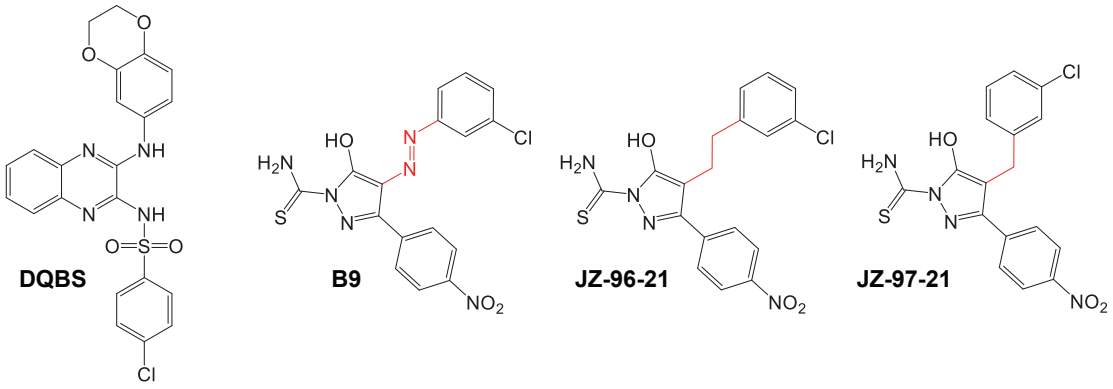
Supplemental Materials:

Supplemental Table 1: Subject characteristics

Donor Identifier	HAART	Plasma VL	CD4 Count	Duration on HAART (years)
OM2	Yes	49	410	9.0
OM4	No	49	600	
OM9	No	88	550	
OM25	No	1024	250	
OM32	Yes	16000	953	1.8
OM33	Yes	49	350	0.7
OM35	No	1566	300	
OM44	No	8459	710	
OM99	Yes	49	450	4.0
OM101	No	82693	340	
OM214	No	38600	310	
OM265	Yes	49	540	9.0
OM275	Yes	49	840	9.0
OM288	Yes	49	690	9.0
OM292	Yes	49	600	9.0
OM298	Yes	49	830	5.0
OM357	No	1582	610	
OM359	Yes	49	830	1.3
OM363	Yes	204	740	1.1
OM442	Yes	49	430	0.6
OM522	Yes	49	450	0.6
OM680	No	996	670	
OM727	No	149256	621	
OM5028	No	91	820	
OM5039	No	2420	460	
OM5218	Yes	49	470	0.7
OM5220	Yes	49	650	2.0
OM5223	No	28955	610	
OM5245	Yes	49	780	1.0
OM5265	Yes	49	870	0.5
OM5267	Yes	94617	290	0.5
OM5314	No	55	1070	
OM5318	Yes	49	570	2.0
OM5327	Yes	49	810	2.0
OM5328	No	95554	490	
OM8010	No	1786	703	
OM8011	No	49	1267	
OM8017	No	14000	272	
OM8020	No	1318	1010	
OM8026	No	453	580	
OM8027	No	46000	598	
Median (IQR)	No	1582 (272-33778)	600 (400-600)	-
Median (IQR)	Yes	49 (49-49)	625 (450-825)	1.9 (0.7-8.0)
Median (IQR)	-	88 (49-5440)	600 (450-795)	1.9 (0.7-8.0)

Supplemental Figure Legends:

Supplemental Figure 1

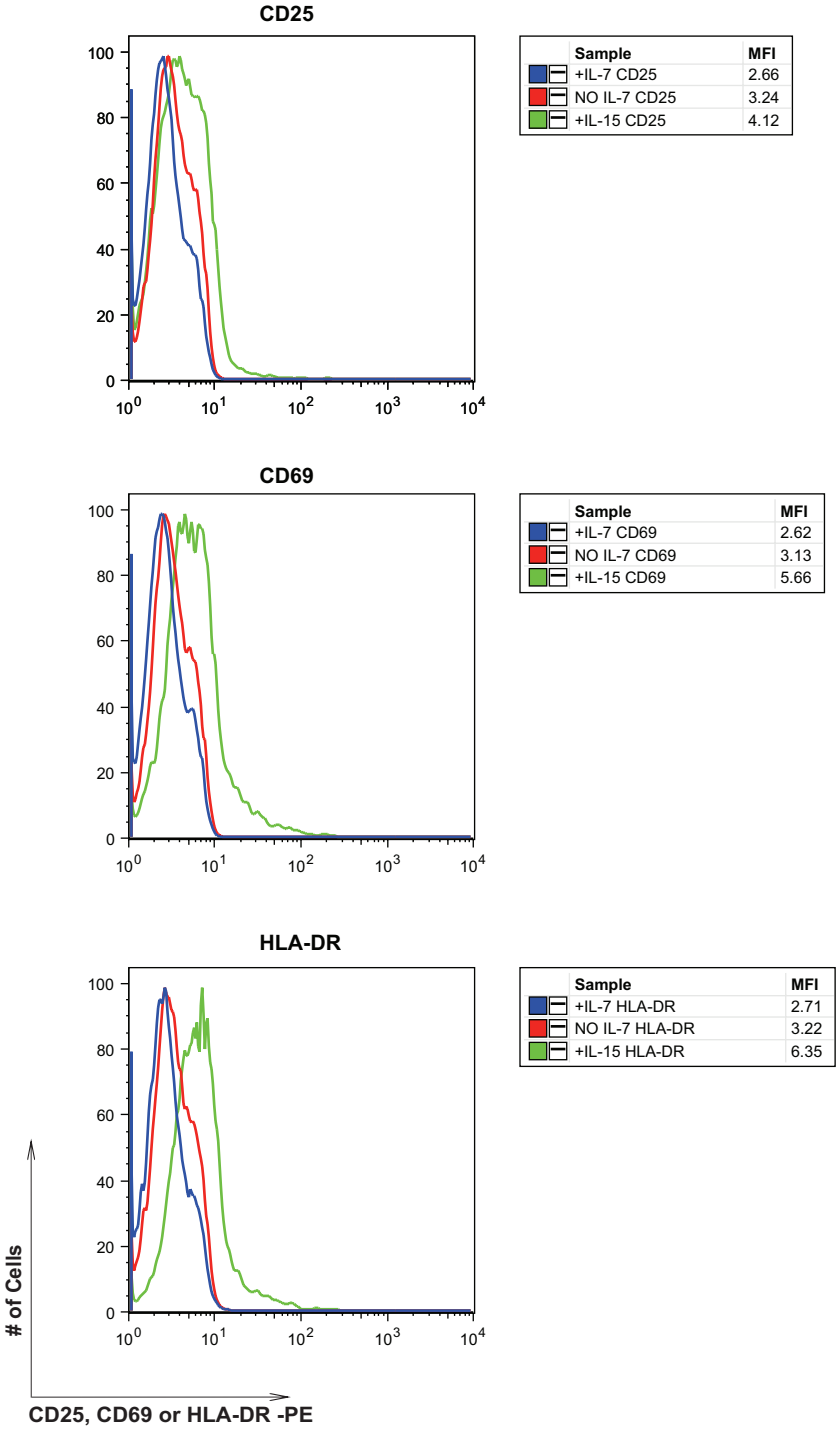


Compound	Chemical class	MW	Nef binding (K_D, nM)[a]	Infectivity IC_{50}, μM[b]	Cytotoxicity CC_{50}, μM[b]	Replication % inhibition[c]	Reference (PMID)
DQBS	Diamino-quinoxaline	468.9	N/D	>3.0	>3.0	52.2%	*Retrovirology,* 2013 (24229420)
B9	Diphenyl-pyrazole	402.8	82	2.53	10	54.6%	*Bioog Med Chem Lett*, 2016 (26852364)
JZ-96-21		402.9	159	0.69	>10	64.5%	
JZ-97-21		388.8	150	3.54	>10	51.6%	

[a] Binding constants determined *in vitro* by surface plasmon resonance with recombinant Nef; N/D, not determined by SPR. Direct interaction of DQBS with recombinant Nef has been demonstrated by differential scanning fluorimetry.
[b] Inhibition of Nef-dependent infectivity enhancement and cytotoxicity determined in TZM/bl reporter cells infected with HIV-1 NL4-3.
[c] Inhibition of Nef-dependent HIV-1 NL4-3 replication determined in U87MG/CD4/CXCR4 cells. Each compound tested at 3.0 μM; no cytotoxicity observed at this concentration.

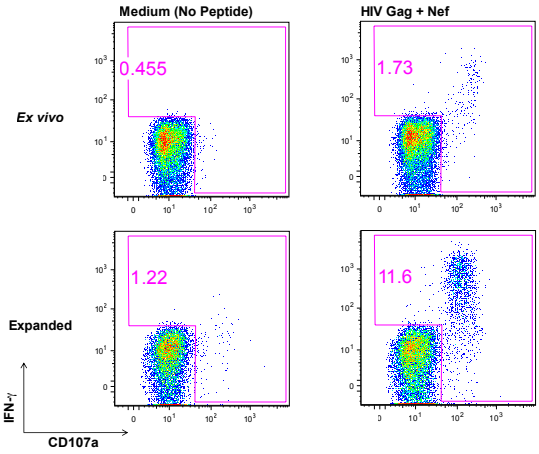
Supplemental Figure 1. Chemical structures of the Nef inhibitors used in the study. Structures of each of the small molecule inhibitors of Nef are shown at the top. The compounds JZ-96-21 and JZ-97-21 are B9 analogs in which the diazene linked (red) has been substituted with a one or two carbon bridge. The table summarizes important features of each compound, including in vitro binding data and effects on Nef-dependent enhancement of HIV-1 infectivity and replication in cell lines. Additional details can be found in the citations.

Supplemental Figure 2



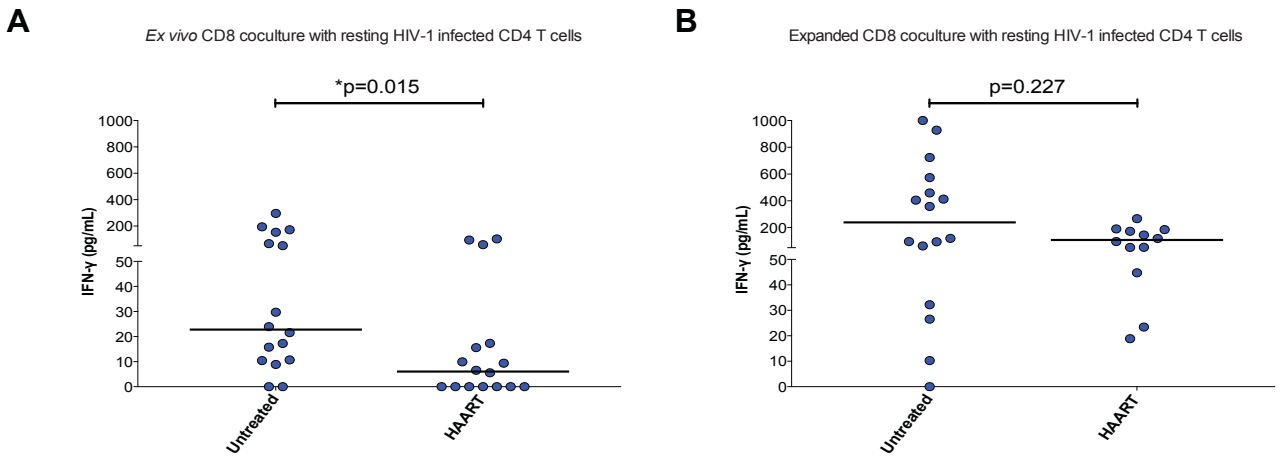
Supplemental Figure 2. Low dose of IL-7 does not induce activation of in vitro generated latently HIV-1 infected cells. Primary resting CD4 T cells were generated as described in the *Methods* section. IL-7 (1 ng/mL) was added to the CD4 T cell cultures for survival in each experiment. At this dose, IL-7 did not induce upregulation of the activation markers CD25, CD69 or HLA-DR. Shown are the histograms comparing expression of each marker on the surface of resting CD4 T cells with IL-7 (blue line) compared to similar cells cultured without IL-7 (red line) for 7 days. IL-15 stimulated cells (green line) are also included as a positive control and exhibit elevated expression of each of the activation markers probed.

Supplemental Figure 3



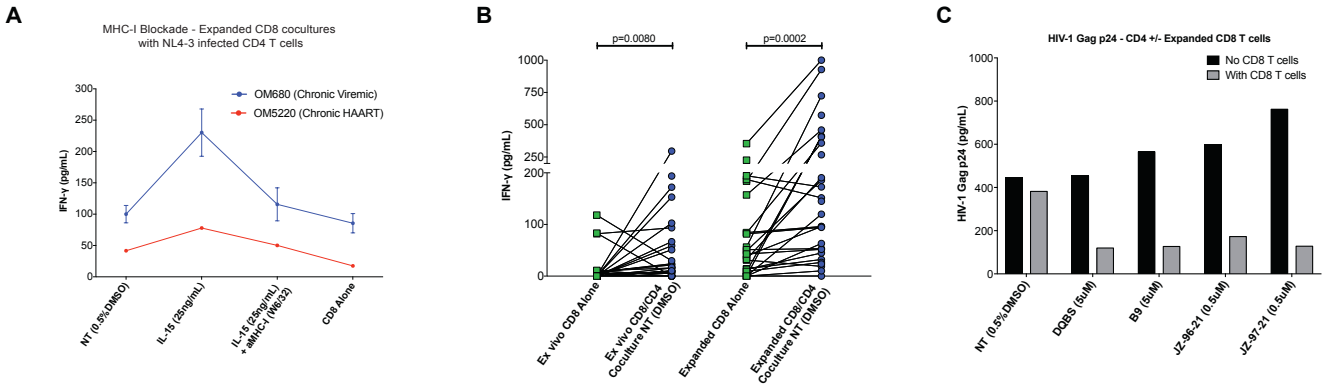
Supplemental Figure 3. HIV-1 Gag and Nef peptide stimulation expands antigen specific CD8 T cells. PBMC from an HIV-1 infected donor on suppressive HAART (plasma viral load undetectable) were stimulated with HIV-1 Gag (80 ng/mL/peptide) and Nef (200 ng/mL/peptide) peptide pools as described in the *Methods*. Six days later cells were re-stimulated with 1μg/mL/peptide of Gag and Nef peptide pools for six hours in the presence of Brefeldin A and an antibody against CD107a. Intracellular staining for IFN-γ was performed and cells were analyzed via flow cytometry. Numbers inset represent the frequency of CD8 T cells degranulating (CD107a+) and producing cytokines in response to peptide stimulation. For comparison, the ex vivo frequencies of HIV-1 Gag and Nef specific cells are shown (top row). HIV-1 peptide stimulation over 6 days resulted in a robust expansion of antigen specific CD8 T cells compared to ex vivo frequencies.

Supplemental Figure 4



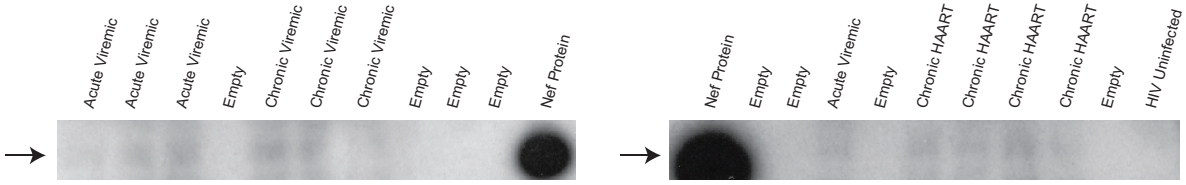
Supplemental Figure 4. Detectable responses from cocultures with autologous isolated ex vivo CD8 T cells are dependent on treatment status and can be restored by HIV-peptide expansion. Data from the IFN-γ coculture experiments reported in the manuscript were split based on donors' antiretroviral treatment statuses i.e. on HAART therapy or not ('Untreated'). Reported data were generated from cocultures of autologous CD8 T cells with wild-type HIV-1 latently infected CD4 T cells. Shown in **(A)** and **(B)** are the IFN-γ levels detected following overnight coculture with freshly isolated ex vivo or HIV-peptide stimulated 'expanded' CD8 T cells, respectively. Mann-Whitney U tests were performed for each analyses between the 'Untreated' and the HAART subsets within each group. Ex vivo CD8 cocultures indicated a greater detectable response from the 'Untreated' fraction compared to the 'HAART' treated group due to a greater frequency of HIV-specific CD8 T cells. Cocultures with HIV-peptide expanded CD8 T cells negated this difference.

Supplemental Figure 5



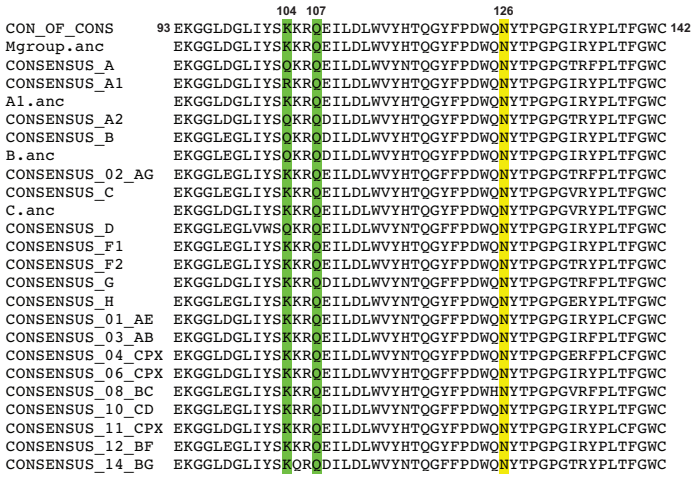
Supplemental Figure 5. Detected immune response detected are dependent upon CD8 CTL-mediated recognition of autologous HIV-1 infected CD4 T cell targets. (A) Autologous HIV-peptide expanded CD8 T cells were cocultured with HIV-1 NL4-3 latently infected cells, generated as described in the *Methods* that had been stimulated with IL-15 for 6 days. CD8 T cell cocultures were performed in the presence or absence of the MHC-I blocking antibody, W6/32 (10 μg/mL). IFN-γ was quantitated in the supernatant following overnight coculture as described in the *Methods*. Each individual experiment is depicted by coloured lines: blue lines illustrate cocultures between a chronically infected therapy naïve donor's T cells, OM680; red lines indicate cocultures with T cells from OM5220, a chronically infected donor on suppressive HAART. Cocultures of IL-15 stimulated CD4 T cells yielded maximal production of IFN-γ from autologous CD8 T cells as these produce maximal viral antigens (Figure 2A in the manuscript). Recognition of IL-15 stimulated NL4-3 infected CD4 T cells was completely abrogated in the in the presence of the MHC-I blocking antibody as indicated by the IFN-γ levels diminishing to basal levels in the cultures with the W6/32 antibody indicating that CD8 CTL recognition of antigens presented on MHC-I induced IFN-γ production by CTL. **(B)** In several experiments of the IFN-γ release assay (n=24 with each, ex vivo and expanded CD8 T cells; n=48 total) we cultured purified CD8 T cells alone at equivalent cell numbers as in the CD4:CD8 cocultures and measured IFN-γ following overnight incubation in the supernatants in parallel with cocultures. In these CD8 T cell alone wells we detected minimal IFN-γ due to the absence of antigen. However, coincubation of these CD8 T cells with resting latently infected autologous CD4 T cell targets resulted in basal IFN-γ production in response to antigen which was statistically significant (Wilcoxon matched pairs test performed between groups). **(C)** HIV-1 Gag p24 was quantitated by ELISA in an individual following viral outgrowth in cultures of latently HIV-1 infected CD4 T cells with or without expanded CD8 T cells. The addition of CD8 CTL to cultures induced the elimination of latently HIV-1 infected CD4 T cells compared to paired cultures without CD8 T cells that did not exhibit any reduction in p24 levels from DMSO control. Furthermore, coincubation of CD8 T cells with Nef inhibitor treated CD4 T cells exhibit enhanced clearance relative to the DMSO control. Collectively, these results demonstrate that IFN-γ detected in our assays is CTL-dependent and due to recognition of antigen via MHC-I presentation from HIV-1 infected CD4 T cells.

Supplemental Figure 6



Supplemental Figure 6. Nef protein is detectable in PBMC of HIV-1 infected donors on or off HAART therapy. CD8 T cell-depleted PBMC ($5x10^6$) from various HIV-1 infected and uninfected donors were lysed with RIPA buffer and protease inhibitor and lysates were cleared by centrifugation. Nef protein was immunoprecipitated (IP) via Protein A complexes utilizing a rabbit polyclonal Nef antiserum. IP samples were run on a denaturing SDS PAGE gel along with lane of recombinant purified Nef protein and transferred onto a PVDF membrane. PVDF blots were probed with mouse anti-HIV-1 Nef monoclonal antibodies EH1 and JR-CSF and developed on X-ray film. Two separate experiments are shown with their respective positive control band (recombinant purified Nef protein); a two-hour exposure is shown. The arrow indicates bands corresponding to the HIV-1 Nef protein.

Supplemental Figure 7



Supplemental Figure 7. Critical residue N126 for inhibitor binding is conserved across multiple consensus HIV-1 Nef sequences. Docking models with the four inhibitors and a crystal structure of the Nef dimer (PDB:1EFN) have identified Nef residues Q104, Q107 and N126 (HXB2 numbering; GenBank K03455) as possible inhibitor contacts within the Nef structures (references [25-27] in main text). Of these, N126 (highlighted yellow) has been validated as a point of inhibitor contact by mutagenesis and conserved across multiple global clades of HIV-1 as shown. The residues Q104 and Q107 (highlighted green) may help stabilize the protein:inhibitor complex. Consensus sequence data was obtained from the Los Alamos HIV Database (http://www.hiv.lanl.gov/). The most recently updated sequence database (2014) available at the time of writing this manuscript is displayed.

Supplemental Materials and Methods:

HIV-1 Nef western blot

Frozen PBMC from HIV-1 infected and uninfected donors were thawed at 37^0C, washed and counted. CD8 T cells were depleted from PBMC using StemCell CD8 Positive Selection kit as per manufacturer's protocol. $5x10^6$ CD8 depleted PBMC were lysed in 100μL of 1X RIPA buffer (Cell Signalling) containing 1X Protease Inhibitor, EDTA free (Roche) for 15 minutes at 4^0C with rocking. Lysates were cleared by centrifugation at 21,000xg for 30minutes and supernatant was collected. Nef protein was immunoprecipitated from the lysates using Dynabeads Protein A kit (Life Technologies) with a rabbit polyclonal serum against HIV-1 Nef (Catalog #2949, NIH AIDS Reagent Program). Immunoprecipitated samples were loaded on a 12% Bis-Tris SDS PAGE gel (Life Technologies) and transferred onto a PVDF membrane. Blocking was performed with a 3% BSA solution in 1X PBS. Anti HIV-1 Nef mouse antibodies EH1 and JR-CSF (Catalog #3689 and 1539 respectively, NIH AIDS Reagent Program) were used at 0.25μg/mL each in a 1% BSA solution in TBS-Tween20 to probe for Nef and a goat anti-mouse HRP antibody (Life Technologies) served as the secondary antibody. ECL Plus (Thermo Fisher) chemiluminescent substrate was used to develop and image the blot on X-Ray film.