Supporting Information

Pizzato 10.1073/pnas.1001554107

SI Text

VDJ Junction Sequence of the *tcr* of JJ6 and JTAg Is Identical. DNA was extracted from JM, JJ6, and CEM cells. A PCR to amplify a TCR gene region encompassing the VDJ junction was performed by using primers 5′-AGTCGGTGACCCAGCTTGGC-3′ and 5′-ATCTTGGAAGCACTGCTGTTCGG-3′. A PCR product was only obtained from DNA derived from JTag and JJ6. The DNA sequence of the PCR products was determined and found to be 100% identical in the two cell lines and to the sequence of the Jurkat T cell receptor active α-chain precursor mRNA (accession no. M12423).

Effect of gPr80 and Nef on Cell Surface Expression Levels of CD4 and MHC-I. JTAg cells and mouse immortalized fibroblasts MC57 were transfected with vectors encoding HA-gg189 or Nef-HA in combination with a plasmid encoding EGFP. Forty-eight hours after transfection, surface human CD4, and human and mouse MHC-1 expression levels on cells gated for GFP expression were determined by two-color flow cytometry after staining with mouse anti-CD4, anti-human HLA-ABC, and anti-mouse H-2K^b (BD Pharmingen) followed by APC-conjugated anti-mouse (Jackson ImmunoResearch).

Virus Binding Assay. Virus binding assay for detection of mCAT-1 surface expression levels was performed on JTAg cells stably expressing mCAT-1 or control parental JTAg cells. Cells were transfected with vectors encoding HA-gg189 or Nef-HA in combination with a plasmid encoding EGFP. Forty-eight hours after transfection, cells were incubated with a MoMLV suspension produced from 293T cells transfected with pNCA. at 4 °C for 60 min. Virus–cell complexes were stained with anti-RLV SU (Quality Biotech) followed by APC-conjugated anti-goat, and cells gated for GFP expression were analyzed.

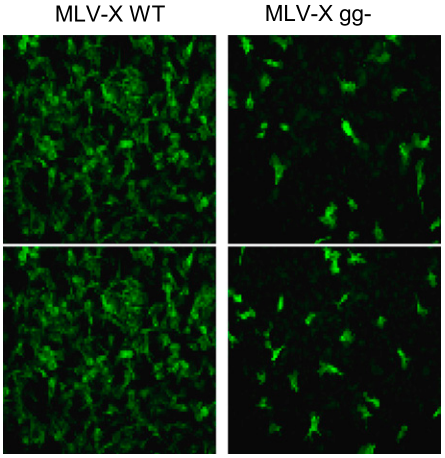

Fig. S1. gPr80 is required for optimal infectivity of MLV. MLV-X restricted to a single cycle of replication produced from JTAg cells and normalized for RT activity was inoculated onto HT10180 cells. The figure shows immunofluorescence staining of infected target cells performed 48 h after infection by using an anti-RLV P30 antibody followed by Alexa-488 conjugated anti-goat antibodies.

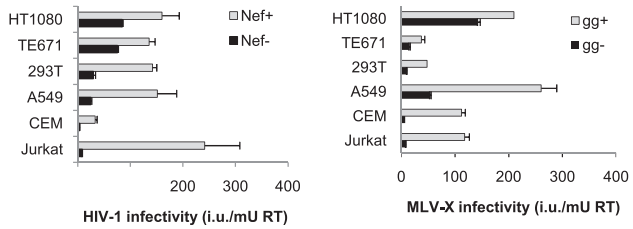

Fig. S2. Absolute infectivity of HIV-1 and MLV-X derived from different producer cell types. Results expressed as percentage of WT in Fig. 5*B* are here shown as absolute infectivity values.

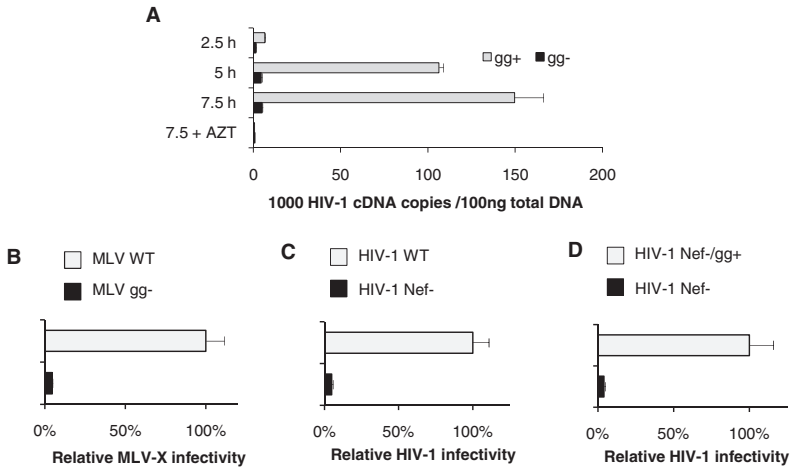

Fig. S3. gPr80 affects progression of HIV-1 reverse transcription. (*A*) gPr80 affects the steady-state levels of Nef-negative HIV-1 full-length viral cDNA, resembling the effect of Nef (Fig. 5*C*). (*B–D*) Relative infectivity of HIV-1 and MLV-X used in Fig. 5*C*. Viruses produced in JTAg cells and used in Fig. 5*C* and in *A* were serially diluted and inoculated onto HT1080 (*B*) or NP2-CD4/CXCR4 (*C* and *D*) respectively. Infected target cells were stained with anti RLV P30 anti HIV-1 p55/p2 and followed by Alexa-488 conjugated antibodies and quantified by scoring clusters of infected cells by fluorescence microscopy.

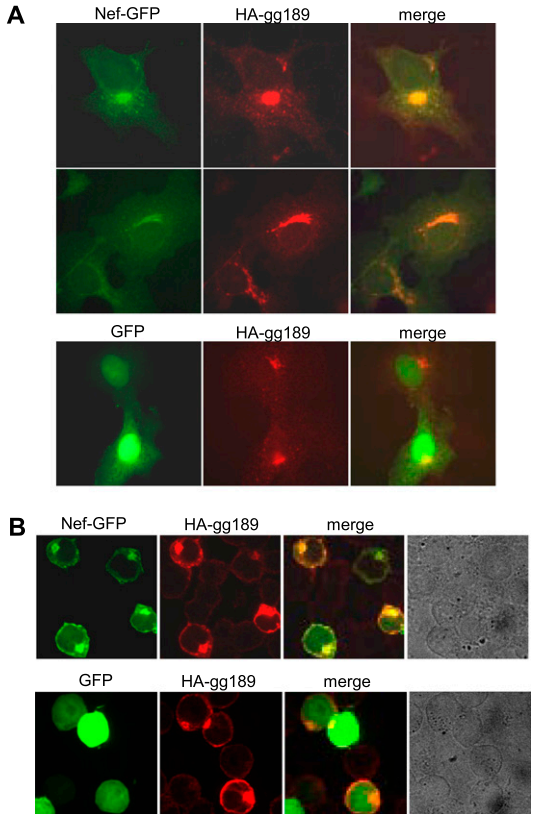

Fig. S4. Glycogag and Nef colocalize. Fluorescence microscopy shows colocalization of HA-gg189 with Nef-GFP but not with untagged GFP in Cos-7 cells (*A*) and in Jurkat TAg cells (*B*). Cells are cotransfected with vectors encoding HA-gg189 and Nef-GFP or GFP. Colocalization was observed in every cell found to coexpress HA-gg189 and Nef-GFP.

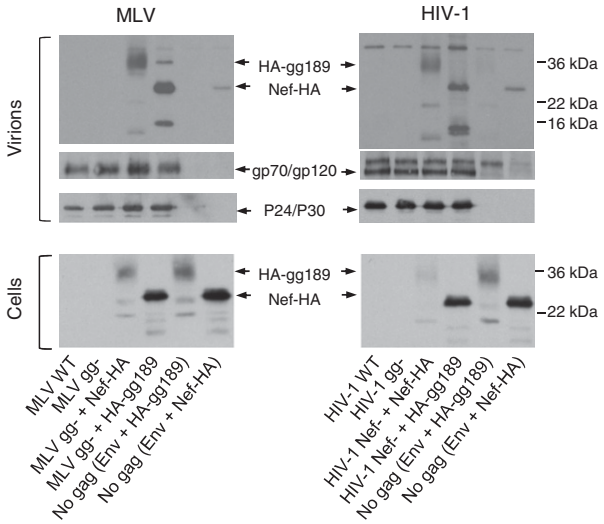

Fig. S5. Nef-HA and HA-gg189 are efficiently incorporated in to MLV and HIV-1 particles. Neither Nef nor glycogag affect the incorporation of Env on HIV-1 and MLV. Env defective MLV and HIV-1 NL4-3 provirus constructs were transfected into JTAg cells together with Env-expressing plasmids and vectors encoding Nef-HA or HA-gg189 as indicated. Virus supernatants were harvested 48 h after transfection and pelleted through a 25% sucrose cushion. Virus pellets and producer cell lysates were analyzed by SDS/PAGE followed by Western blotting using mouse anti-HA (HA.11; Covance), anti-HIV-1 p55/p2 and anti-HIV-1 gp120 ARP423 (National Biological Standards Board), anti-RLV P30 (Quality Biotech) and anti-β-actin (Sigma). No virus controls were obtained by replacing provirus constructs with empty vectors to provide evidence that Nef-HA and HA-gg189 are genuinely detected in virus particles.

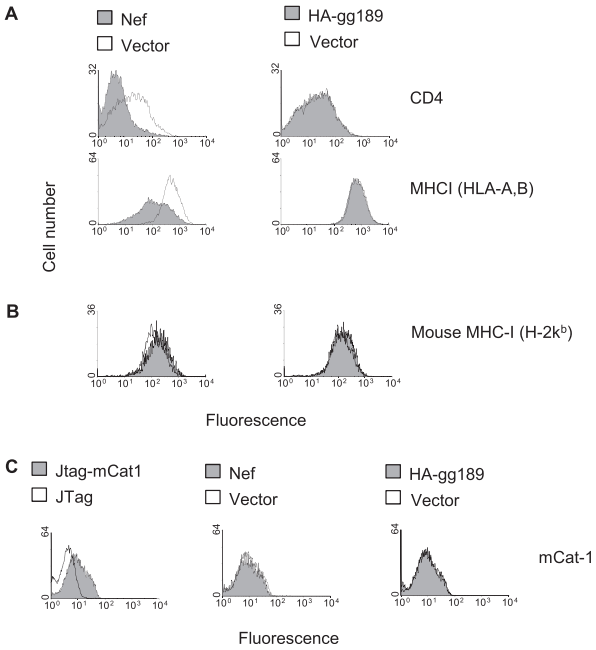

Fig. S6. Unlike Nef, glycogag does not down-regulate retrovirus receptors and MHC-I. (*A*) gPr80 does not affect cell surface expression levels of human CD4 and MHC-I in JTAg cells. (*B*) Neither gPr80 nor Nef affect surface levels of mouse MHC-I (H-2K^b) in MC57 mouse fibroblasts. Of note, the failure of HIV-1 Nef to down-regulate MHC-I in mouse cells has been reported (1) (*C*) Virus binding assay for detection of cell surface mCAT-1 on JTAg/mCat-1 cells (*SI Text*). (*Left*) The presence of mCat-1 on JTAg cells promotes virus binding detected by using an anti-SU antibody. (*Center* and *Right*) Nef and gPr80 expression in JTAG/mCat1 do not affect efficiency of SU binding and, therefore, of mCat-1 surface expression level.

1. Fleis R, Filzen T, Collins KL (2002) Species-specific effects of HIV-1 Nef-mediated MHC-I downmodulation. *Virology* 303:120–129.

Table S1. Properties and origin of the cell lines used in Fig. 1

Cell line	Origin	Comments	Source
C8166	Human T cell leukemia	Parental	Imperial College London
CEM	Acute lymphoblastic leukemia	Parental	University College London
CEM/A3.01	Acute lymphoblastic leukemia	Derivative of CEM, highly permissive to HIV-1 replication	NIH AIDS Research & Reference Reagent Program
CEM/A3.01-F7	Acute lymphoblastic leukemia	Derivative of CEM/A3.01, ectopic expression of CCR5	National Biological Standards Board
CEM-SS	Acute lymphoblastic leukemia	Derivative of CEM, highly susceptible for fusion with HIV	NIH AIDS Research & Reference Reagent Program
DG75/HAD	Burkitt's lymphoma	Parental, EBV-negative	Medical College of Wisconsin
DG75/UW	Burkitt's lymphoma	Parental, EBV-negative	Hadassah Medical Center
H9	Human T cell lymphoma	Derivative of HUT-78, highly permissive for HIV-1 replication	Imperial College London
HBS-2	Acute lymphoblastic leukemia	parental, CD4-negative	NIH AIDS Research & Reference Reagent Program
Jurkat D1.1	Acute T cell leukemia	Derivative of JM, CD4-negative	American Type Culture Collection
Jurkat E6.1	Acute T cell leukemia	Derivative of JM, high producers of IL-2	American Type Culture Collection
Jurkat J6	Acute T cell leukemia	Derivative of JM, high expression of CD3	London Research Institute
Jurkat JM	Acute T cell leukemia	Parental	NIH AIDS Research & Reference Reagent Program
Jurkat TAg	Acute T cell leukemia	Derivative of JM, express SV40 Large T antigen	Dana-Farber Cancer Institute
RAJI	Burkitt's lymphoma	Parental, EBV-positive	Imperial College London
Ramos	Burkitt's lymphoma	Parental, EBV-negative	Imperial College London
SupT1	Lymphoblastic leukemia	Parental	Imperial College London
U937	Histiocytic lymphoma	Parental	Imperial College London