Figure S1

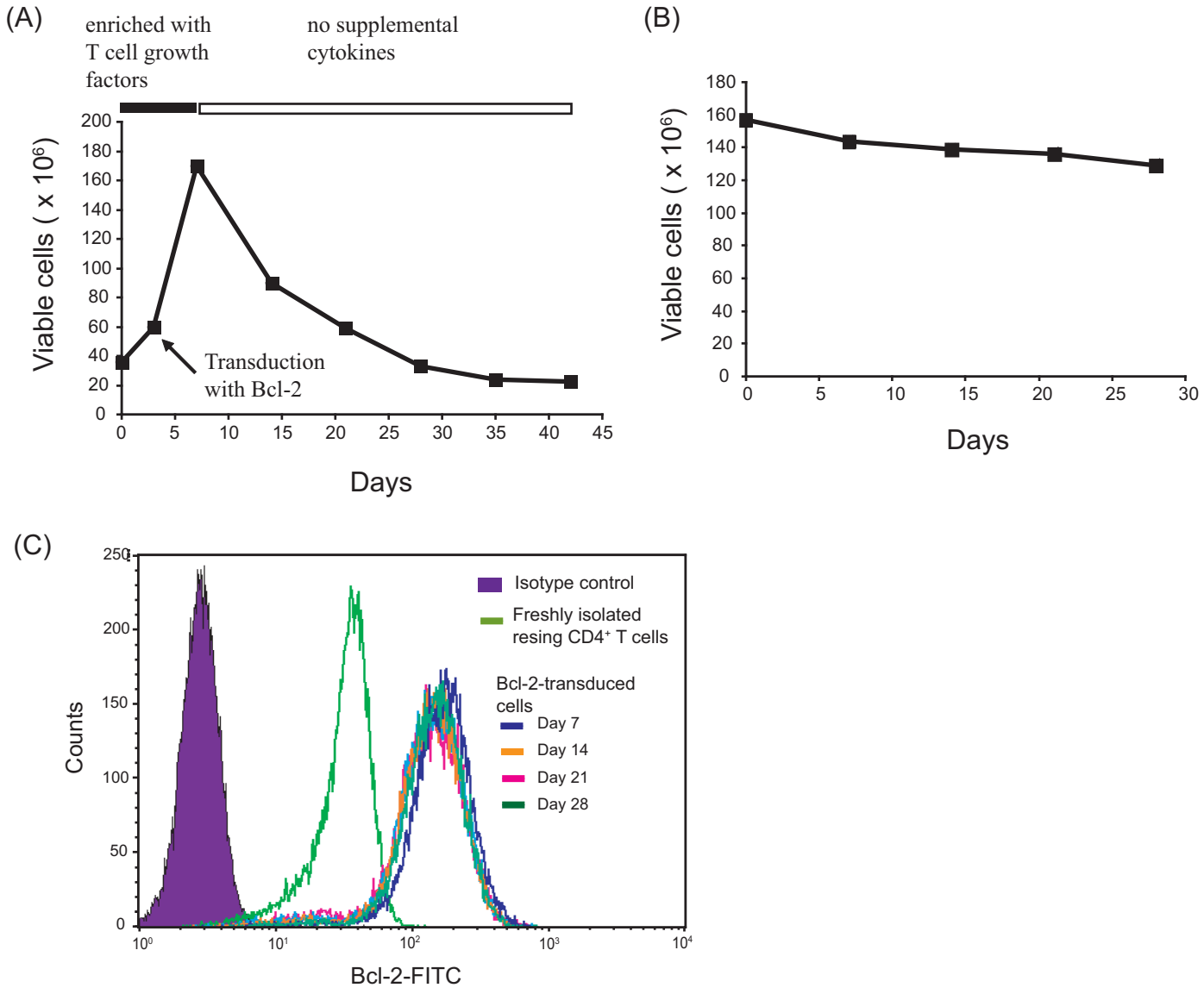

Figure S1.

The longevity and the stable expression of Bcl-2 of Bcl-2-transduced cells (A) The viability of primary $CD4^+$ T cells following transduction of Bcl-2. Freshly isolated $CD4^+$ T cells were activated with anti-CD3 and anti-CD28 antibodies and were grown in T cell growth factors-enriched medium. Three days following transduction, cells were maintained in the medium without any supplemental cytokines. The number of viable cells was measured weekly using tryptan blue exclusion method. (B) The viability of Bcl-2-transduced cells. The Bcl-2-transduced cells were generated and expanded as described in Methods. They were then maintained in the medium without any supplemental cytokines. The number of viable cells was counted weekly. (C) The expression levels of Bcl-2 in freshly isolated resting $CD4^+$ T cells and Bcl-2-transduced cells. The Bcl-2-transduced samples were weekly collected from the same cell population as (B). The Bcl-2-expression levels were determined using intracellular staining. For the purpose of comparison, the Bcl-2 expression levels of freshly isolated CD4+ T cells were also measured. For control, freshly isolated resting $CD4^+$ T cells were stained with an isotype-control antibody conjugated with FITC.

Figure S2

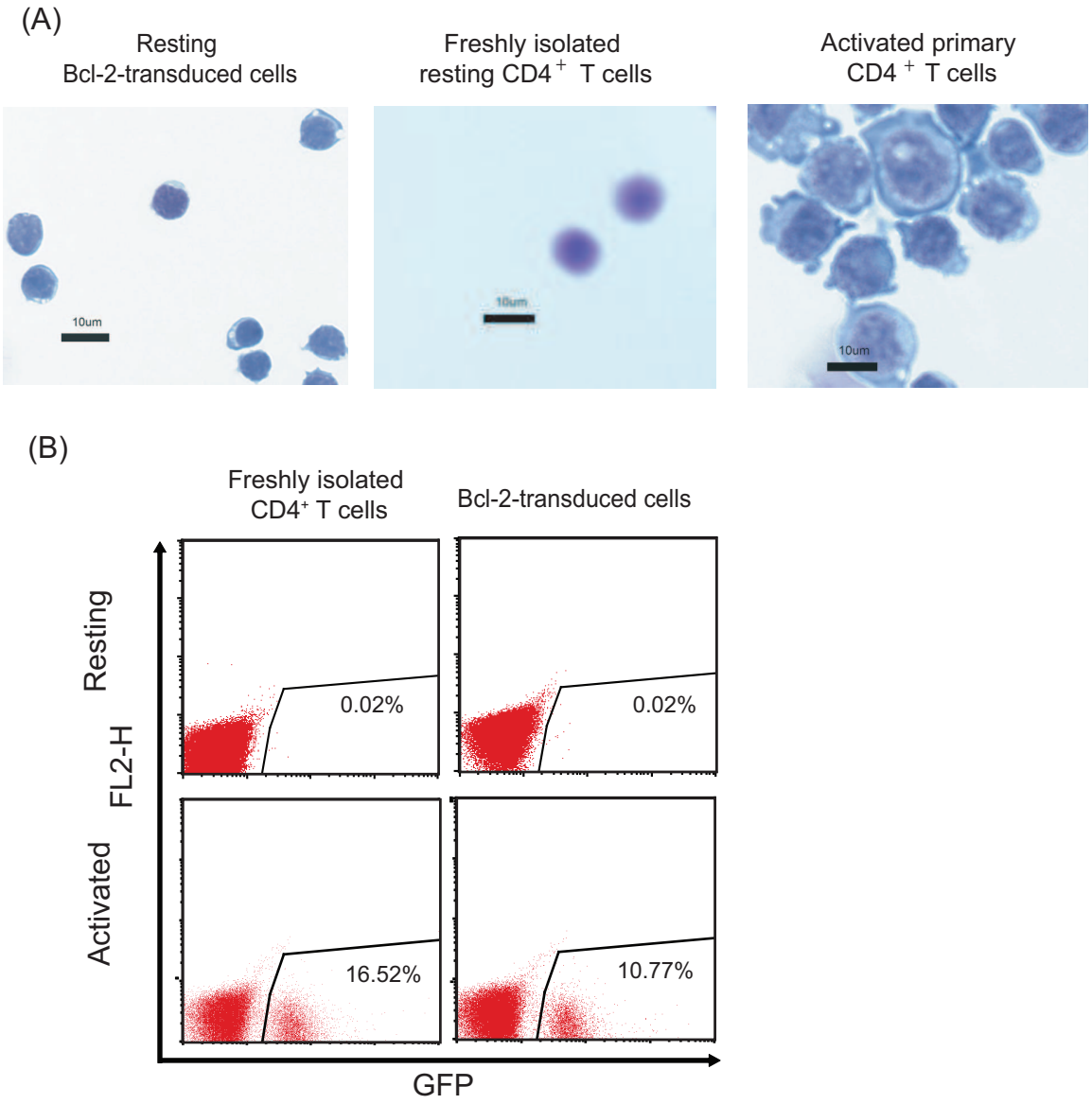

Figure S2. Additional characteristics of resting Bcl-2-transduced cells. (A) Morphology of resting Bcl-2-transduced cells, freshly isolated resting CD4$^+$ T cells, and activated primary CD4$^+$ T cells. Cells were stained with Hema 3* Wright-Giemsa Staining system (Fisher Healthcare, USA). Micrographs were taken with 400x power. Scale bar: 10 μm. (B) The susceptibility of activated andresting freshly isolated and Bcl-2-transduced CD4$^+$ T cells to HIV-1 infection. Cells were infected with reporter virus NL4-3-Δ6-drEGFP pseudotyped with VSV-G envelope. The number in each plot indicates the percentage of infected (GFP-positive) cells. The number in each plot indicates the frequency of infected cells (GFP-positive) cells.

Figure S3

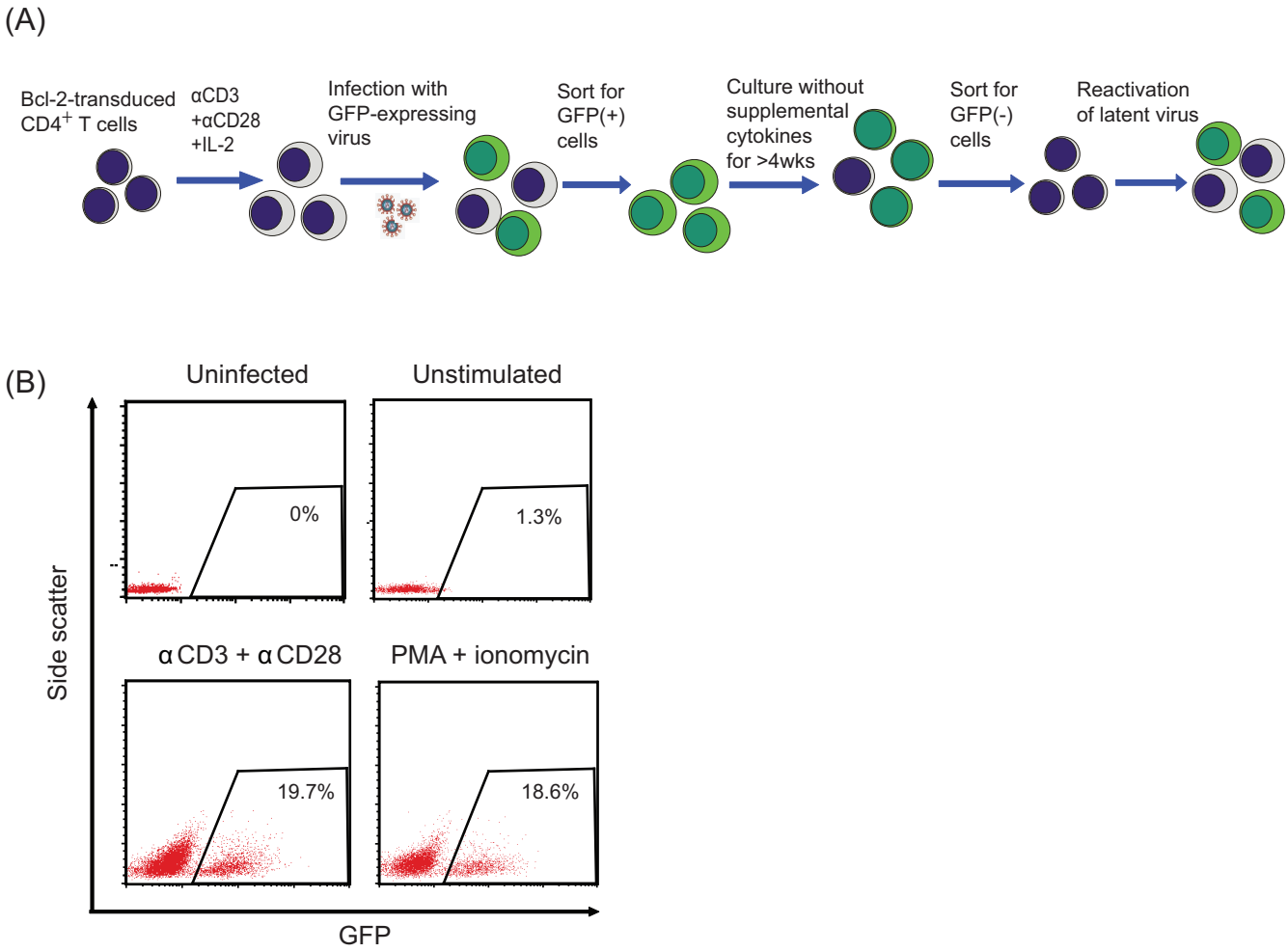

Figure S3. Establishment of *in vitro* HIV-1 latency in the Bcl-2-transduced CD4$^+$ T cells with the enrichment strategy. (A) Strategy for generating latently infected Bcl-2-transduced cells by enriching the HIV-1-infected cells. (B) Detection of latently infected cells in the sorted GFP-negative population from the enrichment strategy. The sorted GFP-negative cells were activated with 2.5μg anti-CD3 plus 1μg anti-CD28 monoclonal antibodies or 10ng/ml PMA plus 1 μM ionomycin for 2 days and then analyzed by flow cytometry to quantify the number of GFP-positive cells. The number in each plot indicates the frequency of GFP(+) cells.

Figure S4

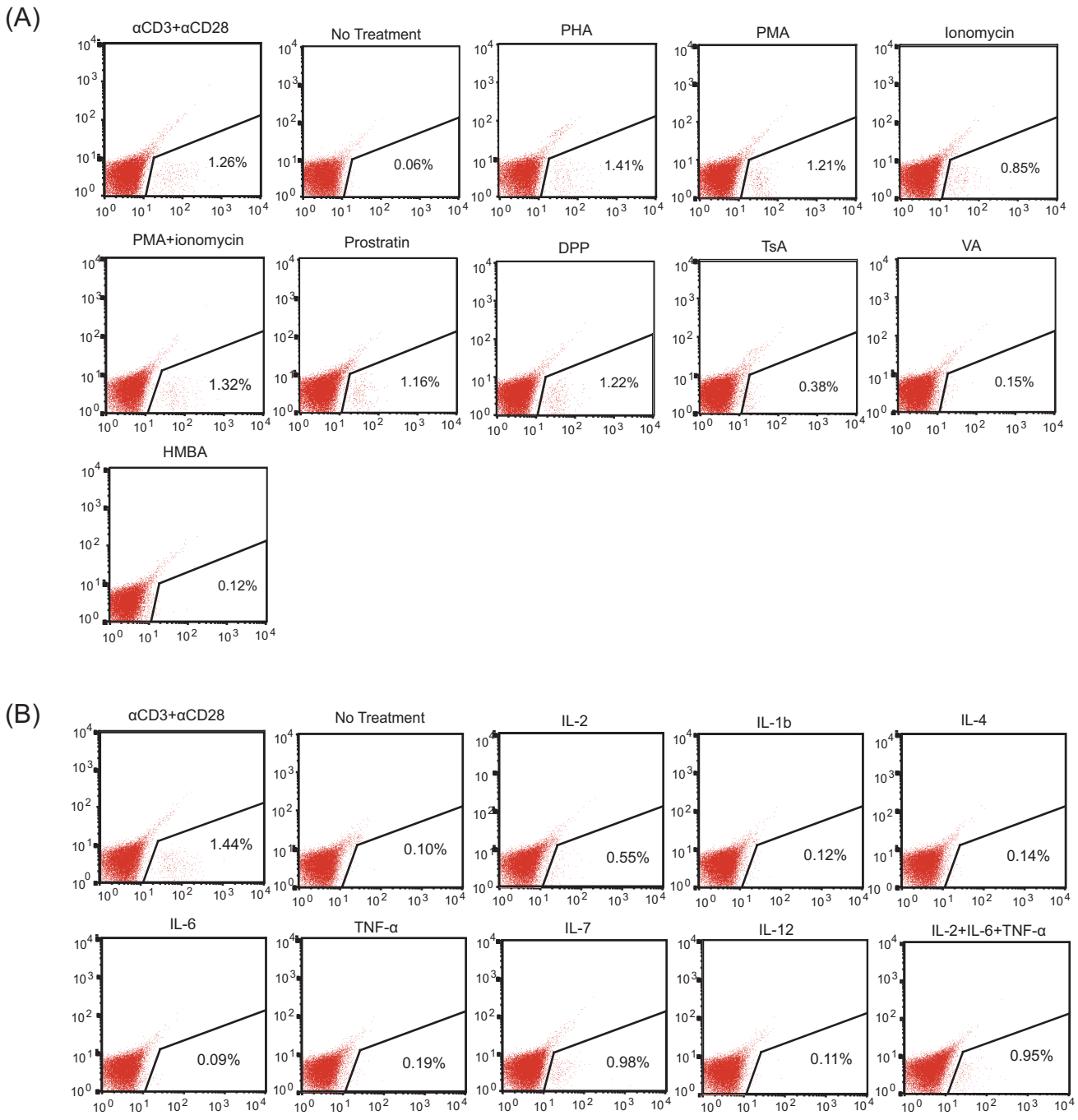

Figure S4. Latent HIV-1 can be activated by a variety of activators from latently infected Bcl-2-transduced cells. Latently infected cells were treated with small molecules (A) or cytokines (B). The number in each plot indicates the frequency of GFP (+) cells. Flow cytometric plots shown here came from the same experiments in Figures 4A and B. Each flow cytometric plot represents one of triplicate samples in an experiment.

Figure S5

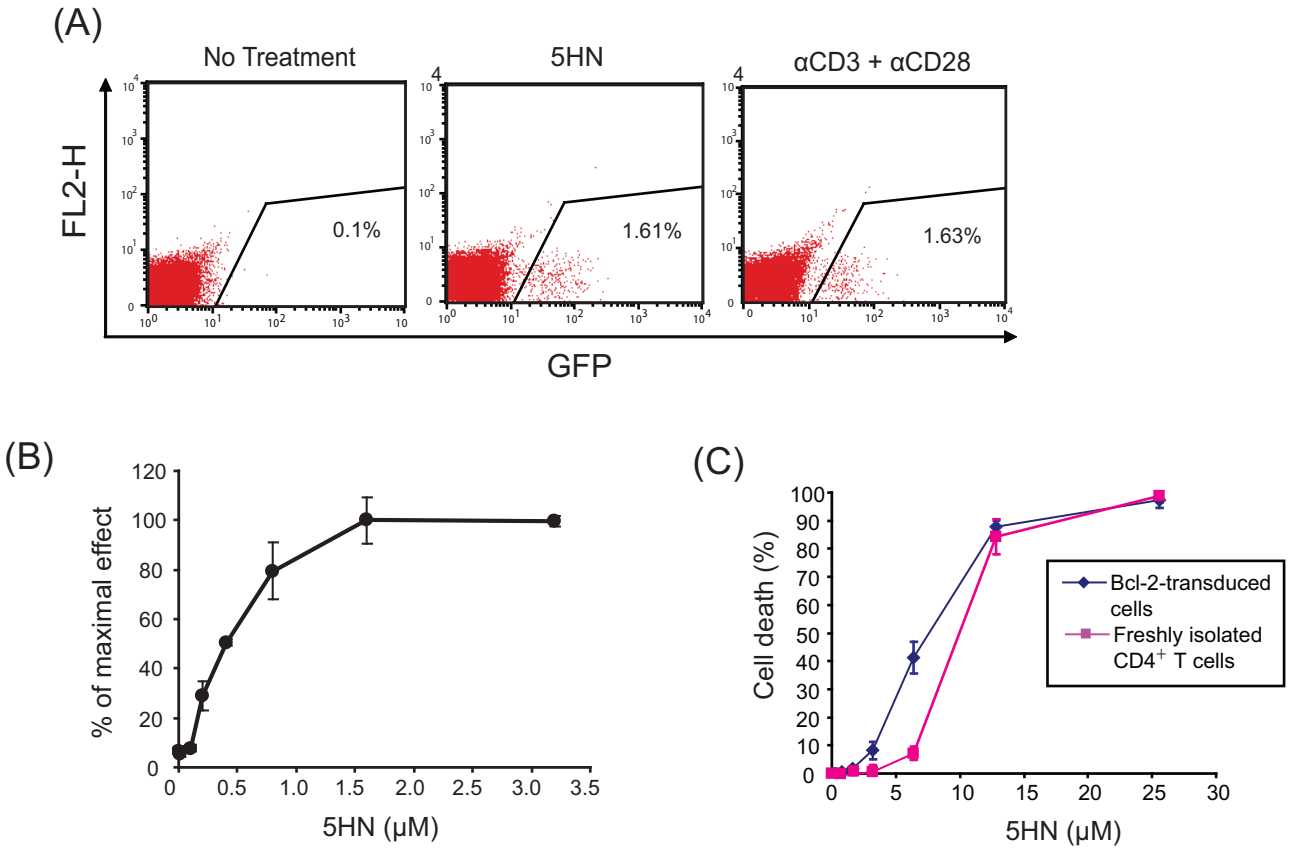

Figure S5. Effects of 5HN on reactivation of latent HIV-1 and cell viability (A) The latently infected Bcl-2-transduced cells were treated with 5HN or anti-CD3 plus anti-CD28 antibodies and analyzed for percentage of the GFP-positive cells using flow cytometry. The number in each plot indicates the frequency of GFP (+) cells. (B) The dose-response curve for the reactivation of latent HIV-1 in latently infected Bcl-2-transduced cells by 5HN. The effect of 5HN was determined by quantifying the GFP-positive cells using flow cytometry and was normalized to the maximal effect of 5HN. Data are means ± s.d. of triplicate samples of an representative experiment from three independent experiments. (C) The percentage of apoptosis in Bcl-2-transduced cells and freshly isolated $CD4^+$ T cells receiving indicated concentrations of 5HN. The fraction of apoptotic cells were determined using annexin V-PE and 7-AAD and analyzed by flow cytometry. Data are means ± s.d. of triplicate samples of a representative experiment from two independent experiments.

Figure S6

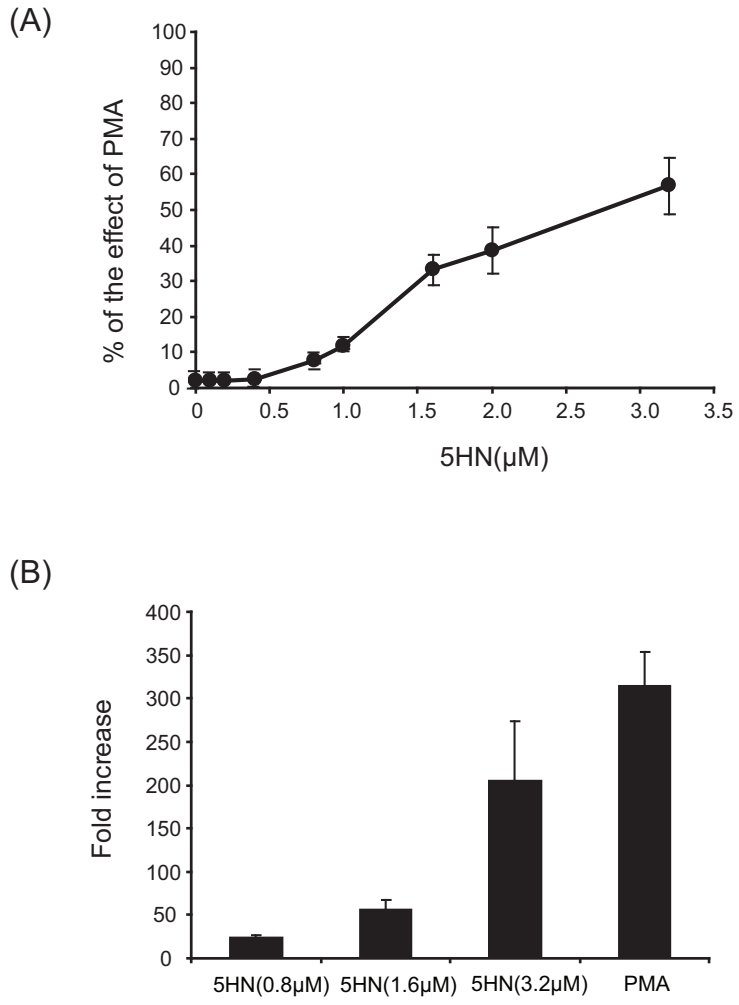

Figure S6. The effects of 5HN on the reactivation of latent HIV-1 in J-Lat cells. (A) The dose-response curve of 5HN in reactivation of latent HIV-1 in J-Lat cells. The effect of 5HN was determined by quantifying the GFP-positive cells using flow cytometry. The result was normalized to the effect of PMA. (B) Effects of 5HN on the transcription of viral genes in J-Lat cells. The J-Lat cells were left unstimulated or stimulated with the indicated concentrations of 5HN or PMA. The levels of viral mRNA were quantified using real time RT-PCR, and were normaliazed to beta-actin mRNA levels. The results were compared to unstimulated samples and showed as fold change. Data are means ± s.d. of triplicate samples and represent two independent experiments.

Figure S7

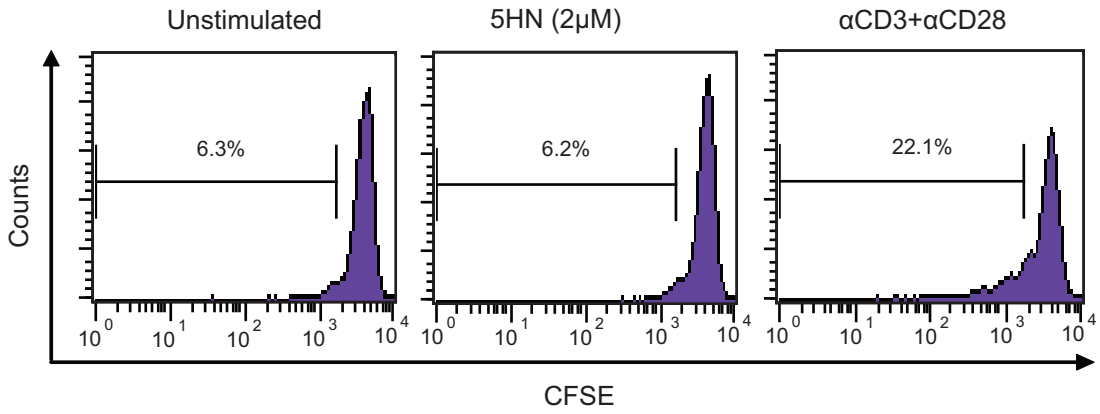

Figure S7. The effects of 5HN or anti-CD3 plus anti-CD28 antibodies on the proliferation of primary $CD4^+$ T cells. Cells were stained with CFSE and then treated with 2μM 5HN or anti-CD3 plus anti-CD28 antibodies for 3 days. Cell division was measured by the dilution of CFSE using flow cytometry. The percentage indicated the number of cells that had undergone celluar division.

Table S1: The mutations of amino acids of viral genes and the primers used for mutagenesis in the viral vector pNL4-3-Δ6-drEGFP

Mutation of viral genes	Amino acid change	Primers
Gag	S6 Stop	GAGATGGGTGCGAGAGCGTAATAATTAAGCGGGGGAG AATTAG CTAATTCTCCCCCGCTTAATTATTACGCTCTCGCACCCA TCTC
Gag	M142 Stop	CAGAACCTCCAGGGGCAAATCTAACATCAGGCCATATC ACC GGTGATATGGCCTGATGTTAGATTTGCCCCTGGAGGTTC TG
Vif	Y30 Stop	GATTAGTAAAACACCATTAGTATATTTCAAGGAAAGC GCTTTCCTTGAAATATACTAATGGTGTTTTACTAATC
Vpr	Q8 Stop	CAAGCCCCAGAAGACTAAGGGCCACAGAGGG CCCTCTGTGGCCCTTAGTCTTCTGGGGCTTG
Vpu	I4 frame shift	GTACATGTAATGCAACCTTAATAGTAGCAATAGTAGC GCTACTATTGCTACTATTAAGGTTGCATTACATGTAC

The underlined letters indicate the mutated nucleotides.

Measurement of the residual integrated provirus in latently HIV-1-infected cells after activation of T cells

Because the reporter virus we used can only undergo single-round infection and because the latently infected cells have been in culture for more than one month, the majority of the latently infected cells should contain integrated provirus and not the unintegrated virus because the latter has very short half life (~ 1 day) [1]. Therefore, we measured the total HIV-1 DNA, which should represent the integrated provirus, in the GFP(-) population.

We first utilized anti-CD3 and anti-CD28 antibodies to activate latently infected Bcl-2-transduced cells for three days, after which we sorted for GFP(-) cells. Genomic DNA of the sorted GFP(-) cells was extracted using QIAamp DNA micro kit (Qiagen). The amount of total HIV-1 DNA was quantified using real-time PCR with SYBR Green PCR Master Mix and a pair of primers (6F (5'-CATGTTTTCAGCATTATCAGAAGGA-3') and 84R (5'-TGCTTGATGTCCCCCCACT-3')) that bind to a conserved region of gag [2]. For measuring the HIV-1 copies per genome, we used a cell line that only harbors a single copy of integrated provirus. The integration site is within human HPRT gene located on the X chromosome [3]. The frequency of integrated HIV-1 in this male cell line can be viewed as 1 per human genome. To calculate the percentage of cells with integrated HIV-1, this cell line served as a standard. The frequency of residual HIV (per genome) can be calculated by determining the ratio of the amount of HIV in GFP(-) cells to the cells carrying a single-copy provirus, normalized to the copies of HPRT gene. The copy number of HPRT was also measured using real-time PCR with a pair of primers: 5'-ACGTCTTGCTCGAGATGTGA-3' and 5'-AATCCAGCAGGTCAGCAAAG-3'. Of note, the latently infected cells were generated from a female donor, while the cell line that harbor a single-copy integrated provirus was derived from a male individual. Therefore, the copy number of HPRT needs to be adjusted for the differential number of X chromosomes.

Based on the method mentioned above, we calculated the frequency (GN) of residual HIV genome copies in the GFP(-) cells were 0. 275% ± 0.021% (per human genome equivalent). The percentage (GP) of GFP(+) cells following activation of latently infected cells was 0.267% ± 0.025%. Therefore, the percentage (R) of residual HIV in latently infected cells after activation was ~50% based on the following equation:

R (%) = GN / (GN+GP) x 100 (%)

In this case:

R (%) = 0.275% / (0.275%+0.267%) x 100 (%) = 50.74%

Reference List

1. Zhou,Y., Zhang,H., Siliciano,J.D., & Siliciano,R.F. Kinetics of human immunodeficiency virus type 1 decay following entry into resting CD4+ T cells. *J. Virol.* **79**, 2199-2210 (2005).
2. Palmer,S. *et al.* New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. *J. Clin. Microbiol.* **41**, 4531-4536 (2003).
3. Han,Y. *et al.* Orientation-dependent regulation of integrated HIV-1 expression by host gene transcriptional readthrough. *Cell Host. Microbe* **4**, 134-146 (2008).