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Supplemental Information

Stimulation of HIV-1-Specific Cytolytic T Lymphocytes Facilitates Elimination of Latent Viral Reservoir after Virus Reactivation

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Inventory of Supplemental Information

Table S1: Characteristics of study patients.

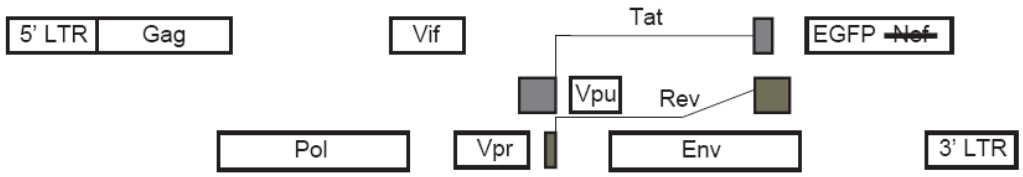
Patient	Year of Diagnosis	CD4 count*	Viral load*	Years on HAART
EC32	2009	1289	<50	0
EC18	1998	1330	<50	0
EC06	1992	1139	<50	0
HP03	1996/1997	718	<50	6
HP05	2001	500	<50	9
HP06	1996	977	<50	15
HP11	1994	1182	<50	7
HP12	1997	1074	<50	14
HP14	<1999	475	<50	11
HP16	2000	687	<50	8
HP17	2007	867	<50	3
HP18	1998	773	<50	>3
HP19	2006	620	<50	5
HP21	2002	1207	<50	9
HP22	2008	509	<50	3
HP24	2007	668	<50	3
HP27	1987	784	<50	3

EC: elite controller; HP: patient on HAART.

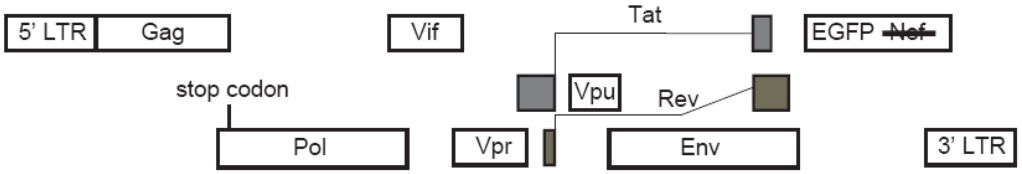
* Patient CD4 count was measured during our study. Viral load of all patients has been undetectable for at least 3 years.

For viral CPE study

NL4-3-Δnef-EGFP (replication-competent)

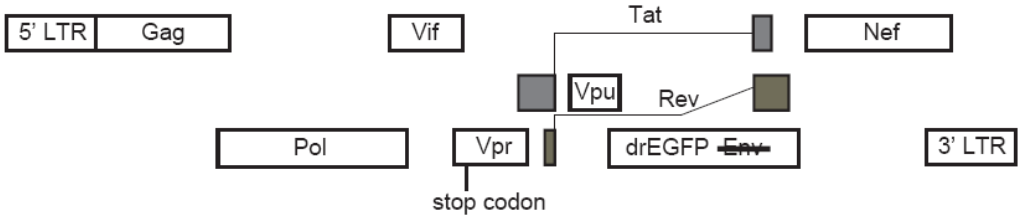


NL4-3-Δnef-Δpol-EGFP (replication-deficient)

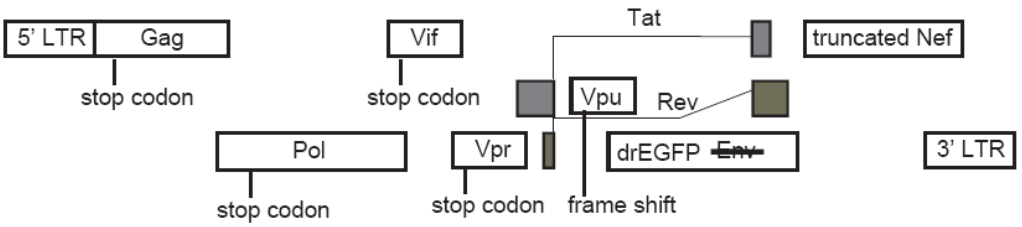


For CTL study

NL4-3-Δvpr-Δenv-drEGFP (replication-deficient)



NL4-3-Δ6-drEGFP (replication-deficient)



NL4-3-Δ5-drEGFP (replication-deficient)

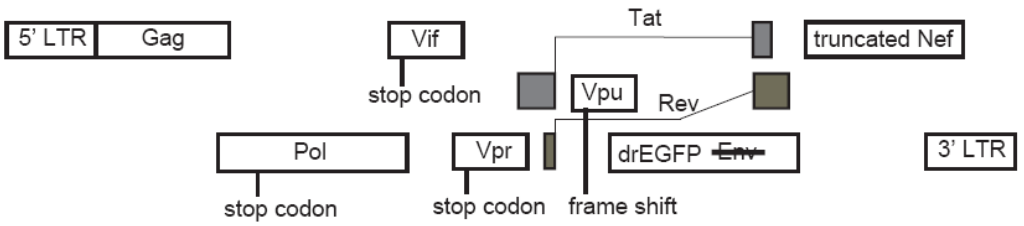


Figure S1. Genome structure of the HIV-1 reporter viruses. Premature stop codons, frame shift mutations or truncations were introduced into indicated ORFs.

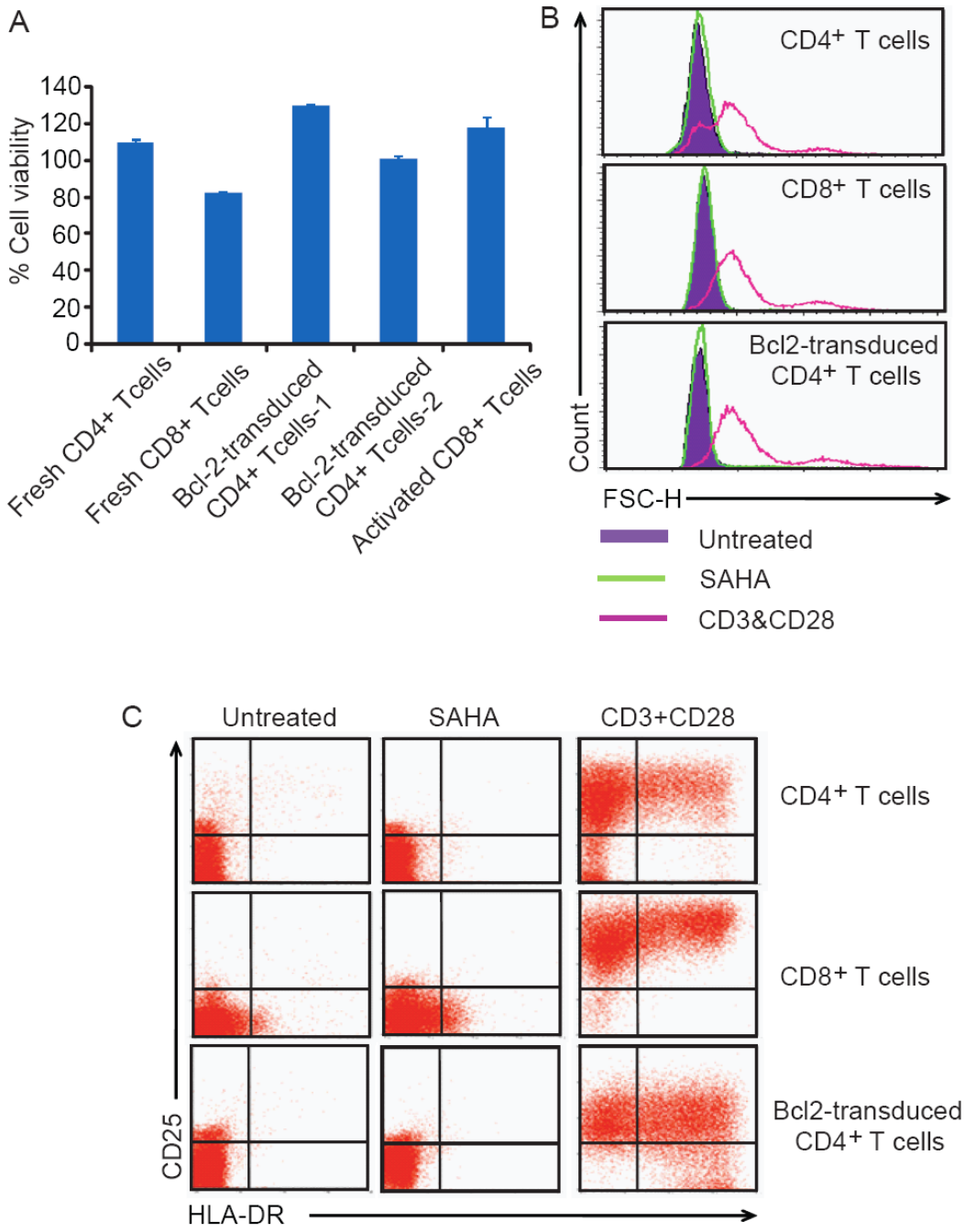


Figure S2. SAHA at a concentration of 500 nM does not affect T cell viability and T cell activation. (A) SAHA does not affect T cell viability. Freshly isolated $CD4^+$ and $CD8^+$ T cells, Bcl-2-transduced $CD4^+$ T cells, and $CD8^+$ T cells stimulated with anti-CD3 and anti-CD28 antibodies were cultured in the presence or absence of 500 nM SAHA for one week. Cell viability was determined by MTS assay (Promega) and the value was normalized to no SAHA controls. **(B and C)** SAHA does not cause T cell activation. Freshly isolated $CD4^+$ or $CD8^+$ T cells or Bcl-2-transduced $CD4^+$ T cells were treated with 500 nM SAHA or anti-CD3 and anti-CD28 antibodies for 3 days. Cell size and expression of the activation markers CD25 and HLA-DR were analyzed with FACS. Error bars represent SEM, n = 3.

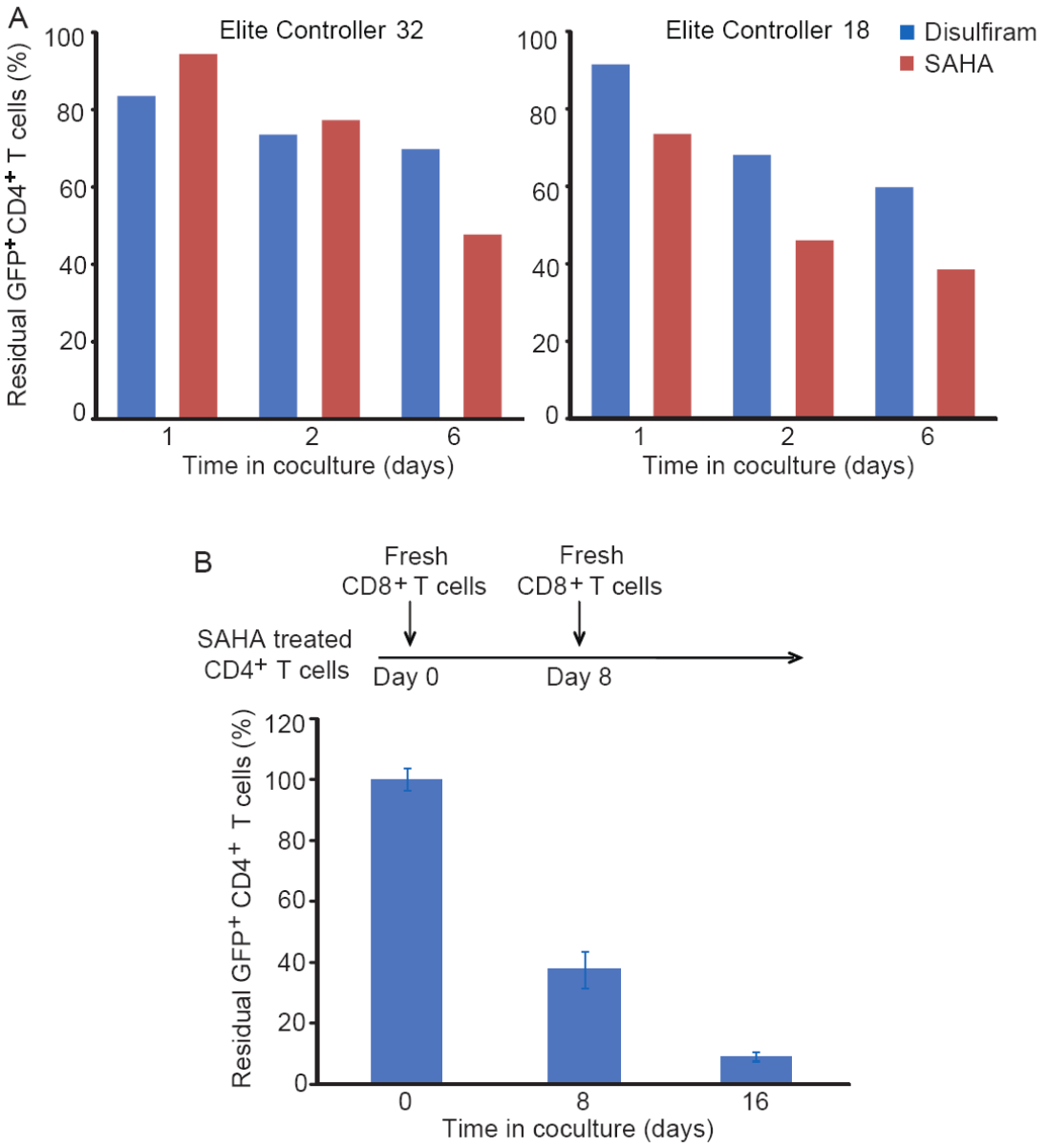


Figure S3. Killing of latently infected CD4$^+$ T cells after virus reactivation. Primary CD4$^+$ T cells isolated from Elite controllers were transduced with Bcl-2-expressing lentiviral vector and then infected with NL4-3-Δvpr-Δenv-drEGFP to generate latent infection *in vitro*. **(A)** Comparison between SAHA and Disulfiram. To reactivate latent virus, CD4$^+$ T cells were treated with 500 nM SAHA or 1uM disulfiram and then cocultured with freshly isolated autologous CD8$^+$ T cells at a 1:1 ratio. Fraction of GFP$^+$ cells were analyzed with FACS. **(B)** Residual infected CD4$^+$ T cells are not resistant to CTL-mediated killing. After virus reactivation with 500 nM SAHA for two days, CD4$^+$ T cells were cocultured with freshly isolated autologous CD8$^+$ T cells at a 1:1 ratio at day 0 for 8 days. On day 8, additional freshly isolated autologous CD8$^+$ T cells were added to the culture at a 1:1 ratio. Fraction of GFP$^+$ cells were analyzed with FACS. Error bars represent SEM, n = 3.

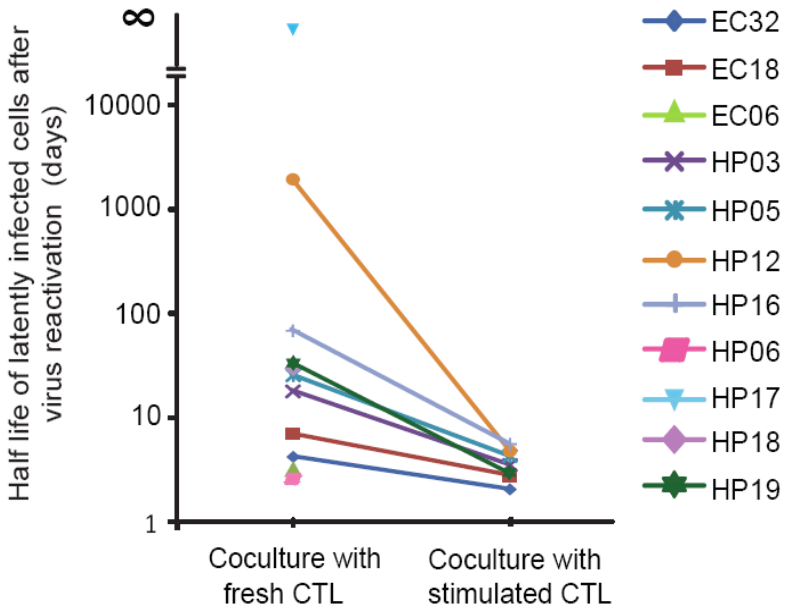


Figure S4. Half life of latently infected CD4$^+$ T cells with the presence of autologous CD8$^+$ T cells. The half-life of GFP$^+$ CD4$^+$ T cells was calculated by fitting the number of GFP$^+$ CD4$^+$ T cells overtime (Figure 4) to an exponential decay formula. To do so, it was assumed that the decay rate of the GFP$^+$ CD4$^+$ T cells is constant for the duration of the experiment. The half-life of GFP$^+$ CD4$^+$ T cells was calculated at E:T ratio 1:1.

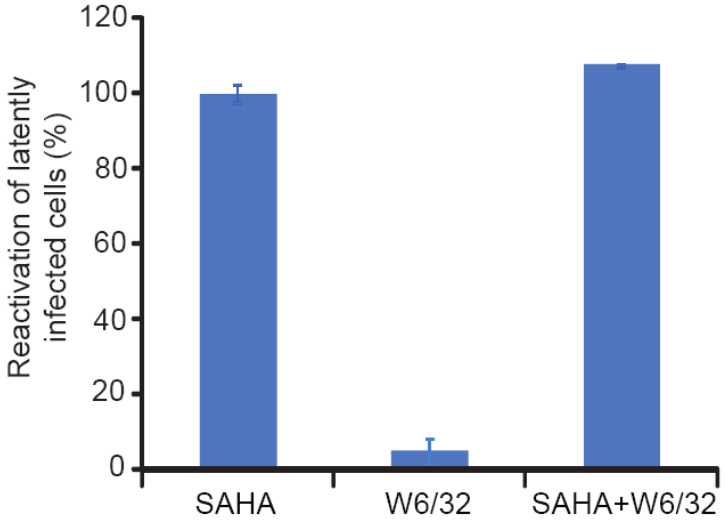


Figure S5. Anti-MHC-I antibody W6/32 does not have effect on reactivation of latent HIV-1. Bcl-2 transduced CD4$^+$ T cells were infected with NL4-3-Δvpr-Δenv-EGFP. Cells were treated with 500nM SAHA, or 5 µg/ml W6/32, or both. Fraction of GFP$^+$ cells were analyzed with FACS 3 days after treatment. Effect of each treatment was normalized to the effect of SAHA alone. Error bars represent SEM, n = 3.