and assayed for  $\beta$ -galactosidase activity, whose expression is driven by a Tat sensitive promoter. (G) Dosage of p24 Gag in the 24 hr culture supernatants harvested as indicated in A. \*\*\* indicates that the difference between the two histogram bars is statistically significant (p value below 0.001).

25

**Figure 3. KIF3A is dispensable for HIV-1 production by infected T cells**

(A) Schematic representation of the experimental design. (B) Immunoblot analysis of KIF3A and Tsg101 expression in Jurkat T cells expressing the indicated shRNA. All 30 shRNA specific for KIF3A reduced after 3 days its level of expression down to less than 25% in Jurkat T cells. (C) Dosage of p24 Gag in 20-hr culture supernatants of NL-4.3  $\Delta$ Env-infected Jurkat T cells harvested as indicated in A. Values presented have been corrected by the number of HIV-infected cells present in each sample, as calculated using

CellTiter-Glo (for cell viability) and anti-Gag staining analyzed by flow cytometry (for percentage of infection). (D) Confocal micrographs of primary T cells infected with VSV-g-pseudotyped HIV-1 NL-4.3 for 24hr and stained for the indicated markers. Note that Gag distribution is highly polarized at the plasma membrane whereas KIF3A distribution is  
5 totally different. Bars, 5  $\mu$ m. (E) Ultrathin cryosection of Jurkat T cells infected with HIV-1 NL-4.3 and immunogold labelled for Pr55Gag with PAG10. Bar, 200 nm.

**Figure 4. The Virus-Containing Compartments are associated with tubulin and the kinesin KIF3A.**

10 (A) Confocal micrographs of HIV-1-infected macrophages. Cells were fixed and stained with antibodies specific for the indicated proteins. A Z-projection of a stack of images is presented on the top row, while the bottom row shows one confocal plane of the magnification of the boxed area. Bar, 10 $\mu$ m. (B) Structured Illumination Microscopy of HIV-1-infected macrophages stained for p24 and KIF3A. (C) 3D reconstruction with half  
15 opacity of the region boxed in B. (D) Immuno-EM of ultrathin cryosection of macrophages infected with HIV-1 NLAD8 for 7 days labelled for the indicated proteins. Arrowheads point to KIF3A staining associated with  $\alpha$  tubulin at the limiting membrane of a VCC. Bar, 500nm.

20 **Figure 5: KIF3A depletion results in intracellular accumulation of VCCs**

(A) Examples of 3D reconstructions obtained after thresholding of image stacks acquired by confocal microscopy of HIV-1-infected macrophages transfected with the indicated siRNA and stained for Gag. (B to E) Effects of KIF3A depletion on the indicated parameters within HIV-1-infected macrophages. Reconstructions as seen in (A) were  
25 segmented and quantified using Imaris software. KIF3A knock down was estimated in parallel by immunoblot to be between 50 to 80 % efficiency in the 8 donors used for these experiments. n.s. = not statistically significant.

**Figure 6: KIF-3A-iCh is recruited to active Virus-Containing Compartments**

30 (A) Schematic representation of the KIF3A-iCh construct. (B and C) Correlative light and electron microscopy. (B) Primary macrophages grown on coverslips with coordinates were co-infected with HIV-1 Gag-iGFP and KIF3A-iCherry lentiviral vector, fixed and imaged

by spinning disk microscopy at day 7 p.i. (C) The same coverslips were then embedded in epon and processed for EM. An overview of the macrophage imaged in B is presented in the inset. The VCCs are in the top third of the cell. (D) Two magnifications of the VCC rich region, with a viral budding profile shown in the inset.

5

**Figure 7: Dynamics of internally tagged forms of KIF3A and Gag in primary macrophages**

General view of a macrophage co-infected with HIV-1 Gag-iGFP ΔEnv and KIF3A-iCherry lentiviral vector and cultured for 5 days. The cell was imaged for 5 hr by spinning disc microscopy. Time 0, 1, 2 and 3h40 of a 5D reconstruction (x, y, z, t, c) of the boxed area are presented with the tracking of the VCCs visible on the 3h40 panel.

**Figure 8, related to Figure 1C. The distribution of Gag+ compartments depends on the integrity of the microtubule network.**

(A) Schematic representation of the algorithm used to quantify the effect of nocodazole treatment on VCC spatial distribution. HIV-1-infected macrophages for 7 days, untreated or treated with nocodazole for 1h at 37°C, were stained for p24, revealed by a secondary antibody coupled to Alexa 488. Confocal images were acquired, thresholded and dilated to count Gag+ vesicles. Examples of images before and after numeric treatments are presented. The spreading index corresponds to the number of vesicles counted after dilatation divided by their number before dilatation and is plotted for each cell treatment. Spreading index close to 0 means that Gag vesicles are clustered whereas a value close to 1 means that they are dispersed. (B) Confocal micrographs of HIV-1-infected macrophages exposed to DMSO or Nocodazole (10 μM) for 1 hr (Noco) or Nocodazole for 1 hr followed by washes and reincubation for 15 min at 37°C (Noco + WO). Cells were fixed and stained with antibodies specific for the indicated proteins  $\alpha$  tubulin and p24 Gag). Z-projections of image stacks are presented. Upon Nocodazole exposure, Gag+ compartments were redistributed in the perinuclear area. This effect was reversible as upon wash out of the Nocodazole, microtubule growth was accompanied by spreading of the Gag+ compartments. Bar, 5μm.

**Figure 9, related to Figure 2. siRNA-based screen of the production of HIV-1 by primary macrophages.**

Monocytes from 3 donors were pooled and seeded in 96 well glass bottom plates (Iwaki), and differentiated for 7 days with M-CSF. Then, monocyte-derived macrophages were 5 infected with HIV-1 NL4-3 ΔEnv, VSV-G pseudotyped, with an MOI of 2. siRNA transfection was performed as described in Experimental Procedure. The screen included 4 individual siRNA per gene (Qiagen), targeting 45 kinesins and associated proteins. The experiment was performed in duplicate. A full plate control was included in which half of the wells were transfected with a Tsg101-specific siRNA, as a positive control, while the 10 other half with a luciferase-specific siRNA, as a negative control. In addition, on each experimental plate we included the same positive and negative controls (6 of each). Cells were washed at day 6 post-infection and supernatant harvested 24h later to measure their p24 content by Elisa. In parallel, the cells were fixed, permeabilized and stained with Goat anti p24 (Abcam) revealed by donkey anti goat antibodies coupled to Alexa 488 15 (Molecular Probes) and Dapi. Image acquisition was performed using a 96 well-plate reader automatized microscope (GE Healthcare IN Cell Analyzer 1000). Acquisition of 8 fields per well was carried out with a 10x objective. The total number of cells and the number of infected cells (Gag<sup>+</sup>) present in the 8 fields were estimated by applying an automatic segmentation algorithm to the images. The amount of p24 (pg/ml) estimated in 20 each well was divided by the number of infected cells found in the 8 fields, and the mean of the duplicate was plotted for each siRNA. Experimental points were considered as outliers when inferior to the value of the mean obtained with all the wells containing the Tsg101-specific siRNA minus two standard deviations. We considered a given gene as a hit when at least two different siRNA out of the 4 generated values below the dash line in 25 the 2 replicates.

**Figure 10, related to Figure 2. Immunoblot analysis of the effects of KIF3A depletion on cell- and virion-associated Gag.**

Macrophages were infected with NLAD8 (VSV-G) and transfected according to the 30 timeline shown in Figure 2A. At day 4 post infection/transfection, supernatant was collected, centrifuged for 5 min at 1000g and then at 150 000g for 1h30. Pellets containing released viruses were suspended in 0.5% NP40 in PBS. Supernatant and cell lysates were

analyzed by immunoblot for p24 and KIF3A contents. Intensity levels of the signals on the immunoblots were normalized to the level of the siLuc controls, and are shown below the corresponding bands. Silencing of KIF3A induced a slight increase of intracellular Gag levels and a decrease of released Gag.

5

**Figure 11, related to Figure 3. Depletion of KIF3A in HIV-infected HeLa cells reduces the release of p24.**

(A) Schematic representation of the protocol used for HeLa cells. (B) Cell lysates were analyzed by immunoblot for their contents in KIF3A and  $\alpha$ -tubulin. Efficiency of KIF3A 10 knockdown was above 90%. (C) p24 contents in the supernatants of VSV-G-pseudotyped HIV NLAD8-infected HeLa cells were measured by ELISA. Values represented here have been corrected by the number of infected cells in each sample, as calculated using CellTiter-Glo (for number of cells) and anti-Gag staining analyzed by flow cytometry (for percentage of infection). (D and E) Epon sections of HeLa cells infected with NLAD8 15 (VSV-G) for 3 days. Arrowheads point to budding profiles present at the plasma membrane. Arrows point to internal compartments containing viral particles. Viral buds can be seen at the limiting membrane of the compartment (see inset) indicating that active HIV assembly can take place in compartments.

20 **Figure 12, related to Figure 4. KIF3A and KIF3C, but not KIF3B and KIF15, are associated with the Virus-Containing Compartments**

Confocal micrographs of HIV-1-infected macrophages stained for the indicated markers. (A) Z-projections of image stacks are shown. (B) One confocal plane is shown. Bars, 5 $\mu$ m.

25 **Figure 13, related to Figure 4. The kinesin KIF3A is associated with the Virus-Containing Compartment.**

(A) 3D reconstruction of a Gag+ compartment in green with KIF3A distribution in red. This reconstruction was achieved using the Imaris software to treat a stack of images acquired by confocal microscopy. Bar, 2 $\mu$ m.(B) Confocal micrographs (one plane) of HIV- 30 1-infected macrophages stained with antibodies specific for the indicated proteins. While Gag and Env stainings overlap extensively, KIF3A staining appears partially codistributed with the viral markers. The bottom row of images is a magnification of the box shown on

the overlay in the top row. Bar, 10 $\mu$ m. (C) Disruption of the microtubule network leads to redistribution of KIF3A in infected macrophages. Confocal micrographs of HIV-1-infected macrophages exposed to Nocodazole (10 $\mu$ M) for 1 hr. Cells were fixed and stained with antibodies specific for the indicated proteins. A z-projection of an image stack is presented.

5 Bar, 10 $\mu$ m.

**Figure 14, related to Figure 5. KIF3A depletion does not affect MIP-1 $\beta$  secretion by HIV-infected macrophages.**

(A) Macrophages were uninfected or infected with HIV NLAD8 (VSV-G). MIP-1 $\beta$  released in the supernatant was measured at 4h, 1, 3 and 7 days post-infection by Cytometric Bead Array (BD Biosciences). (B) Macrophages were transfected with siRNA and infected 2 days later. MIP-1 $\beta$  released in the supernatant was measured 24 hr post-infection for 2 healthy donors.

15 **Figure 15.** related to Figure 6. Characterization of the KIF3A-iCherry construct.

(A) 293T cells were transfected or not with KIF3A-iCherry, and analyzed by immunoblot revealed by antibodies specific for KIF3A or actin. (B) Confocal micrographs (one plane) of 293T cells expressing KIF3A-iCherry and stained for the indicated proteins. (C) Confocal spinning disk micrograph (one plane) of RPE1 cells co-expressing KIF3A-iCherry and GFP-tubulin. KIF3A is aligned on microtubules and moves along them.

**Figure 16. HIV Gag-iGFP characterization**

Monocyte derived macrophages were infected with HIV1 NLAD8 (AD8) or with HIV 25 Gag-iGFP (GG). (A) Amounts of p24 released in the culture supernatant were determined by ELISA at various times after infection. HIV Gag-iGFP-infected cells produced significant but lower amounts of p24 than NLAD8-infected cells. The kinetics of p24 production was similar in both conditions. (B) Cytotoxicity was measured in both types of culture at the indicated times post infection. (C) Western blot analysis of HIV proteins 30 revealed with IVIG (AIDS Research and Reference Reagent Program) and present in the supernatant and cell lysates of macrophage cultures not infected (NI), NLAD8-infected, HIV Gag-iGFP-infected (NLGG). The Gag-iGFP fusion protein was well expressed in

macrophages and in virions secreted and was well processed into p24. (D) Infectivity assay using the TZM-bl reporter cell line of the virions produced by infected macrophages. HIV Gag-iGFP produced by macrophages were infectious although less than NLAD8. We concluded that HIV Gag-iGFP behaved like normal HIV virus although its production and infectivity were lower.

**Figure 17. The distribution of Gag+ compartments depends on the integrity of the microtubule network.**

Confocal micrographs of HIV-1-infected macrophages exposed to DMSO- or Nocodazole (10 $\mu$ M) for 1 hr (Noco) or Nocodazole for 1 hr followed by washes and re-incubation for 15 min at 37°C (Noco + WO). Cells were fixed and stained with antibodies specific for the indicated proteins ( $\alpha$ -tubulin and p24 Gag). Upon Nocodazole exposure, Gag+ compartments were redistributed in the perinuclear area. This effect was reversible as upon wash out of the Nocodazole, microtubule growth was accompanied by spreading of the Gag+ compartments.

**Figure 18. KIF3A knockdown does not affect virus production in T cells.**

JLTRG cells were infected or not with lentivirus carrying shRNA scramble or against KIF3A. After puromycin selection, cells were infected with HIV for 8 hr and washed. Supernatant was collected 16 hr after wash. (A) Cell lysates were used for western blot with anti-KIF3A and anti-b-tubulin. Knockdown was quantified in relation to untreated cells and corresponded to approximately 57% and 93% (for shKIF3A #1 and #2, respectively). (B) p24 contents in the supernatant was measured by ELISA. Values represented here have been corrected by the number of infected cells in each sample, as calculated using CellTiter-Glo (for number of cells) and GFP expression (for percentage of infection). (C) Infectivity assay was performed by incubating supernatant collected from JLTRGs containing equal amounts of p24 (2ng) with TZM-bl cells.

Methods: JLTRG are Jurkat T-derived cells which have been stably transfected with an LTR-GFP construct and were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: JLTRG (Cat #11587), from Dr. Olaf Kutsch, (Kutsch O, et al. Antimicrob Agents Chemother. 2004; 48(5):1652-63). JLTRG cells were infected with lentivirus carrying shRNA scramble (MISSION Non-Target shRNA Control

Vector, Sigma-Aldrich, catalogue number SHC002) or against KIF3A (MISSION shRNA, Sigma-Aldrich. Sh1 TRC number TRCN0000116812, Sh2 TRC number TRCN0000116814) and selected with puromycin (3 µg/ml). Similar numbers of untreated or lentivirus-infected JLTRG were washed and infected with HIV NL-AD8 VSV-g for 8 hr  
5 in puromycin-free medium, washed in PBS and cultured for 16 hr, when supernant was collected for p24 quantification and infectivity assay. Cells were also harvested and used for cell quantification by CellTiter-Glo (Promega), lysed for western blot assay or fixed for flow cytometric analysis.

10 **Figure 19. Characterization of the KIF3A-iCherry construct**

(A) Schematic representation of the various KIF3A constructs carried out. (B) 293T cells were transfected with the indicated constructs and analyzed by immunoblot revealed by antibodies specific for KIF3A. (C) Confocal micrographs of 293T cells expressing KIF3A-iCh and stained for the indicated proteins.  
15

**Figure 20. Amino acid sequence KIF3A-iCherry.**

Spacers are underlined. Cherry protein in grey. KIF3A motor domain is between amino acids N°s 1-350; KIF3A stalk domain between amino acid N°s 351-594; KIF3A cargo domain between amino acids N°s 862-968 of SEQ ID N°31, spacers between amino acids  
20 595-609 and 847-861 and iCherry protein between amino acid 610-846.

**Figure 21. Nucleic acid sequence: SEQ ID N°32 KIF3A-iCherry.** The coding sequence is indicated in capital letters.

25 **Detailed description of the invention**

The inventors showed, in a surprising way, that kinesin depletion, in particular KIF3A, KIF3C or KIFAP3 depletion, led to a strong reduction of HIV virion production in primary macrophages infected with full-length HIV. The HIV production could indeed be decreased by means of an inhibitor of kinesin, in particular by means of a nucleic acid  
30 blocking the expression of KIF3A, KIF3C or KIFAP3. They indeed observed, using 3 different siRNA that had been validated in Hela cells by studying KIF3A protein expression by Western blot, that the depletion of KIF3A in primary macrophages led to a

strong reduction (75%) of the amounts of HIV secreted in the supernatant. Depletion of others kinesins, KIF5B, KIF3C or KIFAP3, also resulted in reduction of secreted HIV virions. The inventors thus revealed that inhibiting the expression and/or activity of a host factor, KIF5B, KIF3A, KIF3C or KIFAP3, could decrease or inhibit HIV production by 5 infected macrophages.

#### 1. Kinesine inhibitors as therapeutic agents in HIV infection.

In a first aspect, the present invention provides new therapeutic agents for treating HIV infection, namely kinesin inhibitors and in particular KIF3A inhibitors, KIF5B inhibitors, 10 KIF3C inhibitors and/or KIFAP3 inhibitors.

The term "HIV infection" defines a condition caused by the human immunodeficiency virus (HIV). Infection with HIV-1 is associated with a progressive decrease of the CD4<sup>+</sup> T cell count and an increase in viral load. The stage of infection can be determined by 15 measuring the patient's CD4<sup>+</sup> T cell count, and the level of viral load in the blood. HIV infection has four basic stages: incubation period, acute infection, latency stage and AIDS. The initial incubation period upon infection is asymptomatic and usually lasts between two and four weeks. The second stage, acute infection, lasts an average of 28 days and can include symptoms such as fever, lymphadenopathy, pharyngitis, rash, myalgia, malaise, 20 and mouth and esophageal sores. The latency stage, which occurs third, shows few or no symptoms and can last anywhere from two weeks to twenty years and beyond. AIDS, the fourth and final stage of HIV infection is characterized by a very low level of CD4+ T cells and a severe immunodeficiency, and is accompanied by various opportunistic infections.

In the context of the invention, the terms "treatment", "treat" or "treating" are used herein 25 to characterize a therapeutic method or process that is aimed at (1) slowing down or stopping the progression, aggravation, or deterioration of the symptoms of the disease state or condition to which such term applies; (2) alleviating or bringing about ameliorations of the symptoms of the disease state or condition to which such term applies; and/or (3) reversing or curing the disease state or condition to which such term applies.

30 As used herein, the term "subject" or "patient" refers to an animal, preferably to a mammal, even more preferably to a human, including adult, child and human at the prenatal stage. However, the term "subject" can also refer to non-human animals, in

particular mammals such as cats, horses, and non-human primates, among others, that are in need of treatment.

In the present description, the terms "nucleic acid," "nucleic sequence," "polynucleotide," "oligonucleotide" and "nucleotide sequence" are used interchangeably and refer to a  
5 sequence of deoxyribonucleotides and/or ribonucleotides.

As used herein the term "polypeptide" refers to any chain of amino acids linked by peptide bonds, regardless of length or post-translational modification. Polypeptides include natural proteins, synthetic or recombinant polypeptides and peptides (i.e. polypeptides of less than 50 amino acids) as well as hybrid, post-translationally modified polypeptides, and  
10 peptidomimetic.

As used herein, the term "amino acid" refers to the 20 standard alpha-amino acids as well as naturally occurring and synthetic derivatives. A polypeptide may contain L or D amino acids or a combination thereof.

As used herein the term "peptidomimetic" refers to peptide-like structures, which have  
15 non-amino acid structures substituted but which mimic the chemical structure of a peptide and retain the functional properties of the peptide. Peptidomimetics may be designed in order to increase peptide stability, bioavailability, solubility, etc.

All kinesins share a conserved core motor domain implying a common mechanism for  
20 generating force from ATP hydrolysis. Kinesins belong to a class of functional proteins generally called "molecular motors" and represent a superfamily of 15 classes including kinesin-related proteins of various functions.

Kinesins are a large superfamily of microtubule motor proteins that use the energy of ATP hydrolysis to produce force. They are defined by the presence of a catalytic core motor  
25 domain (historically known as the 'head' domain), which hydrolyzes ATP and binds to microtubules (MTs). Kinesins are classified into three subfamilies based on the position of their motor domain within the primary sequence of the protein. The Kin C (Kinesin 14) subfamily comprises kinesins with a C-terminally located core motor domain; Kin N kinesins (Kinesin-1 to 12) have an N-terminally located core motor domain; and Kin M  
30 (Kinesin 13) kinesins possess an internally located core motor domain. (Nobutaka Hirokawa et al, (2009) Kinesin superfamily motor proteins and intracellular transport) All kinesins share a high degree of sequence identity within the core motor domain. The

crystal structures of the Kin N and Kin C core motor domains are clearly similar to each other. Functionally, the position of a kinesin's motor domain within the polypeptide chain usually predicts its directionality. Kin N kinesins walk toward the plus ends of MTs, whereas Kin C kinesins translocate toward the minus ends of MTs. Unexpectedly, the Kin 5 I kinesins are not able to translocate along MTs in the conventional sense but, instead, depolymerize MT filaments from both ends. Kinesin is an oligomeric complex composed of two heavy chains and two light chains. The heavy chain is composed of three structural domains: a large globular N-terminal domain which is responsible for the motor activity of kinesin (it is known to hydrolyse ATP, to bind and move on microtubules), a central alpha-helical coiled coil domain, the stalk domain, that mediates the heavy chain dimerisation; and a small globular C-terminal domain, the cargo domain, which interacts with other proteins (such as the kinesin light chains), vesicles and membranous organelles.

As used herein in the context of the invention, the terms "kinesin", and KIF used interchangeably throughout the specification refer to all motor-type kinesins including both 15 N-type and C-type kinesins but excluding kinesins unable to transport cargo proteins along microtubules. In particular, such terms refer to all naturally-occurring forms of motor kinesins including allelic variants, splice variants and isoforms.

In the context of the invention, we focus essentially on kinesins belonging to the kin N family, so-called "N-kinesin" or "N-type kinesins", more particularly on kinesins 20 belonging to either the kinesin-1 and/or kinesin-2 subfamilies, even more particularly to KIF5B, KIF3A, KIF3C and KIFAP3.

Kinesin-2 subfamily is one of the most ubiquitously expressed KIFs and has been implicated in the intracellular transport of membrane-bound organelles and protein complexes in various tissues such as neurons, melanosomes, and epithelial cells. Kinesin-2 25 molecules are expressed ubiquitously and transport cargoes along microtubules (MTs) from the nucleus to the cell periphery.

Kinesin-2 is a heterotrimeric complex composed of a KIF3A/3B heterodimer and KIF-associated protein 3 (KAP3 also known as KIFAP3). The KIF3A/3B heterodimer possesses a plus-end-directed microtubule sliding activity that uses energy derived from 30 ATP hydrolysis. On the other hand, KAP3 links KIF3A/3B with various cargo proteins.

KIF3A can form heterodimers either with KIF3B or KIF3C to walk along microtubules towards their plus end (Yamakasi, 1995 4547) (Yang, 1998 4548). KIF3A comprises of

an N-terminal head (motor) domain that attaches to and migrates along MTs and C-terminal tail that presumably functions as the cargo-binding domain. KIF17, an NH2-terminal motor domain-type motor, is also a member of the kinesin-2 family.

As used herein in the context of the invention, the terms “kinesin-2”, “kinesins 2”, “kinesin II” or “kinesin-2 family”, used interchangeably throughout the specification, refer to all kinesins belonging to the kinesin 2 subfamily including KIF3A, KIF3B, KIF17 (also known as OSM3) and KIFAP3. Unigene Cluster for kinesin 2 include KIF3A, KIF3B, which representative mRNA and protein sequences are AB002357, and 015066, respectively; KIF17, which representative mRNA and protein sequences are AY48442, and Q9P2E2 respectively; KAP3, which representative mRNA and protein sequences are M31158 and P31323 respectively.

As used herein, the terms “kinesin 3A”, “KIF3A” and ” Kinesin-like protein KIF3A” are used interchangeably throughout the specification and refer to all naturally-occurring forms of KIF3A including allelic variants, splice variants and isoforms. The Unigene Cluster for Kif3A is Hs AC004039, representative mRNA is AF041853, and Swissprot Q9Y496 (KIF3A\_HUMAN), respectively.

As used herein, the terms “kinesin 3C”, “KIF3C” and Kinesin-like protein KIF3C” are used interchangeably throughout the specification and refer to all naturally-occurring forms of KIF3C including allelic variants, splice variants and isoforms. The Unigene Cluster for KIF3C is Hs AJ002223 representative mRNA is AAC05302, and Swissprot O14782 (KIF3C\_HUMAN), respectively.

As used herein, the terms “KIFAP3”, “KIFA3”, “KI3AP” and “Kinesin-associated protein 3” are used interchangeably throughout the specification and refer to all naturally-occurring forms of KIFAP3 including allelic variants, splice variants and isoforms. The Unigene Cluster for KIFAP3 is Hs AL121714 representative mRNA is BC048012, and Swissprot Q92845 (KIAP3\_HUMAN), respectively.

As used herein in the context of the invention, the terms “kinesin-1”, “kinesins 1”, “kinesin I” or “kinesin-1 family”, used interchangeably throughout the specification, refer to all kinesins belonging to the kinesin 1 subfamily (see Hirokawa et al., Mol. Cell biol. (2009), 30 10:682) including but not limited to KIF5A, KIF5B, and KIF5C.

As used herein, the term “kinesin inhibitor” refers to any molecule able to decrease or inhibit the expression and/or activity of a kinesin of interest according to the invention. Preferably, such a kinesin inhibitor is a direct inhibitor, meaning that it interacts directly with either a kinesin protein or a nucleic acid encoding a kinesin.

- 5 In one embodiment, the kinesin inhibitor is selective for a given kinesin of interest, preferably for KIF3A, for KIF5B, for KIF3C or for KIFAP3, and/or a subset of kinesins, preferably N-type kinesins, more preferably kinesins 1 or kinesins 2. Selectivity means that the kinesin inhibitor inhibits preferentially the expression and/or activity of the kinesin of interest (or subgroup of kinesins), or with a greater efficacy compared to other kinesins
- 10 10 (or groups of kinesins), for instance by a factor of at least 10, 100 or 1000. If an inhibitor is selective for kinesin Kif3A, meaning it binds Kif3A with a greater efficacy compared to other kinesins, it is referred to throughout the specification as a Ki3fA selective inhibitor. Similarly inhibitors selective for either N-type kinesin or kinesin 2 are referred to as N-type kinesin selective inhibitors and kinesin 2 selective inhibitors respectively.
- 15 In a preferred embodiment of the invention, said kinesin inhibitor is a selective kinesin inhibitor, preferably a N-kinesin selective inhibitor, more preferably a kinesin 2 selective inhibitor or a kinesin 1 selective inhibitor. In an even preferred embodiment, said inhibitor is a KIF3A selective inhibitor, a KIF5B selective inhibitor, a KIF3C selective inhibitor or a KIFAP3 selective inhibitor.
- 20 However, in another embodiment, the kinesin inhibitor of the invention is an inhibitor able to inhibit the expression and/or activities of several kinesins of interest. Such multiple-specificity kinesin inhibitor can be useful especially in cases where Gag could be transported on microtubules by more than one kinesin. For example, kinesin inhibitors showing selectivity for KIF3A, KIF5B, KIF3C and/or KIFAP3 are contemplated.

25

A kinesin inhibitor of the invention may act by blocking and/or inhibiting the activity of the kinesin of interest, preferably the activity of N-type kinesins, more preferably the activity of a kinesin 1 or a kinesin 2, even more preferably the activity of KIF3A, KIF5B KIF3C or KIFAP3.

30 This may for example be achieved by interfering with the motor activity of the kinesin of interest. Alternatively, this may be achieved by interfering with the binding of the kinesin

of interest with its cargo protein. Both options ultimately result in blocking or reducing transport of the cargo protein, hence in blocking or reducing the kinesin functional activity.

The kinesin inhibitors according to the invention are capable of inhibiting or decreasing the functional activity of the kinesin *in vivo* and/or *in vitro*. The inhibitor may inhibit the

5 functional activity of the kinesin by at least about 10%, preferably by at least about 30%, preferably by at least about 50%, preferably by at least about 70, 75 or 80%, still preferably by 85, 90, 95, or 100%.

Functional activity of the kinesin may be readily assessed by the one skilled in the art according to known methods. Indeed, since, MT activated kinesin ATPase is a major

10 parameter in kinesin motor function, functional activity of a kinesin may be assessed by measuring the level of ATPase activity. For example, Kinesin activity can be tested using the Kinesin ELIPAT<sup>TM</sup> (Enzyme Linked Inorganic Phosphate Assay) Biochem Kit. The assay is an adaptation of a method originally described by Webb, M. R. (Pathway of the microtubule-kinesin ATPase. Biophys. (1992)) for the measurement of glycerol kinase plus

15 D-glyceraldehyde ATPase activity and for actin activated myosin ATPase. The assay is based upon an absorbance shift (330 - 360 nm) that occurs when 2-amino-6-mercaptopurine ribonucleoside (MESG) is catalytically converted to 2-amino-6-mercaptopurine in the presence of inorganic phosphate (Pi). The reaction is catalyzed by purine nucleoside phosphorylase (PNP). One molecule of inorganic phosphate will yield 20 one molecule of 2-amino-6-mercaptopurine in an essentially irreversible reaction. Thus, the absorbance at 360 nm is directly proportional to the amount of Pi generated in the kinesin ATPase reaction.

Functional activity of a kinesin of interest may also be assessed by measuring its ability to transport a cargo protein, along microtubules. For example, the method described in

25 Example 5 of the present invention may be used wherein a cargo protein tagged with GFP is coexpressed with a tagged kinesin (i.e. KIF3A-iCh) and the movements and eventual colocalization of both tagged proteins are determined using time lapse confocal microscopy. Analysis of the respective movements of both proteins in various conditions allows to characterize actual kinesin-dependent transport of the cargo protein.

30

The kinesin inhibitor of the invention may also act by blocking and/or inhibiting the expression of the kinesin of interest, preferably the expression of N-type kinesins, more

preferably the expression of a kinesin 2 or of a kinesin 1; even more preferably the expression of KIF3A, KIF5B, KIF3C or KIFAP3.

The decrease or the inhibition of kinesin expression can be evaluated by any means known to those skilled in the art including but not limited to assessing the level of the kinesin protein of interest using Western Blot analysis, for example using an Anti KIF3A antibody (Abcam), and assessing the level of mRNA for the kinesin of interest using any available technique such as qPCR for example. By a “decrease” in expression is meant, for example, a decrease of at least 30%, preferably at least 50%, more preferably at least 70%, or 80%, even more preferably at least 90%, 95% or 100% of the gene expression product.

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Accordingly, a kinesin inhibitor according to the invention may be identified as a molecule which reduces the level of activity and/or expression of the kinesin of interest or subgroup of kinesins of interest using any of the methods and assays above mentioned and comparing the activity and/or expression of the kinesin(s) of interest in presence of the molecule whose kinesin inhibiting activity is to be assessed with the expression and/or activity of the kinesin(s) of interest in the absence of any such molecule.

15

Of particular interest, a kinesin inhibitor according to the invention may be further assessed for its ability to prevent Gag transport along microtubules and/or HIV virions release by any technique known to those skilled in the art including those described in the Example section of the present specification.

20

The kinesin inhibitor of the invention can be of various structural natures and include, without being limiting thereto, small molecules, aptamers, antibodies, nucleic acids, lipids, and peptides, polypeptides or proteins, such as kinesin dominant negative peptides or proteins, able to decrease kinesin expression and/or activity. The kinesin inhibitor may for instance be a peptide or polypeptide, such as an antibody or aptamer directed against the kinesin of interest; a nucleic acid molecule which reduces or prevents kinesin expression, such as an aptamer, a kinesin anti-sense oligonucleotide, a kinesin interfering RNA (iRNA) such as siRNA, or a ribozyme; or a small molecule inhibitor of kinesin activity.

30 The kinesin inhibitor of the invention is preferably selected from the group consisting of a small molecule, an anti-kinesin antibody, and a nucleic acid molecule interfering specifically with kinesin expression.

In an even preferred embodiment, said kinesin inhibitor is a kinesin siRNA, a kinesin antisens nucleic acid or a ribozyme interfering specifically with kinesin expression.

### 1.1 Inhibitors of kinesin activity

5 It is known that Pr55Gag coordinates the recruitment of different host proteins necessary for at least its transport to the assembly site and the formation of the viral particles. The inventors have shown that KIF3A is involved in the transport of Gag<sup>+</sup> compartments containing virions.

Therefore the invention relates to the use of kinesin activity inhibitor, in particular KIF3A,  
10 KIF5B, KIF3C and/or KIFAP3 inhibitors, in HIV treatment.

The kinesin inhibitor of the invention can act through direct binding to a kinesin molecule.  
In this case, the inhibitor can be an antagonist which binds to the cargo binding domain or  
to another domain of the molecule which modifies the activity of the kinesin by steric  
hindrance or modification. This inhibitor can be, for instance, a small molecule, an  
15 aptamer, a dominant negative peptide, or an antibody directed against the active domain of  
the kinesin.

In one embodiment, a kinesin inhibitor according to the invention may be a small molecule  
inhibiting the activity of the kinesin(s) of interest. As used herein, the term “small  
molecule inhibiting the kinesin activity” refers to small molecule that can be an organic or  
20 inorganic compound, usually less than 1000 daltons, with the ability to inhibit or reduce  
the activity of the kinesin. Structural design in chemistry might help to find such a small  
molecule.

Small kinesin-inhibiting molecules are also known in the art and include, without being  
limiting thereto, tetracaïne, Lidocaïne, acepromazine, chlorfenethazine, chlorpromazine,  
25 N-methyl chlorpromazine, cyamemazine, fluphenazine, mepazine, methotriimeprazine,  
methoxypromazine, norchlorpromazine, perazine, perphenazine, phenothiazine,  
prochlorperazine, promethazine, propiomazine, putaperazine, thiethylperazine,  
thiopropazate, thioridazine, trifluoperazine, triflupromazine as well as inhibiting molecules  
described in patent applications and patents WO 01/98278, WO 02/057244, WO  
30 02/079169, WO 02/057244, WO 02/056880, WO 03/050122, WO 03/050064, WO  
03/049679, WO 03/049678, WO 03/049527, WO 03/079973, US 6,890,933, WO  
2008/070739, WO 2006/060737, WO 2006/119146 and US 6,777,200.

In another embodiment, the kinesin inhibitor is an aptamer. The aptamers are nucleic acids, preferably RNA, generally comprising between 5 and 120 nucleotides (Osborne et al., 1997, Curr Opin Chem Biol. 1, 5-9).

As used here, the term “aptamer” means a molecule of nucleic acid or a peptide able to bind to a kinesin of interest or to a subgroup of kinesins of interest. It refers to a class of molecule that represents an alternative to antibodies in term of molecular recognition. Aptamers are oligonucleotide or oligopeptide sequences with the capacity to recognize virtually any class of target molecules with high affinity and specificity.

Such ligands may be isolated through Systematic Evolution of Ligands by EXponential enrichment (SELEX) of a random sequence library, as described in Tuerk C. and Gold L., Science, 1990, 249(4968):505-10. The random sequence library is obtainable by combinatorial chemical synthesis of DNA. In this library, each member is a linear oligomer, eventually chemically modified, of a unique sequence. Possible modifications, uses and advantages of this class of molecules have been reviewed in Jayasena S.D., Clin. Chem., 1999, 45(9):1628-50. Peptide aptamers consist of a conformationally constrained antibody variable region displayed by a platform protein, such as E. coli Thioredoxin A that are selected from combinatorial libraries by two hybrid methods (Colas et al., Nature, 1996, 380, 548-50).

As used here, the term “kinesin dominant negative peptide” refers to a peptide comprising at least part of a kinesin interacting with a cargo protein and able to suppress or decrease its activity.

In a preferred embodiment, the dominant negative peptide is only made up of the part of kinesin interacting with the protein cargo, i.e the part corresponding to the cargo domain of kinesin. This peptide is thus to interact with kinesin but, in the absence of the segment corresponding to the motor domain of kinesin, is unable to move along microtubules and thus to fulfill its function. Example of a KIF3A dominant negative peptide would consists essentially of or consists of the amino acid acids between positions 862 to 968 of SEQ ID N°13.

The inhibitor can be a specific antibody of the kinesin(s) of interest, preferably a N-type kinesins, more preferably a kinesin 2, even more preferably KIF3A, KIF5B, KIF3C or KIFAP3.

As used in the present invention, the term "antibody" includes monoclonal antibodies, chimeric antibodies, humanized antibodies, recombinant antibodies and fragments thereof. Antibody fragment means, for example F(ab)2, Fab, Fab' or sFv fragments. This small molecule can be derived from any known organism (including, but not limited to animals, 5 plants, bacteria, fungi and viruses) or from a library of synthetic molecules immunologic binding agent such as IgG, IgM, IgA, IgD and IgE, and humanized or chimeric antibody. In certain embodiments, IgG and/or IgM are preferred because they are the most common 10 antibodies in the physiological situation and they are most easily manufactured. The term "antibody" is used to refer to any antibody-like molecule that has an antigen binding region, and includes antibody fragments such as Fab', Fab, F(ab')<sub>2</sub>, single domain antibodies (DABs), Fv, scFv (single chain Fv), and the like. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art. Means for preparing and characterizing antibodies are also well known in the art (See, e.g., Harlow and Lane, 1988).

15 A "humanized" antibody is an antibody in which the constant and variable framework region of one or more human immunoglobulins is fused with the binding region, e.g. the CDR, of an animal immunoglobulin. "Humanized" antibodies contemplated in the present invention are chimeric antibodies from mouse, rat, or other species, bearing human constant and/or variable region domains, bispecific antibodies, recombinant and 20 engineered antibodies and fragments thereof. Such humanized antibodies are designed to maintain the binding specificity of the non-human antibody from which the binding regions are derived, but to avoid an immune reaction against the non-human antibody.

A "chimeric" antibody is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable 25 region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity such as Mouse anti KIF3A (Becton Dickinson) .

The inhibition can also be due to the reduction or suppression of the expression of the gene product coding for the kinesin of interest, for example by using specific siRNA, antisense or ribozyme, which ultimately induce a decrease of the protein expression.

- In a preferred embodiment of the invention, the kinesin inhibitor is a nucleic acid  
5 comprising or consisting of a sequence capable of hybridizing specifically with a nucleic acid (for example a gene or a mRNA) coding for a kinesin, preferably a N kinesin, more preferably a kinesin 2, even more preferably KIF3A, KIF5B, KIF3C or KIFAP3, and to decrease or suppress the expression of said kinesin. Such nucleic acids are more amply detailed below.
- 10 In the present invention, a “nucleic acid molecule specifically interfering with Kinesin gene expression” is a nucleic acid molecule which is able to reduce or to suppress the expression of gene coding for kinesin, in a specific way. It includes, but is not limited to siRNA, antisense and ribozyme molecules. The nucleic acid used according to the invention generally has a length of 15 to 50 nucleotides, preferably from 15 to 30  
15 nucleotides in length.

In the present invention, the nucleic acid is capable of hybridizing specifically to a gene or transcripts coding for a kinesin, in particular KIF3A, KIF5B, KIF3C or KIFAP3. Nevertheless, it is understood that the nucleic acid according to the invention does not need to have 100% complementarity with the target sequence to hybridize specifically. In particular, a nucleic acid with a degree of complementarity at least equal to approximately 20 90% is capable of hybridizing specifically. Preferably, the degree of complementarity between the nucleic acid according to the invention and the target sequence is equal to at least 95%, 96%, 97%, 98%, 99% or 100%.

- 25 In a particular embodiment, the nucleic acid molecule specifically interferes with a KIF3A sequence selected from the group consisting of
- KIF3A\_1 target sequence of SEQ ID No 16 (GTGCCTTATCGTAACCTCA),
  - KIF3A\_2 target sequence of SEQ ID No 17 (GCCGATCAATAATCAGAG),
  - KIF3A\_3 target sequence of SEQ ID No 18 (AAGACCTGATGTGGGAGTT),
  - 30 - KIF3A\_4 target sequence of SEQ ID No 19 (CTGGTTCAGAAAGACAGGC),
  - KIF3A\_2bis target sequence of SEQ ID N°20 (GGTCAGAAAGACAGGCAA),
  - KIF3A\_8 target sequence of SEQ ID N° 21 (GACCTGATGTGGGAGTTA), and

- KIF3A\_9 target sequence of SEQ ID N°22 (GCCTAAAGGAAGCTACAAA).

- In a more particular embodiment, the nucleic acid molecule specifically interfering with KIF3A gene expression comprises or consists of a sequence selected from the group consisting of
- siKIF3A\_1 of SEQ ID No 1, (GUGCCUUAUCGUACUCUAAA), preferentially interfering with the KIF3A\_1 target sequence of SEQ ID No 16 (GTGCCTTATCGTAACCTCA),
  - siKIF3A\_2 of SEQ ID No 2, (GCCGAUCAAUAAAUCAGAGAA), preferentially interfering with the KIF3A\_2 target sequence of SEQ ID No 17 (GCCGATCAATAAACATCAGAG),
  - siKIF3A\_3 of SEQ ID No 3, (AAGACCUGAUGUGGGAGUUTA), preferentially interfering with the KIF3A\_3 target sequence of SEQ ID No 18 (AAGACCTGATGTGGGAGTT),
  - siKIF3A\_4 of SEQ ID No 4 (CUGGUUCAGAAAGACAGGCAA) preferentially interfering with the KIF3A\_4 target sequence of SEQ ID No 19 (CTGGTTCAGAAAGACAGGC),
  - siKIF3A\_2bis of SEQ ID N°5 (GGUUCAGAAAGACAGGCAATT) preferentially interfering with the KIF3A\_2bis target sequence of SEQ ID N°20 (GGTCAGAAAGACAGGCAA),
  - siKIF3A\_8 of SEQ ID N°6 (GACCUGAUGUGGGAGUUUATT) preferentially interfering with the KIF3A\_8 target sequence of SEQ ID N° 21 (GACCTGATGTGGGAGTTA), and
  - siKIF3A\_9 of SEQ ID N°7 (GCCUAAAGGAAGCUACAAATT) preferentially interfering with the KIF3A\_9 target sequence of SEQ ID N°22 (GCCTAAAGGAAGCTACAAA).

- In a particular embodiment, the nucleic acid molecule specifically interferes with a KIF3C sequence selected from the group consisting of
- KIF3C\_1 target sequence of SEQ ID N°23, (CAGGCCGACCTGTATGACG),
  - KIF3C\_2 target sequence of SEQ ID N°24, (AAGGGCCTTGATTAGGACC),
  - KIF3C\_3 target sequence of SEQ ID N°25, (AACGAAGATATTAACATC), and

- KIF3C\_4 target sequence of SEQ ID N°26, (ATCATGGATCACACCAACG).

In a more particular embodiment, the nucleic acid molecule specifically interfering with KIF3C gene expression comprises or consists of a sequence selected from the group consisting of

- siKIF3C\_1 of SEQ ID No 8, (CAGGCCGACCUGUAUGACGAA) preferentially interfering with the KIF3C\_1 target sequence of SEQ ID N°23, (CAGGCCGACCTGTATGACG),
- siKIF3C\_2 of SEQ ID No 9 (AAGGGCCUUGAUUAGGACCAA), preferentially interfering with the KIF3C\_2 target sequence of SEQ ID N°24, (AAGGGCCTTGATTAGGACC),
- siKIF3C\_3 of SEQ ID No 10 (AACGAAGAUUAACAUACTA), preferentially interfering with the KIF3C\_3 target sequence of SEQ ID N°25, (AACGAAGATATTAACATC), and
- siKIF3C\_4 of SEQ ID No 11 (AUCAUGGAUCACACCAACGAA) preferentially interfering with the KIF3C\_4 target sequence of SEQ ID N°26, (ATCATGGATCACACCAACG).

In a particular embodiment, the nucleic acid molecule specifically interferes with a KIFAP3 sequence selected from the group consisting of

- KIFAP3\_1 target sequence of SEQ ID N°27, (CCACATCTTGATGCTAAGT),
- KIFAP3\_2 target sequence of SEQ ID N°28, (TCGAGTTAGCTACAAACAT),
- KIFAP3\_3 target sequence of SEQ ID N°29, (AAGCCCTTGATCGGGACAA), and
- KIFAP3\_4 target sequence of SEQ ID N°30, (CAGCATGATGGACCAACTA).

25

In a more particular embodiment, the nucleic acid molecule specifically interfering with KIFAP3 gene expression comprises or consists of a sequence selected from the group consisting of

- siKIFAP3\_1 of SEQ ID No 12, (CCACAUCUUGAUGCUALGUAA) preferentially interfering with the KIFAP3\_1 target sequence of SEQ ID N°27, (CCACATCTTGATGCTAAGT),

- siKIFAP3\_2 of SEQ ID No 13 (UCGAGUUAGCUACAAACAUAA) preferentially interfering with the KIFAP3\_2 target sequence of SEQ ID N°28, (TCGAGTTAGCTACAAACAT),
- siKIFAP3\_3 of SEQ ID No 14 (AAGCCCUUGAUCGGGACAATT) preferentially interfering with the KIFAP3\_3 target sequence of SEQ ID N°29, (AAGCCCTTGATCGGGACAA), and
- siKIFAP3\_4 of SEQ ID No 15 (CAGCAUGAUGGACCAACUAAA) preferentially interfering with the KIFAP3\_4 target sequence of SEQ ID N°30, (CAGCATGATGGACCAACTA).

10

The term "siRNA" or "interfering RNA" means any RNA which is capable of down-regulating the expression of the targeted protein. It encompasses small interfering RNA (siRNA), double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules. RNA interference designates a 15 phenomenon by which dsRNA specifically suppresses expression of a target gene at post-translational level. In normal conditions, RNA interference is initiated by double-stranded RNA molecules (dsRNA) of several thousands of base pair length. In vivo, dsRNA introduced into a cell is cleaved into a mixture of short dsRNA molecules called siRNA. The enzyme that catalyzes the cleavage, Dicer, is an endo-RNase that contains RNase III 20 domains (Bernstein, Caudy et al. 2001 Nature. 2001 Jan 18;409(6818):363-6). In mammalian cells, the siRNAs produced by Dicer are 21-23 bp in length, with a 19 or 20 nucleotides duplex sequence, two-nucleotide 3' overhangs and 5'-triphosphate extremities (Zamore, Tuschl et al. Cell. 2000 Mar 31;101(1):25-33; Elbashir, Lendeckel et al. Genes Dev. 2001 Jan 15;15(2):188-200; Elbashir, Martinez et al. EMBO J. 2001 Dec 25;20(23):6877-88).

A number of patents and patent applications have described, in general terms, the use of siRNA molecules to inhibit gene expression, for example, WO 99/32619, US 20040053876, US 20040102408 and WO 2004/007718.

30 siRNA are usually designed against a region 50-100 nucleotides downstream the translation initiator codon, whereas 5'UTR (untranslated region) and 3'UTR are usually avoided. The chosen siRNA target sequence should be subjected to a BLAST search

against EST database to ensure that the only desired gene is targeted. Various products are commercially available to aid in the preparation and use of siRNA.

In a preferred embodiment, the RNAi molecule is a siRNA of at least about 15-50 nucleotides in length, preferably about 20-30 base nucleotides, preferably about 20-25 nucleotides in length.

In a particular embodiment, the siRNA molecule comprises a sequence selected from the group consisting of siKIF3A\_1 of SEQ ID No 1, KIFF3A\_2 of SEQ ID No 2, siKIF3A\_3 of SEQ ID No 3, siKIF3A\_4 of SEQ ID No 4, siKIF3A\_2bis of SEQ ID No 5, siKIF3A\_8 of SEQ ID No 6 and siKIF3A\_9 of SEQ ID No 7.

10 In a particular embodiment, the siRNA molecule comprises a sequence selected from the group consisting of siKIF3C\_1 of SEQ ID No 8, siKIF3C\_2 of SEQ ID No 9, siKIF3C\_3 of SEQ ID No 10, siKIF3C\_4 of SEQ ID No 11.

15 In a particular embodiment, the siRNA molecule comprises a sequence selected from the group consisting of siKIFAP3\_1 of SEQ ID No 12, siKIFAP3\_2 of SEQ ID No 13, siKIFAP3\_3 of SEQ ID No 14, siKIFAP3\_4 of SEQ ID No 15.

20 siRNA can comprise naturally occurring RNA, synthetic RNA, or recombinantly produced RNA, as well as altered RNA that differs from naturally-occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end of the molecule or to one or more internal nucleotides of the RNAi, including modifications that make the RNAi resistant to nuclease digestion.

RNAi may be administered in free (naked) form or by the use of delivery systems that enhance stability and/or targeting, e.g., liposomes, or incorporated into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, bioadhesive microspheres, or 25 proteinaceous vectors (WO 00/53722), or in combination with a cationic peptide (US 2007275923). They may also be administered in the form of their precursors or encoding DNAs.

30 Antisense nucleic acid can also be used to down-regulate the expression of the kinesin. The antisense nucleic acid can be complementary to all or part of a sense nucleic acid encoding a kinesin e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence, and it thought to interfere with the translation of the target mRNA

In a preferred embodiment, the antisense nucleic acid is a RNA molecule complementary to a target mRNA encoding a kinesin.

An antisense nucleic acid can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. Particularly, antisense RNA molecules are usually 18-50 nucleotides  
5 in length.

An antisense nucleic acid for use in the method of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. Particularly, antisense RNA can be chemically synthesized, produced by in vitro transcription from linear (e.g. PCR products) or circular templates (e.g., viral or non-viral  
10 vectors), or produced by in vivo transcription from viral or non-viral vectors.

Antisense nucleic acid may be modified to have enhanced stability, nuclease resistance, target specificity and improved pharmacological properties. For example, antisense nucleic acid may include modified nucleotides designed to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate  
15 derivatives and acridine substituted nucleotides.

Ribozyme molecules can also be used to decrease levels of functional kinesin. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes can be used to catalytically cleave mRNA transcripts to thereby  
20 inhibit translation of the protein encoded by the mRNA. Ribozyme molecules specific for functional KIF3A, KIF3C or KIFAP3 can be designed, produced, and administered by methods commonly known to the art (see e.g., Fanning and Symonds, 2006, reviewing therapeutic use of hammerhead ribozymes and small hairpin RNA).

The interfering RNA, the antisense nucleic acids and the ribozyme molecule used  
25 according to the invention can be administered in the form of DNA precursors or molecules coding for them.

The kinesin inhibitors according to the invention are able, when they are introduced into infected macrophages, to induce decrease or suppression of the expression or activity of a  
30 kinesin, in particular to KIF3A, KIF5B, KIF3C or KIFAP3, with a consequence being a significant decrease in the HIV production specific of infected macrophages.

1.3 Use of kinesin inhibitors of the invention.

The inventors showed that inhibition of KIF3A, KIF3C or KIFAP3 led to a strong reduction of HIV virions production by macrophages, and thus to a diminution of HIV viral load in the supernatant. The invention thus relates to a method for treating HIV infection by administering a therapeutically effective amount of a kinesin inhibitor, preferably a N-type kinesin inhibitor, more preferably a kinesin 1 and/or kinesin 2 inhibitor, even more preferably a KIF3A inhibitor, a KIF5B inhibitor, a KIF3C inhibitor and/or a KIFAP3 inhibitor, and to the use of such kinesin inhibitor for treating HIV infection in patients in need thereof. In particular, the treatment allows the improvement of the clinical outcome of a HIV-infected patient, through the diminution of HIV viral load.

10 The term “anti-HIV therapy” as used herein means any anti-HIV drug found useful for treating HIV infection in man alone, or as part of multidrug combination therapies, especially the triple and quadruple combination therapies called HAART. Typical suitable anti-HIV therapies include, but are not limited to multidrug combination therapies such as

15 (i) at least those described in EP 1034790 A2.

10 The term “highly active antiretroviral therapy” or HAART as used herein means the multi-drug therapies used in current clinical treatment of HIV infections, including but not limited to the multi-drug therapies, e.g., the triple and quadruple drug therapies such as disclosed by A-M. Vandamme et al., in Antiviral Chemistry & Chemotherapy, 9: 187-203  
20 (1998) which describes the current clinical treatments of HIV infections, including when to start multi-drug therapy and which drugs to combine. The triple drug therapy may include two nucleoside and nucleotide reverse transcriptase inhibitors (“NRTIs”) and one protease inhibitor (“PI”), but there are many issues to be considered in the choice of the precise HAART for any patient. The highly active antiviral therapy (HAART) usually includes a  
25 combination of reverse transcriptase (RT) and protease inhibitors that induce undetectable levels of viral RNA in the peripheral blood plasma.

10 The term “nucleoside and nucleotide reverse transcriptase inhibitors” (“NRTI”) as used herein means nucleosides and nucleotides and analogues thereof that inhibit the activity of HIV reverse transcriptase, the enzyme which catalyzes the conversion of viral genomic  
30 HIV RNA into proviral HIV DNA.

Typical suitable NRTIs include zidovudine (AZT) available under the RETROVIR tradename from Glaxo-Well-come Inc., Research Triangle(EP 1034790 A2)

The term “non-nucleoside reverse transcriptase inhibitors (“NNRTI’s)” as used herein means non-nucleosides that inhibit the activity of HIV reverse transcriptase.

Typical suitable non nucleoside reverse transcriptase inhibitors include nevirapine (EP 1034790 A2)

- 5 The term “protease inhibitor” (“PI”) as used herein means inhibitors of the HIV protease, an enzyme required for the proteolytic cleavage of viral polyprotein precursors (e.g., viral GAG and GAG Pol polyproteins), into the individual functional proteins found in HIV infection. HIV protease inhibitors include compounds having a peptidomimetic structure, high molecular weight (7600 daltons) and substantial peptide character, e.g. CRIXIVAN  
10 (available from Merck) as well as nonpeptide protease inhibitors e.g., VIRACEPT (available from Agouron).

Typical suitable protease inhibitors. (EP 1034790 A2)

- “HIV viral load” tests are reported as the number of HIV copies in a milliliter (copies/mL) of blood. If the viral load measurement is high, it indicates that HIV is reproducing and  
15 that the disease will likely progress faster than if the viral load is low. During treatment and monitoring, a high viral load can be anywhere from 5,000 to 10,000 copies/mL. Initial, untreated, and uncontrolled HIV viral loads can range as high as one million or more copies/mL. A low viral load is usually between 40 to 500 copies/mL, depending on the type of test used. This result indicates that HIV is not actively reproducing and that the risk  
20 of disease progression is low. A viral load result that reads “undetectable” does not mean that you are cured. It may mean that either the HIV RNA is not present in your blood at the time of testing or that the level of HIV RNA is below the threshold needed for detection. Even though HIV may be undetectable in the blood, it persists in cells and tissues throughout the body as “HIV provirus.” HIV provirus refers to virus that has moved into  
25 cells and into the nucleus, where it has become integrated with the DNA of the host cell. This is also call “HIV proviral DNA”. Change in viral load is also a very important measurement. A rising count indicates either that the infection is getting worse or that you have developed resistance to the drugs that are being used for therapy, while a falling count indicates improvement and suppression of the HIV infection. Good clinical practice to  
30 minimize detectable HIV RNA plasma levels are known to those skilled in the art. See for example A-M. Vandamme et al., in Antiviral Chemistry & Chemotherapy, 9: 187-203

(1998) and “Drugs for HIV Infection” in The Medical Letter Vol.39 (Issue 1015) December 5, 1997, pages 111-116.

Compared to current anti-HIV treatments, one advantage of the use of a kinesin inhibitor according to the invention is its lesser vulnerability to virus mutation and subsequent escape from chemotherapy.

Accordingly, the present invention relates to

- a pharmaceutical composition comprising a kinesin inhibitor, and optionally a pharmaceutically acceptable carrier, in particular for use in the treatment of HIV infection, optionally in combination with another anti-viral treatment;
- 10 - a kinesin inhibitor, and optionally a pharmaceutically acceptable carrier, for use in the treatment of HIV infection, optionally in combination another anti-viral treatment;
- the use of a kinesin inhibitor for the manufacture of a medicament for the treatment of HIV infection, optionally in combination with another anti-viral treatment;
- a method for treating HIV infection in a subject in need thereof, comprising 15 administering an effective amount of a pharmaceutical composition comprising a kinesin inhibitor and optionally a pharmaceutically acceptable carrier;
- a combined preparation, product or kit containing (a) a kinesin inhibitor and (b) an anti-viral treatment as a combined preparation for simultaneous, separate or sequential use, in particular in the treatment of HIV infection; and
- 20 - a method for treating HIV infection in a subject in need thereof, comprising administering an effective amount of a pharmaceutical composition comprising a kinesin inhibitor, and an effective amount of a pharmaceutical composition comprising an anti-viral agent.

In one aspect, the present invention relates to a pharmaceutical composition comprising at least one kinesin inhibitor according to the invention optionally with a pharmaceutically acceptable carrier.

In one embodiment, said inhibitor is a N-kinesin inhibitor. More preferably, said kinesin inhibitor is a kinesin 2 and/or a kinesin 1 inhibitor. Even more preferably, said inhibitor is a KIF3A, KIF5B, KIF3C or KIFAP3 inhibitor.

30 Preferably, said inhibitor is a N-kinesin selective inhibitor. More preferably, said kinesin is a kinesin 2 selective inhibitor. Even more preferably, said inhibitor is a Kif3A selective

inhibitor, a KIF5B, a KIF3C, a KIFAP3 selective inhibitor or an inhibitor selective for KIF3A, KIF3C and KIFAP3.

According to a preferred embodiment, said inhibitor is a nucleic acid comprising or consisting of a sequence able to hybridise with a gene or mRNA coding for a kinesin, in particular KIF3A, KIF5B, KIF3C or KIFAP3, and to decrease or suppress the expression of said kinesin.

The kinesin inhibitor used in the pharmaceutical composition of the invention is present in a therapeutically effective amount. The term "therapeutically effective amount" as used in the present application is intended an amount of therapeutic agent, a kinesin inhibitor, administered to a patient that is sufficient to constitute a treatment of HIV infection as defined above.

The pharmaceutical composition comprising the kinesin inhibitor is formulated in accordance with standard pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy (20th ed.), ed. A. R. Gennaro, Lippincott Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York) known by a person skilled in the art.

Possible pharmaceutical compositions include those suitable for oral, rectal, intravaginal, mucosal, topical (including transdermal, buccal and sublingual), or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. For these formulations, conventional excipient can be used according to techniques well known by those skilled in the art.

The compositions for parenteral administration are generally physiologically compatible sterile solutions or suspensions which can optionally be prepared immediately before use from solid or lyophilized form. Adjuvants such as a local anesthetic, preservative and buffering agents can be dissolved in the vehicle and a surfactant or wetting agent can be included in the composition to facilitate uniform distribution of the active ingredient.

For oral administration, the composition can be formulated into conventional oral dosage forms such as tablets, capsules, powders, granules and liquid preparations such as syrups, elixirs, and concentrated drops. Non toxic solid carriers or diluents may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, magnesium, carbonate, and the like. For compressed tablets, binders, which are agents which impart cohesive

qualities to powdered materials, are also necessary. For example, starch, gelatine, sugars such as lactose or dextrose, and natural or synthetic gums can be used as binders. Disintegrants are also necessary in the tablets to facilitate break-up of the tablet. Disintegrants include starches, clays, celluloses, algins, gums and crosslinked polymers.

- 5 Moreover, lubricants and glidants are also included in the tablets to prevent adhesion to the tablet material to surfaces in the manufacturing process and to improve the flow characteristics of the powder material during manufacture. Colloidal silicon dioxide is most commonly used as a glidant and compounds such as talc or stearic acids are most commonly used as lubricants.
- 10 For transdermal administration, the composition can be formulated into ointment, cream or gel form and appropriate penetrants or detergents could be used to facilitate permeation, such as dimethyl sulfoxide, dimethyl acetamide and dimethylformamide. For transmucosal administration, nasal sprays, rectal or vaginal suppositories can be used. The active compound can be incorporated into any of the known suppository bases by 15 methods known in the art. Examples of such bases include cocoa butter, polyethylene glycols (carbowaxes), polyethylene sorbitan monostearate, and mixtures of these with other compatible materials to modify the melting point or dissolution rate.
- 20 Pharmaceutical compositions according to the invention may be formulated to release the active drug substantially immediately upon administration or at any predetermined time or time period after administration.
- Pharmaceutical compositions according to the invention can comprise one or more kinesin inhibitor(s) associated with pharmaceutically acceptable excipients and/or carriers. These excipients and/or carriers are chosen according to the form of administration as described above. Other active compounds can also be associated with kinesin inhibitors, in particular 25 antiviral drugs such as the ones used in anti-HIV therapy, preferably used in HAART therapy including the ones disclosed above. The initiation of the HAART may occur before, after or concurrently with administering a therapeutically effective amount of a kinesin inhibitor according to the invention, preferably a N-type kinesin inhibitor, more preferably a kinesin 2 inhibitor, even more preferably a KIF3A, KIF5B, KIF3C or KIFAP3 30 inhibitor in accordance with the present invention.

Such combination with other antiviral treatments is aimed at reinforcing the desired effects. Indeed, adding a kinesin inhibitor to such an anti-HIV therapy allowing a

significant decrease of the viral load should allow to clear viral reservoirs in macrophages, with the aim of eradicating the virus.

The amount of kinesin inhibitor to be administered has to be determined by standard procedure well known by those of ordinary skill in the art. Physiological data of the patient  
5 (e.g. age, size, and weight), the routes of administration and the disease to be treated have to be taken into account to determine the appropriate dosage.

The kinesin inhibitor may be administered as a single dose or in multiple doses. If the kinesin inhibitor is a small molecule inhibiting the kinesin activity, each unit dosage may contain, for example, from 200 to 1000 mg/kg of body weight, particularly from 500 to  
10 800 mg/kg of body weight. If the kinesin inhibitor is an anti-kinesin antibody, each unit dosage may contain, for example, from 0.1 to 20 mg/kg of body weight, particularly from 4 to 10 mg/kg of body weight. If the kinesin inhibitor is a kinesin RNAi molecule, each unit dosage may contain, for example, from 2 to 50 mg/kg of body weight, particularly from 5 to 20 mg/kg of body weight. If the kinesin inhibitor is a kinesin dominant negative  
15 receptor such as a cargo-only kinesin domain, each unit dosage may contain, for example, from 5 to 100 mg/kg of body weight, particularly from 15 to 70 mg/kg of body weight.

## 2. Tagged kinesin

It has been up to now difficult to characterize more precisely the intracellular trafficking  
20 and targeting of kinesin intracytoplasmic protein. Therefore, one aspect of the present invention is to provide new tools allowing study of the intracellular trafficking of kinesins, identification of intracellular partners as well as identification and screening of molecules able to inhibit such intracellular trafficking.

Kinesins have often been tagged by replacing the cargo domain with a fluorescent protein  
25 (Douglas S. Martin et al, PNAS 2010 March 23; 107(12): 5453-5458; Jennetta W Hammond et al, PLoS Biol.2009 March; 7(3)). However, such constructions are not functional as they do not transport anymore their cargo protein and therefore prevents any definitive conclusions on the role of such cargo protein when transported along microtubules.

30 To further elucidate the role of KIF3A in HIV infection, the inventors investigated the dynamics of KIF3A in infected macrophages using an original construct where the fluorescent protein mCherry has been inserted between the stalk and the cargo domain of

Kif3A, flanked by two spacers derived from antibody fragment to provide flexibility. This KIF3A-internal mCherry construct (KIF3A-iCh) was readily well expressed as seen by western blot and immunofluorescence using an antibody specific for the 20 last amino acids of KIF3A (i.e. within the cargo domain). The fusion Kif3A-iCh protein was observed  
5 to move on microtubules (identified by co-expression of EGFP- $\alpha$ -tubulin).

Primary macrophages were then co-infected with HIV Gag-iGFP $\Delta$ Env virus and KIF3A-iCh lentiviral vector. Thus, the inventors were able to show that KIF3A was colocalized with Gag+ compartments-containing virions, using such tagged Kif3A-iCh kinesin.

The inventors have indeed unexpectedly showed that a tag protein such as the fluorescent  
10 protein mCherry, inserted between the stalk and the cargo domain of KIF3A kinesin protein, did not impair the cargo domain activity and enabled to follow the location or targeting of the protein inside the cell.

The present invention thus features tagged kinesin proteins which have a variety of different uses including being used as a tool to study kinesin function, transport of its cargo  
15 proteins, and identification of intracellular partners. In particular, such tagged kinesin can be used to study the dynamics of viral infection, in particular HIV infection, as well as to screen for kinesin inhibitors. Such tagged kinesins according to the invention are able to maintain the functional activity of a kinesin as above defined and in particular are able to move cargo proteins along microtubules. In addition, they can be easily detected using  
20 their tag portion.

Therefore, the invention relates to the use, particularly *in vitro* or *ex vivo*, of a tagged kinesin according to the invention, preferably a N-type tagged kinesin, more preferably a tagged kinesin 1 or a tagged kinesin 2, even more preferably KIF3A, KIF5B, KIF3C or KIFAP3 , to screen for kinesin inhibitors. Of particular interest is the use of a tagged  
25 KIF3A kinesin to screen for KIF3A kinesin inhibitors useful in the treatment of HIV infection.

#### Recombinant hybrid kinesin polypeptide

The invention thus relates to a tagged kinesin comprising a kinesin motor domain, a stalk  
30 domain, a cargo domain and a tag allowing said tagged kinesin to be detected, and wherein said tag is located between said stalk domain and said cargo domain. In a preferred

embodiment, said tag is flanked at least on one side, preferably on both sides, by spacers providing flexibility.

In one embodiment, said tagged kinesin is a chimeric kinesin, wherein at least one of the 3 major domains (motor, stalk and cargo) belong to a native kinesin different from the 5 kinesin from which at least one of the other 2 domains are derived. For example, such a chimeric kinesin may comprise a kinesin 2 motor domain, (e.g. amino acid sequence comprised between amino acids N°s 1-350 of SEQ ID N°31), whereas the cargo and/or stalk domain belong to a kinesin 1.

In another embodiment, the tagged protein of the invention comprises a motor domain, a 10 stalk domain and a cargo domain all belonging to the same native kinesin, preferably to a N-type kinesin, more preferably to a kinesin 2, even more preferably to KIF5B, KIF3A, KIF3C or KIFAP3. In a further preferred embodiment, said motor domain comprises an amino acid sequence at least 75, 80, 85, 90, 95, or 98 % identical to the amino acid sequence comprised between amino acids 1-350 of SEQ ID N°31, said stalk domain 15 comprises an amino acid sequence at least 75, 80, 85, 90, 95, or 98 % identical to the amino acid sequence comprised between amino acids 351-594 of SEQ ID N°31 and cargo domain comprises an amino acid sequence at least 75, 80, 85, 90, 95, or 98 % identical to the amino acid sequence comprised between amino acids 862-968 of SEQ ID N°31. Therefore, the invention particularly relate to a tagged kinesin comprising a KIF3A motor 20 domain, preferably comprising an amino acid sequence at least 75, 80, 85, 90, 95, or 98 % identical to the amino acid sequence comprised between amino acids 1-350 of SEQ ID N°31, a KIF3A stalk domain, preferably comprising an amino acid sequence at least 75, 80, 85, 90, 95, or 98 % identical to the amino acid sequence comprised between amino acids 351-594 of SEQ ID N°31, a KIF3A cargo domain preferably comprising an amino 25 acid sequence at least 75, 80, 85, 90, 95, or 98 % identical to the amino acid sequence comprised between amino acids 862-968 of SEQ ID N°31, and a tag allowing said tagged kinesin to be detected, and wherein said tag, preferably a fluorescent tag protein, more preferably a mCherry protein, is located between said KIF3A stalk domain and said KIF3A cargo domain protein.

30 Within the scope of the invention are kinesin motor domains, kinesin stalk domains and kinesin cargo domains derived from naturally-occurring kinesin proteins as well as those

comprising amino acid changes (addition, deletion, mutation) that do not affect significantly the functional activity of said kinesin.

Tag protein

- 5 The tagged kinesin according to the invention comprises a so-called “tag” peptide, polypeptide or protein which could advantageously be used for detection and localisation of said tagged kinesin. Any type of tag known to those skilled in the art may be used provided it does not affect significantly the kinesin activity including BCCP-tag, c-myc-tag, calmodulin-tag, FLAG-tag, HA-tag, His-tag, Maltose binding protein-tag, Nus-tag,
- 10 Glutathione-S-transferase-tag, Green fluorescent protein-tag, Thioredoxin-tag, S-tag, Strep-tag, human protein C tag, Chitin binding protein tag, T7-tag, Myc-tag, V5-tag, VSV-tag, Avi.tag, BioEase-tag, SNAP-tag, FlaSH-tag, Nus A-tag, DsbA-tag , GFP, EGFP, mCherry, Emerald, Topaz, W1b. (EGFP, Emerald , Topaz, and W1b are derivatives of GFP.)
- 15 In a preferred embodiment said tag is a fluorescent peptide or protein such the Green Fluorescent Protein (GFP) and the red fluorescent protein mCherry, more preferably selected from the group consisting of GFP-tag, EGFP, mCherry, Emerald, Topaz, W1b, and their fluorescent derivatives.  
A “fluorescent protein” region contains a chromophore that fluoresces. Preferably, the  
20 fluorescent protein region is the red cherry fluorescent protein. Preferred derivatives have a sequence similarity of at least about 75%, at least about 85%, or at least about 95% to the cherry protein.  
Said tag can be of any length compatible with its function as a tag, generally between 5 and 100 amino acids.

25

Spacer sequence

- Additionally, the tagged kinesin according to the invention may contain a spacer peptide or polypeptide. Indeed, the tag polypeptide region is linked either directly, i.e. contiguously, to the stalk domain on one side and to the cargo domain on the other side, or indirectly, i.e. through a polypeptide linker, to at least one of the kinesin stalk or cargo domain. Tagged kinesin fusion proteins contain a kinesin polypeptide region and a fluorescent protein

region either directly joined together or joined together through a linker. In one embodiment, said tag, preferably a fluorescent tag, is flanked by spacers at both ends. Such spacers (also called linkers) provide flexibility to the overall construct, allowing the tagged kinesin to still function as a kinesin while being able to be detected through its tag portion.

Such spacers are known to those skilled in the art. They usually comprise, or consist essentially of non polar amino acids such as Glycine, Serine or Alanine residues. Some of them derive from immunoglobulin amino acid sequences. In one specific embodiment, said spacers comprise or consists in a series of reiterated glycine and/or alanine residues, such as repetitions of Gly-Gly-Gly-Gly-Ser peptide or an analogous derived sequence.

In a preferred embodiment, the invention relates to a tagged kinesin comprising a motor domain, a stalk domain, a cargo domain and a tag, preferably a fluorescent tag, even more preferably mCherry, allowing said tagged kinesin to be detected, wherein said tag is located between said stalk domain and said cargo domain, and is flanked by spacers, preferably by spacers comprising or consisting in chains of GGGGS residues. Said tagged kinesin is preferably a N-type kinesin, more preferably a kinesin 1 or kinesin 2, even more preferably KIF3A, KIF5B, KIF3C or KIFAP3. In one embodiment, said kinesin comprises the amino acid sequence of SEQ ID N°31.

The various polypeptides of the present invention can be prepared in any suitable manner including isolation from natural sources, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods.

#### Nucleic acid comprising tag

25 The present invention also relates to a nucleotide sequence coding for a tagged kinesin such as defined above.

Another aspect of the present invention describes a nucleic acid encoding a tagged kinesin protein according to the invention. Such nucleic acid comprises either a contiguous nucleotide sequence that codes for the protein or a sequence that is processed by a host cell 30 to produce a contiguous nucleotide sequence encoding for the protein. Another aspect of the present invention describes an expression vector comprising a nucleic acid encoding a tagged kinesin protein described herein. Nucleic acids able to encode any of the tagged

kinesins described in the present specification are contemplated. In an embodiment, said nucleic acid comprises the nucleic acid sequences of a tagged KIF3A, KIF5B, KIF3C or KIFAP3, particularly the nucleic acid sequence of SEQ ID N°32.

5    Vector

The present invention also relates to a vector, particularly a plasmid, containing a nucleotide sequence such as defined above. In particular, the invention relates to an expression vector comprising a nucleic acid sequence encoding a tagged kinesin according to the invention.

- 10   The term “vector” refers to DNA molecule used as a vehicle to transfer recombinant genetic material into a host cell. The four major types of vectors are plasmids, bacteriophages and other viruses, cosmids, and artificial chromosomes. The vector itself is generally a DNA sequence that consists of an insert (a heterologous nucleic acid sequence, transgene) and a larger sequence that serves as the “backbone” of the vector. The purpose  
15   of a vector which transfers genetic information to the host is typically to isolate, multiply, or express the insert in the target cell. Vectors called expression vectors (expression constructs) are specifically adapted for the expression of the heterologous sequences in the target cell, and generally have a promoter sequence that drives expression of the heterologous sequences.
- 20   Generally, the regulatory elements that are present in an expression vector include a transcriptional promoter, a ribosome binding site, a terminator, and optionally present operator. Another preferred element is a polyadenylation signal providing for processing in eukaryotic cells. Preferably, an expression vector also contains an origin of replication for autonomous replication in a host cell, a selectable marker, a limited number of useful  
25   restriction enzyme sites, and a potential for high copy number.

An expression vector contains recombinant nucleic acid encoding for a polypeptide along with regulatory elements for proper transcription and processing. The recombinant nucleic acid contains two or more nucleic acid regions not naturally associated with each other. Exogenous regulatory elements such as an exogenous promoter can be useful for  
30   expressing recombinant nucleic acid in a particular host. Examples of expression vectors are cloning vectors, modified cloning vectors, specifically designed plasmids and viruses.

Expression vectors providing suitable levels of polypeptide expression in different hosts are well known in the art. Mammalian expression vectors well known in the art include pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene). Bacterial expression vectors well known in the art include pET11a (Novagen), lamda gt11 (Invitrogen). Fungal cell expression vectors well known in the art include pYES2 (Invitrogen) and Pichia expression vector (Invitrogen).

Examples of techniques for introducing nucleic acid into a cell and expressing the nucleic acid to produce protein are provided in references such as Ausubel, Current Protocols in molecular biology, John Wiley, 1987-1998, and Sambrook, et al., in Molecular cloning, A laboratory Manual 2<sup>nd</sup> Edition, Cold Spring Harbor Laboratory Press, 1989.

Expression vectors may be introduced into host cells using standard techniques. Examples of such techniques include transformation, transfection, lipotransfection, protoplast fusion, and electroporation.

15 Host cell

The present invention relates to a host cell chosen among any type of cells, particularly human cells, transformed with a vector such as defined above. Of particular interest are macrophages and monocytes, either primary cells or cell lines, transformed with an expression vector encoding a tagged kinesin according to the invention.

20 Another aspect of the present invention describes a host cell comprising a nucleic acid encoding a tagged kinesin fusion protein, particularly an expression vector encoding a tagged kinesin according to the invention. The nucleic acid may be part of the host genome or may exist independently of the host genome.

Another aspect of the present invention describes a non-human transgenic animal comprising such a nucleic acid encoding a tagged kinesin protein.

Method for expressing a recombinant polypeptide

Tagged kinesin proteins can be produced using techniques well known in the art. Preferably, such proteins are produced by recombinant expression inside a host cell by way of an expression vector or by way of nucleic acid integrated into the host genome.

Typically, natural isolated nucleic acid encoding the mCherry proteins and the KIF3A kinesin protein are spliced into expression construct using conventional methodologies, see

e.g Molecular cloning, a laboratory Manual (Sambrook, et al. Cold Spring Harbor Laboratory), current protocols in Molecular Biology (Eds. Ausubel, et al., Greene Publ. Assoc., Wiley-Interscience, NY). Alternatively, the amino acid sequences of the subject peptides are used to back-translate peptide-encoding nucleic acids optimized for selected expression systems (Holler et al. (1993) Gene 136, 323-328; Martin et al. (1995) Gene 154, 150-166). In either instance, the constructs are designed for expression in any conventional system, such as bacteria or eukaryotic cells.

### 3. Screening of kinesin inhibitors for their use against HIV infection.

10

The present invention also concerns a method of screening of molecules useful in the treatment of HIV infection based on the ability of such molecules to inhibit the expression and/or activity of a kinesin of interest, preferably N-type kinesins, more preferably kinesin-1 or kinesins 2, even more preferably KIF3A, KIF3C, KIFAP3 or KIF5B. It relies on the inventors' observations that the host cell KIF3A kinesin protein pathway influences retroviral infection.

20 The recombinant protein, described above could be used to analyse the recruitment of different host proteins necessary for at least its transport to the virions formation, and the formation of the viral particles and/or to screen for molecules able to inhibit the transport of various cargo virus proteins, in particular HIV proteins along microtubules. As indicated here above, several kinesin inhibitors are already known to the skilled in the art. However, it may be desirable to isolate new kinesin inhibitors being suitable for the treatment of HIV infection.

#### 25 3.1 Methods of screening

In one embodiment, the present invention is a method of screening test agents as inhibitors of HIV virions formation comprising the step of determining whether the test agent is a kinesin, preferably a kinesin-1 or a kinesin-2, even more preferably a KIF3A, KIF5B, KIF3C or KIFAP3 kinesin inhibitors. If the test agent is a KIF3A, KIF5B, KIF3C or KIFAP3 inhibitor, then the agent is a suitable inhibitor of HIV virions formation. In a preferred embodiment, the present invention is a method of screening test chemical or compounds for inhibition of HIV infection, comprising the steps of exposing the test

chemical or compound to a cell, and determining whether said test compound inhibits the gene expression or activity of a kinesin, preferably a kinesin-2 and even more preferably kinesin KIF3A, KIF5B, KIF3C or KIFAP3, as measured by the HIV virions release in the supernatant or localisation of known KIF3A, KIF5B, KIF3C or KIFAP3 fluorescent protein partners.

In another embodiment, the present invention is a method of screening test chemicals or compounds for inhibition of HIV virions secretion. In one embodiment of the first aspect, the method comprises the steps of (a) primary cells infection, (b) exposing a test chemical or compound to the primary infected cells, (c) examining the results of step (a) and (b).

10 The present invention is thus a method of screening test agents as inhibitors of HIV new virions formation. If the test agent is a kinesin activity or expression inhibitors, then the agent is a suitable inhibitor of HIV infection, in particular in HIV new virions formation.

In a second embodiment, the present invention is a method of screening test agent as inhibitors of kinesin expression or activity. If the test agent inhibits the localisation of a

15 known KIF3A fluorescent protein partners at the cell periphery, then the agent is a suitable inhibitor of HIV infection, in particular in HIV new virions formation.

### 3.1.1 Test agents, chemicals, or compounds

One embodiment of the present invention relates to a screen for kinesin inhibitors, 20 preferably kinesin 2 inhibitors, even preferably KIF3A, KIF5B, KIF3C or KIFAP3 kinesin inhibitors. The identified inhibitors are expected to inhibit a step coincident with virus establishment of HIV in human cells and inhibit subsequent HIV virions formation.

One of skill in the art would understand that many different chemicals or compounds could be screened for inhibition of kinesin, preferably kinesin 2, even preferably KIF3A, KIF5B,

25 KIF3C or KIFAP3, including small molecules, natural products, peptides, and proteins. Also included would be nucleic acids, such as siRNAs, small hairpin RNAs (shRNAs), antisense oligonucleotides, and ribozymes.

Suitable groups of compounds would include the Hembridge collection (16000 compounds), ChemDiv collection (20000 compounds), and the NCI open collection

30 (140000 compounds). All of the terms indicate a test substance that one would evaluate as a screen for HIV inhibition.

### 3.1.2 Cell lines

In a preferred embodiment one would use macrophage primary cells. One of skill in the art would understand that other human and mammalian cells could replace macrophage primary cells. Any mammalian cell line that is able to be infected by HIV would be useful,

5 especially monocytes/macrophages cell lines such as U937.

### 3.1.3 Screening

In particular, the invention is drawn to a method for screening for compounds useful for the treatment of HIV infection comprising the steps of:

- 10a) providing or obtaining a candidate compound; and
- b) determining whether said candidate compound inhibits the activity and/or expression of a kinesin, preferably a N-type kinesin, more preferably a kinesin 2, even more preferably kinesin KIF3A, KIF5B, KIF3C or KIFAP3,  
wherein the ability of said candidate compound to inhibit the expression or activity of said  
15 kinesin indicates that said candidate compound is indicative of its usefulness for the treatment of HIV infection.

The candidate compound to be tested in the frame of this method may be of any molecular nature, for example it may correspond to a chemical molecule (preferably a small molecule), an antibody, a peptide, a polypeptide, an aptamer, a siRNA, a sense or antisense  
20 oligonucleotide, or a ribozyme.

The ability of said candidate compound to inhibit the expression or activity of a kinesin of interest may be tested using any of the methods known to those skilled in the art, including those mentioned in the present specification, for example those mentioned in the section entitled “1. Kinesin inhibitors as therapeutic agents in HIV infection”.

- 25 In one embodiment of such screening method, the chimeric kinesin described in the above section might be used. In particular, to screen for kinesin inhibitors, the original construct where the fluorescent protein mCherry has been inserted between the stalk and the cargo domains of native Kif3A, KIF5B, KIF3C or KIFAP3, flanked by two spacers derived from antibody fragment to provide flexibility may be used. Therefore, in one embodiment of the  
30 screening method of the invention, step b) of said method consists in determining whether said candidate compound inhibits the activity of a tagged kinesin preferably a N-type kinesin, more preferably a kinesin 2, even more preferably a KIF3A, KIF5B, KIF3C or

KIFAP3 according to the invention. In a further embodiment, said step b) consists in determining whether said candidate compound inhibits the binding of said tagged kinesin to a cargo protein.

The screening method according to the invention may further comprise, for such candidate compound having the ability to inhibit the expression and/or activity of a kinesin of interest, the step of determining whether said candidate compound is able to inhibit and/or decrease HIV infection. In one embodiment, such step c) comprises administering said candidate compound to a cellular model or to a non-human animal model of a HIV infection in order to confirm that the candidate compound is suitable for the treatment of HIV infection. In a further embodiment, said step c) comprises determining whether said candidate compound interferes with HIV virions formation, preferably using primary macrophages/monocytes or macrophage/monocyte cell lines infected with HIV, more preferably of human origin.

In another embodiment, step c comprises determining whether said candidate compound has an impact on viral load.

Furthermore the present invention also comprises a method of screening for candidate substances for their ability to modulate viral load using a fluorescent KIF3A-iCherry protein.

In one embodiment, the invention therefore relates to a method of screening for candidate compounds able to modulate viral load, comprising the steps of

- a) contacting said candidate compound with a cell infected with a retrovirus, preferably HIV, wherein said candidate compound is a kinesin inhibitor,
  - b) measuring viral load in said cell, and
  - c) determining whether said kinesin inhibitor candidate decrease said viral load.
- 25 In one embodiment, said method comprises a first step prior to step a) of determining whether said candidate compound is a kinesin inhibitor, preferably a kinesin-2 or a kinesine-1 inhibitor, even preferably KIF3A, KIF5B, KIF3C or KIFAP3.

The following examples are given for purposes of illustration and not by way of limitation.

### **Examples**

### **Materials et Methods**

*Plasmids, Antibodies and Reagents*

pBR NL4-3 Gag-iGFP T-tropic envelope was a gift from B. Chen<sup>29</sup>. Original envelope has been replaced by th\_174 V3 loop macrophage-tropic envelope. pENTR KIF3A (NM\_007054.1) has been purchase from Genecopoeia. To construct KIF3A-iCherry, the 5 cDNA for the mCherry fluorescent protein, flanked by linkers (amino acid sequence GGGGSGGGSGGGGS), has been inserted between the stalk and the cargo domain region of KIF3A. The resulting cDNA KIF3A-iCherry has been cloned into a pExp plasmid using Gateway technology.

Antibodies specific for KIF3A (Abcam), CD81 (Abcam), Gag p24 (KC57-FITC, Beckman Coulter), Gag p24 (Abcam), α-Tubulin (Serotec) were used according to the manufacturers' recommendations. Antibodies specific for p17 (NIH4811) and gp120 (NIH1476) were provided by the NIH AIDS Reagent Program. Secondary antibodies conjugated with Alexa Fluor 488 (Invitrogen), Cy3 or Cy5 (Jackson) were used according to the manufacturers' instructions. Nocodazole (Sigma) was dissolved in DMSO at a 15 10mM stock concentration. M-CSF (Calbiochem) was dissolved in ultrapure water at a 25 μM stock.

*Primary Cells and Cell Lines*

PBMC were purified from blood from healthy donors and monocytes were isolated by 20 positive selection using CD14+ Microbeads from Miltenyi. Monocytes were differentiated for 7 days in macrophages by culture on non treated culture plastic (Nunc) in RPMI 1640 supplemented with 10% FCS (Gibco) and M-CSF (25 mg/ml final). Ghost, HEK293T, TZM-Bl and HeLa cells were cultured in DMEM glutamax whereas Jurkat T cells were cultured in RPMI 1640. Both mediums were supplemented with 10% FCS (Gibco).

25

*Virus Preparations and Infections*

The following virus strains have been produced and used in the present study: NL4-3 AD8 (NLAD8), NL4-3 ΔEnv, HIV Gag-iGFP, HIV Gag-iGFP ΔEnv. Viruses were produced by transfection of the corresponding proviral cDNA in 293T cells by calcium phosphate/DNA 30 co-precipitation. Pseudotyping was eventually achieved by co-transfection of the plasmid encoding the VSV-G envelope protein. Supernatant were harvested 48-72 hr after transfection, and ultracentrifuged at 100,000g for 90 min. Pellets were resuspended in 2 %

BSA in PBS. Virus preparations were titrated by infecting the Ghost reporter cell line and infectivity was measured 24 hr post-infection by flow cytometry.

NLAD8-infected macrophage samples were prepared for immuno-electron microscopy as previously described<sup>12</sup>. For the other infection experiments, macrophages were infected at

5 a Multiplicity of Infection (MOI) of 2 and kept in culture for indicated times.

#### *Viral production and Infectivity assays*

Viral release was monitored in supernatants by p24 ELISA (Ingen). Virion-containing supernatants were normalized for equal amounts of p24 and infectious titers were

10 determined using TZM-bl indicator cells as described<sup>30</sup>. Briefly, the volume of supernatant equivalent to 2 ng of p24 was incubated with 10.000 TZM-bl cells for 24 hr and assay was developed using the GalScreen kit (Applied Biosystems) for chemo luminescent detection of reporter β-galactosidase.

15 *RNA interference*

The sequences of the various siRNA used in the present study are given in Table 1.

Macrophages were transfected using Interferin (Polyplus). Briefly, siRNA (100nM final) were diluted in 250 µl of OptiMEM (Gibco) mixed with 5 µl of Interferin and left at room temperature for 15 minutes. Complexes were added drop-wise onto the macrophage

20 culture. Cells were assayed 3 days later.

#### *Western blotting*

Cells were directly lysed into the well in 0.5% NP40 lysis buffer for 30min at 4°C. Cell

lysates were analyzed on 4-12% Bis-Tris Acrylamid gels (NuPAGE, Invitrogen) followed

25 by immunoblotting.

#### *Immunofluorescence*

Cells were fixed in methanol at -20°C for 5 sec and incubated in 0.2 % BSA in PBS.

Antibody staining and washes were performed in the same buffer, before mounting

coverslips in moviol. Samples were imaged on a Nikon Ti Inverted Microscope fitted with

30 a confocal A1R system. Using a 60x oil immersion objective with a numerical aperture of 1.4, confocal images were collected as a 3D stack with a focal step size from 0.25 to 0.5 µm.

*Live imaging*

Long time-lapse movies (days) were acquired using a Nikon Biostation system using 20x and 40x dry objective. Fast imaging was performed on a Nikon Ti Inverted Microscope 5 fitted with a video-rate confocal system consisting of a spinning disk confocal head (Yokogawa). Using a 100x oil immersion objective with a numerical aperture of 1.4 and confocal images were collected with a HQ2 camera.

*Correlative light-electron microscopy*

10 Purified monocytes were plated with M-CSF on CELlocate coverslips (eppendorf) that etched grids with coordinates, allowing the cell of interest to be found through all of the steps of the procedure. After 7 days, macrophages were co-infected with HIV-1 Gag-iGFP V3\_loop and KIF3A-iCherry lentiviral vector. At day 7 post-infection, cells were fixed in 2.5% glutaraldehyde in 0.2M Cacodylate buffer and imaged with a spinning disk confocal 15 microscope. Then, coverslips were embedded in epon and processed for electron microscopy<sup>12</sup>.

*Image Analysis*

Micrographs were processed with either NIS Element, MetaMorph, Imaris or ImageJ 20 software according to the kind of analysis to be performed.

	siRNA sequences	target sequences
Luc	CGUACGCGGAUACUUUCGATT	
Tsg101	CAGUUUAUCAAUCAAGUGUAA	
KIF3A_1	GUGC CUUAUCGUAACUCUAAA	GTGCCTTATCGTAACCTCA
KIF3A_2	GCCGAUCAAUAAAUCAGAGAA	GCCGATCAATAATCAGAG
KIF3A_3	AAGACCUGAUGUGGGAGUUTA	AAGACCTGATGTGGGAGTT
KIF3A_4	CUGGUUCAGAAAGACAGGCAA	CTGGTTCAGAAAGACAGGC
KIF3B_1	CAGAAAUGCAUGGGUAAGGTA	CAGAAATGCATGGTAAGG
KIF3B_2	AAGGAUGAUUACUGGCAGGGAA	AAGGATGATTACTGGCGGG
KIF3B_3	UUAGGGAAAAGCUUCUUUA	TTAGGGAAATAGCTTCTTT
KIF3B_4	AAUCCGUGGUGACCCUGAAAA	AATCCGTGGTGACCCTGAA
KIF3C_1	CAGGCCGACCUGUAUGACGAA	CAGGCCGACCTGTATGACG

KIF3C_2	AAGGGCCUUGAUUAGGACCAA	AAGGGCCTGATTAGGACC
KIF3C_3	AAACGAAGAUUAUUAACAUCA	AAACGAAGATATTAACATC
KIF3C_4	AUCAUGGAUCACACCAACGAA	ATCATGGATCACACCAACG
KIFAP3_1	CCACAUCUUGAUGCUALGUAA	CCACATCTTGATGCTAAGT
KIFAP3_2	UCGAGUUAGCUACAAACAUAA	TCGAGTTAGCTACAAACAT
KIFAP3_3	AAGCCCUGAUCGGGACAATT	AAGCCCTGATCGGGACAA
KIFAP3_4	CAGCAUGAUGGACCAACUAAA	CAGCATGATGGACCAACTA
KIF15_1	AACGAGCAGAUUAUGAUCA	AACGAGCAGATATATGATC
KIF15_2	GACGUGUGGCAUCAACAUCA	GACGTGTGGCATCACATC
KIF15_3	CAGGAUUCCUAUGACAAACUTA	CAGGATTCCATGACAAC
KIF15_4	UUGAGAUUGACCAACUUUCAA	TTGAGATTGACCAACTTC
KIF3A_2bis	GGUUCAGAAAGACAGGGCAATT	GGTCAGAAAGACAGGCCA
KIF3A_8	GACCUGAUGUGGGAGUUUATT	GACCTGATGTGGAGTTA
KIF3A_9	GCCUAAAGGAAGCUACAAATT	GCCTAAAGGAAGCTACAAA

Table 1. Name and target sequences of the siRNA and sequences of the siRNA used

TT DNA modifications have been added at the 3' end of all siRNA sequences, which were  
5 purchased from Eurogentec. KIF4A and Tsg101 siRNA have been previously shown to efficiently knockdown their targets (Martinez NW et al, 2008; Garrus JE et al, 2001).

### 10      Example 1 Microtubule-dependent distribution of virus-containing compartments (VCCs)

We first aimed at studying the role of the microtubule cytoskeleton in the intracellular transport and spatial organization of the virus-containing compartments (VCCs). Analysis of HIV-1 infected macrophages by Immuno-Electron Microscopy (EM) revealed VCCs with viral budding profiles at their limiting membrane typical of viral assembly (Figure 1A). Examination by confocal microscopy of HIV-1-infected macrophages revealed that large Gag<sup>+</sup> compartments were present and frequently scattered in the central area of the cells (Figure 1B and C). Of note, the distribution of these compartments was rather heterogeneous among the infected cells, ranging from clustered to spread. Treatment of

HIV-1-infected macrophages for 1 hr with nocodazole disrupted most of the microtubule network, while all Gag<sup>+</sup> compartments concentrated in the perinuclear area (Figure 1C). Quantification of the effect of the nocodazole treatment on the spatial distribution of the Gag<sup>+</sup> compartments by automated image analysis generating a spreading index (see details 5 in Figure 8B), fully confirmed this observation. Upon nocodazole washout, we observed that within 15 min the Gag<sup>+</sup> compartments became spatially dispersed (Figure 8A), supporting the idea that the movement of Gag<sup>+</sup> compartments towards the cell periphery is directed by microtubules.

At the ultrastructural level, microtubules showing specific  $\alpha$ tubulin staining were 10 present in the vicinity of compartments containing Gag<sup>+</sup> particles but mostly absent from the viral particles (Figure 1D). Strikingly, 85% of the VCCs had at least one microtubule at less than 100 nm from their limiting membrane ( $n = 32$ ) (Figure 1D, arrowheads). The mean number of microtubules associated per compartment was of  $3 \pm 2$ . These results indicated that each VCC was closely surrounded by several microtubules. Thus, in HIV-15 infected macrophages, microtubules appear to play a critical role in the spatial distribution of VCCs.

## Example 2 HIV production by macrophages is dependent on the kinesin KIF3 complex

If transport of VCCs occurs along microtubules in macrophages, then kinesins must be involved in their movement. Thus, we screened a collection of human kinesins and associated proteins (45 genes) by RNA interference in HIV-1-infected primary macrophages and quantified the production of p24 in the supernatant. The details of the 20 procedure and an example of the results obtained for a subset of kinesins are given in Figure S2. The knockdown of KIF3A, KIF3C and KIFAP3, but not KIF3B and KIF15, inhibited the release of p24. Of note KIF3B was not considered as a hit according to our criteria as only 1 out its 4 siRNA significantly reduced the p24 production(see Figure 9 legend). KIF3A can form heterodimers with KIF3C and both are kinesin-2 members(Hirokawa, 2000), while the accessory subunit KIFAP3 may stabilize the motor 30 complex (Doodhi et al., 2009).

To evaluate the role of KIF3A on HIV-1 secretion by macrophages, transfection of two different siRNA specific for KIF3A was performed in infected primary macrophages following the timeline presented in Figure 2A. Negative and positive controls were included, i.e. siRNA specific for Luciferase and Tsg101 (Garrus et al., 2001), respectively.

5 Efficiency of KIF3A depletions ranged from 50% to 80% as determined by immunoblot analysis (Figure 2B). Cell viability and percentages of infected macrophages were similar in the different cell populations upon viral infection and siRNA transfection (Figure 2C and D). Early steps of infection were not affected by KIF3A depletion (Figure 2F). No significant difference in the infectivity of the virions produced by macrophages under these

10 conditions was observed when using normalized amounts of p24 (Figure 2E). Finally, KIF3A depletion by RNAi led to a strong reduction (up to 75%) of the amounts of p24 secreted in the supernatant (Figure 2G). The extent of the inhibition was similar to that obtained when the expression of the ESCRT protein Tsg101, which is crucial for viral budding in 293T cells (Garrus et al., 2001), was knocked down. These results were

15 confirmed by immunoblot analysis (Figure 10). Macrophages depleted for KIF3A exhibited in their lysates reduced levels of KIF3A, slightly more Gag (p55 + p24) protein, and a higher ratio of p24/p55. Importantly, the cell supernatant contained less than 50% of the p24 present in macrophages transfected with a control siRNA. Thus, silencing of KIF3A inhibits the release of HIV-1 particles by infected macrophages.

20

### **Example 3 KIF3A requirement for HIV-1 production is celltype-specific**

To determine whether the involvement of KIF3A in the HIV-1 cycle was cell type-specific, similar experiments were performed first on HeLa cells (Figure 11A). Knock down efficiency of KIF3A expression was superior to 95% as judged by immunoblot (Figure 11B) and led to a reduction of p24 release which was less pronounced than in macrophages (compare Figure 11C and figure 2G). This may reflect the fact that HeLa cells share some features with both natural targets of HIV, T cells and macrophages. Indeed, HeLa cells can resist for several days to the infection, and electron microscopy analysis revealed that viral budding events occur at the plasma membrane but also in internal compartments (Figure 11D and E), in agreement with previous work (Dong et al., 30 2005; Joshi et al., 2009).

The role of KIF3A was next assessed in the Jurkat T cell line. To achieve efficient silencing in these cells, we used shRNA delivered by lentiviral vectors (see Figure 3A). Knockdown induced in Jurkat cells by this mean was very efficient (from 78% to 97% depletion) (Figure 3B) but did not impact on p24 release, whereas Tsg101 depletion led to 5 efficient reduction (80%) of p24 released. In addition, staining of HIV-1-infected Jurkat T cells (not shown) and primary CD4+ T cells (Figure 3D) revealed that Gag and KIF3A had totally different intracellular distributions. Ultrastructural analysis of HIV-1-infected Jurkat T cells confirmed that viral assembly only takes place at the plasma membrane where zones of intense budding can be seen (Figure 3E). We conclude that KIF3A does not play a 10 role in the HIV-1 cycle in T cells. Taken together these data may suggest that KIF3A is required when viral assembly takes place in internal compartment but is dispensable when it occurs at the plasma membrane.

#### 15      **Example 4 The kinesin KIF3A associates with microtubules at the limiting membrane of VCCs**

To approach the role of the KIF3 complex in infected macrophages, we first analyzed KIF3A/C intracellular distribution in HIV-infected macrophages. The tetraspanin CD81 was used as a VCC marker. KIF3A and KIF3C exhibited a similar pattern of association 20 with CD81+Gag+VCCs (Figure 12A). In contrast, the distribution of KIF15, a kinesin-12 member and of KIF3B, a kinesin-2 member able to bind KIF3A, appeared completely different from that of Gag (Figure 12A and B), as expected from the results of our screen.

Focusing on KIF3A, we observed that while Gag+ compartments were heterogeneous in size and often rather large (up to several  $\mu\text{m}$  of diameter), KIF3A staining tended to 25 localize to one or several restricted zones at the periphery of the Gag+ compartments together with tubulin (Figure 4A). 3D reconstructions from confocal images further showed association of patches of KIF3A staining with the edge of Gag+ compartments (Figure 13A). The Gag+KIF3A+ compartments were co-stained for Env (Figure 13B), suggesting that they contained viral particles and thus, were *bona fide* VCCs.

30      Similar samples were examined by Structured Illumination Microscopy (SIM) that provides a higher resolution (Schermelleh et al., 2010). 3D reconstructions from SIM images showed that KIF3A was localized along the VCC and further suggested that KIF3A

was aligned on longitudinal structures that, in all likelihood, were microtubules (Figure 4B and C). Finally, immuno-EM unambiguously established that compartments containing both immature and mature virions had at their limiting membrane microtubules that stained positive for KIF3A (Figure 4D). Nocodazole exposure of HIV-1-infected macrophages led  
5 to the redistribution of Gag<sup>+</sup> compartments together with KIF3A towards one pole of the nucleus (Figure 13C). Wash out of nocodazole allowed growth of microtubules and spreading of the Gag<sup>+</sup> KIF3A<sup>+</sup> compartments (not shown). Thus, analysis of HIV-1 infected macrophages by confocal microscopy, SIM and immuno-EM, all indicated that KIF3A is associated with microtubules that contact the limiting membrane of the VCC.

10

#### **Example 5 KIF3A silencing leads to intracellular accumulation of VCCs**

We reasoned that if KIF3A knockdown prevents transport of VCC and thus release of the virions they contain, then VCCs should accumulate intracellularly. Thus, we compared  
15 HIV-1-infected macrophages from 8 different donors treated with siRNA specific for KIF3A or for Luciferase (control) and stained for Gag by immunofluorescence. Large sets of images were collected for both cell populations by confocal microscopy. 3D reconstruction of Gag<sup>+</sup> compartments (Figure 5A), image segmentation and quantification allowed extracting multiple parameters related to these compartments. The average volume  
20 of Gag<sup>+</sup> compartments per cell, as well as the sum of Gag intensity in VCC per cell, were significantly increased upon KIF3A depletion (Figure 5B and C). Similarly, the ratio of the volume of Gag<sup>+</sup> compartment/cytosolic volume per cell also increased in KIF3A knocked down cells (not shown). In contrast, Gag mean intensity within the VCCs remained the same (Figure 5D), suggesting that the density of Gag was similar and thus that the viral  
25 assembly process itself was not affected by the depletion. Finally, the cell area (not shown) and the total cell volume (Figure 5E) were not statistically different between both cell populations.

We also noticed that the secretion of MIP-1 $\beta$ , which is produced by macrophages upon HIV-1 infection (Figure 14A), was not affected by KIF3A depletion (Figure 14B),  
30 indicating that KIF3A depletion did not affect the classical secretory pathway.

We conclude that in the absence of KIF3A, VCCs accumulate intracellularly explaining the observed reduction of p24 release.

**Example 6 Dynamics of KIF3A and VCC transport in live macrophages**

To analyze the dynamics of VCC and KIF3A in live macrophages, we used two fusion proteins to simultaneously follow Gag and KIF3A. First, we recently derived HIV Gag-iGFP, a replication-competent and macrophage tropic version of an internally GFP tagged HIV-1 (Hubner et al., 2007). We have shown that HIV Gag-iGFP shared essential features with wild type HIV-1 and was suitable for kinetic studies in macrophages (Gaudin et al. submitted). Second, we generated a construct in which the fluorescent protein mCherry was inserted between the stalk and the cargo domains of KIF3A, flanked by two spacers to provide flexibility (Figure 6A). This KIF3A-internal mCherry construct (KIF3A-iCh) was correctly expressed as seen by immunoblot and immunofluorescence in 293T cells (Figure 15A and B). In non-infected cells, the KIF3A-iCh fusion protein was observed to move along microtubules that we identified by co-expression of EGFP- $\alpha$  tubulin (Figure 15C). From the analysis of time-lapse recording by spinning disk microscopy, we estimated the average speed of KIF3A-iCh+ structures at  $0.59\mu\text{m/sec} \pm 0.65$  ( $n=643$ ), and the average length of displacement at  $5.3 \mu\text{m} \pm 4.6$ , as expected for kinesin-2 movements (Cai et al., 2009; Loubry et al., 2008).

To establish whether KIF3A-iCh associated with VCCs like the wild type protein, we performed correlative light and electron microscopy. Primary macrophages were co-infected with HIV Gag-iGFP virus and KIF3A-iCh lentiviral vector. After 7 days of infection, macrophages were fixed on coverslips containing etched grids with coordinates and imaged by spinning disk microscopy. Co-expression of both fluorescent proteins was visualized in large intracellular compartments (Figure 6B). Embedding followed by sectioning of the samples allowed us to perform correlative EM of the very same cell previously imaged by fluorescence microscopy. At low magnification, EM revealed the position of the VCCs within the cell (Figure 6C). At high magnification, we observed that compartments corresponding to Gag-iGFP+ KIF3A-iCh+ contained numerous viral particles (Figure 6C and D). These compartments were active in HIV assembly, as budding profiles were visible at their limiting membrane (Figure 6D, inset). They contained both immature and mature viral particles. These results establish that the Gag-iGFP+ KIF3A-iCh+ compartments, observed in infected macrophages, are indeed VCCs where assembly and storage of viral particles take place.

Time-lapse spinning disk microscopy was finally carried out on primary macrophages co-infected with HIV Gag-iGFP ΔEnv virus and KIF3A-iCh lentiviral vector for 5 days. Observation at t = 0 h of a z projection revealed that KIF3A-iCh was associated in a polarized manner with the periphery of Gag-iGFP+ compartments (Figure 7), in agreement 5 with our observations regarding endogenous KIF3A distribution in HIV-1-infected macrophages (Figure 4). The 5-dimensional pictures revealed that both fluorescent proteins remained associated over time and moved together in a directional manner (Figure 7B, see tracking). Thus, our results indicate that VCCs might be transported by KIF3A towards the periphery of macrophages, suggesting that KIF3A is required for their release to the 10 extracellular milieu.

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- 25

Claims

1. A kinesin inhibitor for use in the treatment of HIV infection.

5

2. The kinesin inhibitor of claim 1, wherein said kinesin inhibitor is a N-type kinesin inhibitor, preferably a kinesin-2 inhibitor.

10 3. The kinesin inhibitor of any one of claims 1 or 2, wherein said inhibitor is selective for said kinesin, and preferably acts through direct binding to said kinesin.

4. The kinesin inhibitor of any one of claim 1 to 3, wherein said kinesin inhibitor is a KIF3A inhibitor and/or a KIF3C inhibitor and/or KIFAP3 inhibitor.

15 5. The kinesin inhibitor according to any one of claim 1 to 4, wherein said inhibitor is selected from the group consisting of a small molecule, a kinesin aptamer, an anti-kinesin antibody, a nucleic acid or a dominant negative peptide.

20 6. The kinesin inhibitor according to any one of claims 1 to 5, wherein said inhibitor inhibits the expression of said kinesin, and is preferably selected from the group consisting of an antisense nucleic acid, a ribozyme and an interfering RNA.

7. A pharmaceutical composition comprising at least one kinesin inhibitor according to any one of the preceding claims for use in the treatment of HIV infection.

25

8. The composition of claim 7, further comprising at least another anti-viral treatment, preferably an anti-HIV drug used in highly active antiretroviral therapy (HAART).

30 9. A tagged kinesin comprising a kinesin motor domain, a stalk domain, a cargo domain and a tag allowing said tagged kinesin to be detected, and wherein said tag is located between said stalk domain and said cargo domain.

10. The tagged kinesin of claim 9, wherein said kinesin motor domain, stalk domain and cargo domain all belong to the same kinesin, preferably a N-type kinesin, more preferably a kinesin 2, even more preferably to KIF3A, to KIF3C or to KIFAP3.
- 5    11. The tagged kinesin according to any one of claim 9 or 10, wherein said tag is a fluorescent tag protein, more preferably a mCherry protein
12. The tagged kinesin according to any one of claims 9 to 11, wherein said tag is flanked at least on one side, preferably on both sides, by spacers providing flexibility.
- 10    13. The tagged kinesin according to any one of claims 9 to 12, comprising the amino acid sequence of SEQ ID N°31.
14. A nucleic acid sequence comprising a sequence which codes for a tagged kinesin
- 15    according to any one of the preceding claim 9 to 13.
15. Use of a tagged kinesin according to any one of claim 9 to 13 to screen for kinesin inhibitors.
- 20    16. A method of screening for compounds useful for the treatment of HIV infection comprising the steps of:
  - a) providing or obtaining a candidate compound; and
  - b) determining whether said candidate compound inhibits the activity of expression of a kinesin, preferably a N-type kinesin, more preferably a kinesin-1 or a kinesin-2 , even more preferably kinesin KIF3A and/or KIF3C and/or KIFAP3,

25    wherein the ability of said candidate compound to inhibits the expression or activity of said kinesin indicates that said candidate compound is indicative of its usefulness for the treatment of HIV infection.

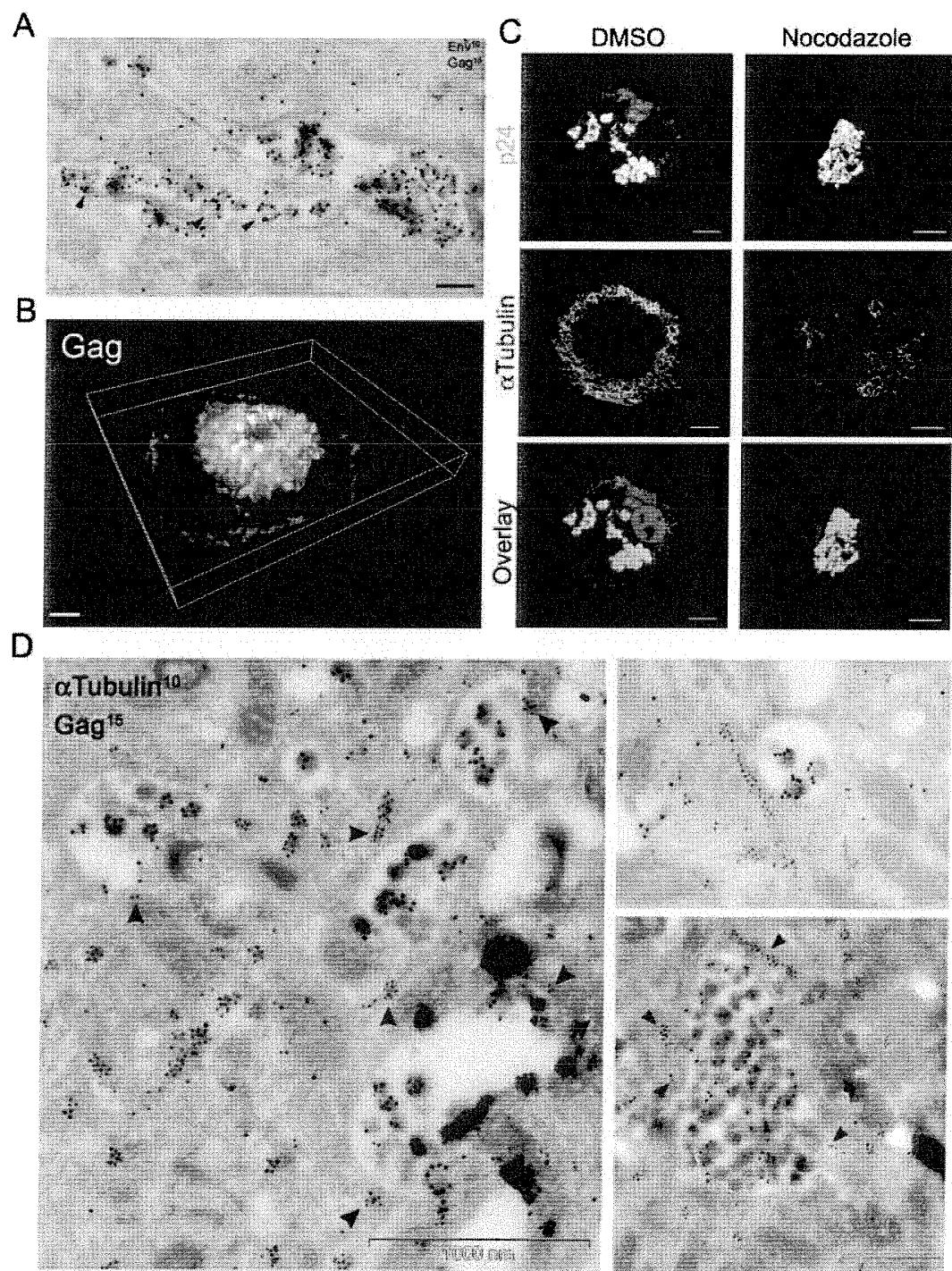
- 30    17. The screening method of claim 16 further comprising, for such candidate compound having the ability to inhibit the expression and/or activity of a kinesin of interest, a step c)

comprising determining whether said candidate compound is able to inhibit and/or decrease HIV infection.

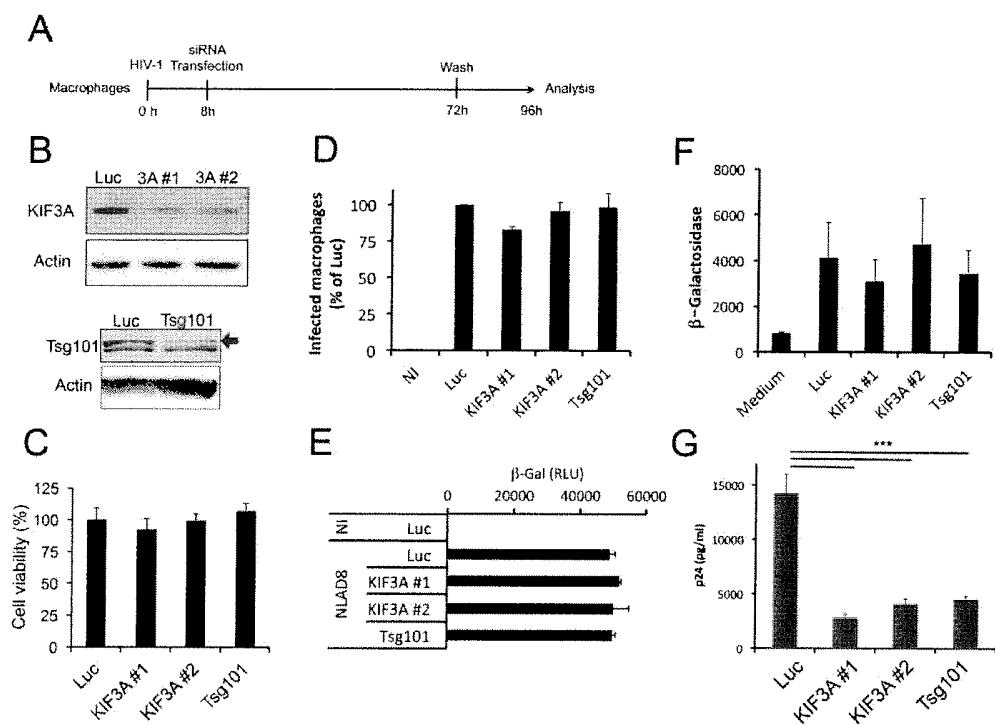
18. The screening method of claim 17, wherein said step c) comprises determining whether  
5 said candidate compound interferes with HIV virions formation, preferably using primary  
macrophages/monocytes or macrophage/monocyte cell lines infected with HIV.

19. The screening method of claim 16, wherein step b) comprises determining whether  
said candidate compound inhibits the activity of a tagged kinesin according to any one of  
10 claims 9 to 13.

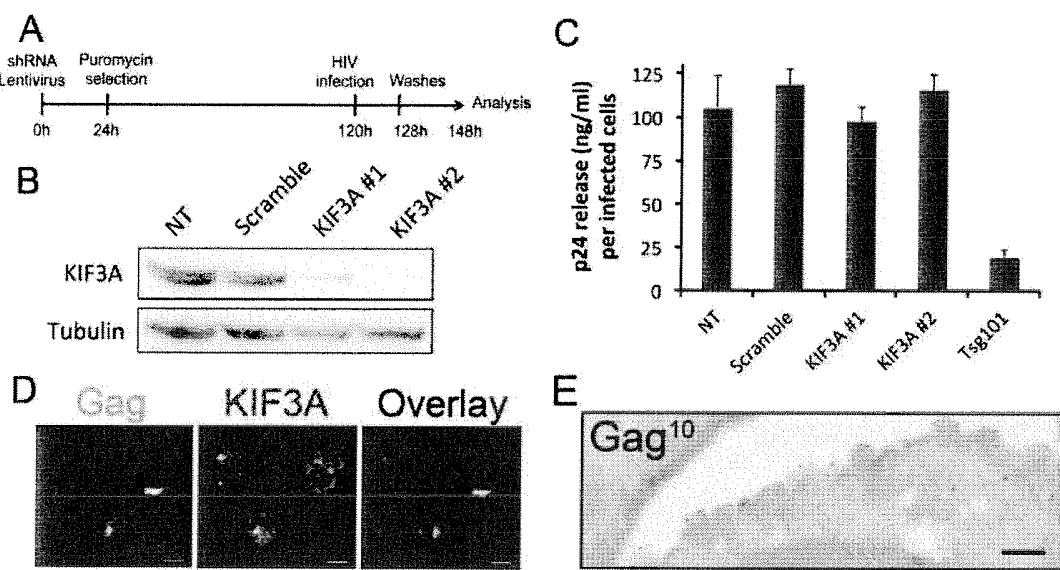
1/20

**Figure 1**

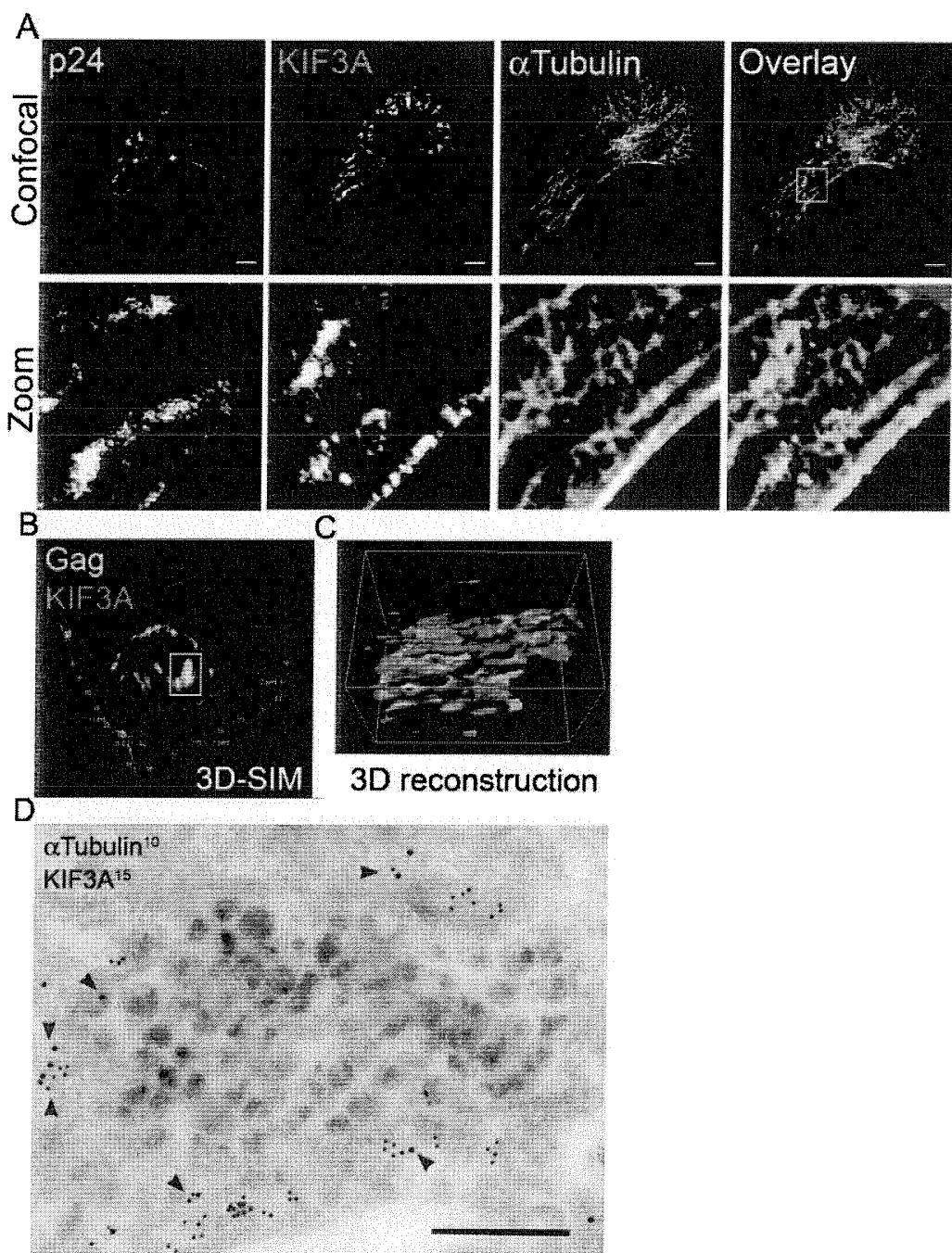
2/20

**Figure 2**

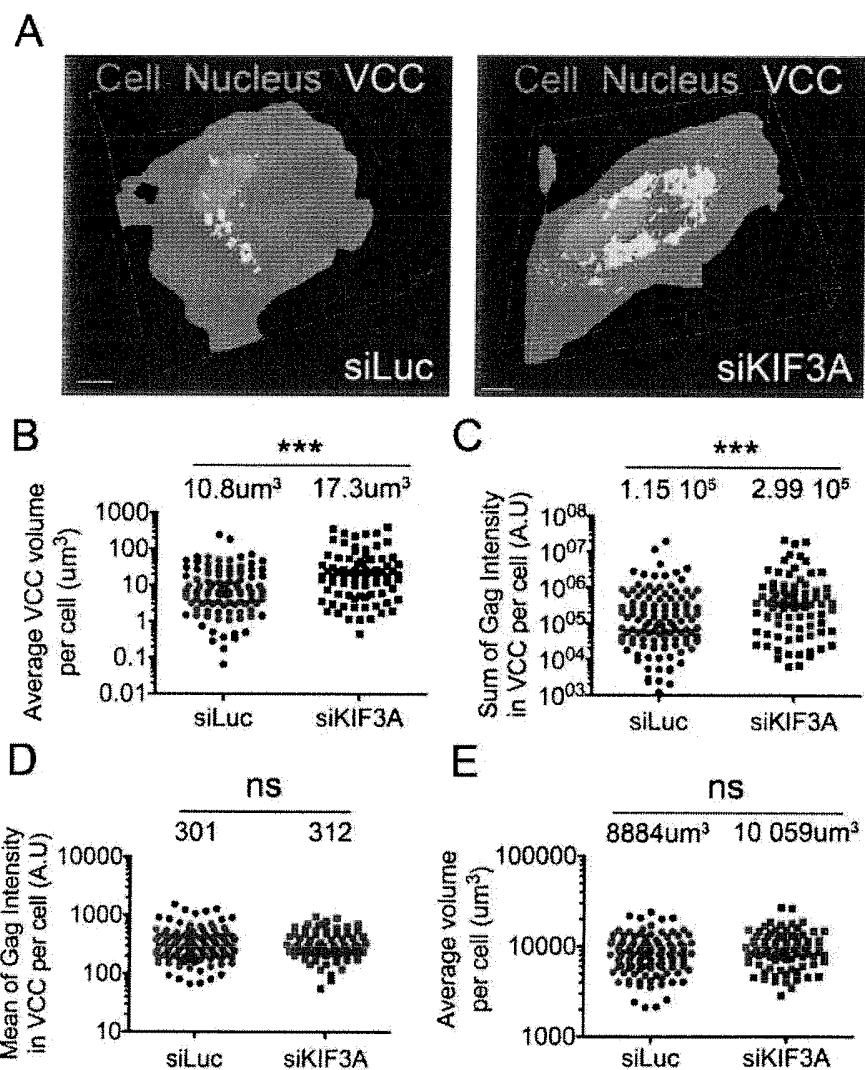
3/20

**Figure 3**

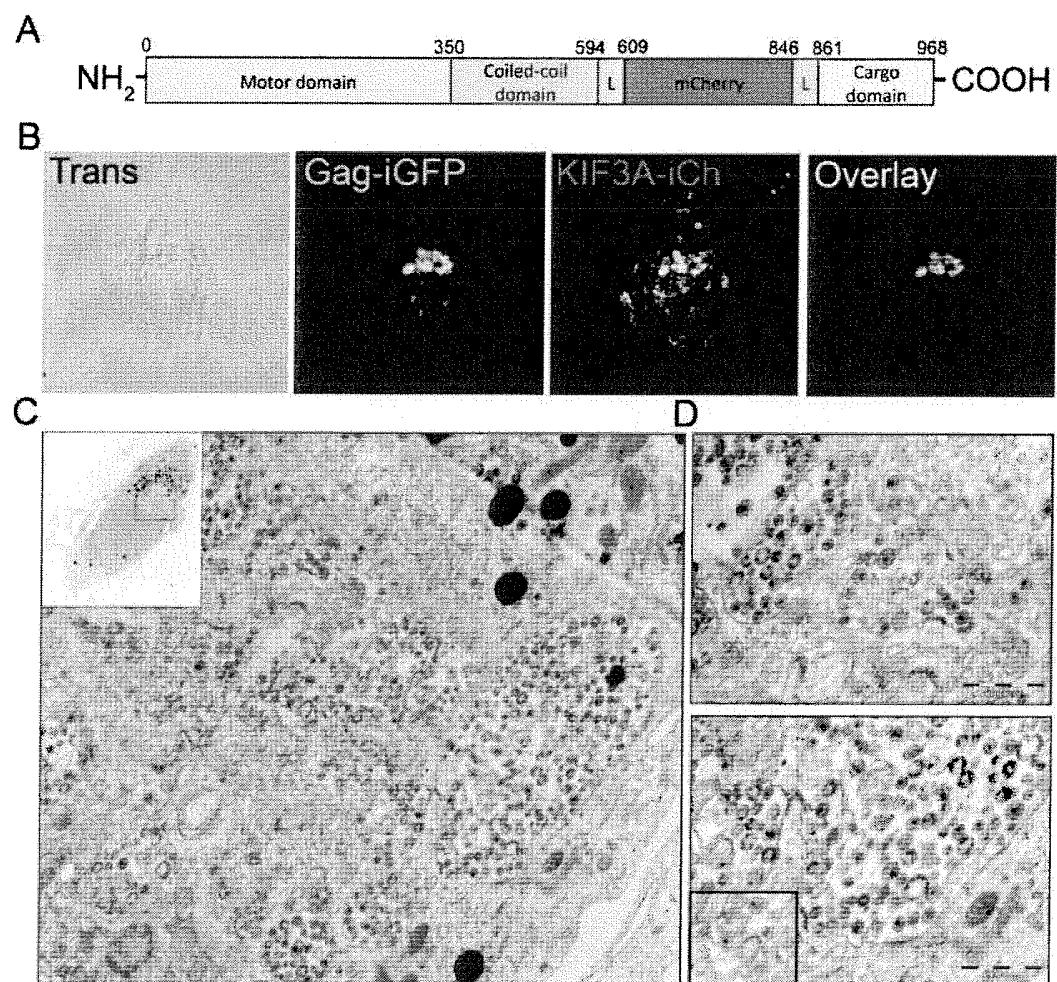
4/20

**Figure 4**

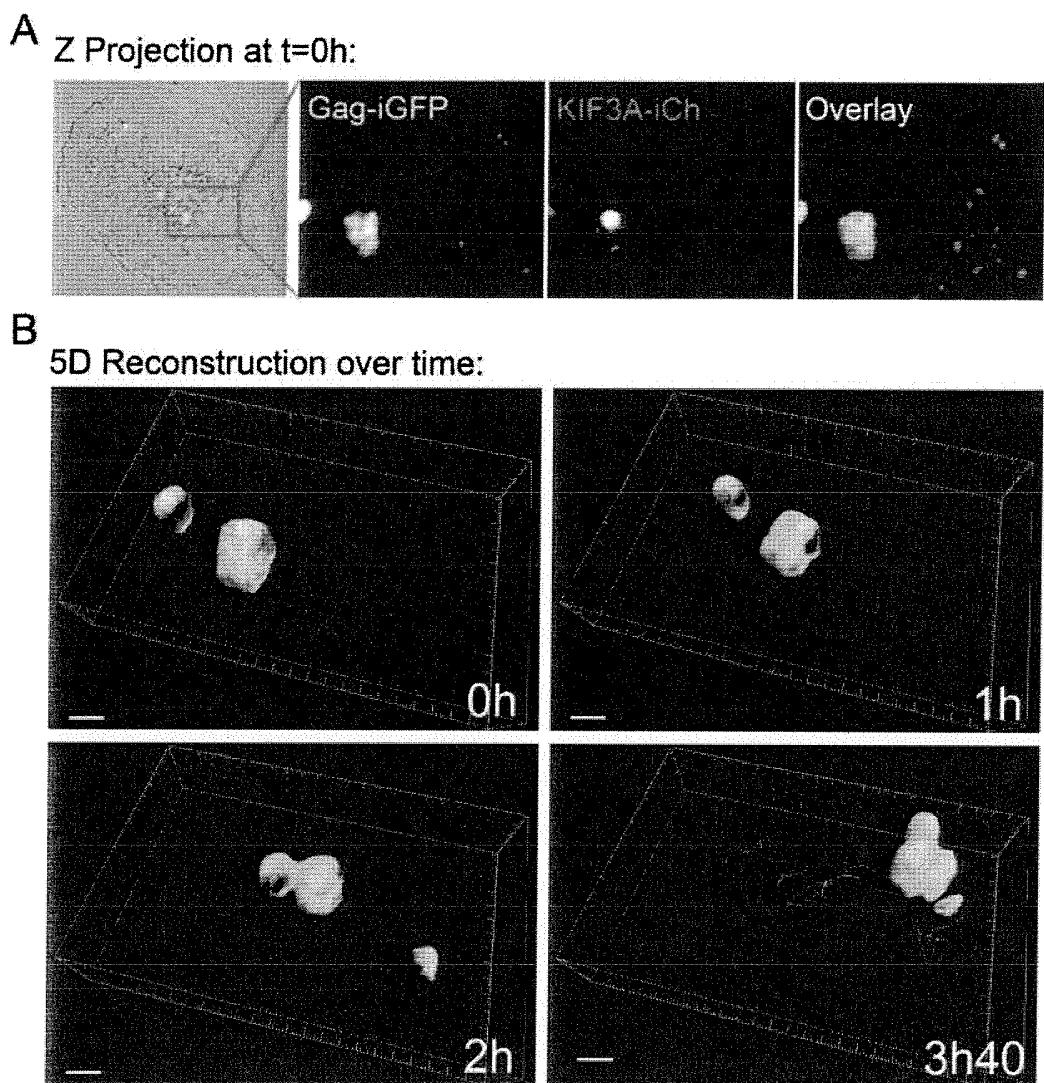
5/20

**Figure 5**

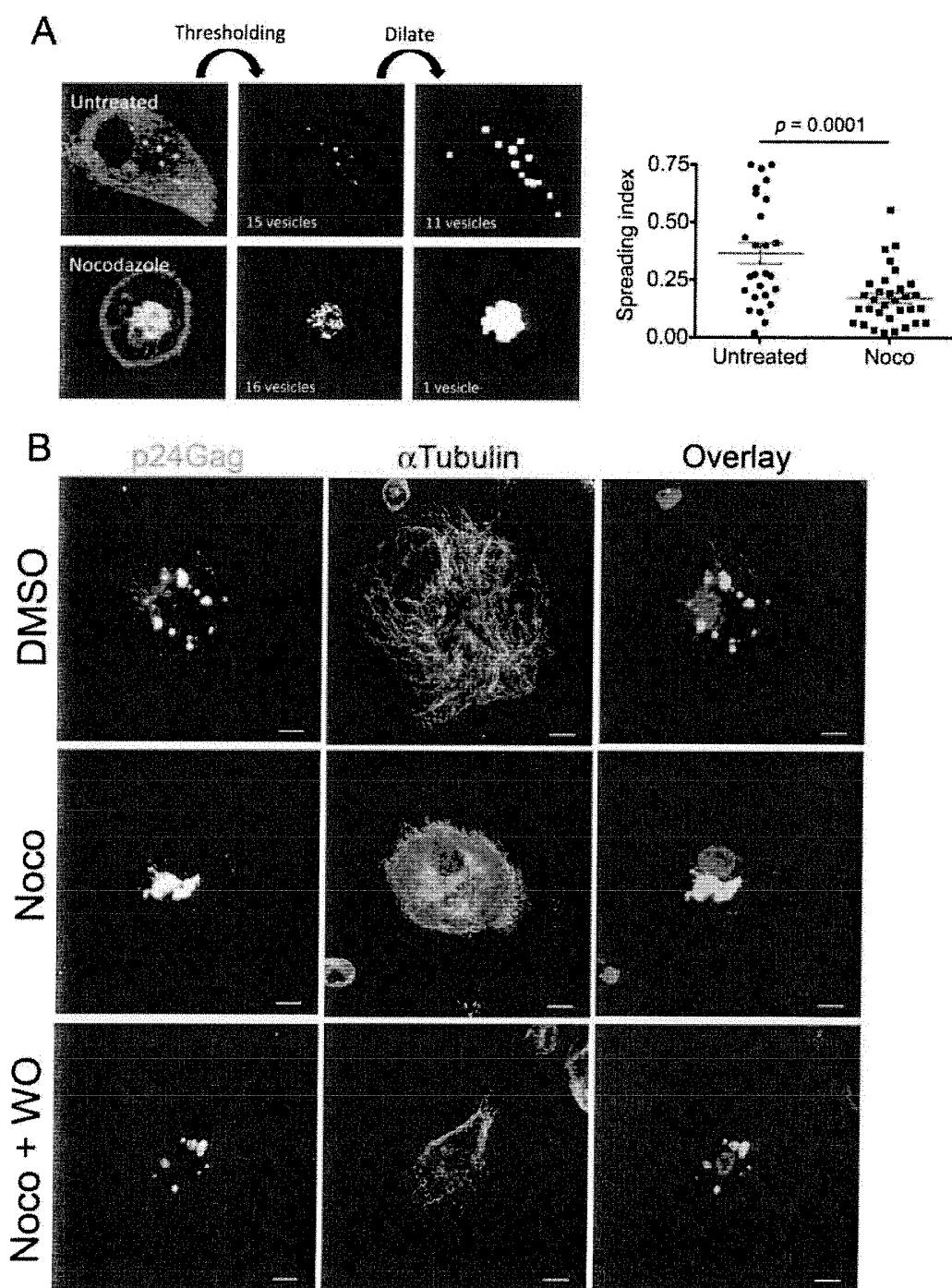
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**Figure 6**

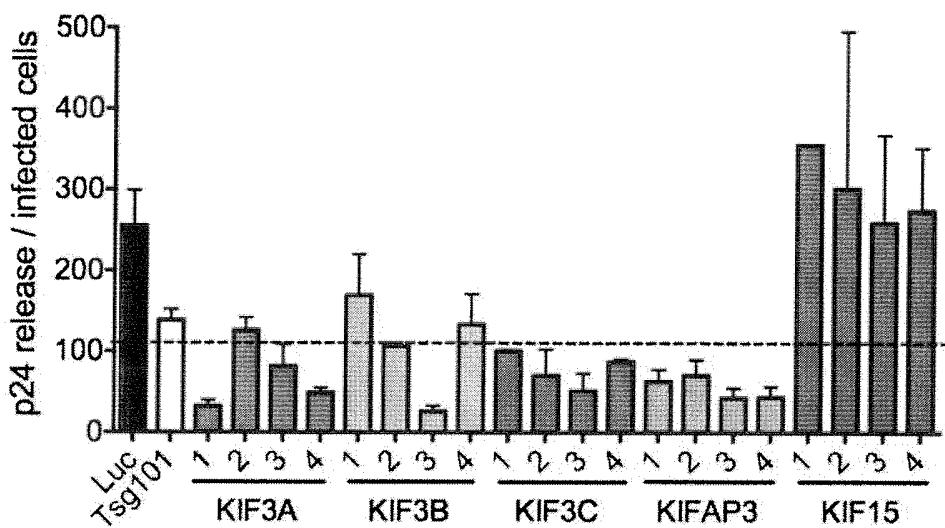
7/20

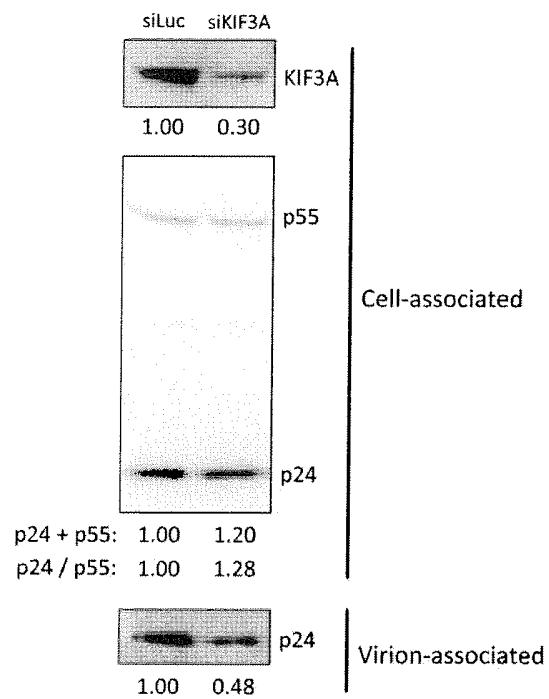
**Figure 7**

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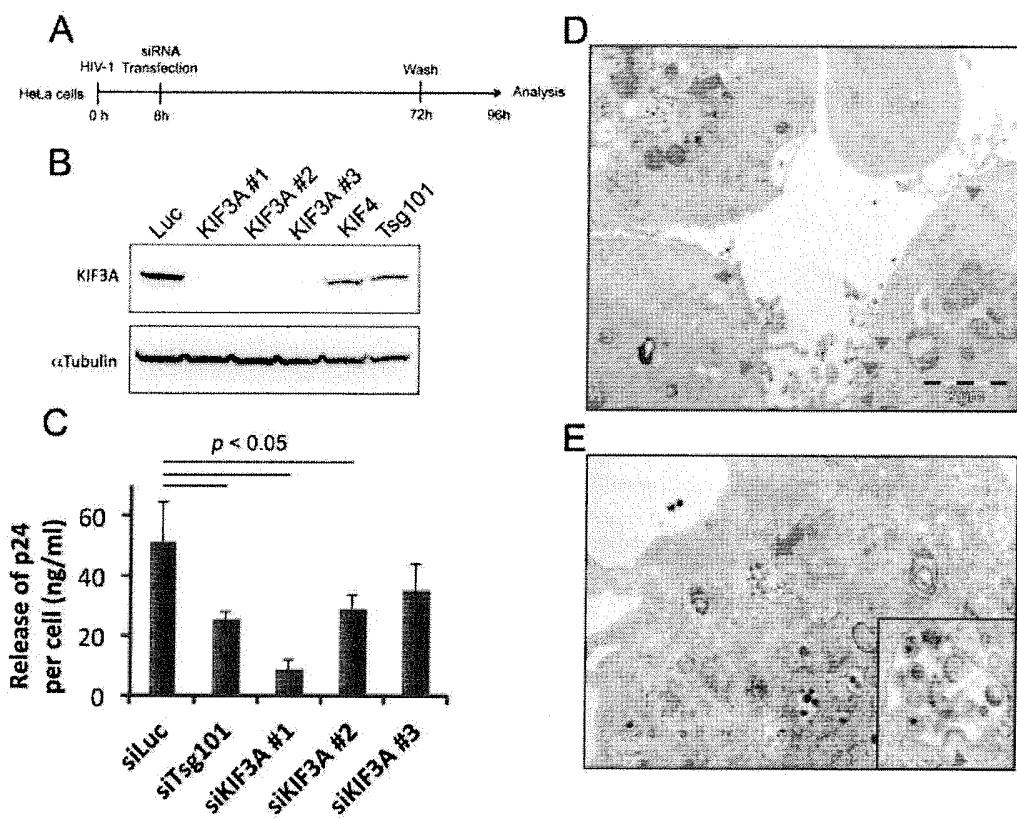
**Figure 8**

9/20

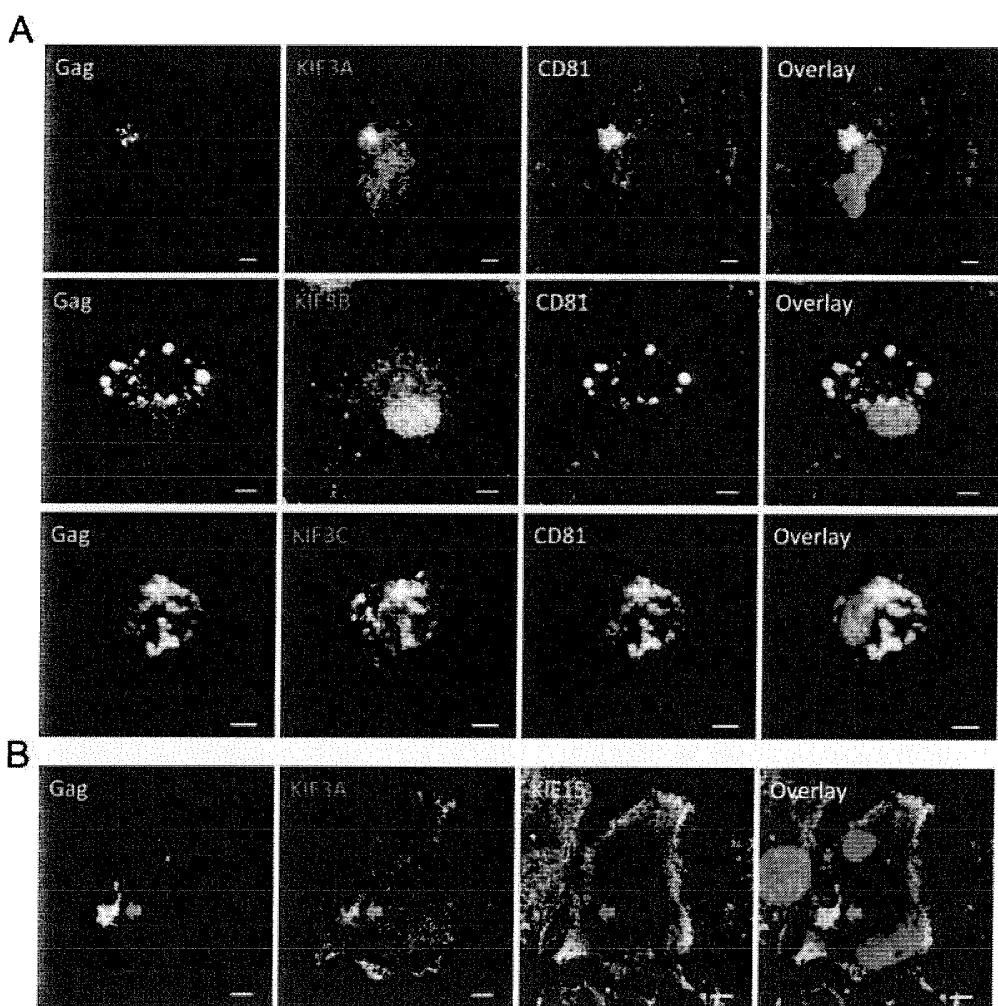
**Figure 9**

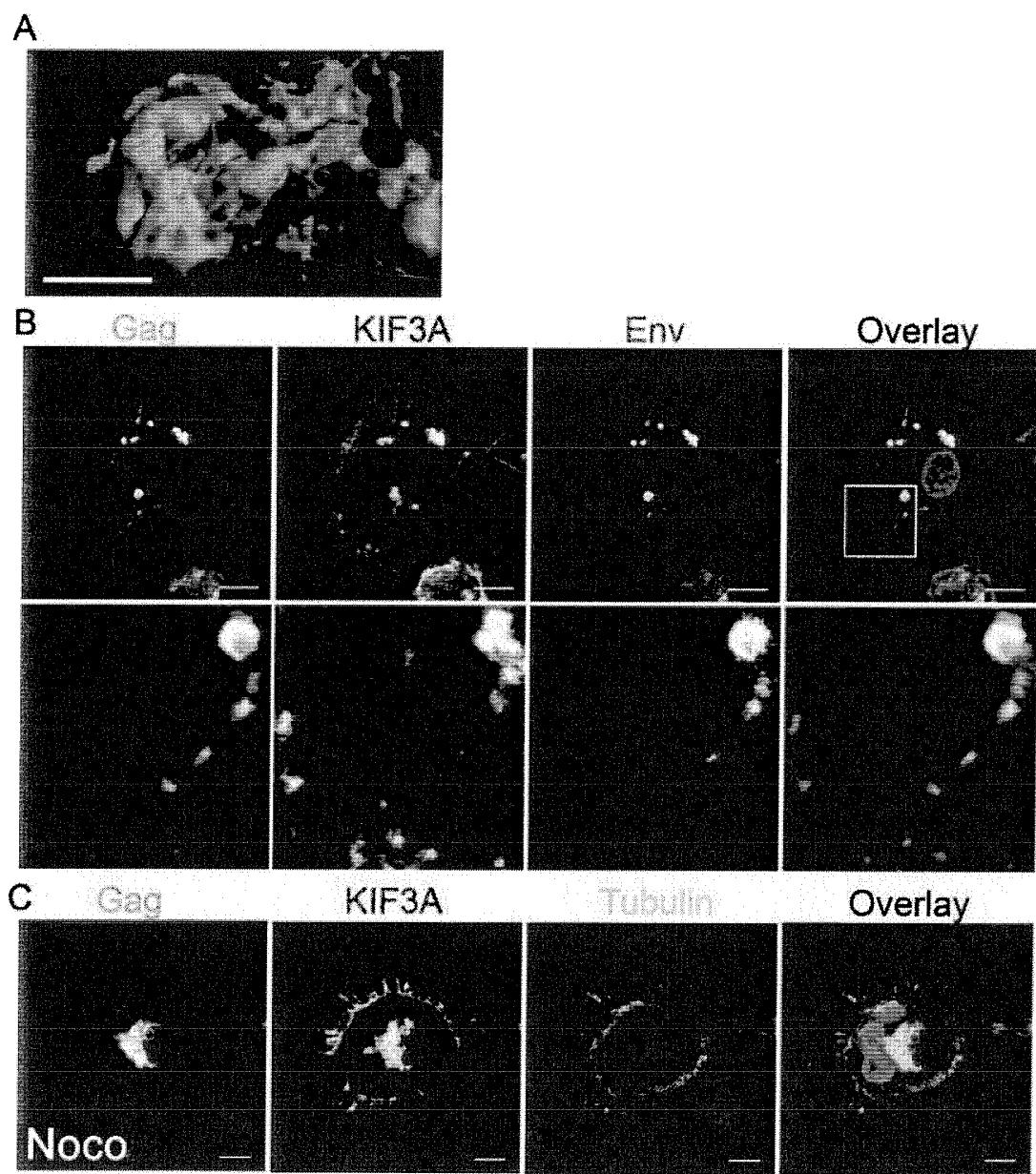
**10/20****Figure 10**

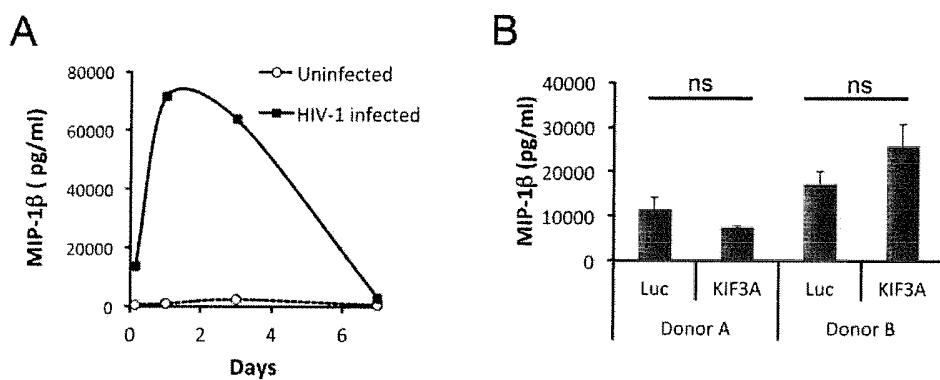
11/20

**Figure 11**

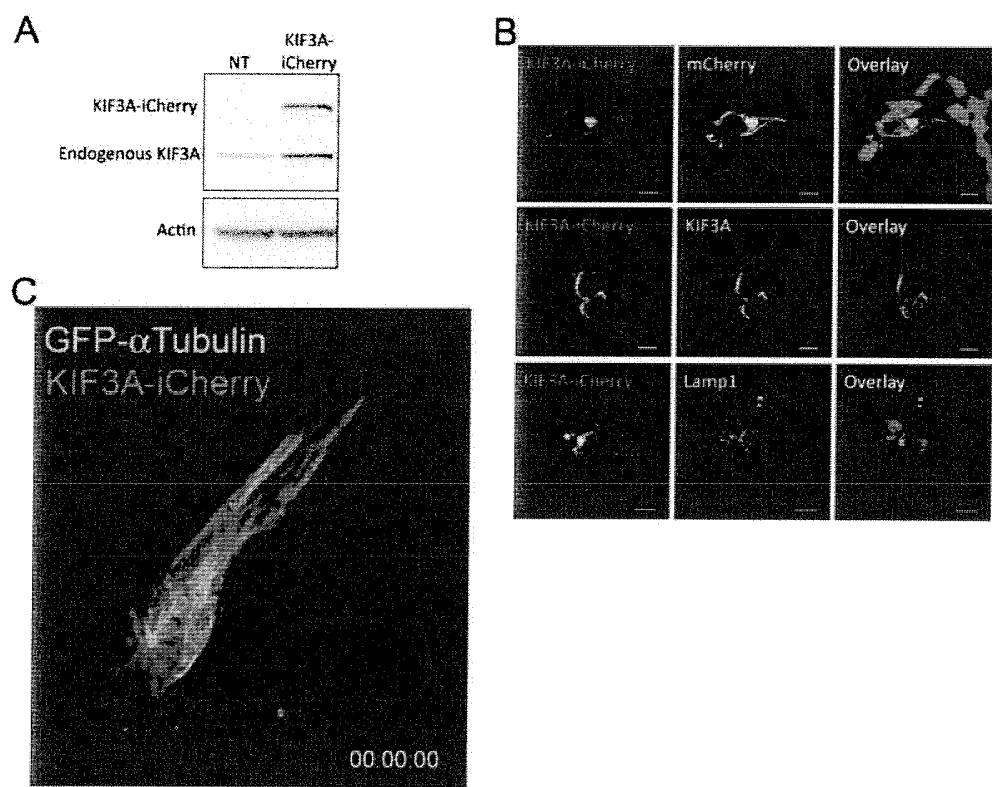
12/20

**Figure 12**

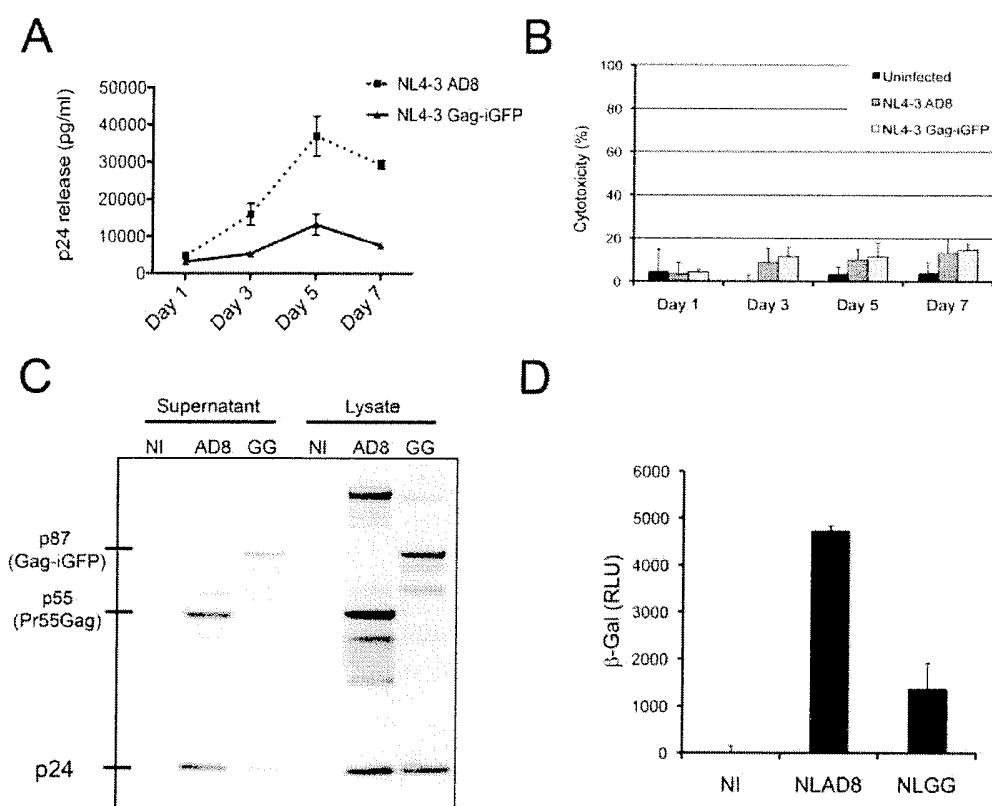
**13/20****Figure 13**

**14/20****Figure 14**

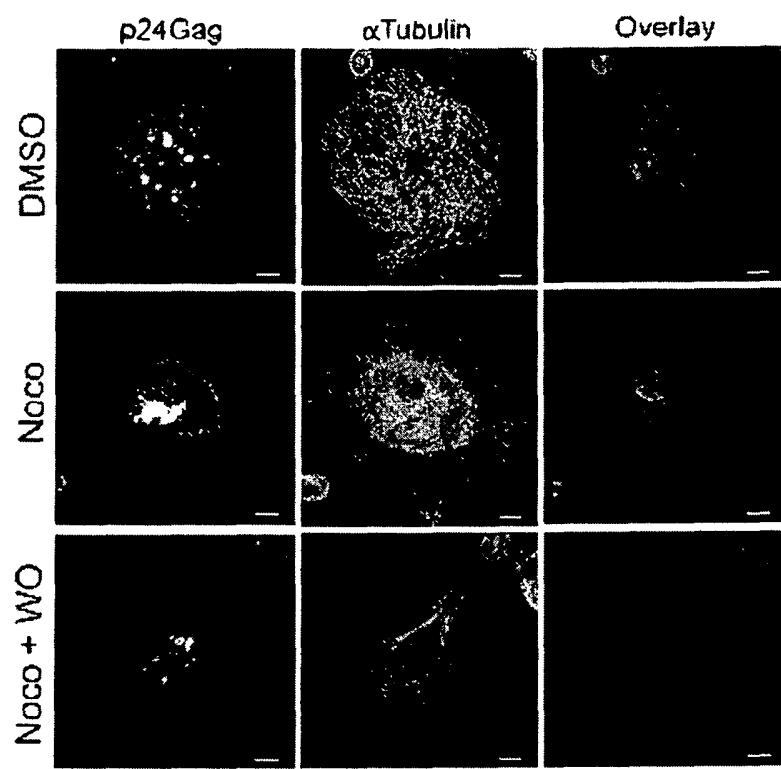
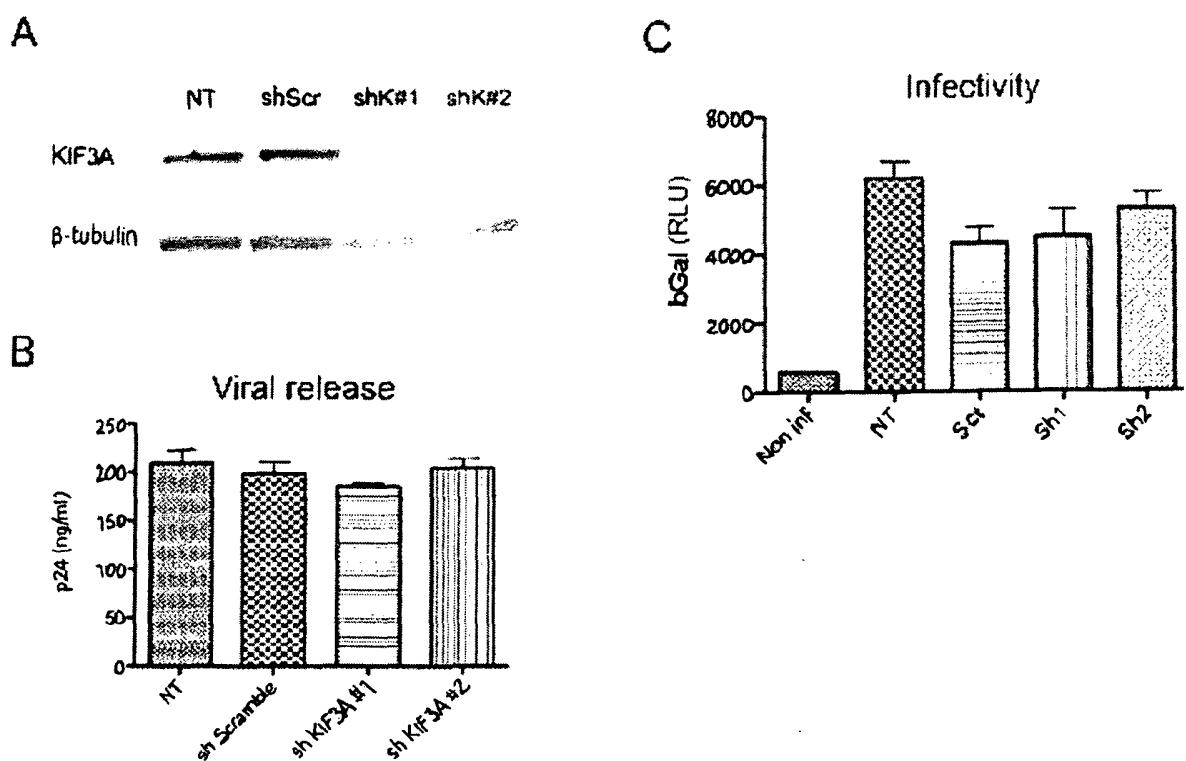
15/20

**Figure 15**

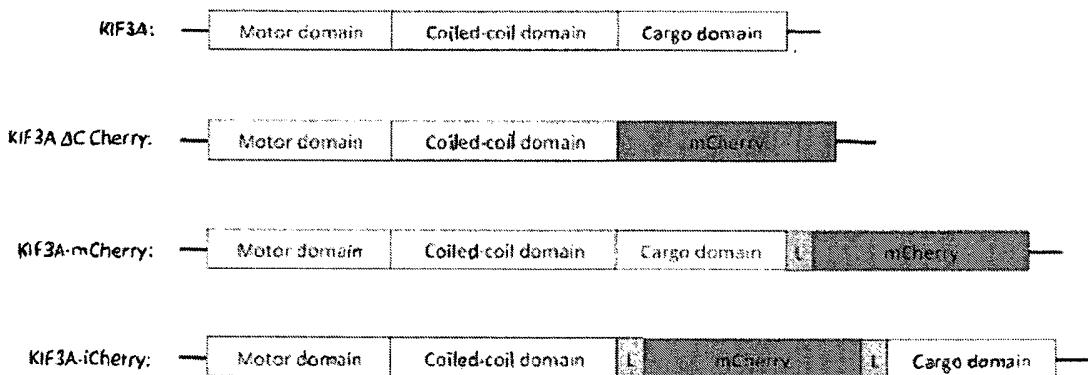
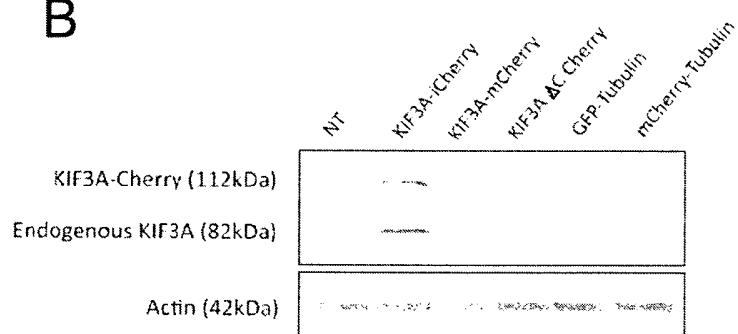
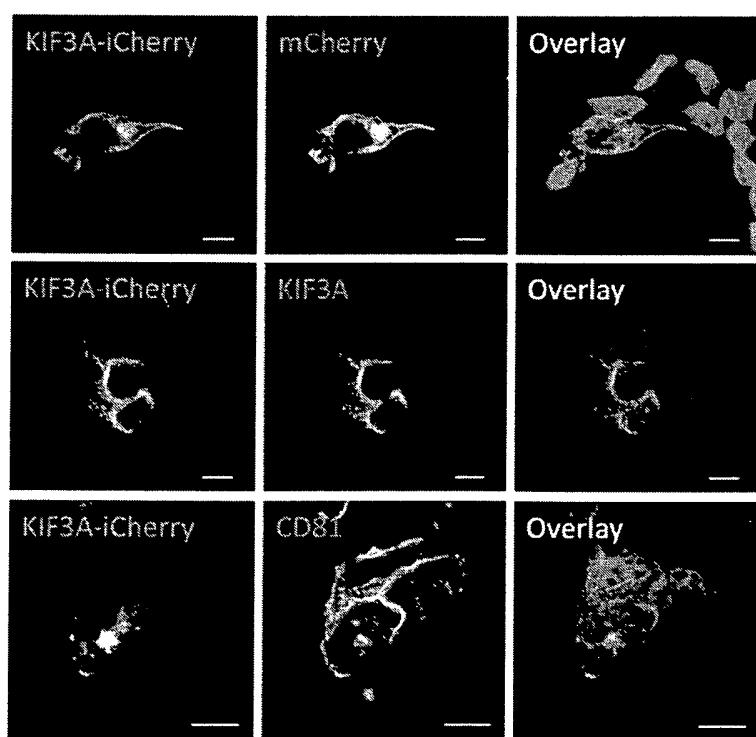
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**Figure 16**

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**Figure 17****Figure 18**

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**Figure 19****A****B****C**

**19/20****Figure 20**

Amino acid sequence KIF3A-iCherry: SEQ ID N°31

MPINKSEKPESCDNVVVRCPLNEREKSMCYKQAVSVDEMRGTTVHKTDSSNEP  
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PELRGIIPNSFAHIFGHIAKAEGDTKFLVRVSYLEIYNNEVRDLLGKDQTQRLEVKERP  
DVGVYIKDLSAVVNNADDMDRIMTLGHKNRSVGATNMNEHSRSRAIFTITIECSE  
KGIDGNMHVRMGKLHLVDLAGSERQAKTGATGQRLKEATKINLSLSTLGNVISALV  
DGKSTHVPYRNSKLTRLLQDSLGGNSKMMCANIGPADNYDETISTLRYANRAKNI  
KNKARINEDPKDALLRQFQKEIEELKKLEEGEEISGSDISGSEEDDDEEGEVGEDGE  
KRKKRRGKKVSPDKMIEMQAKIDEERKALETKLDMEEEERNKARAELEKREKDLL  
KAQQEHQSLLEKLSALEKKVIVGGVDLLAKAEEQEKLLEESNMELEERRKRAEQLRR  
ELEEKEQERLDIEEKYTSLQEEAQGKTKLKKVWTMLMAAKSEMADLQQEHQREIE  
GLLENIRQLSRELRLQMLIIDNGGGSGGGSGGGSASVSKGEEDNMAIIKEFMRFK  
VHMEGSVNGHEFEIEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAY  
VKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGFIYKVKLRTNF  
PSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAK  
KPVQLPGAYNVNICKLDTSHNEDYTIVEQYERAEGRHSTGGMDELYKGGGGSGGG  
GGGGSVPFIPRDYQEMIENYVHWNEDIGEWQLKCVAYTGNMRKQTPVPDKKEK  
DPFEVDSLHVYLAYTEESLRQSLMKLERPRTSKGKARPKTGRRKRSAKPETVIDFLLQ

\*

20/20

**Figure 21**

Nucleic acid sequence: SEQ ID N°32

### KIF3A-iCherry

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2011/070603

A. CLASSIFICATION OF SUBJECT MATTER	INV. C12N9/14	C12N15/113	A61K31/7105	C12N15/62	A61P31/18
	C12Q1/34	G01N33/68			

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
C12N A61K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, Sequence Search, WPI Data, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARTINEZ NATHANIEL W ET AL: "Kinesin KIF4 Regulates Intracellular Trafficking and Stability of the Human Immunodeficiency Virus Type 1 Gag Polyprotein", JOURNAL OF VIROLOGY, vol. 82, no. 20, October 2008 (2008-10), pages 9937-9950, XP002634434, ISSN: 0022-538X cited in the application the whole document -----	1-3,5-8, 16-18
Y	US 6 440 684 B1 (BERAUD CHRISTOPHE [US] ET AL) 27 August 2002 (2002-08-27) column 16, line 38 - line 52 column 12, line 41 - column 15, line 19 column 10, line 31 - column 11, line 18 -----	4
X	-----	1-3,5-8, 16-18
Y	-----	4
	-/-	

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search                      Date of mailing of the international search report

9 January 2012

23/03/2012

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Fax: (+31-70) 340-3016

Authorized officer

Wiame, Ilse

## INTERNATIONAL SEARCH REPORT

International application No PCT/EP2011/070603
---

## C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AZEVEDO CRISTINA ET AL: "Inositol pyrophosphate mediated pyrophosphorylation of AP3B1 regulates HIV-1 Gag release", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 106, no. 50, December 2009 (2009-12), pages 21161-21166, XP002634435, ISSN: 0027-8424 abstract page 21163, column 2, paragraph 2; figure 4 -----	4
A	BENAROCH PHILIPPE ET AL: "HIV-1 assembly in macrophages.", RETROVIROLOGY, vol. 7, no. 29, 7 April 2010 (2010-04-07), pages 1-10, XP002634436, ISSN: 1742-4690 cited in the application abstract page 3, column 1, line 3 - page 4, column 1, line 16; figure 1 -----	1-8
A	DOHNER K ET AL: "Viral stop-and-go along microtubules: taking a ride with dynein and kinesins", TRENDS IN MICROBIOLOGY, vol. 13, no. 7, 1 July 2005 (2005-07-01), pages 320-327, XP004961600, ELSEVIER SCIENCE LTD., KIDLINGTON, GB ISSN: 0966-842X, DOI: 10.1016/J.TIM.2005.05.010 abstract page 323, column 1, paragraph 2 page 325, column 1, line 2 - line 5 -----	1-8
A	ZHAOHUAI YANG ET AL: "Characterization of the KIF3C neural kinesin-like motor from mouse", MOLECULAR BIOLOGY OF THE CELL, vol. 9, no. 2, 1 January 1998 (1998-01-01), pages 249-261, XP055015928, ISSN: 1059-1524 cited in the application abstract page 258, column 2, paragraph 2 ----- -/-	4

## INTERNATIONAL SEARCH REPORT

International application No PCT/EP2011/070603
---

## C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Houff SA and Major EO: "Neuropharmacology of HIV/AIDS" In: Portegies P and Berger J R: "Handbook of Clinical Neurology: HIV and the nervous system", 2007, Elsevier, Amsterdam, XP008137523, ISBN: 978-0-444-52010-4 pages 319-364, page 350, column 1, paragraph 1 ----- A HAMMOND JENNETTA W ET AL: "Autoinhibition of the kinesin-2 motor KIF17 via dual intramolecular mechanisms", JOURNAL OF CELL BIOLOGY, vol. 189, no. 6, June 2010 (2010-06), pages 1013-1025, XP002634437, ISSN: 0021-9525 page 1014, column 1, last paragraph - column 2, paragraph 1 -----	5  1
1		

## INTERNATIONAL SEARCH REPORT

### Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-8(completely); 16-18(partially)

#### Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-8(completely); 16-18(partially)

A kinesin inhibitor for use in the treatment of HIV infection.

A method of screening for compounds useful for the treatment of HIV infection comprising the steps of:

- a) providing or obtaining a candidate compound; and
- b) determining whether said candidate compound inhibits the activity of expression of a kinesin, wherein the ability of said candidate compound to inhibits the expression or activity of said kinesin indicates that said candidate compound is indicative of its usefulness for the treatment of HIV infection.

---

2. claims: 9-15, 19(completely); 16-18(partially)

A tagged kinesin comprising a kinesin motor domain, a stalk domain, a cargo domain and a tag allowing said tagged kinesin to be detected, and wherein said tag is located between said stalk domain and said cargo domain.

A nucleic acid sequence encoding said tagged kinesin.

Use of said tagged kinesin to screen for kinesin inhibitors.

A method of screening for compounds useful for the treatment of HIV infection comprising the steps of:

- a) providing or obtaining a candidate compound; and
- b) determining whether said candidate compound inhibits the activity of expression of said tagged kinesin, wherein the ability of said candidate compound to inhibits the expression or activity of said kinesin indicates that said candidate compound is indicative of its usefulness for the treatment of HIV infection.

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**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No  
PCT/EP2011/070603

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6440684	B1 27-08-2002	NONE	