Supplementary methods

Antibodies, plasmid and reagents. The following monoclonal antibodies were used: AZN-D1 and AZN-D2 (anti-DC-SIGN), DCGM4 (anti-Langerin, Beckman Coulter Inc., Miami, Florida, USA), OKT6 (anti-CD1a, IgG1), NA1/34 (anti-CD1a, IgG2a, Dako Cytomation Denmark, Glostrup, Denmark), HI149FITC (anti-CD1a, Pharmingen, San Diego, CA, USA), 2D7 (anti CCR-5, BD biosciences, Oxnard, CA, USA), goat anti-mouse IgG-PO (Jackson Immunoresearch, West Grove, PA, USA), goat anti-mouseFITC (Zymed Laboratories Inc., South San Fransisco, Ca. USA), HLA-DRPE, CD80PE, CD83PE, CD86PE (Immunotech, Marseille, France), D7230 (sheep anti-HIV-1- P24 polyclonal antibody, Aalto Bioreagents, Dublin, Ireland). Plasmid encoding the gp120-Fc from the JR-FL isolate was obtained from the NIH AIDS Research and reference Reagent Program. Human DC-SIGN and human Langerin were cloned into lentiviral vectors pLOX (LV-DC-SIGN and LV-lang), replacing the green fluorescent protein (GFP) marker gene, as previously described (Arrighi et al. 2004 J. Virol 78; 10848-55).

Cell lines. Raji cells expressing either wild type DC-SIGN or Langerin were generated by retroviral transduction with the respective lentiviral constructs (LV-DC-SIGN and LV-lang). Stable expression of the gene of interest was determined by flow cytometric analysis. The positive population, highly expressing the gene, was sorted on a cell sorter (Moflow; DakoCytomation) and thus, contains a mixture of clones. The CD34+ human AML cell line MUTZ3 (Deutsche Sammlung von Mikroorganismen und Zellkulturen (DMSZ), Braunschweig, Germany) was maintained in (MEM)-Alpha medium containing ribonucleasides and deoxyribonucleosides (Life technologies, Paisley, United Kingdom) supplemented with 20% FCS, 0.01mM 2-mercaptoethanol (Merck, Darmstadt, Germany) and 10% of culture supernatant of the 5637 cell line (American Type Culture collection [ATCC], Manassa, VA)[17]. A LC phenotype was induced as previously described. In short, the cells were seeded in 12 well plates (Costar, Cambridge, MA, USA) at a concentration of 0.1x106/ml and stimulated with GM-CSF 800 U/ml, recombinant human TGF-beta-1 (5ng/ml, Biovision, Mountain View, CA, USA) and recombinant TNF-α(2.5 ng/ml, Strathmann biotec, Hamburg, Germany) and restimulated at day 4 and 7.

Primary cells. Monocyte derived immature DCs were cultured as described before[2]. In short, human blood monocytes were isolated from buffy coats by a Ficoll gradient and a subsequent CD14-selection step using CD14 labeled Immunomagnetic Microbeads (MACS system; Miltenyi Biotec. Bergisch, Gladbach, Germany).

Purified monocytes were differentiated into immature DCs in the presence of IL-4 and GM-CSF (700 and 400 U/ml, respectively; Biosources, Nivelles, Belgium).

Virus. HIV-1 stocks were generated by the passage of viruses through CD4+ lymphocytes, isolated from individuals who did not carry the Δ32 CCR5 allele (CCR5+/+), screened for by standard polymerase chain reaction technique. All viral stocks were assayed for tissue culture infectious dose ($TCID_{50}$) on $CD4^+$-enriched lymphocytes. $CD4^+$-enriched lymphocytes were plated at 2×10^5 cells/well in 96-well plates with 5-fold serial dilutions of the virus. The cells were fed on day 7 with fresh media and scored on day 14 for p24 levels, with the number of positive wells being used to identify the $TCID_{50}$ value for each virus. A subtype B molecular cloned virus JR-CSF (R5) was used in the experiments.

Fluorescent bead adhesion assay. The fluorescent bead adhesion assay was performed as described before[2]. In short, streptavidin was covalently coupled to the carboxylate-modified TransFluorSpheres (488/645 nm, 1.0 μm; Molecular Probes, Eμgene, OR, USA). The streptavidin-coated beads were incubated with a biotinylated F(ab')2 fragment goat anti-human (6 μg/ml; Jackson Immunoresearch), followed by an overnight incubation with gp120-Fc at a concentration of 10 μg/ml at 4°C[2]. The adhesion assay was performed as follows: 50,000 cells were incubated with beads for 45 min at 37 °C. To determine specificity of adhesion, cells were pre-treated with mannan (1 mg/ml), EGTA (10 mM), blocking antibodies against DC-SIGN (20 μg/ml) fucose or mannose (50mM) for 15 min at 37 °C. Binding was measured by FACS analysis.

Immunofluorescence microscopy. Cryosections of healthy tissues (7μ) were fixed with 100% acetone and stained with primary antibodies (10 μg/ml) for 1 h at 37 °C. Sections were counterstained with isotype-specific Alexa488- or Alexa594-labeled anti-mouse antibodies (Molecular probes, Eugene, OR). Nuclei were stained with DAPI (Molecular Probes, Eugene, OR, USA). Epidermal sheets were cut into pieces of 1 cm^2 and stained in 500 ul PBS in a 24 well plate. Sheets were counterstained with a mouse-specific Alexa594 anti-mouse antibody and spread out on glass slides for microscopy.

Electron Microscopy. Migratory LCs or MUTZ3/LCs ($2x10^6$) were incubated with 1μg of HIV-1 (JR-CSF) for 4 h. Cells were fixed in 2% paraformaldehyde and 0.2% glutaraldehyde in 0.1 phosphate buffer for 2 h at room temperature. Cells were pelleted in 12% gelatine, cryoprotected in 2.3M sucrose and snap-frozen in liquid nitrogen. Ultrathin cryosections were double immunolabeled with sheep polyclonal antibody P24 (15nm protein

A goldlabel) and DCGM4 (anti-Langerin) antibody (10nm protein A goldlabel). After incubation, the sections were stained with uranylacetate and embedded in methylcellulose. Sections were examined with a transmission electron microscope (model Philips CM100 Bio Twin).

Flowcytometry. For determining cell surface expression, cells were incubated with primary antibody (5 μg/ml) at 4 ^{o}C, followed by staining with a secondary FITC-labeled anti-mouse antibody (Zymed, San Francisco, CA) and analyzed on FACS calibur (BD Pharmingen, San Diego, CA, USA).