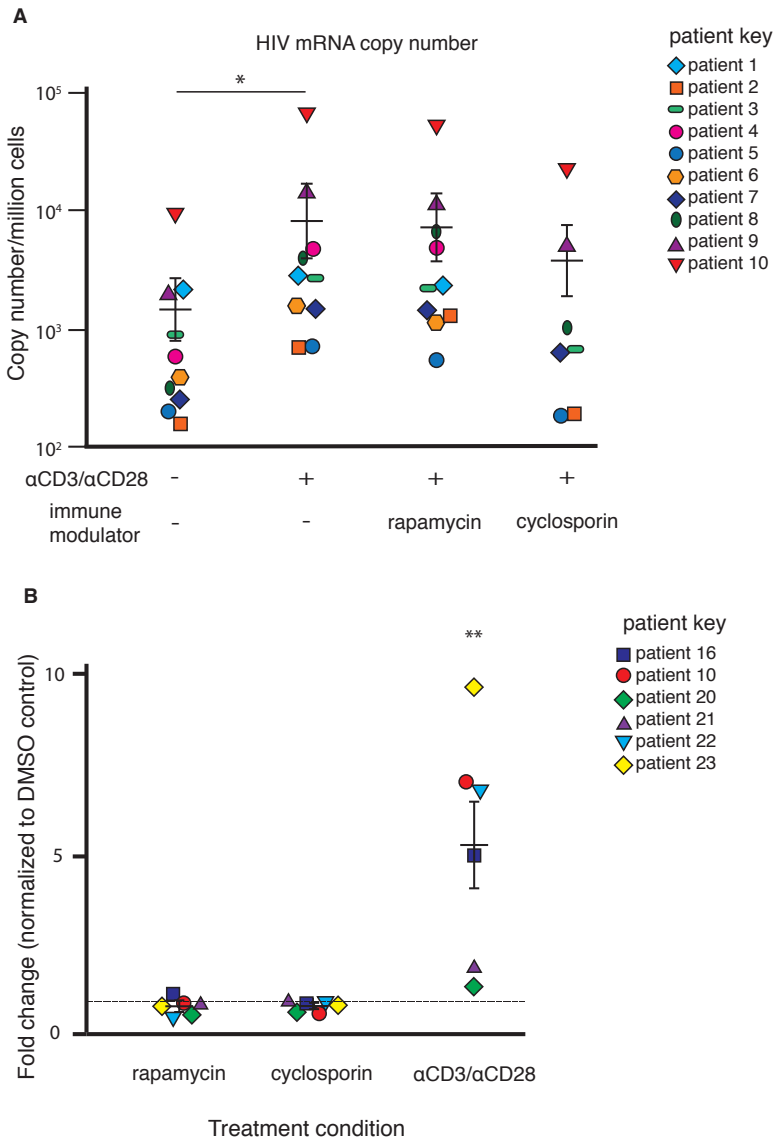
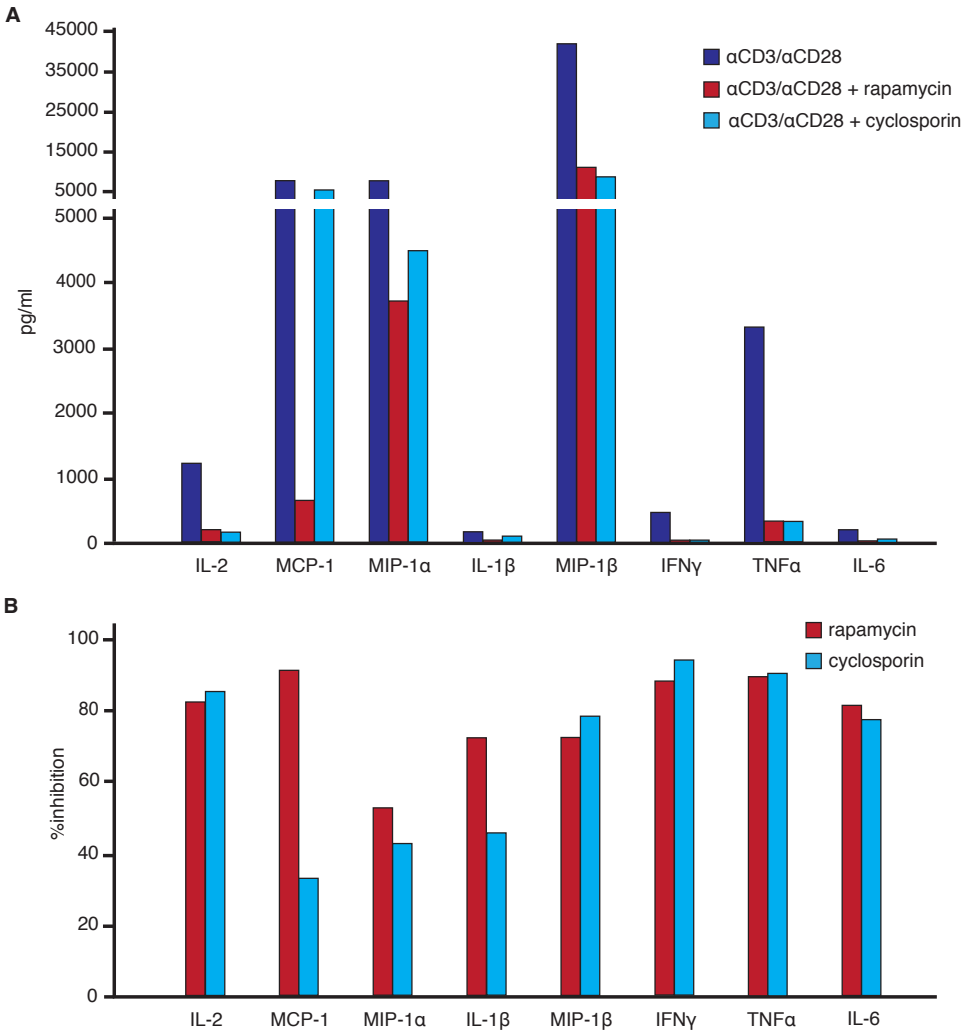


Supplemental Figure 1: RT-qPCR results for various immunosuppressant treatments.

A: RT-qPCR results for intracellular HIV-1 mRNA in rCD4s purified from infected individuals on cART. Copy number represents copies of HIV-1 mRNA detected per million cells. Symbols represent results from different donors (n=10).

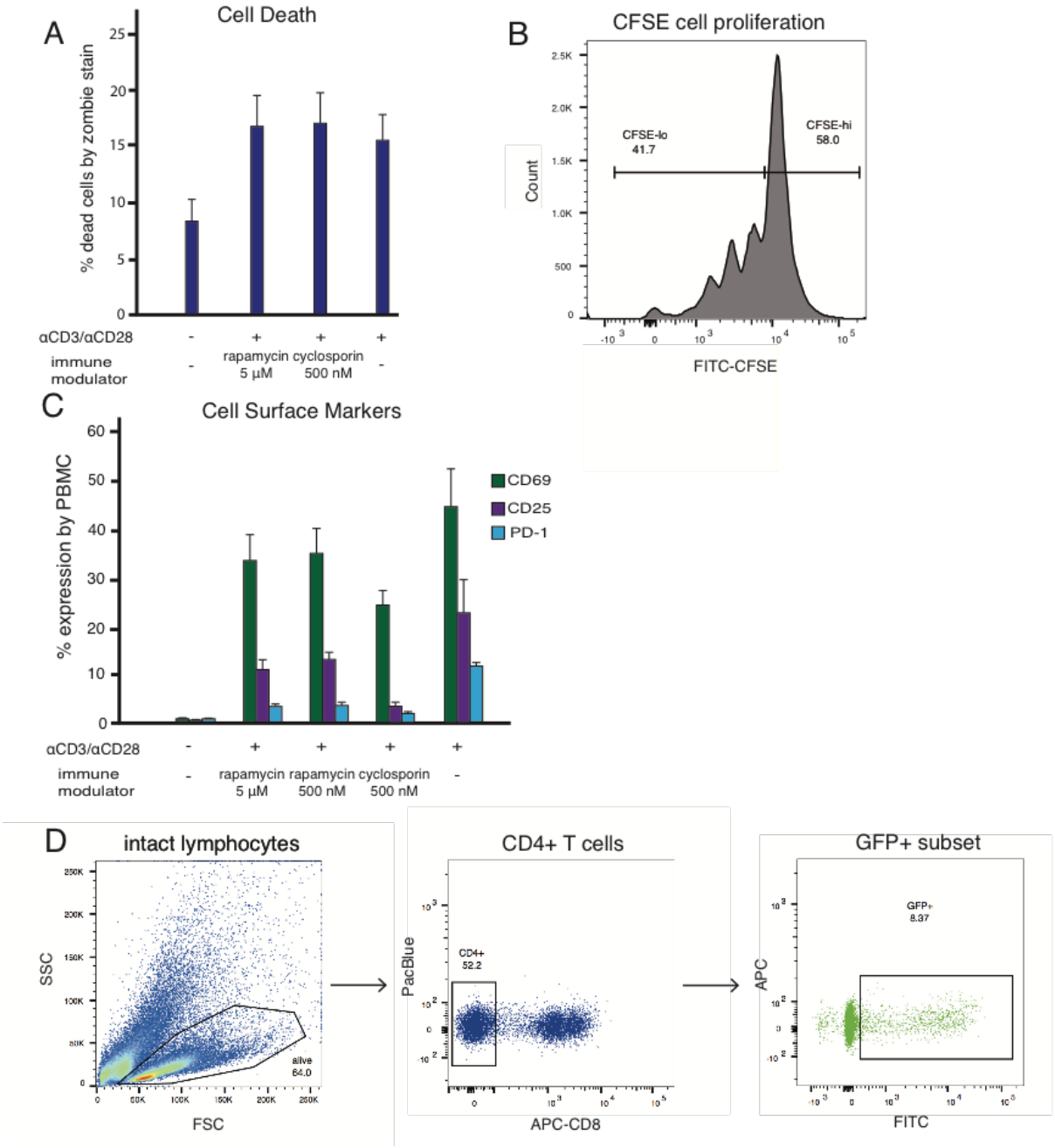
B: Intracellular HIV-1 mRNA levels from cells treated with immunosuppressants alone. Results are shown as fold change over the no stimulation control. Dotted line represents no change from baseline (DMSO alone). Each symbol represents results from a different donor (n=6). Data points are the average of duplicate experiment conditions. Error bars represent mean +/- SEM. (*=$p < 0.05$, **=$p < 0.01$, ***=$p < 0.001$



Supplemental Figure 2: αCD3/αCD28 induced cytokine release by PBMCs is downregulated by immunosuppressant treatment.

A: Quantification of cytokines induced by αCD3/αCD28 alone or cotreatment with rapamycin or cyclosporin. Induced cytokines: IL-2, MCP-1, MIP-1a, IL-1b, MIP-1b, IFNg, TNFa, IL-6. Bars represent the mean of 3 separate experiments.

B: Inhibition of αCD3/αCD28 induced cytokines by cotreatment with rapamycin or cyclosporin (n=3).



Supplemental Figure 3: Immunosuppressant effects on cell surface markers and cell viability; representative flow cytometry plots.

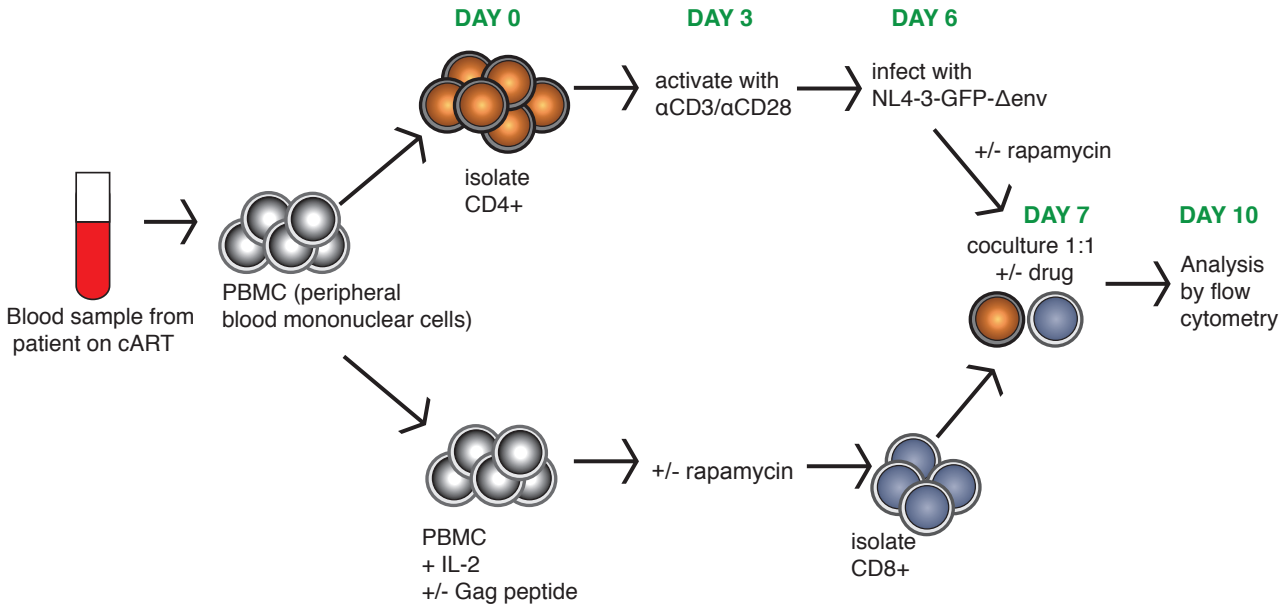
A: Effect of immunosuppressant cotreatment on cell viability. PBMC isolated from healthy donors (n=4) were treated according 24 hours with the indicated drugs, and viability assessed using Zombie Aqua.

B: Representative example of flow cytometry gating for cellular proliferation analysis. Healthy donor derived PBMC (n=3) were stained with CFSE and treated for 24 hours as shown.

C: Activation and exhaustion cell surface markers. PBMC isolated from patients (n=3) were treated for 24 hours with the indicated conditions before cell surface marker measurement with flow cytometry.

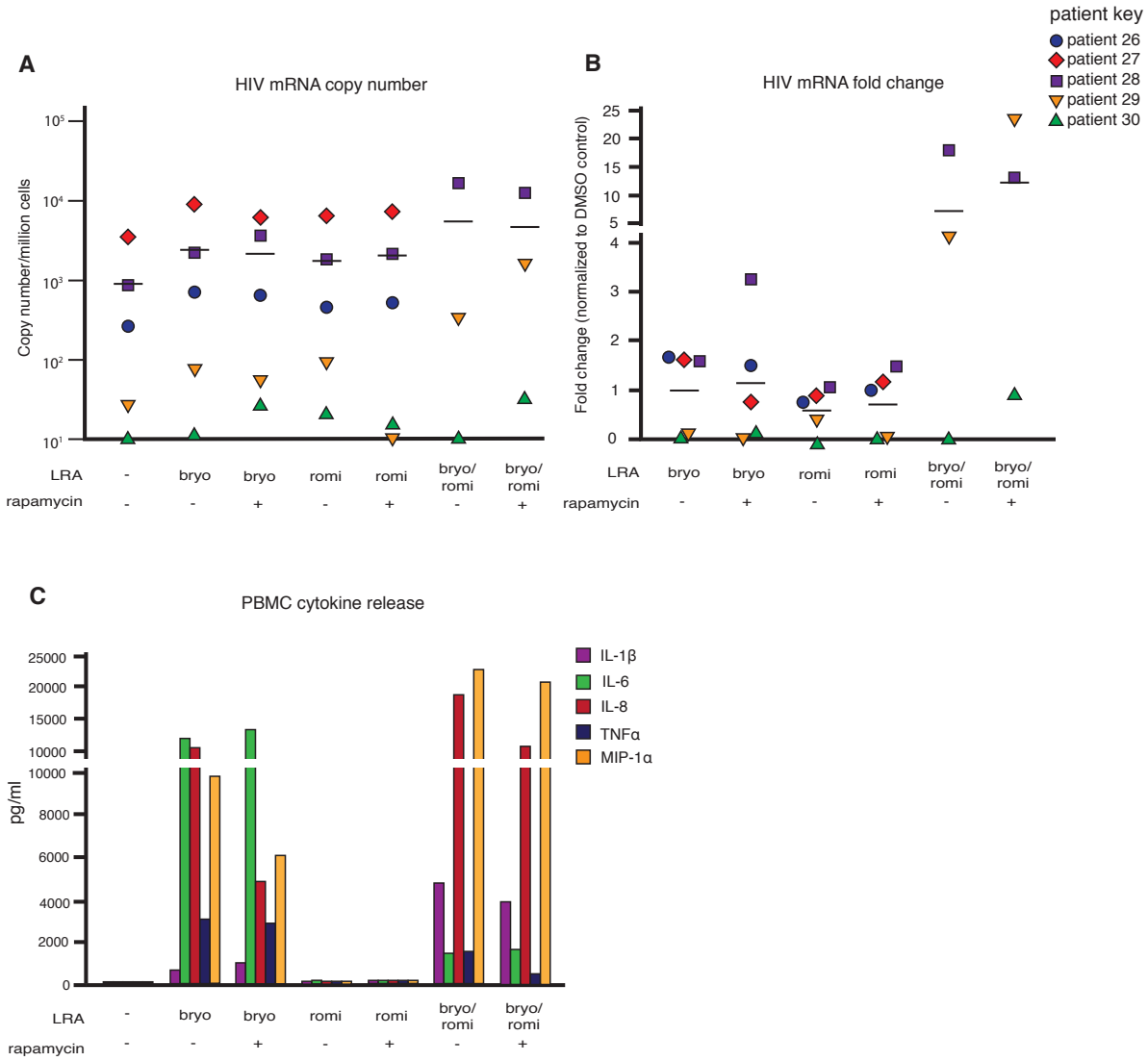
D: Representative example of flow cytometry gating for CTL killing assay. Cells were cocultured for 3 days then stained with CD8-APC to gate $CD4^+$ T cells (APC-). % GFP+ was calculated as a subset of total $CD4^+$ T cells. Fluorescent markers were gated against fluorescent channels to exclude autofluorescent cells.

Data points are the average of duplicate experiment conditions. Error bars represent mean +/- SEM. (*=$p < 0.05$, **=$p < 0.01$, ***=$p < 0.001$)



Supplemental Figure 4: CTL killing assay schematic

Whole blood was collected from HIV-1 infected individuals on suppressive cART and PBMC purified. PBMC samples were split in half; one half was purified for $CD4^+$ T cells and infected with NL4-3-Δenv-GFP for flow cytometry readout. The other half of PBMCs was prestimulated with IL-2 alone or plus gag peptide mixture for 6 days, then purified for $CD8^+$ T cells. Cells were cocultured at a 1:1 E:T ratio and analyzed via flow cytometry.



Supplemental Figure 5: The effect of rapamycin on LRA induction of HIV-1 mRNA and PBMC cytokine release

A: RT-qPCR measurements of intracellular HIV-1 mRNA in rCD4s purified from infected individuals on cART, shown as copies of HIV-1 mRNA detected per million cells. Symbols represent results from different donors (n=5). Treatments include bryostatin-1 (bryo), romidepsin (romi), a combination of bryostatin-1 and romidepsin (bryo/romi), all with or without rapamycin. Data points are the average of duplicate experiment conditions.

B: Relative amounts of induced HIV-1 mRNA from A shown as fold change relative to no stimulation (DMSO alone). Data points are the average of duplicate experiment conditions.

C: Effect of immunosuppressants on LRA induced production of cytokines by healthy donor PBMC. Cytokines shown have been previously suggested to be associated with bryostatin administration to patients. Bars represent the mean of 3 separate experiments.

Supplemental methods

Human Subjects

Whole blood was obtained from both healthy donor and HIV-1 infected participants. All HIV-1 infected individuals enrolled were on a suppressive antiretroviral therapy regimen and maintained undetectable plasma HIV-1 RNA levels (<50 copies per mL) for at least 6 months prior to enrollment. Characteristics of HIV-1 infected donors are listed in Supplemental Table 1.

Isolation of resting CD4+ T cells

Whole blood or leukapheresis blood samples were collected and isolated as described previously.[7] Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density gradient centrifugation. Resting $CD4^+$ T cells (CD4+, CD69-, CD25-, HLA-DR-) were enriched using magnetic microbeads through negative depletion (Miltenyi Biotec).

Drug treatment conditions

All treatments were performed in basic media (RPMI with 10% fetal bovine serum) unless otherwise noted. Drug concentrations were as follows: rapamycin 5.47 μM (5 μg/ml, rounded to 5 μM) or 547 nM (0.5 μg/ml, rounded to 500 nM) as indicated (Millipore, 553211), cyclosporin A 500 nM (Sigma, C3662), bryostatin-1 10 nM (Sigma, B7431), romidepsin 40 nM (Selleck Chemicals, S3020). All drug treatments, including vehicle alone, had a final DMSO concentration of <.05%. αCD3/αCD28 treatment was set up as follows: 10 μL αCD3 antibody (BD Biosciences, 555366) plus 1 mL PBS each was added to wells on a 12 well plate and incubated at 37° C for 1.5 hours. After incubation, wells were washed twice with PBS to remove excess antibody. 1 μL αCD28 antibody (BD Biosciences, 555725) was added per 1 mL media at final treatment.

Quantification of HIV-1 mRNA transcripts

All drug treatments were performed in 1 mL media total with duplicate treatments of $5x10^6$ cells each. RNA isolation and cDNA synthesis were performed as previously described[7]. Real-time PCR was then performed in triplicate using Taqman Fast Advanced MasterMix (Life Technologies, 4444556) on an ViiA7 Real-Time PCR System (Life Technologies). Primers used were specific for correctly terminated polyadenylated HIV-1 mRNA, as described by Shan et al. (1).

Supernatant cytokine analysis

Cytokine release was quantified directly from supernatants of cells used for viral RNA quantification. Supernatants were collected after 24 hours of drug treatment, when cells were used separately for intracellular RNA measurement. Samples were kept at -80 until use and then analyzed according to a previously described protocol (2). Supernatant cytokines were measured using two kits: CBA Human Th1/Th2/Th17 kit (BD Biosciences, 551811) to quantify 7 cytokines in total, shown are the 4 pro-inflammatory cytokines induced to highest levels by αCD3/αCD28 stimulation (IL-2, TNFα, IFNγ, IL-6); or Human Soluble Protein Master Buffer Kit (BD Biosciences, 558264) with MIP-1α, MIP-1β, IL-8, MCP-1, and IL-1β Flex Sets (BD 558325, 558288, 558277, 558287, and 558279 respectively).

Cell proliferation assay

Cellular proliferation was quantified using CellTrace CFSE (Invitrogen, C34554) in PBMC derived from healthy donor blood, according to manufacturer's protocol. Briefly, cells were stained for 5 minutes at room temperature with CFSE at a final concentration of 5 uM. Cells were washed to remove excess stain, and then treatments set up. After treatment, cells were resuspended in PBS and analyzed with a FACSCanto II (BD Biosciences). Treatments were set up as follows: 5 million cells per condition, in duplicate, for 24 hours at drug concentrations described above.

Cell death assay

Cell death was determined in healthy donor PBMC after 24-hour treatment of 5 x 10^6 cells per condition using the Zombie Aqua Fixable Viability Kit (Biolegend, 423101) according to the manufacturer's standard cell staining protocol. Briefly, cells were stained post-treatment using 1 uL stain per 1 x 10^6 cells in 100 uL PBS at room temperature for 15 minutes. Cells were then washed and dead cells quantified on a FACSCanto II.

Measurement of cell surface markers

Surface marker expression was determined in healthy donor PBMC 24-hour after treatment of 1 million cells per condition. Stains used were: PD-1 PE (BD 560795), CD3 Pacific Blue (BD 558117), CD4 FITC (BD 555346), CD69 APC-Cy7 (BD 556656), and CD25 APC (BD 555434) according to manufacturer's protocols. Cells were analyzed by flow cytometry using a FACSCanto II.

CTL coculture

Whole blood samples from HIV-1 infected individuals were collected and processed as described previously (3). Briefly, PBMCs were isolated from whole blood and isolated to $CD4^+$ T cells or prestimulated to prime a $CD8^+$ response. Prestimulated PBMC were cultured with IL-2 (10 U/ml) with or without a mixture of 129 Gag peptides (80 ng/ml for each) (NIH AIDS Reagent Program) for 6 days. Rapamycin (500 uM) was added to some PBMC cultures during the last 3 days of prestimulation without media change. $CD4^+$ T cells were cultured for 3 days, activated as described in cytokine enriched media for an additional 3 days, then infected with NL4-3-Δenv-GFP reporter virus (NIH AIDS Reagent Program). Cells remained in cytokine-enriched media for 1 hour before washing, replating in basic media, and addition of rapamycin to some cell populations as indicated. On the day of coculture, $CD8^+$ T cells were isolated from prestimulated PBMC by negative selection (Miltenyi Biotec, 130-096-49). At coculture rapamycin (500 nM) or cyclosporin (500 nM) were added to some wells as indicated. Cells were

cocultured for 3 days at a 1:1 effector:target (E:T) ratio before staining with CD8 APC (Biolegend 300912) and CD3 Pacific Blue (Biolegend 300330) according to the manufacturer's protocol. Samples were read on a FACSCanto II.

References

1. Shan L, Rabi SA, Laird GM, et al. A novel PCR assay for quantification of HIV-1 RNA. *J Virol*. 2013;87(11):6521-6525.

2. Laird GM, Bullen CK, Rosenbloom DI, et al. Ex vivo analysis identifies effective HIV-1 latency-reversing drug combinations. *J Clin Invest*. 2015;125(5):1901-1912.

3. Shan L, Deng K, Shroff NS, et al. Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation. *Immunity*. 2012;36(3):491-501.