



**HAL**  
open science

## IL-7 Induces SAMHD1 Phosphorylation in CD4+ T Lymphocytes, Improving Early Steps of HIV-1 Life Cycle

M Coiras, M Bermejo, B Descours, E Mateos, J Garcia-Perez, Mr Lopez-Huertas, Mm Lederman, M Benkirane, J Alcamí

► **To cite this version:**

M Coiras, M Bermejo, B Descours, E Mateos, J Garcia-Perez, et al.. IL-7 Induces SAMHD1 Phosphorylation in CD4+ T Lymphocytes, Improving Early Steps of HIV-1 Life Cycle. Cell Reports, 2016, 14 (9), pp.2100-2107. 10.1016/j.celrep.2016.02.022 . hal-01287804

**HAL Id: hal-01287804**

**<https://hal.science/hal-01287804>**

Submitted on 31 May 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License



Published in final edited form as:

Cell Rep. 2016 March 8; 14(9): 2100–2107. doi:10.1016/j.celrep.2016.02.022.

## IL-7 Induces SAMHD1 Phosphorylation in CD4+ T Lymphocytes, Improving Early Steps of HIV-1 Life Cycle

Mayte Coiras<sup>1,\*</sup>, Mercedes Bermejo<sup>1</sup>, Benjamin Descours<sup>2</sup>, Elena Mateos<sup>1</sup>, Javier García-Pérez<sup>1</sup>, María-Rosa López-Huertas<sup>1</sup>, Michael M. Lederman<sup>3</sup>, Monsef Benkirane<sup>2</sup>, and José Alcamí<sup>1,\*</sup>

<sup>1</sup>AIDS Immunopathogenesis Unit, Instituto de Salud Carlos III, Madrid 28220, Spain

<sup>2</sup>Institut de Génétique Humaine CNRS UPR1142, Université de Montpellier, Laboratoire de Virologie Moléculaire, Montpellier 34000, France

<sup>3</sup>Case Western Reserve University, University Hospitals Case Medical Center, Cleveland, OH 44106, USA

### SUMMARY

HIV-1 post-integration latency in CD4+ lymphocytes is responsible for viral persistence despite treatment, but mechanisms involved in the establishment of latent viral reservoirs are not fully understood. We determined that both interleukin 2 (IL-2) and IL-7 induced SAMHD1 phosphorylation in T592, abrogating its antiviral activity. However, IL-7 caused a much more profound stimulatory effect on HIV-1 reverse transcription and integration than IL-2 that required chemokine co-stimulation. Both cytokines barely induced transcription due to low NF- $\kappa$ B induction, favoring the establishment of latent reservoirs. Effect of IL-7 on SAMHD1 phosphorylation was confirmed in IL-7-treated patients (ACTG 5214 study). Dasatinib—a tyrosine-kinase inhibitor—blocked SAMHD1 phosphorylation induced by IL-2 and IL-7 and restored HIV-1 restriction. We propose that  $\gamma$ c-cytokines play a major role in the reservoir establishment not only by driving homeostatic proliferation but also by increasing susceptibility of CD4+ lymphocytes to HIV-1 infection through SAMHD1 inactivation.

### In Brief

Coiras et al. show that IL-2 and IL-7 induce SAMHD1 phosphorylation, abrogating its antiviral activity. This increases susceptibility of CD4+ lymphocytes to HIV-1 infection, contributing to the establishment of the reservoir. These  $\gamma$ c-cytokines also maintain the reservoir through homeostatic

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

\*Correspondence: mcoiras@isciii.es (M.C.), ppalcami@isciii.es (J.A.).

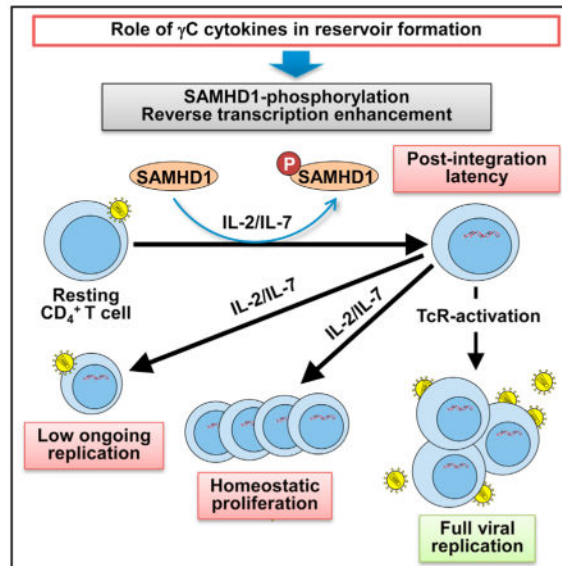
### SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and five figures and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2016.02.022>.

### AUTHOR CONTRIBUTIONS

M.C., M. Benkirane, and J.A. conceived the study and interpreted the results. M.C. and E.M. performed the immunoblotting and qPCR assays. M. Bermejo performed the flow cytometry analysis. B.D. and M. Benkirane developed and provided the antibodies against SAMHD1 phosphorylated at T592 and performed VLP experiments. J.G.-P. performed nucleofection experiments, M.-R.L.-H. performed complementary cytoskeleton analysis, and M.M.L. conducted A5214 and helped analyze and interpret the human data. All authors contributed in writing and revisions of the paper. All authors have read and approved the final submission of the manuscript.

proliferation. The tyrosine-kinase inhibitor dasatinib blocked SAMHD1 phosphorylation induced by IL-2 and IL-7, restoring HIV-1 restriction.



## INTRODUCTION

Persistent HIV-1 infection is due to its capacity to integrate into the host cell DNA where the provirus may persist in a latent form. Antiretroviral treatment (ART) controls viral replication but cannot eliminate this latent reservoir (Finzi et al., 1999; Siliciano et al., 2003). A very small number of these residual, latently infected cells may be sufficient to refuel viral replication and replenish the reservoir if ART fails or is interrupted (Henrich et al., 2014). Resting memory CD4<sup>+</sup> T cells, preferentially stem cell memory (Buzon et al., 2014) and central memory (Chomont et al., 2009), constitute the main reservoir for HIV-1. This reservoir is established very early after infection (Whitney et al., 2014), but its size is quite low in patients on ART. It has been recently estimated between 10 and 100 replication-competent latent provirus per million resting CD4<sup>+</sup> T cells (Ho et al., 2013).

In latent state, HIV-1 persists unscathed by immune response or ART, but T cell activation resumes viral production. Among the inducers of HIV reactivation are T cell receptor (TCR) activation and inflammatory cytokines such as tumor necrosis factor (TNF) or interleukin 6 (IL-6) (Chun et al., 1998), whereas the role of common gamma chain ( $\gamma$ C) cytokines such as IL-7 and IL-2 is controversial (Bosque et al., 2011). IL-7 is crucial for T cell development and homeostasis and participates in survival and maintenance of memory CD4<sup>+</sup> T cells (Kondrack et al., 2003). During HIV infection, increased levels of IL-7 can be detected, but they are not sufficient to maintain T cell homeostasis and CD4<sup>+</sup> count progressively declines (Napolitano et al., 2001). Several clinical trials with IL-7 have been performed in HIV-infected individuals with the goal of increasing the number and function of lymphocytes, particularly memory cells (Levy et al., 2009; Lévy et al., 2012; Sereti et al., 2009; Katlama et al., 2016). When tested as a potential anti-latency drug, IL-7 produced T cell proliferation plus low-level viral reactivation (Bosque et al., 2011), failing to deplete the viral reservoir

and even increasing, at least transiently, the proviral load (Katlama et al., 2016; Sereti et al., 2009).

HIV-1 infects and replicates in activated CD4<sup>+</sup> lymphocytes, but once the drivers of T cell activation diminish, resting CD4<sup>+</sup> lymphocytes are mostly non-permissive for HIV-1 replication. This is largely due to the absence of essential transcription factors such as NF- $\kappa$ B and NF-AT (Coiras et al., 2009) and to the SAMHD1 protein (Descours et al., 2012; Baldauf et al., 2012). SAMHD1 is a key regulator of cell-cycle progression and a major viral restriction factor that blocks early reverse transcription of HIV-1 by depleting the intracellular dinucleotide triphosphate (dNTP) pool (Lahouassa et al., 2012). It has been proposed that HIV restriction of SAMHD1 can also be related with degradation of viral RNA through its RNase activity (Ryoo et al., 2014), but this concept remains controversial (Seamon et al., 2015). The function of SAMHD1 is regulated through the phosphorylation of threonine 592 (T592) by cyclin A2/Cdk1, an event that is induced by T cell activation and that renders the cells susceptible to infection by HIV-1 (Cribier et al., 2013). The accessory protein Vpx of HIV-2 and the simian immunodeficiency virus (SIV) target SAMHD1 for ubiquitination and proteasomal degradation (Laguette et al., 2011). As HIV-1 does not encode Vpx, it remains sensitive to SAMHD1-mediated restriction until T cells receive an activation signal. We evaluated the effect of  $\gamma$ c-cytokines such as IL-2 and IL-7 and determined that they induced SAMHD1 phosphorylation in CD4<sup>+</sup> T cells, promoting susceptibility to HIV-1 infection. Moreover, as tyrosine kinases are essential during T cell activation driven by IL-7 (Johnston et al., 1996), we observed that the tyrosine kinase inhibitor dasatinib interfered with SAMHD1 phosphorylation mediated by IL-7, which preserves its antiviral activity and identifies this factor as a potential therapeutic target.

## RESULTS

### TCR-Mediated Activation and Homeostatic Stimuli Such as IL-2 and IL-7 Induced the Phosphorylation of SAMHD1 at T592 in Human CD4<sup>+</sup> T Cells

Stimuli such as TCR-mediated activation and  $\gamma$ c-cytokines were analyzed to determine their role in SAMHD1 phosphorylation at T592 in human primary CD4<sup>+</sup> T cells. When CD4<sup>+</sup> lymphocytes were treated with anti-CD3/CD28 for 5 days, SAMHD1 phosphorylation initiated after 1 day of TCR-mediated activation (Figure 1A, left), reaching a peak at day 2 that rapidly decayed. Simultaneous treatment with IL-2 permitted a sustained SAMHD1 phosphorylation that remained constant for at least 5 days (Figure 1A, right). Treatment with IL-2 or IL-7 only induced SAMHD1 phosphorylation at day 3, which was sustained for at least 5 days (Figure 1B). A dose-response experiment (Figure 1B, bottom) did not show significant difference between treatments with low (1 nM) or high (10 nM) concentrations of IL-7. SAMHD1 phosphorylation induced by IL-2 or IL-7 was quite stable as it persisted for at least 48 hr after removing the cytokines from the culture medium (Figure 1C, lanes 6 and 7). At the doses employed, IL-2 and IL-7 caused T cell proliferation after 10 days of treatment (Figure S1). Treatment of CD4<sup>+</sup> T cells with chemokines CXCL9, CXCL10, or CXCL12 did not induce SAMHD1 phosphorylation (Figure 1D, lanes 5 and 6). The expression of total SAMHD1 was not modified by the addition of any stimuli to the culture.

## IL-2 and IL-7 Induced Reverse Transcription and Proviral Integration in CD4+ T Cells

To determine the role of IL-2 and IL-7 in HIV-1 reverse transcription and proviral integration, primary CD4+ T cells were treated with IL-2 or IL-7 for 5 days and then infected with X4-tropic NL4-3\_Renilla luciferase strain and cultured for 5 additional days. Viral reverse transcripts (RTs) were analyzed by qPCR 5 hr post-infection. Low levels of early and late RT were induced by IL-2 treatment, whereas IL-7 induced an average 13-fold increase in early RT and 43-fold increase in late RT, compared to IL-2 ( $p < 0.001$ ; Figure 2A). Both cytokines increased two long terminal repeat (2-LTR) circles and proviral integration, but IL-7 was more efficient as it induced an average 2-fold increase in the number of 2-LTR circles (Figure 2B) and 19-fold increase in proviral integration as compared to IL-2 (Figure 2C). Similar increases were obtained with R5-tropic strains (data not shown) and when a wild-type NL4.3 viral clone carrying the *nef* gene was used (Figures S2A–S2C). Treatment with CXCL9/CXCL10 chemokines enhanced IL-2-induced viral integration 27-fold ( $p < 0.01$ ), whereas simultaneous treatment with CXCL9/CXCL10 enhanced integration induced by IL-7 only an average of 2.2-fold ( $p < 0.05$ ; Figure 2D).

Two lines of evidence suggest that IL-7 function was mediated through SAMHD1. First, overexpression of T592A SAMHD1, a phosphorylation-resistant mutant, decreased IL-2- and IL-7-mediated infection when transfected in resting CD4 lymphocytes before cytokine activation (Figure 2E). Second, pretreatment with VLP-Vpx reduced IL7-mediated enhancement of infection of resting CD4 lymphocytes with HIV-cytomegalovirus (CMV)-GFP (Figure S2D).

## LTR- and NF- $\kappa$ B-Dependent Activation in CD4+ T Cells Treated with IL-2 or IL-7

The ability of IL-2 and IL-7 to establish latent proviral integration was determined in primary CD4+ T cells treated with IL-2 or IL-7 for 5 days and infected with NL4-3\_Renilla luciferase strain for 5 additional days. Phorbol 12-myristate 13-acetate (PMA) added 18 hr before cell collection produced an average 14-fold and 17-fold increase in viral transcription in cells treated with IL-2 or IL-7 ( $p < 0.001$ ), respectively (Figure 3A). Treatment with PMA did not increase proviral integration in CD4+ T cells treated with IL-7 (Figure S3).

The effect of IL-2 or IL-7 on HIV-1 transcription was also analyzed in CD4+ T cells treated with IL-2 or IL-7 for 5 days and then nucleofected with luciferase expression vectors under the control of the HIV-1-LTR (pLTR-LUC) or three copies of the NF- $\kappa$ B consensus (p3KB-LUC). Two hours after nucleofection, cells were incubated with PMA and cultured for 18 hr. Both IL-2 and IL-7 barely activate LTR- or NF- $\kappa$ B-dependent transcription that was strongly increased by PMA treatment ( $p < 0.001$ ; Figure 3B).

## SAMHD1 Was Preferentially Phosphorylated in Memory CD4+ T Lymphocytes of HIV-Infected Patients Included in the ACTG 5214 Study Who Received a Single Dose of IL-7

To confirm that IL-7 could phosphorylate SAMHD1 in vivo, sub-populations of CD4+ lymphocytes from nine patients enrolled in the ACTG 5214 study (Sereti et al., 2009) were analyzed by flow cytometry. In this study, patients were treated with a single subcutaneous dose of rhIL-7 with doses ranging from 3 to 30 microg/kg. After selection of living cells (Figure S4), combination of CCR7 and CD45RA markers allowed characterization of naive

(CCR7<sup>+</sup>CD45RA<sup>+</sup>), central memory (CCR7<sup>+</sup>CD45RA<sup>-</sup>), transitional and effector memory (CCR7<sup>-</sup>CD45RA<sup>-</sup>), and terminally differentiated (CCR7<sup>-</sup>CD45RA<sup>+</sup>) CD4<sup>+</sup> lymphocytes. SAMHD1 phosphorylation significantly increased an average 8.0% in central memory cells ( $p < 0.05$ ) and 7.0% in effector memory cells ( $p < 0.05$ ) 4 days after the single injection of rhIL-7 (Figure 4).

### Dasatinib Blocked IL-7-Mediated Reverse Transcription and Proviral Integration

It has been described that  $\gamma$ c-cytokines activate pathways that involve tyrosine kinases (Johnston et al., 1996). Therefore, primary CD4<sup>+</sup> lymphocytes were simultaneously treated for 5 days with IL-7 and dasatinib, an inhibitor of tyrosine kinases—currently used in the treatment of chronic myeloid leukemia (Das et al., 2006). Dasatinib has been described to inhibit HIV-1 replication at a concentration of 75 nM (Pogliaghi et al., 2014), and we demonstrated that, at this concentration, it strongly interfered with SAMHD1 phosphorylation induced by IL-7 (Figure 5A). As a result, dasatinib interfered with IL-7-mediated viral reverse transcription (Figure 5B) and with proviral integration (Figure 5C).

## DISCUSSION

Among the mechanisms for HIV persistence, factors that extend lifetime and homeostatic proliferation of infected cells are of paramount importance (Deeks et al., 2012). In this context, IL-7 is crucial for T cell development and homeostasis and also participates in the survival and maintenance of memory CD4<sup>+</sup> lymphocytes (Kondrack et al., 2003). IL-7 was first reported to increase HIV replication in peripheral blood mononuclear cells (PBMCs) of infected individuals (Smithgall et al., 1996), in resting CD4<sup>+</sup> lymphocytes from patients on ART (Wang et al., 2005), and in chronically infected human cells (Scripture-Adams et al., 2002). As a result, IL-7 was originally proposed as a potential anti-latency agent. Early clinical trials of IL-7 administration were associated with an increased frequency of “blips” of HIV RNA in plasma among HIV-infected patients who were receiving suppressive ART (Imamichi et al., 2011; Lévy et al., 2012). But although IL-7 can activate phosphatidylinositol 3-kinase (PI3K) pathways (Venkitaraman and Cowling, 1994), we and others determined that NF- $\kappa$ B activity was only slightly increased in CD4<sup>+</sup> lymphocytes treated with IL-7 and is not sufficient to reverse viral latency (Bosque et al., 2011). Later, it was demonstrated that IL-7 may contribute to the maintenance of HIV reservoirs via homeostatic proliferation (Chomont et al., 2009). In fact, administration of IL-7 to HIV-infected patients induced expansion of CD4<sup>+</sup> lymphocytes and increased HIV DNA copy numbers (Katlama et al., 2016; Sereti et al., 2009).

Because  $\gamma$ c-cytokines promote the T lymphocytes cell cycling and division and because SAMHD1 is regulated by cyclins (Cribier et al., 2013), we hypothesized that  $\gamma$ c-cytokines might also regulate SAMHD1 activity, leading to an increased susceptibility to HIV-1 infection. We evaluated the effect of IL-2 and IL-7 on SAMHD1 antiviral activity and determined that both cytokines induced SAMHD1 phosphorylation in primary CD4<sup>+</sup> lymphocytes, thereby eliminating its antiviral activity. Consequently, IL-7 promoted successful reverse transcription and subsequent proviral integration more efficiently than IL-2. This effect was mediated through SAMHD1 as demonstrated by the use of SAMHD1



T592A mutant and VLP-Vpx, which reduced the IL7-mediated enhancement of resting CD4 lymphocytes infection. However, because IL-2 and IL-7 produced a similar pattern of SAMHD1 phosphorylation, an additional mechanism should be contributing to the higher efficiency of IL-7. It has been described that actin polymerization dynamics driven by certain chemokines influence different steps of the pre-integration process (Wu and Yoder, 2009). Accordingly, as it has been described (Cameron et al., 2010) that treatment with chemokines CXCL9 and CXCL10, among others, promote post-integration latency, we tested their impact in our model. When CD4+ T cells were treated with IL-2 and IL-7 together with CXCL9/CXCL10, stronger integration in IL-2-cultured lymphocytes was found. However, the impact of chemokine treatment on IL-7 enhancement of proviral integration was lower, suggesting that IL-7 could stabilize or modify actin dynamics as chemokines do. Furthermore, as  $\gamma$ c-cytokines are poor inducers of NF- $\kappa$ B and NFAT, they might be contributing to the establishment of latent reservoirs. In fact, HIV-1 expression was incomplete in CD4+ lymphocytes infected in vitro in the presence of IL-2 or IL-7 as it was further increased with PMA. Thus, although IL-7 efficiently promoted integration, latency persisted until a stronger T cell activation signal was received. TCR-mediated activation induced faster SAMHD1 phosphorylation, which occurs at day 1 after stimulation, but this phosphorylated form rapidly decayed at day 3. On the other hand, IL-2 and IL-7 were slower to induce SAMHD1 phosphorylation, but they induced a more-stable phosphorylation that was maintained even after the stimuli were removed. This suggested that SAMHD1 phosphorylation by TCR-mediated activation or homeostatic cytokines occurred through different mechanisms.

Recent structural work has shown that homotetrameric conformation of SAMHD1 is dependent on diguanosine triphosphate (dGTP) binding, allowing a more-stable interaction with the substrate. Mutations of dGTP-binding residues in the allosteric site affect tetramer formation, dNTPase activity, and HIV-1 restriction (Zhu et al., 2013). It would be important to study whether SAMHD1 phosphorylation at T592 by IL-2 and IL-7 results in decreased tetramer formation. Actually, the total amount of SAMHD1 is not modified by IL-2 or IL-7 treatment, pointing to the generation of full inactive SAMHD1 in the absence of protein degradation, an event that could be related with impairment of multimeric complexes formation due to SAMHD1 phosphorylation.

To assess the effect of IL-7 on SAMHD1 phosphorylation in vivo, we analyzed PBMCs from subjects enrolled in the ACTG 5214 trial, who received a single injection of rhIL-7 (Sereti et al., 2009). A significant increase in SAMHD1 phosphorylation was observed at day 4 in central and effector memory CD4+ lymphocytes from these patients, which was in accordance with the peak of SAMHD1 phosphorylation observed in vitro after 4 days of IL-7 activation. A sharp increase in cycling —measured by Ki67 expression—has been described in these patients at day 4 after IL-7 treatment (Chomont et al., 2009; Sereti et al., 2009), which strongly correlates with our observed induction of SAMHD1 phosphorylation. Our own data confirm (Schmidt et al., 2015) that, in healthy donors, less than 2% of CD4 lymphocytes from peripheral blood display phosphorylated SAMHD1. Basal levels of phosphorylated SAMHD1 in ACTG 5214 patients were higher and could be related to the high level of immune activation described in HIV-infected patients even in the presence of

ART. Actually, ACTG 5214 patients displayed high levels of lymphocyte activation as assessed by CD38/HLA-DR expression (Chomont et al., 2009; Sereti et al., 2009).

It has been proposed that the persistence of the viral reservoir mostly occurs through homeostatic proliferation induced by  $\gamma$ c-cytokines such as IL-7 (Chomont et al., 2009). In fact, IL-7 therapy promotes CD4<sup>+</sup> expansion in HIV-infected patients on ART (Lévy et al., 2012) while concurrently expanding the pool of provirus-containing CD4<sup>+</sup> lymphocytes (Lévy et al., 2012; Vandergeeten et al., 2013; Katlama et al., 2016). Our findings show that IL-7 induced SAMHD1 phosphorylation in CD4<sup>+</sup> lymphocytes, which supported the notion that this cytokine has a role not only in reservoir expansion but also in increasing the infectivity of memory cells, leading to HIV integration and reservoir replenishment. Accordingly, impeding SAMHD1 phosphorylation may represent a valuable target to increase restriction to HIV-1 infection in CD4<sup>+</sup> lymphocytes. Because  $\gamma$ c-cytokines activate JAK/STAT pathways through tyrosine phosphorylation, we assessed whether the tyrosine kinase inhibitor dasatinib also prevented SAMHD1 phosphorylation. We demonstrate that inhibition of SAMHD1 phosphorylation by dasatinib strongly decreased HIV reverse transcription and integration. Consequently, tyrosine-kinase inhibitors may represent potential candidates to be used as adjuvants to ART, in particular in primary infection when viral reservoirs are established.

In conclusion, we propose a model in which  $\gamma$ c-cytokines seed and replenish the reservoir during suboptimal T cell activation. In this model, IL-7 and IL-2 would promote SAMHD1 phosphorylation, making CD4<sup>+</sup> lymphocytes more susceptible to HIV-1 infection by facilitating reverse transcription and, subsequently, proviral integration. However, these  $\gamma$ c-cytokines are weak inducers of viral transcription (Bosque et al., 2011), and therefore, IL-7 and IL-2 would maintain preferentially the provirus in latency. It cannot be discarded that IL-2 and IL-7 contribute to blips or low persistent viremia that can be detected in patients on ART (Coiras et al., 2009; Imamichi et al., 2011). Consequently, IL-7 would fulfill three essential roles in HIV-1 persistence: reservoir seeding through SAMHD1 phosphorylation, low ongoing expression via NF- $\kappa$ B activity, and reservoir maintenance through homeostatic proliferation. In this model, full activation of proviral transcription through TCR-mediated activation or inflammatory cytokine exposure would drive robust viral replication and CD4 depletion.

## EXPERIMENTAL PROCEDURES

### Cells

PBMCs were isolated from healthy donors by Ficoll-Hypaque gradient (GE Healthcare). Primary CD4<sup>+</sup> T cells were isolated using CD4<sup>+</sup> T Cell Isolation Kit (Miltenyi Biotec).

### Patients

Blood samples of patients treated with IL-7 belong to ACTG5214 study. This study was approved by the institutional review boards (IRBs) of all participating sites, and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki (NCT 00099671; as stated in Sereti et al., 2009).



Blood from nine patients enrolled in ACTG 5214 study were collected before rhIL-7 administration (day 0) and 4 days after injection. All participants received a single dose of subcutaneous human recombinant IL-7 on day 0 at doses ranging 3–30 µg/kg. Details of the clinical trial (NCT00099671) have been previously published (Sereti et al., 2009).

### Reagents and Antibodies

IL-2 (Chiron) was used at 300 U/ml. IL-7 (R&D Systems) was used at 1 nM across most experiments. Purified anti-human CD3 (clone OKT3) and CD28 (clone CD28.2) were used for T cell activation (eBiosciences). CXCL9 and CXCL10 (R&D Systems) were used at 0.1 µM. CXCL12 was used at 50 nM and was kindly provided by Dr. Françoise Baleux (Institut Pasteur). PMA (Sigma-Aldrich) was used at 25 ng/ml. A polyclonal antibody against SAMHD1 phosphorylated at T592 was used for immunoblotting, and a monoclonal antibody conjugated with Alexa Fluor 647 (Life Technologies) was used for flow cytometry. Both antibodies were generated at Institute of Human Genetics. Validation of the murine monoclonal antibody against SAMHD1 phosphorylated at T592 was performed in TH1 cells. Titration of the epitope specificity was performed by preincubation of the antibody with immunizing peptide before incubation on cells (Figure S5A). Phosphorylated SAMHD1 showed a predominant nuclear localization (Figure S5B). Antibody against total SAMHD1 was purchased from Bethyl Laboratories. Dasatinib (Sprycel; Bristol-Meyers Squibb) was kindly provided by Dr. Stephen Mason and Dr. Carey Hwang.

### Vectors

pLTR-LUC, p3κB-LUC (Arenzana-Seisdedos et al., 1993), the pNL4.3-Renilla luciferase replication competent viral clone (Garcia-Perez et al., 2007), pOZ-SAMHD1-WT, and T592 mutant expression vectors (Cribier et al., 2013) have been previously described.

### HIV-1 Infection

Primary CD4<sup>+</sup> T cells were infected by spinoculation, as previously described (López-Huertas et al., 2011). Luciferase was measured using Renilla Luciferase Assay System (Promega) with a Sirius luminometer (Berthold).

### Transfection Assays

CD4<sup>+</sup> T cells were transiently transfected in resting conditions by electroporation at 300 V, 1,500 µF, ∞Ω at a concentration of 20 million per cuvette, 1 µg DNA plasmid per million of cells.

### Immunoblotting

Whole protein extracts were fractionated by SDS-PAGE and transferred onto Hybond-ECL nitrocellulose paper (GE Healthcare). Proteins were detected with SuperSignal West Pico Chemiluminescent Substrate (Pierce).

### Flow Cytometry Multicolor Assay

Analysis of different CD4<sup>+</sup> T cells subsets was performed after staining with anti-CD4-PeCy7 (OKT4; BioLegend), anti-CCR7-FITC (3D12), and anti-CD45RA-PE (HI100; BD

Biosciences). Cells were fixed with paraformaldehyde 1%, permeabilized with methanol, and probed with anti-SAMHD1 phosphorylated at T592 conjugated with Alexa Fluor 647. Data acquisition was performed in a fluorescence-activated cell sorting (FACS) Canto flow cytometer (BD Biosciences). Data processing and analysis were done using FACS Diva software and Flowing software v2.5.1.

### qPCR

Strong stop DNA was quantified using primer pairs specific for R and U5 regions of the HIV LTR as described (Mohammadi et al., 2013). Episomal forms of 2-LTR and integrated HIV-1 proviral DNA were quantified by conventional and qPCR, respectively, as described (Buzón et al., 2010). A standard curve of integrated DNA from 8E5 cell line was prepared, and *ccr5* gene was used as housekeeping. Conventional PCR was performed in a C1000 thermal cycler (Bio-Rad), and qPCR was performed in a StepOnePlus Real-Time PCR System, using StepOne v3.2 software (Life Technologies).

### Statistical Analysis

Statistical analysis was performed using Graph Pad Prism 5.0 (Graph Pad Software). Comparisons between two groups were made using two-tailed Mann-Whitney test. Comparisons between more than two groups were made using two-way ANOVA with Bonferroni post test analysis.

### Acknowledgments

The authors thank the patients for their participation in the study. We are indebted to Dr. Irini Sereti (NIAID, NIH), Amanda Zadzilka (Frontier Science and Technology Research Foundation), and Eugenia Aga (Harvard School of Public Health) for assistance with A5214 data and samples. We greatly appreciate the secretarial assistance of Mrs. Olga Palao. We thank the Centro Regional de Transfusión for supplying the buffy coats. We also thank Drs. Javier Martínez-Picado and Maria del Carmen Puertas (IrsiCaixa Institute) for their help with the standardization of qPCR in our laboratory. From Bristol-Myers Squibb, we especially acknowledge Drs. Stephen Mason and Carey Hwang for helpful discussion about this manuscript and Drs. Fred Asare and Frank Lee for kindly providing us with dasatinib. This research was supported by the Spanish Ministry of Economy and Competitiveness (SAF2010-18388, SAF2013-44677-R, and FIS PI12/00506), the SPANISH AIDS Research Network RD12/0017/0015 that is included in the Spanish I+D+I Plan and is co-financed by ISCIII-Subdirección General de Evaluación and European Funding for Regional Development (FEDER), Bristol-Myers Squibb (BMS AI471-041), joined program of the Agence nationale de recherches sur le sida et les hépatites virales (ANRS 2014-2), and the European FP7-HEALTH project HIT HIDDEN HIV. The work of E.M. is supported by Instituto de Salud Carlos III (MPY 1371/12). The work of B.D. is supported by the European FP7-HEALTH project HIT HIDDEN HIV. The ACTG study A5214 was supported by the NIH (AI 069501).

### References

- Arenzana-Seisdedos F, Fernandez B, Dominguez I, Jacqué JM, Thomas D, Diaz-Meco MT, Moscat J, Virelizier JL. Phosphatidylcholine hydrolysis activates NF-kappa B and increases human immunodeficiency virus replication in human monocytes and T lymphocytes. *J Virol.* 1993; 67:6596–6604. [PubMed: 8411362]
- Baldauf HM, Pan X, Erikson E, Schmidt S, Daddacha W, Burggraf M, Schenkova K, Ambiel I, Wabnitz G, Gramberg T, et al. SAMHD1 restricts HIV-1 infection in resting CD4(+) T cells. *Nat Med.* 2012; 18:1682–1687. [PubMed: 22972397]
- Bosque A, Famiglietti M, Weyrich AS, Goulston C, Planelles V. Homeostatic proliferation fails to efficiently reactivate HIV-1 latently infected central memory CD4+ T cells. *PLoS Pathog.* 2011; 7:e1002288. [PubMed: 21998586]

- Buzón MJ, Massanella M, Llibre JM, Esteve A, Dahl V, Puertas MC, Gatell JM, Domingo P, Paredes R, Sharkey M, et al. HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. *Nat Med.* 2010; 16:460–465. [PubMed: 20228817]
- Buzon MJ, Sun H, Li C, Shaw A, Seiss K, Ouyang Z, Martin-Gayo E, Leng J, Henrich TJ, Li JZ, et al. HIV-1 persistence in CD4+ T cells with stem cell-like properties. *Nat Med.* 2014; 20:139–142. [PubMed: 24412925]
- Cameron PU, Saleh S, Sallmann G, Solomon A, Wightman F, Evans VA, Boucher G, Haddad EK, Sekaly RP, Harman AN, et al. Establishment of HIV-1 latency in resting CD4+ T cells depends on chemokine-induced changes in the actin cytoskeleton. *Proc Natl Acad Sci USA.* 2010; 107:16934–16939. [PubMed: 20837531]
- Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio FA, Yassine-Diab B, Boucher G, Boulassel MR, Ghattas G, Brechley JM, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med.* 2009; 15:893–900. [PubMed: 19543283]
- Chun TW, Engel D, Mizell SB, Ehler LA, Fauci AS. Induction of HIV-1 replication in latently infected CD4+ T cells using a combination of cytokines. *J Exp Med.* 1998; 188:83–91. [PubMed: 9653086]
- Coiras M, López-Huertas MR, Pérez-Olmeda M, Alcamí J. Understanding HIV-1 latency provides clues for the eradication of long-term reservoirs. *Nat Rev Microbiol.* 2009; 7:798–812. [PubMed: 19834480]
- Cribier A, Descours B, Valadão AL, Laguette N, Benkirane M. Phosphorylation of SAMHD1 by cyclin A2/CDK1 regulates its restriction activity toward HIV-1. *Cell Rep.* 2013; 3:1036–1043. [PubMed: 23602554]
- Das J, Chen P, Norris D, Padmanabha R, Lin J, Moquin RV, Shen Z, Cook LS, Doweiko AM, Pitt S, et al. 2-aminothiazole as a novel kinase inhibitor template. Structure-activity relationship studies toward the discovery of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-1,3-thiazole-5-carboxamide (dasatinib, BMS-354825) as a potent pan-Src kinase inhibitor. *J Med Chem.* 2006; 49:6819–6832. [PubMed: 17154512]
- Deeks SG, Autran B, Berkhout B, Benkirane M, Cairns S, Chomont N, Chun TW, Churchill M, Di Mascio M, Katlama C, et al. International AIDS Society Scientific Working Group on HIV Cure. Towards an HIV cure: a global scientific strategy. *Nat Rev Immunol.* 2012; 12:607–614. [PubMed: 22814509]
- Descours B, Cribier A, Chable-Bessia C, Ayinde D, Rice G, Crow Y, Yatim A, Schwartz O, Laguette N, Benkirane M. SAMHD1 restricts HIV-1 reverse transcription in quiescent CD4(+) T-cells. *Retrovirology.* 2012; 9:87. [PubMed: 23092122]
- Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, Pierson T, Smith K, Lisziewicz J, Lori F, Flexner C, et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med.* 1999; 5:512–517. [PubMed: 10229227]
- Garcia-Perez J, Sanchez-Palomino S, Perez-Olmeda M, Fernandez B, Alcamí J. A new strategy based on recombinant viruses as a tool for assessing drug susceptibility of human immunodeficiency virus type 1. *J Med Virol.* 2007; 79:127–137. [PubMed: 17177310]
- Henrich TJ, Hanhauser E, Marty FM, Sirignano MN, Keating S, Lee TH, Robles YP, Davis BT, Li JZ, Heisey A, et al. Antiretroviral-free HIV-1 remission and viral rebound after allogeneic stem cell transplantation: report of 2 cases. *Ann Intern Med.* 2014; 161:319–327. [PubMed: 25047577]
- Ho YC, Shan L, Hosmane NN, Wang J, Laskey SB, Rosenbloom DI, Lai J, Blankson JN, Siliciano JD, Siliciano RF. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell.* 2013; 155:540–551. [PubMed: 24243014]
- Imamichi H, Degray G, Asmuth DM, Fischl MA, Landay AL, Lederman MM, Sereti I. HIV-1 viruses detected during episodic blips following interleukin-7 administration are similar to the viruses present before and after interleukin-7 therapy. *AIDS.* 2011; 25:159–164. [PubMed: 21124203]
- Johnston JA, Bacon CM, Riedy MC, O’Shea JJ. Signaling by IL-2 and related cytokines: JAKs, STATs, and relationship to immunodeficiency. *J Leukoc Biol.* 1996; 60:441–452. [PubMed: 8864127]
- Katlama C, Lambert-Niclot S, Assoumou L, Papagno L, Lecardonnel F, Zoorob R, Tambussi G, Clotet B, Youle M, Achenbach CJ, et al. Treatment intensification followed by interleukin-7 reactivates

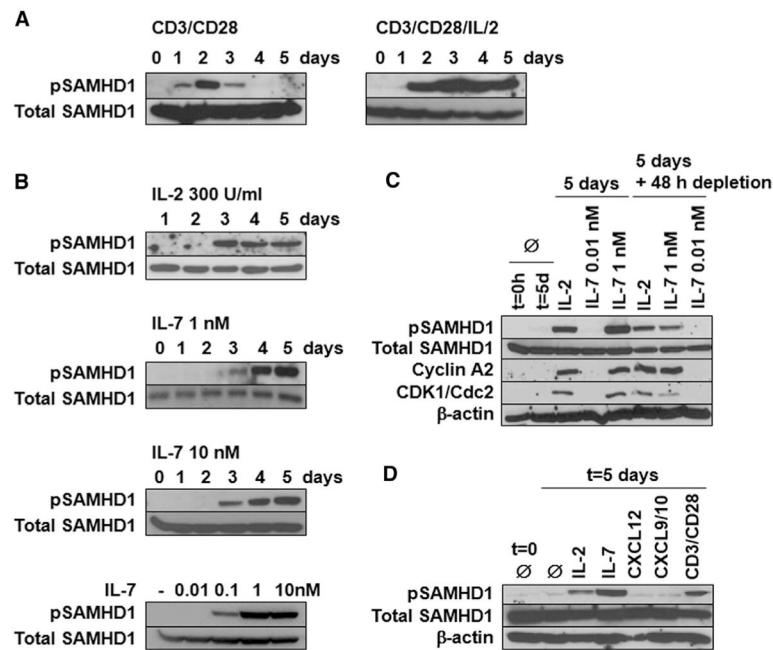
- HIV without reducing total HIV DNA: a randomized trial. *AIDS*. 2016; 30:221–230. [PubMed: 26684819]
- Kondrack RM, Harbertson J, Tan JT, McBreen ME, Surh CD, Bradley LM. Interleukin 7 regulates the survival and generation of memory CD4 cells. *J Exp Med*. 2003; 198:1797–1806. [PubMed: 14662907]
- Laguette N, Sobhian B, Casartelli N, Ringear M, Chable-Bessia C, Ségéral E, Yatim A, Emiliani S, Schwartz O, Benkirane M. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature*. 2011; 474:654–657. [PubMed: 21613998]
- Lahouassa H, Daddacha W, Hofmann H, Ayinde D, Logue EC, Dragin L, Bloch N, Maudet C, Bertrand M, Gramberg T, et al. SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. *Nat Immunol*. 2012; 13:223–228. [PubMed: 22327569]
- Levy Y, Lacabaratz C, Weiss L, Viard JP, Goujard C, Lelièvre JD, Boué F, Molina JM, Rouzioux C, Avettand-Fénoël V, et al. Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment. *J Clin Invest*. 2009; 119:997–1007. [PubMed: 19287090]
- Lévy Y, Sereti I, Tambussi G, Routy JP, Lelièvre JD, Delfraissy JF, Molina JM, Fischl M, Goujard C, Rodriguez B, et al. Effects of recombinant human interleukin 7 on T-cell recovery and thymic output in HIV-infected patients receiving antiretroviral therapy: results of a phase I/IIa randomized, placebo-controlled, multicenter study. *Clin Infect Dis*. 2012; 55:291–300. [PubMed: 22550117]
- López-Huertás MR, Mateos E, Díaz-Gil G, Gómez-Esquer F, Sánchez del Cojo M, Alcamí J, Coiras M. Protein kinase C $\theta$  is a specific target for inhibition of the HIV type 1 replication in CD4+ T lymphocytes. *J Biol Chem*. 2011; 286:27363–27377. [PubMed: 21669868]
- Mohammadi P, Desfarges S, Bartha I, Joos B, Zangger N, Muñoz M, Günthard HF, Beerenwinkel N, Telenti A, Ciuffi A. 24 hours in the life of HIV-1 in a T cell line. *PLoS Pathog*. 2013; 9:e1003161. [PubMed: 23382686]
- Napolitano LA, Grant RM, Deeks SG, Schmidt D, De Rosa SC, Herzenberg LA, Herndier BG, Andersson J, McCune JM. Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: implications for T-cell homeostasis. *Nat Med*. 2001; 7:73–79. [PubMed: 11135619]
- Pogliaghi M, Papagno L, Lambert S, Calin R, Calvez V, Katlama C, Autran B. The tyrosine kinase inhibitor Dasatinib blocks in-vitro HIV-1 production by primary CD4+ T cells from HIV-1 infected patients. *AIDS*. 2014; 28:278–281. [PubMed: 24361684]
- Ryoo J, Choi J, Oh C, Kim S, Seo M, Kim SY, Seo D, Kim J, White TE, Brandariz-Nuñez A, et al. The ribonuclease activity of SAMHD1 is required for HIV-1 restriction. *Nat Med*. 2014; 20:936–941. [PubMed: 25038827]
- Schmidt S, Schenkova K, Adam T, Erikson E, Lehmann-Koch J, Sertel S, Verhasselt B, Fackler OT, Lasitschka F, Keppler OT. SAMHD1's protein expression profile in humans. *J Leukoc Biol*. 2015; 98:5–14. [PubMed: 25646359]
- Scripture-Adams DD, Brooks DG, Korin YD, Zack JA. Interleukin-7 induces expression of latent human immunodeficiency virus type 1 with minimal effects on T-cell phenotype. *J Virol*. 2002; 76:13077–13082. [PubMed: 12438635]
- Seamon KJ, Sun Z, Shlyakhtenko LS, Lyubchenko YL, Stivers JT. SAMHD1 is a single-stranded nucleic acid binding protein with no active site-associated nuclease activity. *Nucleic Acids Res*. 2015; 43:6486–6499. [PubMed: 26101257]
- Sereti I, Dunham RM, Spritzler J, Aga E, Proschan MA, Medvik K, Battaglia CA, Landay AL, Pahwa S, Fischl MA, et al. 5214 Study Team. IL-7 administration drives T cell-cycle entry and expansion in HIV-1 infection. *Blood*. 2009; 113:6304–6314. [PubMed: 19380868]
- Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margolick JB, Kovacs C, Gange SJ, Siliciano RF. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat Med*. 2003; 9:727–728. [PubMed: 12754504]
- Smithgall MD, Wong JG, Critchett KE, Haffar OK. IL-7 up-regulates HIV-1 replication in naturally infected peripheral blood mononuclear cells. *J Immunol*. 1996; 156:2324–2330. [PubMed: 8690924]

- Vandergeeten C, Fromentin R, DaFonseca S, Lawani MB, Sereti I, Lederman MM, Ramgopal M, Routy JP, Sékaly RP, Chomont N. Interleukin-7 promotes HIV persistence during antiretroviral therapy. *Blood*. 2013; 121:4321–4329. [PubMed: 23589672]
- Venkitaraman AR, Cowling RJ. Interleukin-7 induces the association of phosphatidylinositol 3-kinase with the alpha chain of the interleukin-7 receptor. *Eur J Immunol*. 1994; 24:2168–2174. [PubMed: 7522165]
- Wang FX, Xu Y, Sullivan J, Souder E, Argyris EG, Acheampong EA, Fisher J, Sierra M, Thomson MM, Najera R, et al. IL-7 is a potent and proviral strain-specific inducer of latent HIV-1 cellular reservoirs of infected individuals on virally suppressive HAART. *J Clin Invest*. 2005; 115:128–137. [PubMed: 15630452]
- Whitney JB, Hill AL, Sanisetty S, Penaloza-MacMaster P, Liu J, Shetty M, Parenteau L, Cabral C, Shields J, Blackmore S, et al. Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. *Nature*. 2014; 512:74–77. [PubMed: 25042999]
- Wu Y, Yoder A. Chemokine coreceptor signaling in HIV-1 infection and pathogenesis. *PLoS Pathog*. 2009; 5:e1000520. [PubMed: 20041213]
- Zhu C, Gao W, Zhao K, Qin X, Zhang Y, Peng X, Zhang L, Dong Y, Zhang W, Li P, et al. Structural insight into dGTP-dependent activation of tetrameric SAMHD1 deoxynucleoside triphosphate triphosphohydrolase. *Nat Commun*. 2013; 4:2722. [PubMed: 24217394]

**Highlights**

- IL-2 and IL-7 induce SAMHD1 phosphorylation, abrogating its antiviral activity
- IL-7 greatly improves HIV-1 reverse transcription and integration compared to IL-2
- Dasatinib impedes SAMHD1 phosphorylation and HIV replication caused by IL-2 or IL-7
- IL-7 and IL-2 help to establish the reservoir, enhancing susceptibility to infection





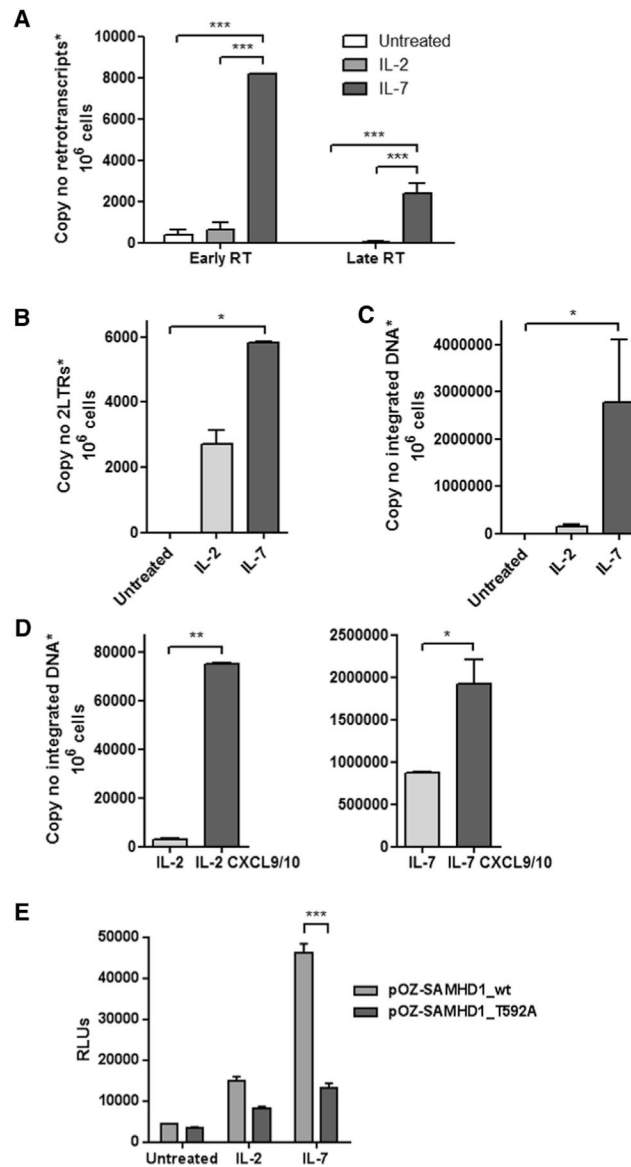
**Figure 1. Kinetics of SAMHD1 Phosphorylation at T592 in Primary CD4+ Lymphocytes in Response to Different Stimuli**

(A) Whole protein extracts from CD4+ T cells treated with anti-CD3/CD28 for 5 consecutive days in the presence or not of IL-2 were analyzed by immunoblotting using a specific antibody against total and phosphorylated (T592) SAMHD1.

(B) Phosphorylation of SAMHD1 was analyzed by immunoblotting in whole protein extracts from CD4+ T cells treated with IL-2 or IL-7 for 5 consecutive days.

(C) Analysis by immunoblotting of the effect on SAMHD1 phosphorylation of IL-2 or IL-7 depletion for 48 hr in CD4+ lymphocytes that were previously treated for 5 days.

(D) Analysis of SAMHD1 phosphorylation by immunoblotting in CD4+ lymphocytes treated with chemokines CXCL12 or CXCL9/10 for 5 days, as well as with IL-2 or IL-7.



**Figure 2. Effect of IL-2 and IL-7 on Reverse Transcription and Proviral Integration in HIV-Infected Primary CD4+ Lymphocytes**

(A) Analysis by qPCR of the reverse transcription of NL4-3\_luciferase strain in CD4+ lymphocytes treated with IL-2 and IL-7 for 5 days and then infected for 5 hr.

(B and C) The same cells were further cultured for 5 days and then 2-LTRs circles (B) and proviral integration (C) were quantified by qPCR.

(D) Analysis by qPCR of proviral integration in CD4+ lymphocytes treated with IL-2 or IL-7 for 5 days and then infected with NL4-3\_Renilla luciferase infectious strain and incubated for 5 days more in the presence or not of CXCL9 and CXCL10.

(E) Purified CD4+ T cells were transfected in resting conditions with pOZ-SAMHD1\_WT or pOZ-SAMHD1\_T592A and then treated with IL-2 or IL-7 for 4 days. Cells were then infected with NL4-3\_Renilla and incubated for 48 hr before measuring the synthesis of Renilla (RLUs). One representative experiment is shown.

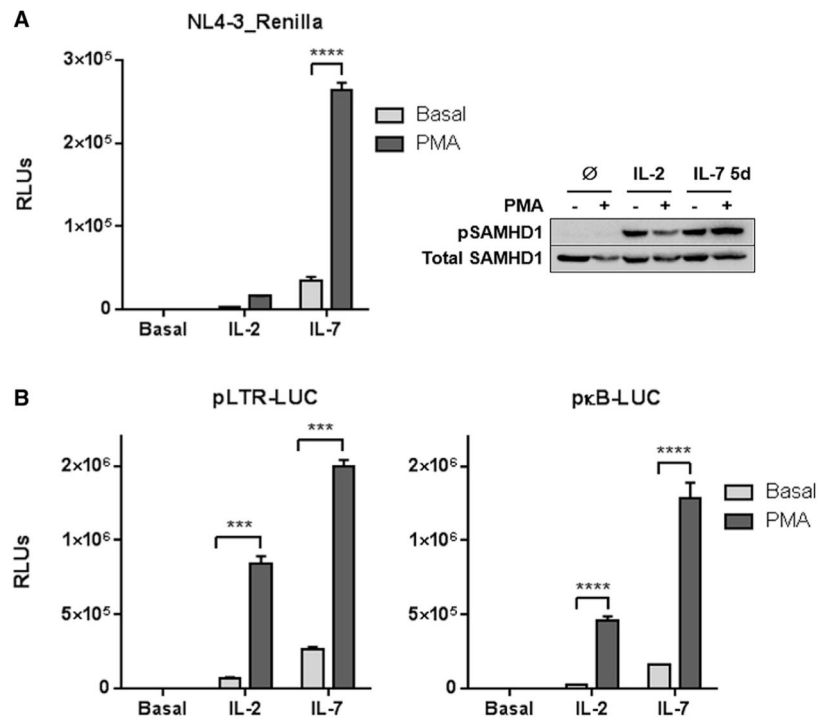
Statistical significance was calculated using two-way ANOVA for (A), one-way ANOVA for (B) and (C), and Mann-Whitney t test for (D). \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . All data are represented as mean  $\pm$  the SEM.

Author Manuscript

Author Manuscript

Author Manuscript

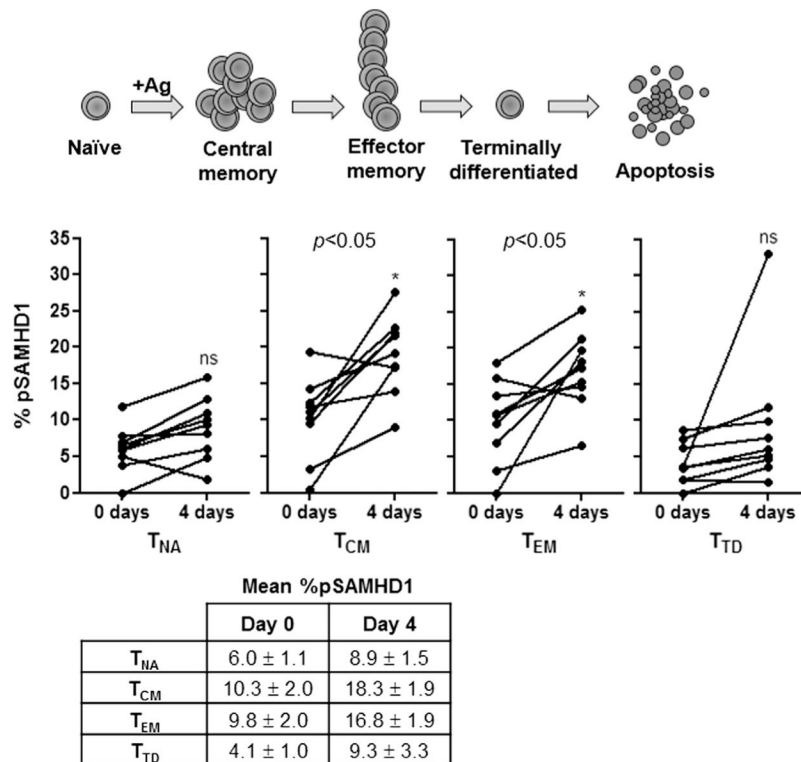
Author Manuscript



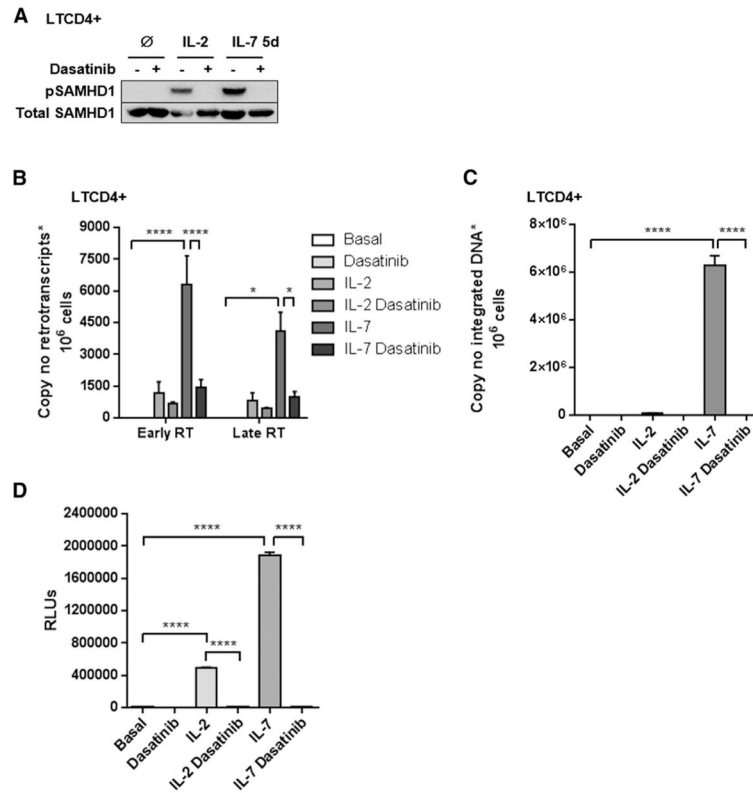
### Figure 3. IL-7 Induced Mostly Latent Proviral Integration

(A) Measurement of luciferase production (RLUs) in primary CD4<sup>+</sup> T cells treated with IL-2 and IL-7 for 5 days and then infected with NL4-3\_Renilla luciferase for 5 days more. PMA was added during the last 18 hr. SAMHD1 levels were analyzed by immunoblotting as described in Figure 1.

(B) Analysis of luciferase expression in resting CD4<sup>+</sup> T cells treated with IL-2 or IL-7 for 5 days and then nucleofected with pLTR-LUC or pκB-LUC vectors. PMA was added 2 hr post-transfection, and cells were incubated for subsequent 18 hr. Statistical significance was calculated using two-way ANOVA. \*\*\* $p < 0.001$ . Data are represented as mean  $\pm$  SEM.



**Figure 4. SAMHD1 Phosphorylation in Different CD4+ T Cell Subsets from HIV-1-Infected Patients Included in the ACTG 5214 Study Who Received a Single Dose of IL-7**  
 Surface staining with antibodies against CCR7 and CD45RA allowed distributing cells in the different CD4+ T cell subsets (depicted in the upper drawing). Subsequent intracellular staining was performed with an antibody against SAMHD1 phosphorylated at T592. Before-after graphs were depicted for each CD4+ T cell subset. Statistical significance was calculated using Mann-Whitney t test. \* $p < 0.05$ ; “ns” for not significant. Data are represented as mean  $\pm$  SEM. The mean percentages and SEM for SAMHD1 phosphorylation at day 0 and day 4 are shown in the lower table.



**Figure 5. Cytostatic Drug Dasatinib Interfered with IL-7-Induced SAMHD1 Phosphorylation**

(A) Analysis by immunoblotting of SAMHD1 phosphorylation in CD4<sup>+</sup> T cells isolated from healthy donors that were treated with IL-7 for 5 days in the presence of dasatinib.

(B and C) Viral reverse transcription (B) and proviral integration (C) were analyzed by qPCR in CD4<sup>+</sup> T cells treated with IL-7 for 5 days in the presence or not of dasatinib and infected with NL4-3\_Renilla luciferase strain for subsequent 5 days.

(D) Luciferase activity was measured in cell extracts 48 hr after infection.

Statistical significance was calculated using two-way ANOVA for (B) and Mann-Whitney t test for (C). \* $p < 0.05$ . Data are represented as mean  $\pm$  SEM.