



Published in final edited form as:

Nat Med. 2010 April ; 16(4): 446–451. doi:10.1038/nm.2109.

## HIV–1 Infects Multipotent Progenitor Cells Causing Cell Death and Establishing Latent Cellular Reservoirs

Christoph C. Carter<sup>1,2,\*\*</sup>, Adewunmi Onafuwa–Nuga<sup>3,\*\*</sup>, Lucy A. McNamara<sup>4,5</sup>, James Riddell IV<sup>3</sup>, Dale Bixby<sup>3</sup>, Michael R. Savona<sup>3,\*</sup>, and Kathleen L. Collins<sup>1,2,3,4,†</sup>

<sup>1</sup>Graduate Program in Cellular and Molecular Biology, University of Michigan, Ann Arbor, MI 48109 USA

<sup>2</sup>Medical Scientist Training Program, University of Michigan, Ann Arbor, MI 48109 USA

<sup>3</sup>Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109 USA

<sup>4</sup>Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI 48109 USA

<sup>5</sup>Department of Epidemiology, University of Michigan, Ann Arbor, MI 48109 USA

### Abstract

HIV causes a chronic infection characterized by depletion of CD4<sup>+</sup> T lymphocytes and development of opportunistic infections. Despite drugs that inhibit viral spread, HIV has been difficult to cure because of uncharacterized reservoirs of infected cells that are resistant to highly active antiretroviral therapy and the immune response. Here we used CD34<sup>+</sup> cells from infected people as well as *in vitro* studies of wild type HIV to demonstrate infection and killing of CD34<sup>+</sup> multipotent hematopoietic progenitor cells (HPCs). In some HPCs, we detected latent infection that stably persisted in cell culture until viral gene expression was activated by differentiation factors. A novel reporter HIV that directly detects latently infected cells *in vitro* confirmed the presence of distinct populations of active and latently infected HPCs. These findings have important implications for understanding HIV bone marrow pathology and the mechanisms by which HIV causes persistent infection.

### Introduction

Despite the host immune response and treatment with highly active antiretroviral therapy (HAART), HIV causes a persistent infection. Viral persistence is due in part to latent HIV reservoirs in resting CD4<sup>+</sup> T cells <sup>1</sup> that do not express viral proteins but can be induced to active infection by a variety of stimuli. However, recent studies of viral genetics have revealed that additional reservoirs likely exist <sup>2</sup>.

Users may view, print, copy, download and text and data- mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use: [http://www.nature.com/authors/editorial\\_policies/license.html#terms](http://www.nature.com/authors/editorial_policies/license.html#terms)

<sup>†</sup>Corresponding author: Phone: 734 615-1320, Fax: 734 -615-5252, [klcollin@umich.edu](mailto:klcollin@umich.edu).

\* current address: Division of Hematology, Blood & Bone Marrow Transplantation, San Antonio Military Medical Center, Department of Internal Medicine, University of Texas Health Science Center, San Antonio, TX 78236

\*\*These authors contributed equally

Hematopoietic progenitor cells (HPCs) have been considered as a possible reservoir, but it has been difficult to establish that these cells are infected by HIV <sup>3-6</sup> because HPCs are difficult to maintain in culture and indirect measurements of infection may be confounded by contamination with other cell types. Here, we utilized flow cytometry and recently developed culture conditions <sup>7</sup> that have allowed us to conclude that a proportion of HPCs become infected following exposure to HIV both *in vivo* and *in vitro*.

## RESULTS

### HIV infects HPCs

To assess the susceptibility of HPCs to HIV, intracellular Gag expression was examined using purified bone marrow (BM) CD34<sup>+</sup> cells treated with HIV 89.6 *Eenv*<sup>89.6</sup> (Fig. 1a). After 3 d in culture, 6% of CD34<sup>+</sup> HPCs expressed intracellular HIV Gag (Fig. 2a, middle right panel). Antiretroviral treatment blocked Gag expression (Fig. 2a, lower right panel) and experiments with five other HIVs yielded similar results (Supplementary Fig. 1a). As previously reported for HIV-infected T cells <sup>8,9</sup>, infected CD34<sup>+</sup> cells downmodulated MHC-I (Fig. 2b).

HPCs are a heterogeneous collection of cells that include multipotent HPCs and stem cells (HSCs). Multipotent HPCs have a Lin<sup>-</sup>CD34<sup>+</sup>CD133<sup>+</sup>CD38<sup>-</sup> surface phenotype, where “Lin” represents markers of specific hematopoietic lineages. Following treatment with wild type HIV 89.6 (Fig. 1b), both Lin<sup>+</sup> and Lin<sup>-</sup> cells expressed intracellular Gag (Fig. 2c).

### HIV is cytotoxic to infected HPCs

A time course analysis revealed that Gag<sup>+</sup> cells were lost rapidly in culture (Fig. 2d and Supplementary Fig. 1b). Moreover, infected cells displayed increased annexin V reactivity (Fig. 2e) and a high fraction of Gag<sup>+</sup> cells had light scatter properties of dead cells (Supplementary Fig. 1c). Cell death required active viral gene expression as transduction of the cells with a reporter virus (Fig. 1c) pseudotyped with an HIV envelope did not result in cell loss unless the HIV LTR actively expressed HIV genes (Supplementary Fig. 1d).

**Multipotent HPCs are susceptible to HIV infection**—To assess the developmental capacity of infected HPCs, we used a minimal HIV genome (HIV-7SF-GFP, Fig. 1d) pseudotyped with 89.6 Env, which “tagged” infected cells without causing cell death. Using this system, we found that a proportion of CD34<sup>+</sup> cells were infected (GFP<sup>+</sup>) [1 to 6% in replicate experiments (e.g. Fig. 3a; **initial sort purity shown in** Supplementary Fig. 2a)] and a more primitive subset of these cells (CD34<sup>+</sup>CD38<sup>-</sup>CD133<sup>+</sup>) had a similar infection rate (Fig. 3b). Infection of CD133<sup>+</sup> HPCs purified from BM yielded similar results (Supplementary Fig. 2f and g). These infection rates were comparable to the fraction of CD34<sup>+</sup> cells expressing both HIV co-receptors (Supplementary Fig. 3a and b).

CD133<sup>+</sup> HPCs from UCB infected with HIV-7SF-GFP<sup>env</sup><sup>89.6</sup> generated GFP<sup>+</sup> colonies of erythroid (CFU-E), myeloid (CFU-M and CFU-GM) and multi-lineage (CFU-GEMM) origin, demonstrating that HIV can infect multipotent HPCs (Fig. 3c). Quantitation revealed similar numbers of total colonies from uninfected and infected cells (Fig. 3d). Similar results

were obtained using a full-length HIV reporter (89.6-SI E-SF-GFP, Fig. 1e) that did not express HIV genes because of an LTR mutation (Fig. 3e,f).

**Induction of latent HIV from infected HPCs**—To assess latent infection, we asked whether induction of differentiation induced viral gene expression. Indeed, BM-derived HPCs (99.5% CD34<sup>+</sup>, Supplementary Fig. 2b) infected with replication defective [HIV HXB-ePLAPenv<sup>VSV-G</sup> (Fig. 1f)] and treated with PMA expressed the reporter in 12-fold more cells (Fig. 4a) and produced more viral particles (Supplementary Fig. 4a) than the control. BM immunodepleted for CD34<sup>+</sup> cells were not viable under these conditions (Supplementary Fig. 4b).

We found similar numbers of integrated genomes plus or minus PMA (Fig. 4b), indicating that PMA-induced gene expression was not due to effects on integration. Consistent with these results, the integrase inhibitor raltegravir blocked initial infection but not PMA-induced gene expression (Supplementary Fig. 5). Similar results were obtained using HXB-ePLAPenv<sup>89.6</sup>, albeit with lower infection rates (Fig. 4c).

Using wild type HIV-89.6 (Fig. 1b), we infected purified BM-derived HPCs (98% CD34<sup>+</sup>, Supplementary Fig. 2d) and cultured them plus or minus GM-CSF and TNF- $\alpha$  to induce myeloid differentiation<sup>10</sup>. GM-CSF-TNF- $\alpha$ -treatment of infected HPCs resulted in rapid release of HIV into the culture supernatant (Fig. 4d). In contrast, BMMCs immunodepleted for CD34<sup>+</sup> cells did not release HIV (Fig. 4d) and rapidly died (Supplementary Fig. 4 b, c, d and f). Flow cytometric analysis of the cells confirmed that GM-CSF-TNF- $\alpha$  stimulated intracellular HIV Gag expression (Fig. 4e) and that cells cultured in GM-CSF-TNF- $\alpha$  acquired myeloid markers (CD83<sup>+</sup>) (Fig. 4f).

To assess the stability of latent HIV in HPCs, we infected CD34<sup>+</sup> BM-derived HPCs (99% pure, Supplementary Fig. 2e) with wild type 89.6. After 7 d, when the culture was uniformly Gag-negative, GM-CSF-TNF- $\alpha$  was added to half the culture. GM-CSF-TNF- $\alpha$  resulted in a resurgence of HIV gene expression compared with the untreated culture (Fig. 4g, h). 89.6 is a dual tropic HIV that can utilize both CCR5 and CXCR4 to enter cells. Similar results were obtained with a wild type virus that only uses CXCR4 although, as expected, there was less viral spread in the differentiated myeloid cells (Supplementary Fig. 6b). Spread of infection in the culture was inhibited by antiretrovirals and supernatant from infected cells could be used to infect T cell lines (Supplementary Fig. 6).

**Direct detection of latency**—To detect latent infection *in situ* without inducing changes in the infected cells, we developed a novel latency reporter virus [89.6- E-SF-GFP (Fig. 1c)] that expresses GFP independently of the HIV LTR. Infection of T cells with 89.6- E-SF-GFPenv<sup>89.6</sup> yields some cells expressing Gag and others expressing only GFP (Fig. 5a). To confirm that GFP<sup>+</sup>Gag<sup>-</sup> cells were latently infected, we demonstrated that CD4 downmodulation, which occurs only when HIV Nef, Vpu or Env is expressed,<sup>11</sup> occurred in Gag<sup>+</sup> but not GFP<sup>+</sup>Gag<sup>-</sup> cells (Fig. 5a). In contrast, when cells were infected with a virus that expressed GFP from the HIV LTR (89.6- E-IRES-GFP, Fig. 1g), GFP-expressing cells downmodulated CD4 (Fig. 5a). Similar results were obtained using peripheral blood mononuclear cells (PBMCs) infected with 89.6- E-SF-GFPenv<sup>89.6</sup> (Fig. 5b). Moreover,

PMA and ionomycin treatment of Jurkat cells infected with the reporter virus increased Gag<sup>+</sup> cell frequency and reduced GFP<sup>+</sup>Gag<sup>-</sup> cell frequency (Fig. 5c).

We observed separate populations of Gag<sup>+</sup> and GFP<sup>+</sup> cells in UCB-derived CD34<sup>+</sup> HPCs infected with the latency reporter virus, indicating that active and latent infection occurred in this cell type (Fig. 5d). In culture, the Gag<sup>+</sup> cells were rapidly lost whereas the GFP<sup>+</sup>Gag<sup>-</sup> cells persisted at least 20 d (Fig. 5e, Supplementary Fig 1d). Analysis of these cells revealed that many had a cell surface phenotype consistent with primitive HPCs (CD34<sup>+</sup>Lin<sup>-</sup> or CD34<sup>+</sup>CD38<sup>-</sup>) (Fig. 5f).

### CD34<sup>+</sup> cells from donors with HIV are Gag<sup>+</sup>

We obtained samples from HIV-infected people (Supplementary Table 1) and found that we could detect Gag<sup>+</sup>CD34<sup>+</sup> cells in three of seven freshly isolated samples (Supplementary Table 1, Fig. 6a, **bottom right panel**). When the cells were cultured in GM-CSF-TNF- $\alpha$ , Gag expression could be detected in samples from all seven donors (Fig. 6b,c). In contrast, donor BMMCs specifically depleted of CD34<sup>+</sup> cells did not express Gag after culturing (Fig. 6c, d). The addition of the anti-HIV drug raltegravir [which inhibits new *in vitro* infection in T cells (Supplementary Fig. 7)] partially suppressed the induction of Gag<sup>+</sup> cells (Fig 6c, d), confirming that a component of the infection we observed was from viral spread. Similar results were also obtained from a donor (number seven) who had undetectable viral loads for 2 years (Supplementary Table 1, Fig. 6e).

### CD34<sup>+</sup> cells from HIV<sup>+</sup> donors with undetectable viral loads contain HIV genomic DNA

Using a real-time PCR assay for integrated HIV DNA, we detected viral genomes in freshly isolated CD34<sup>+</sup> cells from four of nine donors with undetectable viral loads on HAART for more than 6 months (44%) (Fig 6f, g). In these donors, 40 (donor 7), 3.1 (donor 12), 39 (donor 14), and 2.5 (donor 15) HIV genomes per 10,000 CD34<sup>+</sup> cells were detected. We detected HIV genomes in BMMCs immunodepleted of CD34<sup>+</sup> cells only for donor 12, for whom 1.2 HIV genomes per 10,000 CD34<sup>-</sup> cells were detected. The limit of detection for this assay varied by donor, but was approximately 1 genome per 10,000 cells due to the limited number of CD34<sup>+</sup> cells obtained from each donor. Thus, it is likely that the proportion of donors in which we detected HIV genomes underestimates the percentage of HIV<sup>+</sup>, HAART-treated individuals harboring integrated HIV genomes in CD34<sup>+</sup> cells.

## DISCUSSION

Long-lived cellular reservoirs of latent HIV genomes are a critical obstacle to viral eradication. Here, we demonstrate that HIV can infect hematopoietic progenitor cells *in vivo* and *in vitro* to cause an active, cytotoxic infection as well as a latent infection that can be induced to active infection by cytokine treatment.

Our finding that HIV infects HPCs with an immature phenotype, has clear ramifications for HIV disease because some of these cells may be long-lived and could carry latent HIV for extended periods of time. While further studies are needed to demonstrate that CD34<sup>+</sup> stem cells *per se* are infected, the detection of HIV genomes in HPCs isolated from people effectively treated with HAART for more than 6 months confirms that HIV targets some

long-lived HPCs. One might expect these results to predict the presence of identifiable proviral records in differentiated lineages that are known not to be susceptible. However, we show that actively infected HPCs are rapidly killed. Therefore, we expect latently infected HPCs will be killed by viral activation shortly after differentiation is induced.

Further studies are now needed to demonstrate that residual circulating virus in individuals on HAART is derived in part from HPCs, as previously demonstrated for resting memory T cells<sup>2</sup>. Additionally, studies examining the factors influencing HIV infection and latency in CD34<sup>+</sup> cells, as well as limiting dilution experiments to determine the fraction of proviral genomes in these cells that can be reactivated, would further our understanding of this viral reservoir.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

Funded by NIH grant RO1 AI051192, MO1-RR000042, the Burroughs Wellcome Foundation, University of Michigan Molecular Mechanisms in Microbial Pathogenesis Training Grant (CCC), Rackham Predoctoral Fellowship (CCC), a National Science Foundation predoctoral fellowship (LAM), and a Bernard Maas Fellowship (LAM). We thank C. van de Ven for umbilical cord blood and C. McIntyre-Ramm for assistance with human studies. The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: p89.6 from R. G. Collman (University of Pennsylvania), pNL4-3 from M. Martin (Viral Pathogenesis and Vaccine Section at NIAID), p94UG-114.1 and pYU-2 from B. Hahn (University of Alabama at Birmingham), and pMJ4 from T. Ndung'u (Nelson Mandela School of Medicine), B. Renjifo (Harvard School of Public Health), and M. Essex (Harvard School of Public Health). pCMV-G was from N. Hopkins, (Massachusetts Institute of Technology), pCMV-HIV-1 and pHIV-7/SF-GFP were from Shin-Jiing-Kuan Yee (City of Hope National Medical Center), and pEGFP-N2-LAMP1 was from Norma Andrews (Yale University).

C.C.C., L.A.M. and A.N. conducted in vitro experiments and data analysis and assisted with writing the manuscript

J.R. assisted with human subjects

M.R.S. and D.B. obtained bone marrow aspirates and assisted with human subjects

K.L.C. supervised the project and wrote the manuscript

## Appendix

### METHODS

#### Antibodies

CD4: OKT4 conjugated to FITC (Fluorotag, Sigma-Aldrich); CD34: FITC-conjugated (BD Biosciences) or APC-conjugated (Caltag); CD38: PE-Cy7-conjugated (eBioscience); CD133: PE-conjugated (Miltenyi); CCR5: PE-conjugated (eBioscience); CXCR4: PE-conjugated (eBioscience); Gag: clone KC57 conjugated to FITC or PE (Coulter); lineage markers: FITC-conjugated (BD Biosciences) or lineage cocktail (BD Biosciences) conjugated to biotin (EZ Link Sulfo-NHS-Biotin, Pierce Biotechnology) or PE-Cy5.5 (Lightning Link PE-Cy5.5, Innova Biosciences); PLAP (serotec); goat antibody to mouse immunoglobulin: PE-conjugated (Invitrogen).

## Plasmid constructs

p89.6 E was generated by dropping out a BsaBI–StuI fragment from p89.6. The 89.6 *env* expression vector pCDNA–89env was created by cloning the HindIII and EcoRV fragment into pcDNA3.1(+).

To construct p89.6– E–SF–GFP, a BamH–KpnI fragment containing the SFFV promoter from pHIV–7/SF–GFP was sub–cloned into pCDNA3.1(+), replacing the original CMV promoter. A BamHI–XbaI fragment from pEGFP–N2–LAMP1 was inserted downstream of the promoter. Next, a *nef*<sup>89.6</sup> PCR product was ligated into MfeI cut pSFDNA–EGFP, generating pNef–SFFV–EGFP. Then a PCR product generated using the primers: 5′–CACCATTATCGTTTCAGACCCT–3′ and 5′–

TCTCGAGTTTAAACTTATAGCAAAGCCCTTTCCA–3′ with p89.6 as a template was ligated into p89.6 (p89.6 NE). Finally, a PmeI fragment from pNef–SFFV–EGFP was cloned into PmeI–cut p89.6 NE.

Standard PCR mutagenesis approaches were used to delete the 3′ U3 region of the viral LTR of p89.6– E–GFP, analogous to those previously described<sup>15</sup>. The resulting U3 deletion eliminates all consensus AP–1, NF–AT, NF–κB SP–1 and TATA motifs, but maintains the polyA site found in the R region.

## Cell culture

Fresh whole bone marrow aspirates were obtained commercially (AllCells Ltd.). Pre-existing umbilical cord blood lacking patient identifiers was obtained following scheduled cesarean section procedures. Bone marrow mononuclear cells (BMMCs) and cord blood mononuclear cells (UCB) were prepared by density separation using Ficoll–Paque (GE healthcare) according to the manufacturer’s instructions.

CD34<sup>+</sup> cells were prepared from adherence depleted mononuclear cells using commercially available kits [EasySep CD34 positive selection kit, StemCell Technologies and CD34 MACS positive selection, Miltenyi, biotin selection kit with CD34–biotin antibody (Invitrogen)] according to the manufacturer’s instructions. CD133<sup>+</sup> cells were isolated similarly, using CD133 positive selection MACS (Miltenyi).

Following isolation, CD34<sup>+</sup> cells were maintained in StemSpan (StemCell Technologies) or Stemline (Sigma–Aldrich) medium supplemented with CC110 (StemCell Technologies) cytokine cocktail (100 ng ml<sup>–1</sup> SCF, 100 ng ml<sup>–1</sup> Flt3–L and 100 ng ml<sup>–1</sup> TPO). To expand immature progenitor cells for colony formation assays, media was supplemented with STIF cytokine cocktail (CC110 plus 100 ng ml<sup>–1</sup> IGFBP–2<sup>7</sup>). For chemical stimulation, washed cells were incubated with 10 ng ml<sup>–1</sup> PMA (Sigma–Aldrich) in DMEM supplemented with 10% FBS as described elsewhere<sup>16,17</sup>. For cytokine stimulation of HPCs, washed cells were cultured with 100 ng ml<sup>–1</sup> rhGM–CSF (R&D systems) and 2.5 ng ml<sup>–1</sup> TNF–α (Biolegend).

Methylcellulose colony forming assays were conducted according to the manufacturer’s recommendation (Methocult H4034, StemCell Technologies).



## HIV preparation

Infectious supernatants were prepared by transfection of 293T cells using polyethylenimine<sup>17</sup>. For pseudotyped and internal promoter viruses, supernatants were concentrated using high-molecular weight polyethylene glycol (PEG) precipitation<sup>18</sup>. Pellets were resuspended in StemSpan medium and MOIs were calculated using  $MOI = -\ln(1-p)$  where  $p$  is the proportion of CEM T cells infected. HIV infections were conducted using a standard spin infection technique for primary cells.

## Flow cytometry

Cells were stained in FACS buffer (2% FBS, 1% human serum, 2 mM HEPES, 0.025% NaN<sub>3</sub> and PBS) for 20 min on ice, washed and fixed in 2% paraformaldehyde and PBS. For intracellular Gag staining, cells were then incubated for 5 min in 0.1% Triton-X100 in PBS at room temperature. Washed cells were incubated with antibody to Gag in FACS buffer for 30 min on ice and analyzed on a FACScan or FACSCanto flow cytometer. Cell sorting was performed using a FACSVantage SE in Normal-R mode with a sorted drop envelope of 1.0.

## qPCR integration assay

Genomic DNA was isolated with Qiagen DNeasy Blood and Tissue Kit. Assays were similar to those previously described [25]. One primer was modified to utilize a more conserved sequence (2<sup>nd</sup>-LTR-F-univ, GTGTIGAAAATCTCTAGCAGTGGC). Copy number per sample was calculated using a standard curve based on ACH-2 cell DNA<sup>19</sup>.

## Isolation of CD34<sup>+</sup> cells from HIV-infected donors

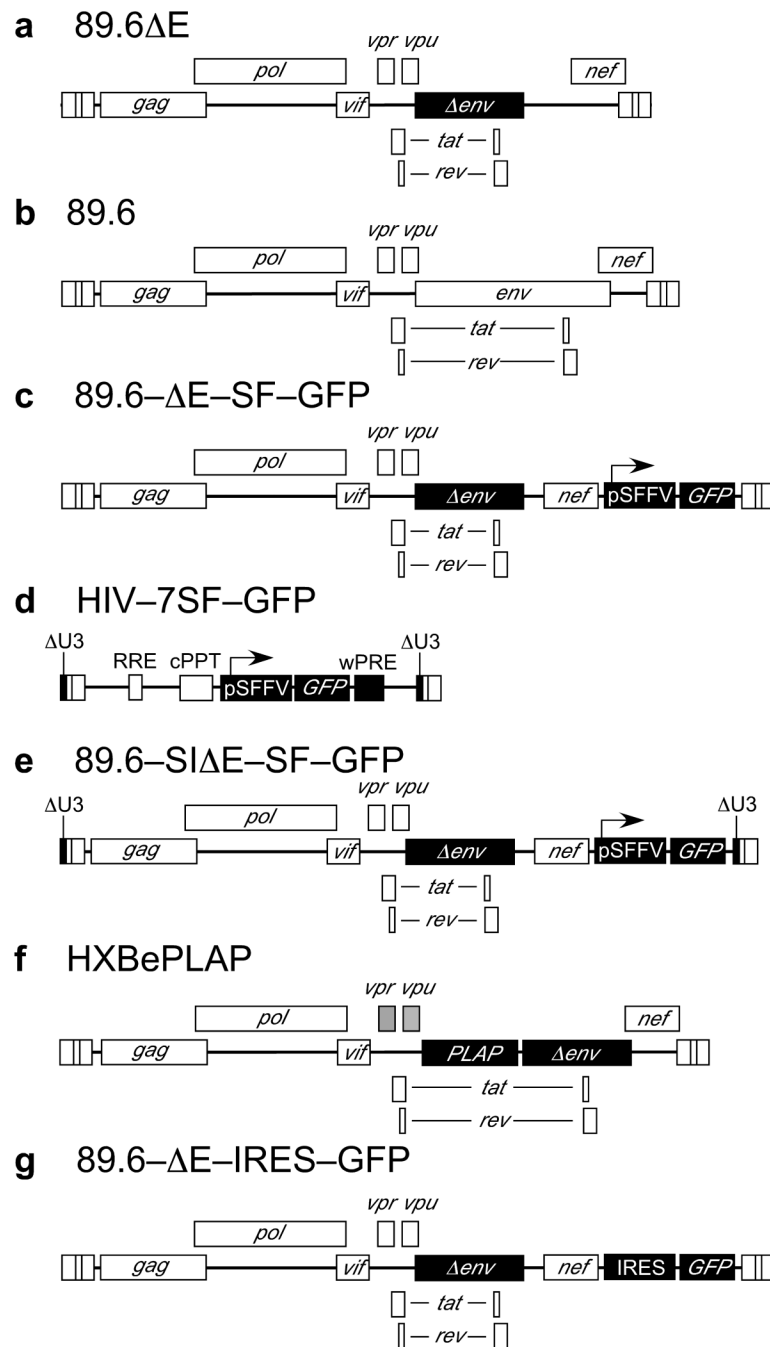
HIV<sup>+</sup> individuals were recruited from The University of Michigan HIV-AIDS Clinic and informed consent was obtained according to a University of Michigan IRB-approved protocol. 10 ml of marrow was obtained in preservative-free heparin. CD34<sup>+</sup> cells were isolated as described above.

## REFERENCES

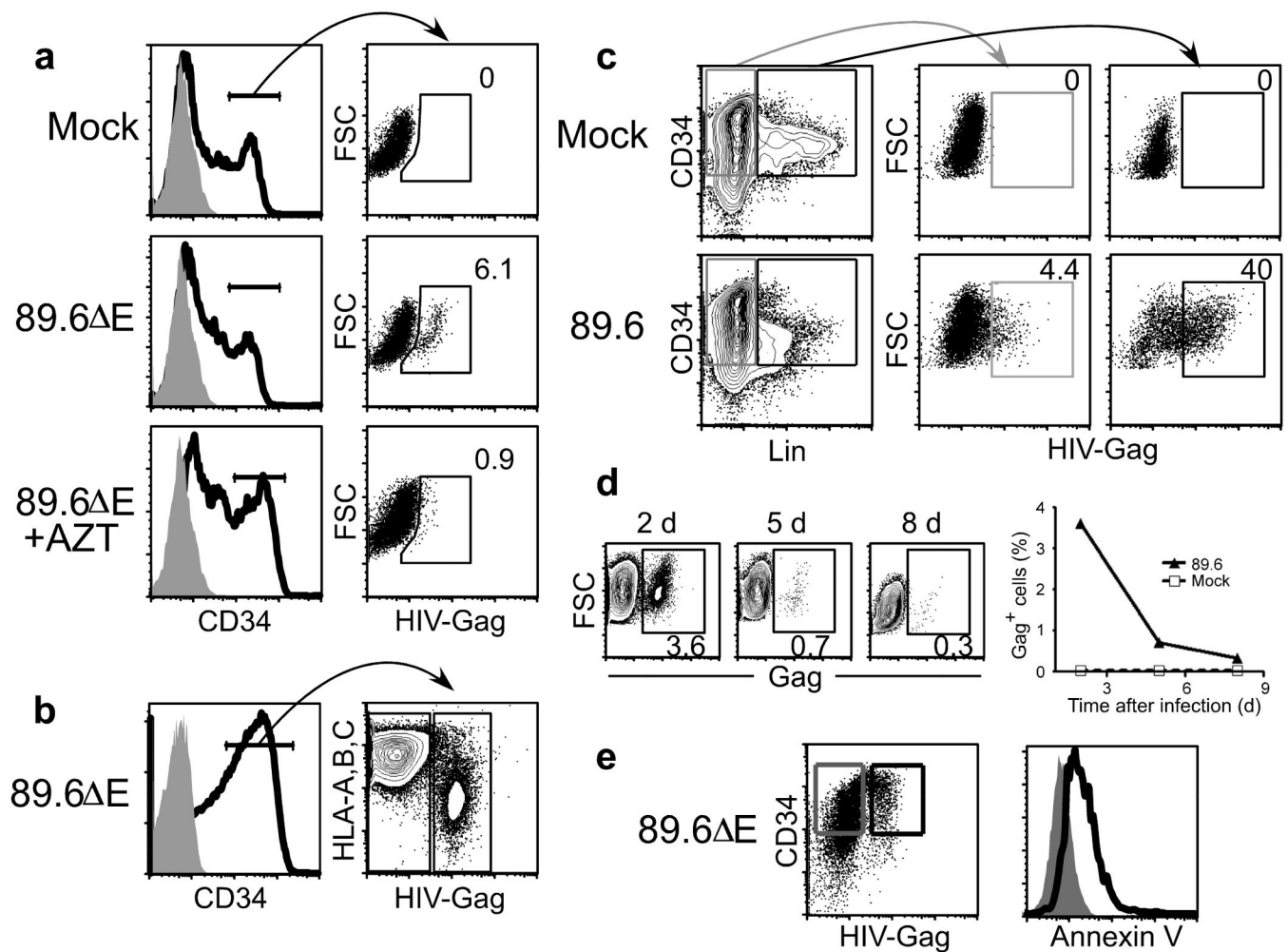
1. Bukrinsky MI, Stanwick TL, Dempsey MP, Stevenson M. Quiescent T lymphocytes as an inducible virus reservoir in HIV-1 infection. *Science*. 1991; 254:423-427. [PubMed: 1925601]
2. Bailey JR, et al. Residual human immunodeficiency virus type 1 viremia in some patients on antiretroviral therapy is dominated by a small number of invariant clones rarely found in circulating CD4<sup>+</sup> T cells. *J Virol*. 2006; 80:6441-6457. [PubMed: 16775332]
3. Neal TF, et al. CD34<sup>+</sup> progenitor cells from asymptomatic patients are not a major reservoir for human immunodeficiency virus-1. *Blood*. 1995; 86:1749-1756. [PubMed: 7544640]
4. Weichold FF, et al. Neither human immunodeficiency virus-1 (HIV-1) nor HIV-2 infects most-primitive human hematopoietic stem cells as assessed in long-term bone marrow cultures. *Blood*. 1998; 91:907-915. [PubMed: 9446651]
5. Majka M, et al. Bone marrow CD34(+) cells and megakaryoblasts secrete beta-chemokines that block infection of hematopoietic cells by M-tropic R5 HIV. *J Clin Invest*. 1999; 104:1739-1749. [PubMed: 10606628]
6. Zhang J, Scadden DT, Crumpacker CS. Primitive hematopoietic cells resist HIV-1 infection via p21. *J Clin Invest*. 2007; 117:473-481. [PubMed: 17273559]
7. Zhang CC, Kaba M, Iizuka S, Huynh H, Lodish HF. Angiopoietin-like 5 and IGFBP2 stimulate ex vivo expansion of human cord blood hematopoietic stem cells as assayed by NOD/SCID transplantation. *Blood*. 2008; 111:3415-3423. [PubMed: 18202223]

8. Schwartz O, Marechal V, Le Gall S, Lemonnier F, Heard JM. Endocytosis of major histocompatibility complex class I molecules is induced by the HIV-1 Nef protein. *Nat Med.* 1996; 2:338–342. [PubMed: 8612235]
9. Collins KL, Chen BK, Kalams SA, Walker BD, Baltimore D. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. *Nature.* 1998; 391:397–401. [PubMed: 9450757]
10. Caux C, Dezutter-Dambuyant C, Schmitt D, Banchereau J. GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells. *Nature.* 1992; 360:258–261. [PubMed: 1279441]
11. Chen BK, Gandhi RT, Baltimore D. CD4 down-modulation during infection of human T cells with human immunodeficiency virus type 1 involves independent activities of vpu, env, and nef. *J Virol.* 1996; 70:6044–6053. [PubMed: 8709227]
12. Gasmı M, et al. Requirements for efficient production and transduction of human immunodeficiency virus type 1-based vectors. *J Virol.* 1999; 73:1828–1834. [PubMed: 9971760]
13. Yam PY, et al. Design of HIV vectors for efficient gene delivery into human hematopoietic cells. *Mol Ther.* 2002; 5:479–484. [PubMed: 11945076]
14. Yamamoto N, et al. Analysis of human immunodeficiency virus type 1 integration by using a specific, sensitive and quantitative assay based on real-time polymerase chain reaction. *Virus Genes.* 2006; 32:105–113. [PubMed: 16525741]
15. Zufferey R, et al. Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. *J Virol.* 1998; 72:9873–9880. [PubMed: 9811723]
16. Davis TA, et al. Phorbol esters induce differentiation of human CD34+ hemopoietic progenitors to dendritic cells: evidence for protein kinase C-mediated signaling. *J Immunol.* 1998; 160:3689–3697. [PubMed: 9558069]
17. Kuroda H, Kutner RH, Bazan NG, Reiser J. Simplified lentivirus vector production in protein-free media using polyethylenimine-mediated transfection. *J Virol Methods.* 2008
18. Kohno T, et al. A new improved method for the concentration of HIV-1 infective particles. *J Virol Methods.* 2002; 106:167–173. [PubMed: 12393147]
19. Clouse KA, et al. Monokine regulation of human immunodeficiency virus-1 expression in a chronically infected human T cell clone. *J Immunol.* 1989; 142:431–438. [PubMed: 2463307]



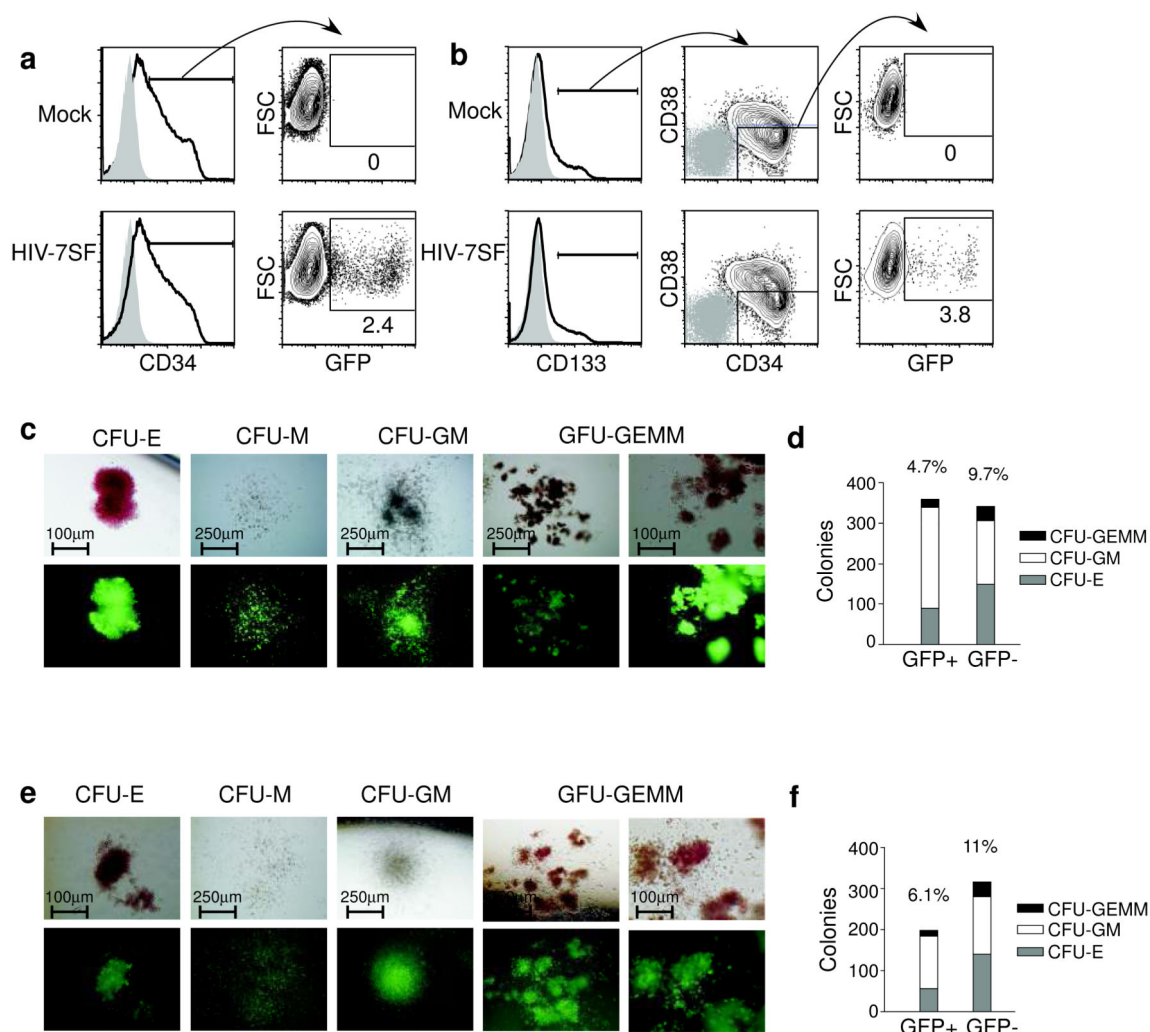
**Figure 1.**

HIV genomes. (a), (b), (c), (e) and (g) are derived from the molecular clone p89.6. (d) and (f) have been described elsewhere and are derived from HXB and NL4-3<sup>11,13</sup>. Expressed viral genes are shown in white, deletions and additions to the genome are shown in black, and non-functional genes are shaded in gray.



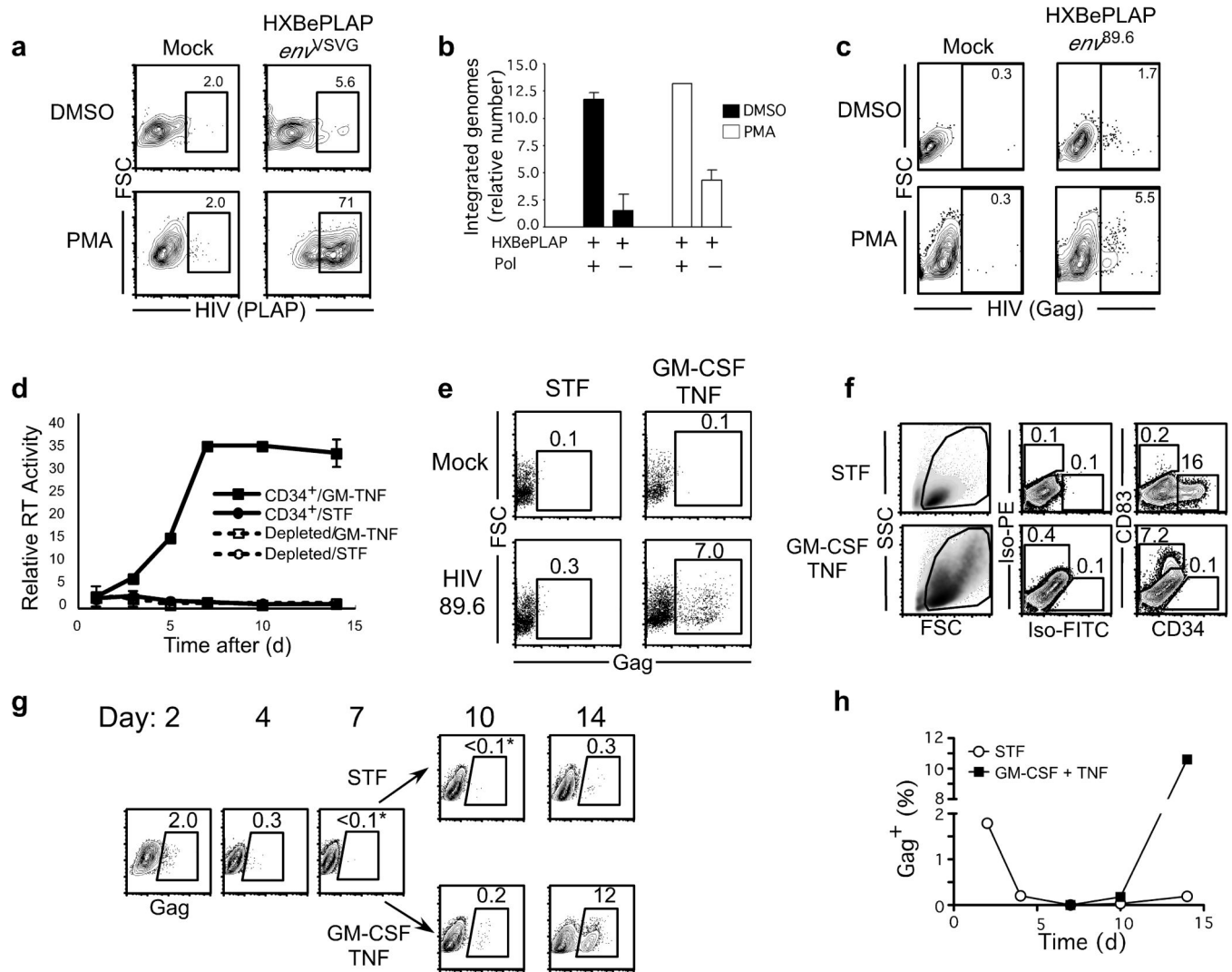
**Figure 2.**

HIV actively infects HPCs, leading to cell death. **(a)** Intracellular Gag in BM-derived CD34<sup>+</sup> HPCs infected with HIV 89.6 *Eenv*<sup>89.6</sup> (Fig. 1a) for 3 d. Gray histograms are isotype controls. **(b)** CD34, MHC-I and intracellular Gag expression in UCB-derived CD34<sup>+</sup> HPCs infected with 89.6 *Eenv*<sup>89.6</sup> for 48 h. **(c)** Gag, CD34 and Lin staining in BM-derived CD34<sup>+</sup> HPCs infected with 89.6. **(d)** Time course of intracellular Gag expression in UCB-derived CD34<sup>+</sup> HPCs infected with 89.6. **(e)** Annexin V reactivity in UCB-derived CD34<sup>+</sup> HPCs infected with 89.6 *Eenv*<sup>89.6</sup> for 48 h. The right panel shows CD34<sup>+</sup>Gag<sup>-</sup> (gray gate and histograms) and CD34<sup>+</sup>Gag<sup>+</sup> cells (black gate and histogram).



**Figure 3.**

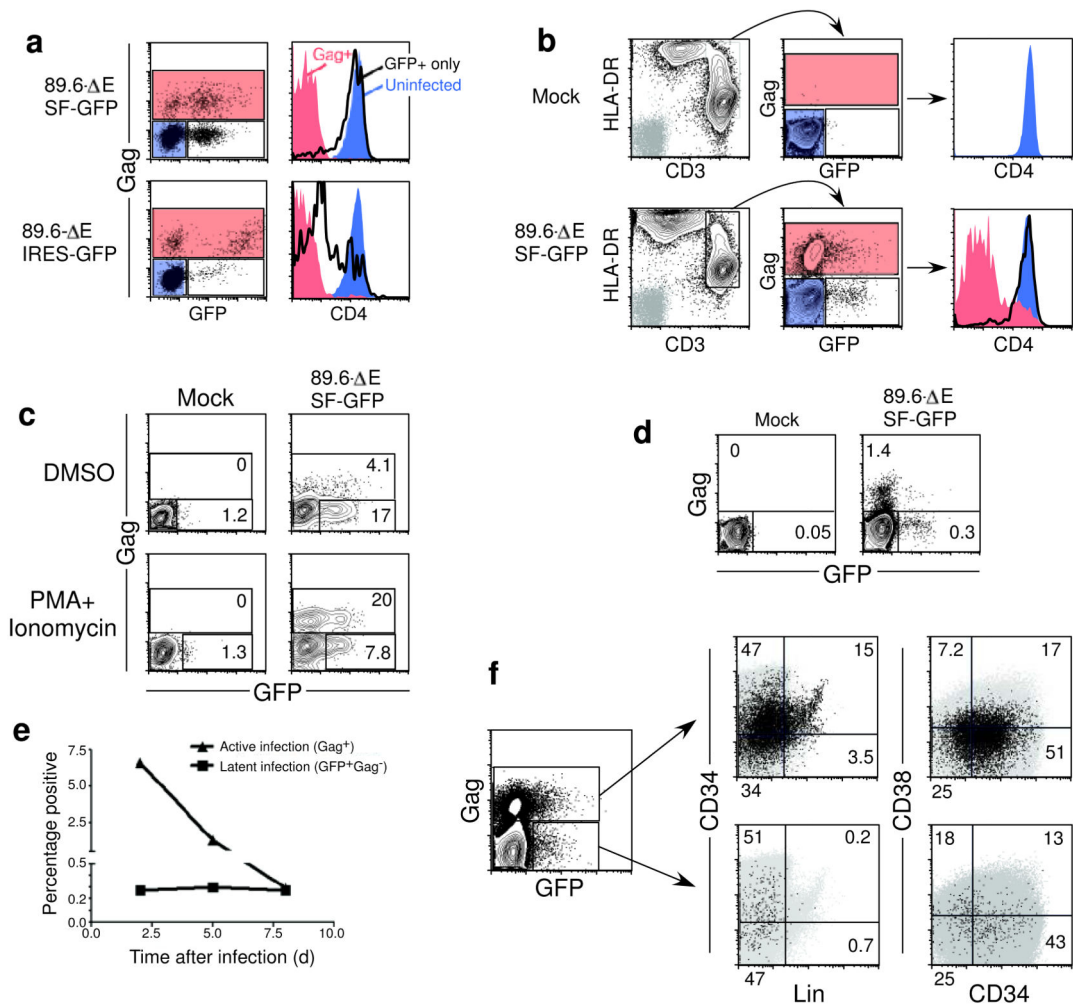
HIV infects multipotent HPCs. **(a)** GFP expression in CD34<sup>+</sup> UCB cells infected with HIV-7SF-GFP $\text{env}^{89.6}$  for 3 d. **(b)** The percentage of CD133<sup>+</sup>,CD34<sup>+</sup>,CD38<sup>-</sup> cells expressing GFP 3 d post-infection with HIV-7SF-GFP $\text{env}^{89.6}$ . Gray histograms and events represent isotype control staining. **(c)** and **(e)**, Colony formation by GFP<sup>+</sup>CD133<sup>+</sup> UCB-derived HPCs infected with HIV-7SF-GFP $\text{env}^{89.6}$  **(c)** or 89.6-SI E-GFP $\text{env}^{89.6}$  **(e)**. **(d)** and **(f)**, The relative number of colonies formed by equal numbers of sorted GFP<sup>+</sup> and GFP<sup>-</sup>, CD133<sup>+</sup> UCBs infected with HIV-7SF-GFP $\text{env}^{89.6}$  **(d)** or 89.6-SI E-GFP $\text{env}^{89.6}$  **(f)**. (erythroid (CFU-E), granulocyte-macrophage (CFU-GM), multi-lineage (CFU-GEMM)).

**Figure 4.**

Induction of HIV from latency. **(a)** The percentage of BM CD34<sup>+</sup> HPCs (See Supplementary Fig. 2b for initial cell purity) expressing an HIV marker gene (PLAP) following infection with HXB-ePLAP $env^{VSVG}$  plus or minus PMA. **(b)** qPCR<sup>14</sup> of integrated DNA from BM CD34<sup>+</sup> cells infected and cultured as in **(a)**. Pol-minus samples lack polymerase in the first round. Data are displayed as mean relative amount of integrated HIV DNA  $\pm$  standard deviation (sd),  $n = 3$ . **(c)** The percentage of BM-derived CD34<sup>+</sup> HPCs (Supplementary Fig. 2c) expressing intracellular Gag following infection with HXB-ePLAP $env^{89.6}$  plus or minus 10 ng ml<sup>-1</sup> PMA. **(d)** Reverse transcriptase activity of CD34<sup>+</sup> BM HPCs (Supplementary Fig. 2c) infected with HIV 89.6, plus or minus GM-CSF-TNF- $\alpha$ . The mean  $\pm$  sd,  $n = 3$  is shown. Control is BMMC immunodepleted for CD34. **(e)** Intracellular Gag expression 14 d post-infection for BM-derived HPCs infected and cultured as in **(d)**. **(f)** CD34 and CD83 expression (right panels) after 2 weeks in CC110 or GM-CSF-TNF- $\alpha$ . Isotype-matched controls are shown (Iso-FITC and Iso-PE). **(g)** Intracellular Gag expression for BM-derived HPCs (Supplementary Fig. 2e) infected with

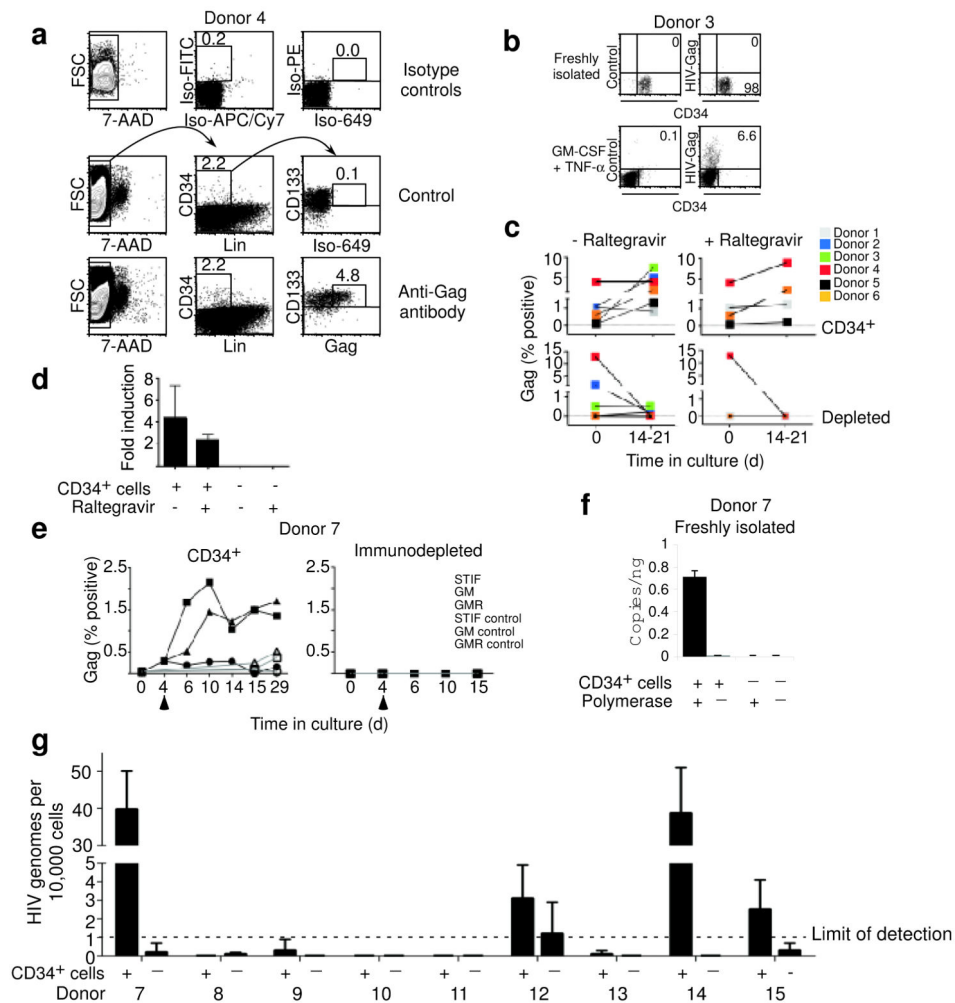
HIV 89.6 and cultured in CC110 cocktail. On day 7, the cells were divided either into CC110 cocktail or GM-CSF-TNF- $\alpha$ . Asterisks indicate Gag reactivity < mock treated cells.

**(h)** Graphical representation of the experiment depicted in part **(g)**.

**Figure 5.**

Active and latent infection in T cells and HPCs. **(a)** Gag, GFP and CD4 expression 7 d after infection in CEM-SS cells infected with 89.6- E-SF-GFP<sup>env</sup><sup>89.6</sup> or 89.6- E-IRES-GFP<sup>env</sup><sup>89.6</sup>. Histogram shading corresponds to cell gate. **(b)** Flow cytometric analysis of PHA-activated PBMC infected with 89.6- E-SF-GFP<sup>env</sup><sup>89.6</sup> for 48 h. The histogram is shaded to match the gated cells. In the left panel, the isotype control is shown in gray. **(c)** Flow cytometric analysis of Jurkat cells infected with 89.6- E-SF-GFP<sup>env</sup><sup>89.6</sup> for 7 d, then split into PMA and ionomycin or DMSO control for 48 h. **(d)** Flow cytometric analysis of UCB-derived CD34<sup>+</sup> HPCs infected with 89.6- E-SF-GFP<sup>env</sup><sup>89.6</sup> for 3 d. **(e)** Time course analysis of Gag<sup>+</sup> and Gag<sup>-</sup> GFP<sup>+</sup> UCB-derived CD34<sup>+</sup> HPCs infected as above and cultured in CC110. **(f)** Flow cytometric analysis of UCB-derived CD34<sup>+</sup> HPCs infected as above and cultured 3 d in CC110 medium. Gag<sup>+</sup> cells and Gag<sup>-</sup>GFP<sup>+</sup> cells were gated on the left plot and overlaid (black dots) on plots of CD34 vs. Lin (middle panels) or CD38 plots (right panels). The grey background shows the total population.



**Figure 6.**

Active and inducible infection in HPCs from HIV<sup>+</sup> people. **(a)** HIV-1 Gag expression in freshly isolated adherence-depleted Lin<sup>-</sup>CD34<sup>+</sup>CD133<sup>+</sup> BMMCs. The middle panel shows background staining using an isotype control for the Gag antibody only. **(b)** CD34 and intracellular Gag expression in CD34<sup>+</sup> cells stained immediately or after culturing (14 d). Control shows background staining with an isotype control antibody. **(c)** Gag expression before and after culturing in GM-CSF-TNF- $\alpha$  plus or minus raltegravir for donors 1-6. **(d)** Summary graph of Gag induction plus or minus raltegravir. Fold induction = (% Gag<sup>+</sup> in cultured cells) ÷ (initial % Gag<sup>+</sup>). Mean  $\pm$  sd is shown. **(e)** Intracellular Gag expression in Donor 7 CD34<sup>+</sup> or CD34-immunodepleted BMMCs cultured as described in c. [GM (GM-CSF-TNF- $\alpha$ ; GMR (GM-CSF-TNF- $\alpha$  plus raltegravir)]. **(f)** Real-time PCR of HIV genomes ng<sup>-1</sup> DNA isolated from fresh CD34<sup>+</sup> or immunodepleted BMMCs. Mean  $\pm$  sd is shown,  $n = 3$ . **(g)** Real-time PCR of HIV genomes from donor CD34<sup>+</sup> or immunodepleted cells. The limit of detection was approximately one HIV genome per 10,000 cells. Means  $\pm$  sd,  $n =$  two independent experiments with three replicates each.