Supplementary Data

Isolation of biological HIV-1 clones

Patient peripheral blood mononuclear cells (PBMCs; at a concentration ranging from 1×10^4 to 8×10^4 cells/well) were cocultivated in 96-well plates with 1×10^5 phytohemagglutinin (PHA, Sigma Aldrich)-stimulated PBMCs from two healthy donors in Iscove's modified Dulbecco's medium (IMDM, Sigma Aldrich) supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin, polybrene (5 μ g/ ml, Sigma Aldrich), and recombinant interleukin 2 (rIL-2; 20 U/ml, Novartis). Every week for 4 weeks, half of the cells were transferred to new 96-well plates, and fresh medium and 10⁵ new healthy donor PHA-stimulated PBMCs were added to propagate the cultures. Virus replication in the culture supernatant was detected by an in-house HIV-1 p24 antigen (Ag) ELISA assay (www.aaltobioreagents.ie). The proportion (F) of infected cells per well was estimated by the formula for the Poisson distribution, $F = -\ln F0$, where F0 is the fraction of negative cultures per total number of cultures.

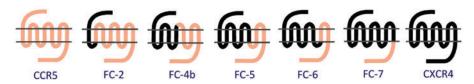
Phenotype determination of HIV-1 biological clones in U87.CD4 cells

Cells were infected in duplicate with each virus containing at least 2 ng/ml HIV-1 p24 Ag. The cultures were kept for 7 days and inspected for syncytia formation at days 1, 3, and 7. Supernatant was collected on day 1, after extensive washing, and on the last day of culture, and tested for the presence of HIV-1 p24 Ag with the in-house p24 Ag ELISA assay.

Cultures were considered for evaluation only if the HIV-1 p24 Ag value at day 1 was below the lower detection limit of the assay (0.065 ng/ml). Viral antigen production was considered positive when the absorbance at day 7 exceeded 0.2 OD. Experiments were performed twice. Cells were grown in Dulbecco's minimal essential medium (DMEM) supplemented with 10% fetal calf serum (FCS), 10% penicillin/streptomycin, and selection antibiotics geneticin (500 μ g/ml) and puromycin (1 μ g/ml).

RANTES and MVC sensitivity assay

PHA-activated PBMCs derived from two HIV-seronegative blood donors were infected with viral supernatant in 96well round-bottom microtiter plates. The infection was performed overnight at 37°C, in the presence or absence of RANTES (R&D Systems, Minneapolis, MN) or maraviroc (MVC; Pfizer, New York, NY) diluted in RPMI 1640, 10% FCS, antibiotics, and 100 U/ml IL-2 (IL-2 medium). Five steps of 2-fold dilutions starting from a concentration of 125 ng/ml of RANTES and 1,000 nM of MVC, respectively, were added to duplicate wells with two dilutions of viral supernatant to obtain a TCID₅₀ between 10 and 50. At days 1 and 3 postinfection the cells were washed and RANTES in fresh IL-2 medium was added at the corresponding final concentration. Culture supernatant from each well was assayed for the presence of HIV-p24 Ag in the in-house ELISA at day 7 after infection.



SUPPLEMENTARY FIG. S1. Schematic pictures of the chemokine receptors CCR5 and CXCR4 and the chimeric receptors FC-2, FC-4b, FC-5, FC-6, and FC-7, where successively larger parts of CCR5 have been replaced with corresponding regions of CXCR4.