Antibodies, Cell lines, viral stocks and plasmids. DCs were stained at day 7 using the following monoclonal antibodies (mAbs): CD4-peridinin chlorophyll protein (PerCP), HLA-DR–PerCP (BD); CD14-flourescein (FITC), CD83- phycoerythrin (PE), and CD86-FITC (Pharmingen); and DCSIGN-PE (R&D). Adequate differentiation from monocytes to iDCs was based on the loss of CD14 and the acquisition of DC-SIGN, while maturation upregulated the expression of CD83, CD86, and HLA-DR in DCs.

The human T cell lines Jurkat, MOLT and MT4 were grown in RPMI (Invitrogen). TZM-BL indicator cell line (obtained through the NIH AIDS Research and Reference Reagent Program, from Dr. Kappes) and HEK-293T cell line were maintained in D-MEM (Invitrogen). Ghost CXCR4/CCR5 (obtained through the NIH AIDS Research and Reference Reagent Program, from Dr. KwalRamani and Dr. Littman) were maintained in D-MEM supplemented with 500 μg/ml of geneticin, 100 μg/ml of hygromycin and 1 μg/ml of puromycin (Invitrogen). All media contained 10% fetal bovine serum, 100 U/ml of penicillin and 10 μg/ml of streptomycin (all from Invitrogen).

$VLP_{HIV\text{-}Gag\text{-}eGFP}$ were obtained transfecting the molecular clone pGag-eGFP (obtained through the NIH AIDS Research and Reference Reagent Program, from Dr. Marilyn D. Resh[1-4]). The plasmid, pHIV-Gag-Cherry was constructed by substituting eGFP sequence for mCherry (derived from pmCherry-N1; Invitrogen) by swapping a BamHI-XbaI fragment. Replication competent full-length HIV_{NL43}, $HIV_{NFN\text{-}SX}$ and HIV_{Lai}, virus lacking the envelope glycoprotein $HIV_{\Delta env\text{-}Bru}$ and $HIV_{\Delta env\text{-}NL43}$ and the fluorescent $HIV_{NL43/eGFP}$ virus have been described elsewhere[5,6]. VLPs $_{MLV\text{-}Gag}$ were obtained

transfecting the molecular clone pCL-Eco (ImGenex) that expresses all of the ecotropic MLV structural genes. HEK-293T cells were transfected with calcium phosphate (CalPhos, BD) in T75 flasks using 20 µg of plasmid DNA. Viral stocks were also generated after HIV_{NL43} infection of MOLT, MT4 or PHA-stimulated PBMCs. Supernatants containing virus and VLPs were filtered (Millex HV, 0.45 µm, Millipore) and frozen at –80ºC until use. The $p24^{Gag}$ content of infectious viral stocks was determined by an ELISA (Perkin-Elmer). The $p24^{gag}$ content of $VLP_{HIV\text{-}Gag\text{-}eGFP}$ stocks was measured with a sandwich $p24^{Gag}$ ELISA using the anti-$p24^{gag}$ monoclonal antibody (183-H12-5C; AIDS Research and Reference Reagent Program) and HIV IG (cat # 3957; AIDS Research and Reference Reagent Program), as described previously[7]. HEK-293T cell derived $VLP_{MLV\text{-}Gag}$ containing supernatants were tested for presence of reverse transcriptase activity as described elsewhere[8].

Indiana strain VSV was grown on BHK cells according to established protocols[9]. Briefly, cells at 70-80% confluence were infected with VSV (MOI = 0.01). Twenty-four h post-infection, media was removed and centrifuged at 2,000 x g for 5 min to remove cell debris. Virions were then pelleted by high-speed centrifugation for 1 h at 4°C (Sorvall Surespin rotor, 100,000 x g). Virus pellets were resuspended in 10mM Tris pH 8.1. Aliquots of virus were checked for purity by SDS-PAGE and coomassie staining for viral proteins. Portions of this enriched virus fraction were either treated with 400 µg/ml of pronase for 1 h at 4°C or mock-digested. The number of infectious viruses remaining in each sample was then determined by dilution and plaque assay on BHK cells[10], and found to be reduced by >90% following proteases treatment. Pronase-treated VSV

particles were used in competitive binding assays with $VLP_{HIV\text{-}Gag\text{-}eGFP}$ as described in the materials and methods section.

Carboxylated beads assays. The yellow-green Carboxylated-Modified FluoSpheres® (Molecular Probes) of 0.1 μm of diameter were sonicated before each use, and the suspension was briefly centrifuged at high speed to further remove agglomerates. A total of $5x10^5$ iDCs and mDCs were incubated at 4ºC and 37ºC for 2 h with approximately $1.8x10^{10}$ beads at a final concentration of $2x10^6$ cells/ml. Pulsed DCs were washed with PBS and fixed with 2% formaldehyde to analyze by FACS. Forward-angle and side-scatter light gating were used to exclude dead cells and debris from all the analysis performed.

Carboxylated bead competition of $HIV_{\Delta env\text{-}NL43}$ binding to mDCs was measured by pre-incubating $5x10^5$ mDCs for 30 min with increasing amounts of beads, and then pulsing the cells additionally for 1 h at 37ºC with 130 ng of $HIV_{\Delta env\text{-}NL43}$ $p24^{Gag}$ in a final volume of 0.5 ml. Cells were washed extensively with PBS, and each sample was divided in half: one-half of the sample was fixed and analyzed by flow cytometry for carboxylated bead capture. The other half was lysed with 0.5% Triton-X100 (at a final concentration of $5x10^5$ cells per ml), cleared of cell debris by centrifugation (10,000 x g for 5 min) and assayed for $p24^{Gag}$ antigen content by an ELISA.

Co-localization analysis. mDCs were pulsed for 12 h with 150 ng of HIV_{NL43} $p24^{gag}$ and 3 mg of $Exosomes_{DiI}$ per $5x10^5$ cells, extensively washed in PBS, and fixed in 2.5% glutaraldehyde for1 h. Cells were then processed as described previously using a Jeol JEM 1010 electron microscope. Co-localization experiments were done pulsing mDCs with $VLP_{HIV\text{-}Gag\text{-}eGFP}$ and $Exosomes_{DiI}$ during 6 h as described in the capture assays

section. Cells were then fixed and cytospun into glass-slides. Single sections were acquired with an Olympus FV1000 confocal microscope. Quantification of co-localization was performed using the FV10-ASW 1.7 software (Olympus), analyzing 35 vesicles in mDCs of 3 different donors. The co-localization signals in percentages of the vesicle's area and the Manders and Pearson coefficients were calculated for each image. To obtain three-dimensional reconstructions, confocal Z stacks were acquired for some vesicles and imported into the three-dimensional visualization software Imaris 6.1.0 (Bitplane AG), employing the isosurface module of Imaris.

Kinetics experiments. Exosome kinetic analysis was assessed by pulsing $2x10^5$ mDCs with 150 μg or 75 μg of $Exosomes_{DiI}$ in a final volume of 0.1 ml. Cells were maintained at 37ºC, and aliquots were periodically harvested over a period of 8 h before fixing them and analyzing exosome capture by FACS. $VLP_{HIV\text{-}Gag\text{-}eGFP}$ kinetic analysis was assessed in a similar way, pulsing cells with 2,500 pg of $VLP_{HIV\text{-}Gag\text{-}eGFP}$ $p24^{Gag}$ per $1x10^5$ cells at a final volume of 0.1 ml. Fate of captured $VLP_{HIV\text{-}Gag\text{-}eGFP}$ was assessed pulsing $0.5x10^6$ mDCs with 12,500 pg of $p24^{Gag}$ for 2 h. Cells were then extensively washed and kept in culture at 37ºC for up to 48 h before fixing them and analyzing mDCs VLP retention capacity by FACS.

Confirmation of pronase activity on exposed cells and particles. mDCs were stained with anti-DC-SIGN, anti-CD4 and anti-CD81 mAbs (BD). Mock treated or pronase-treated mDCs were challenged with 5 ng of His tagged soluble trimeric HIV-1 JFFL gp140 envelope glycoprotein (gracious gift of Dr. Joseph Sodroski, DFCI) for 1 h at 4°C, washed and gp140 binding to cells revealed by staining with serial incubations with biotin-conjugated anti-His mAb and straptavidin-FITC. Mean flourescence intensity

values of staining were used to calculate the efficiency of protease treatment, which did not affect cell viability. To assess protease activity on particles, $VLP_{HIV\text{-}Gag\text{-}eGFP}$ pseudotyped with HIV-1 envelope glycoprotein gp120 were pelleted through 20% sucrose cushions (100,000 x g, 2 h, 4°C), resuspended in Dulbecco-PBS containing Ca^{2+}, Mg^{2+}, and were left untreated or treated with 400 μg/ml pronase for 1 h at 4°C. Pseudotyped VLPs were then diluted with DMEM media containing protease inhibitors (Roche), layered over a 50% Optiprep cushion (0.85% NaCl, 60 mM HEPES, pH 7.4) and centrifuged for 2 h at 100,000 x g. The VLPs present at the media-50% optiprep interface were harvested, pelleted and analyzed for expression of gp120 with a murine anti-gp120 monoclonal antibody (Clone 1121; Immunodiagnostics, Woburn, MA) and Gag-eGFP with mouse anti-HIV $p24^{Gag}$ mAb (Clone AG3.0, Cat#4121, NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) via western blot analysis. The efficiency of pronase activity on infectious virus particles (400 μg/ml of pronase for 1 h at 4ºC) was confirmed by comparing the infectivity of untreated and pronase-treated wild type HIV_{NL43} particles on TZM-BL cells. There was an 18-fold reduction in infectivity following pronase treatment (from a mean $TCID_{50}$/ml of 22,357 to 1,167 after pronase treatment). Note that in these experiments the TZM-BL cells employed had the same final concentration of pronase, regardless of whether or not viral stocks had been previously treated with pronase.

Confocal microscopy of pronase treated mDCs. Cells were left untreated or incubated with 200 μg/ml of pronase for 30 min at 4ºC. Effectiveness of pronase treatment was confirmed as detailed above. Pronase or mock treated mDCs were exposed to $VLP_{HIV\text{-}Gag\text{-}eGFP}$, $HIV_{NL43\text{-}vpr\text{-}eGFP}$ or $Exosomes_{DiI}$ during 4 hours at 37ºC in the presence of 50

μg/ml of pronase to avoid complete recycling of the surface-exposed cellular receptors previously digested by the proteases. Cells were then washed and fixed or allowed to recover for 2 hours before starting a 4-hour co-culture with cell-tracker labeled Jurkat T cells. All samples were analyzed in a confocal microscope (Laser Optic Leica TCS SP2 AOBS).

Amantadine and chlorpromazine treatment of mDCs. Cells were treated with amantadine or chlorpromazine hydrochloride (Sigma) at the concentrations indicated for 30 min at 37ºC before pulsing them with HIV_{NFN-SX}. Pulsed mDCs were extensively washed and assayed for $p24^{Gag}$ with an ELISA. mDCs viability was assessed with FACS, labeling cells with propidium iodide and DIOC-6 (Sigma and MolecularProbes). Amantadine treated mDCs were also pulsed with $VLP_{HIV-Gag-eGFP}$, stained with DAPI and TRITC, fixed with 2% of formaldehyde and cytospun into glass-slides to analyze them by deconvoultion microscopy.

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