Phosphorylation of SAMHD1 by Cyclin A2/CDK1 Regulates Its Restriction Activity toward HIV-1

Alexandra Cribier,[1,*] Benjamin Descours,[1] Ana Luiza Chaves Valadão,[1,2] Nadine Laguette,[1] and Monsef Benkirane[1,*]
[1]Institut de Génétique Humaine, CNRS UPR1142, Laboratoires de Virologie Moléculaire, Montpellier 34000, France
[2]Present address: Laboratório de Virologia Molecular, Departamento de Genética, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
*Correspondence: alexandra.cribier@igh.cnrs.fr (A.C.), monsef.benkirane@igh.cnrs.fr (M.B.)
http://dx.doi.org/10.1016/j.celrep.2013.03.017

SUMMARY

SAMHD1 restricts HIV-1 replication in myeloid and quiescent CD4^{+} T cells. Here, we show that SAMHD1 restriction activity is regulated by phosphorylation. SAMHD1 interacts with cyclin A2/cdk1 only in cycling cells. Cyclin A2/CDK1 phosphorylates SAMHD1 at the Threonine 592 residue both in vitro and in vivo. Phosphorylation of SAMHD1 Thr592 correlates with loss of its ability to restrict HIV-1. Indeed, while PMA treatment of proliferating THP1 cells results in reduced Thr592 phosphorylation, activation of resting peripheral blood mononuclear cells (PBMCs) and purified quiescent CD4^{+} T cells results in increased phosphorylation of SAMHD1 Thr592. Interestingly, we found that treatment of cells by type 1 interferon reduced Thr592 phosphorylation, reinforcing the link between the phosphorylation of SAMHD1 and its antiviral activity. Unlike wild-type SAMHD1, a phosphorylation-defective mutant was able to restrict HIV-1 replication in both PMA-treated and untreated cells. Our results uncover the phosphorylation of SAMHD1 at Thr592 by cyclin A2/CDK1 as a key regulatory mechanism of its antiviral activity.

INTRODUCTION

Among the numerous blocks that oppose the replication of HIV-1 in human cells are restriction factors. Four restriction factors have been identified to date: (1) Tripartite Motif (TRIM) 5 alpha (TRIM5a); (2) apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G (APOBEC3G, A3G); (3) BST-2/Tetherin; and (4) Sterile Alpha Motif (SAM) domain and HD domain-containing protein 1 (SAMHD1). These cellular factors mediate a cell-intrinsic resistance that arrest the viral life cycle at a specific step (Hrecka et al., 2011; Kirchhoff, 2010; Laguette et al., 2011; Malim and Bieniasz, 2012).

Indeed, SAMHD1 has been identified as the cellular protein responsible of the reverse transcription block to HIV-1 infection observed in myeloid cells as well as in quiescent CD4^{+} T cells (Hrecka et al., 2011; Laguette and Benkirane, 2012; Baldauf et al., 2012; Descours et al., 2012). SAMHD1 is constituted of a sterile alpha motif (SAM) and a HD domain, the presence of which suggests a putative phosphohydrolase activity (Aravind and Koonin, 1998) that has been subsequently demonstrated in vitro (Goldstone et al., 2011; Powell et al., 2011). Indeed, SAMHD1 possesses a deoxyguanosine-triphosphate (dGTP)-dependent triphosphate triphosphohydrolase activity, which hydrolyses the deoxynucleoside triphosphates (dNTPs) to deoxynucleoside and inorganic triphosphate. It has thus been proposed that SAMHD1 limits HIV-1 infection by depleting the intracellular pool of dNTPs to a level below the one required to complete the reverse transcription step (Lahouassa et al., 2012; St Gelais et al., 2012). Nucleic-acid binding and processing in vitro by SAMHD1 has also been reported (Goncalves et al., 2012; Beloglazova et al., 2013; Tungler et al., 2013), therefore suggesting additional SAMHD1-associated restriction mechanisms.

Unlike TRIM5a, A3G, and BST-2, whose sole expression is sufficient to block infection by HIV-1, SAMHD1 restriction activity appears to be subjected to additional regulation mechanisms. In fact, SAMHD1 was primarily identified in differentiated myeloid cells, such as monocyte derived dendritic cells (MDDCs), macrophages, or PMA (phorbol-12myristate-13acetate)-treated THP1 as being responsible for their poor infectability by HIV-1 (Berger et al., 2012; Hrecka et al., 2011; Laguette et al., 2011). However SAMHD1 is expressed in various cell types where no block to HIV-1 infection has been witnessed. Indeed, while SAMHD1 is expressed at similar levels in primary resting and activated CD4^{+} T cells (Baldauf et al., 2012; Descours et al., 2012), the latter is fully permissive to HIV-1, suggesting that additional partners are required for SAMHD1 to be fully potent against HIV-1, that would either directly regulate the activity of SAMHD1 or act as cofactors.

Here, we questioned the molecular mechanism underlying the regulation of SAMHD1 activity. We show that SAMHD1 associates with the cyclin A2/CDK1 complex that mediates its phosphorylation at threonine 592. We further demonstrate that this phosphorylation event is dependent on the cell proliferation status and is a key regulatory mechanism that inhibits the ability of SAMHD1 to block HIV-1 infection. Finally, we show that unlike previously described restriction factors that are upregulated (Harris et al., 2012), SAMHD1 Thr592 phosphorylation is reduced after type 1 interferon (IFN) treatment, while its expression level remains insensitive, uncovering a regulation of restriction factors by IFN.
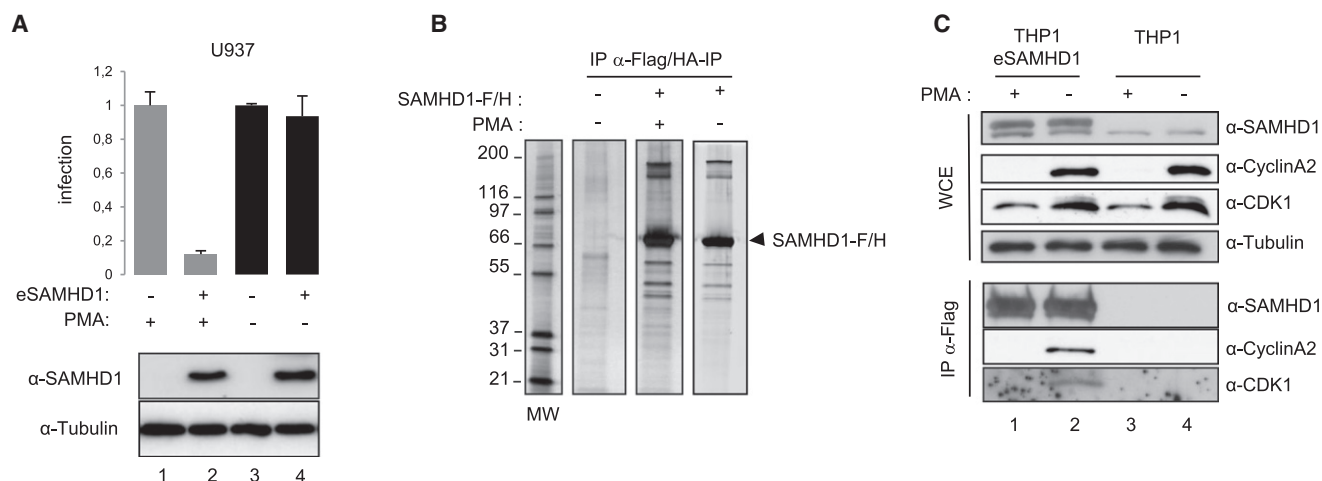
Figure 1. SAMHD1 Interacts with Cyclin A2/CDK1 in Proliferating Cells

(A) Sole expression of SAMHD1 is not sufficient to restrict HIV-1 infection. U937 and U937eSAMHD1 cells were mock- or PMA-treated prior infection with 50 ng of HIV-LUC-G. Luciferase activity was measured at 24 hr p.i. and normalized for protein concentration. The results are expressed relative to infection of mock-treated U937 cell, which was set to 1.

(B) Tandem affinity purification of SAMHD1 and its cellular partners. Nuclear extracts prepared from THP1 and THP1eSAMHD1 which were mock- or PMA-treated were subjected to tandem immunoaffinity purification using anti-Flag and subsequently anti-HA antibodies. Immunoprecipitates were separated on SDS-PAGE. SAMHD1 and its partners were visualized by silver staining.

(C) SAMHD1 interacts with cyclin A2/CDK1 in proliferating cells. Whole-cell extracts (WCEs) were prepared from mock- or PMA-treated THP1 and THP1eSAMHD1 cells and subjected to immunoprecipitation using anti-Flag antibody. The presence of CDK1, cyclinA2 and SAMHD1 was determined by western blot using specific antibodies.

RESULTS

SAMHD1 Interacts with Cyclin A2/CDK1 in Proliferating Cells

To assess for correlation between SAMHD1 expression and its antiviral activity, we generated U937 cells stably expressing Flag- and HA-tagged SAMHD1 (U937eSAMHD1). U937 cells and U937eSAMHD1 were mock- or PMA-treated, and we measured their susceptibility to infection by HIV-1-containing luciferase as a reporter gene in place of *nef* and pseudotyped with the fusogenic envelope G of the vesicular stomatitis virus (HIV1-LUC-G). Proliferating U937 and U937eSAMHD1 were equally permissive to HIV1-LUC-G. Interestingly, when treated with PMA, expression of SAMHD1 rendered U937 cells 8-fold less permissive to HIV1-LUC-G than their parental counterpart, indicating that SAMHD1 restricts HIV-1 infection only in PMA-treated cells (Figure 1A, upper panel). Western blot analysis of whole-cell extracts showed that SAMHD1 was equally expressed in PMA-treated and untreated U937eSAMHD1 cells (Figure 1A, lower panel). These results strongly suggest that the sole expression of SAMHD1 is not sufficient to confer resistance to HIV-1 infection. SAMHD1 restriction activity might require posttranslational modifications or the presence of partner expressed in PMA-treated cells. To test these hypotheses, we immunopurified SAMHD1 and its interacting partners from mock- or PMA-treated THP1 cells expressing Flag- and HA-tagged SAMHD1 (THP1eSAMHD1) (Figure 1B). Proteins that specifically associate with SAMHD1 were identified by mass spectrometry. Interestingly, among the interacting partners identified, cyclin A2 and CDK1 were present only when

SAMHD1 was purified from proliferating THP1 cells (data not shown). To confirm the interaction between SAMHD1 and cyclin A2/CDK1 in proliferating cells, eSAMHD1 was immunoprecipitated from THP1eSAMHD1, which were mock- or PMA-treated. Mock- and PMA-treated THP1 cells were used as controls. The presence of cyclin A2 and CDK1 in the immunoprecipitates was assessed by western blot using specific antibodies. As shown in Figure 1C, cyclin A2 and CDK1 were recovered only when eSAMHD1was immunoprecipitated from PMA-untreated THP1eSAMHD1 (Figure 1C, compare lane 2 to lane 1). Loss of the interaction between SAMHD1 and cyclin A2/CDK1 in PMA-treated cells is likely due to the loss of cyclin A2 expression as a result of cell-cycle arrest induced by PMA. Taken together, these experiments show that SAMHD1 interacts with the cell-cycle regulator cyclin A2/CDK1. This interaction is dependent on cellular proliferation and cyclin A2 expression.

SAMHD1 Is Substrate for Cyclin A2/CDK1

The identification of the cell-cycle regulator cyclin A2/CDK1 as SAMHD1 partner in proliferating cells suggests that SAMHD1 might be a substrate for CDK1. First, we assessed whether SAMHD1 is modified by phosphorylation. Whole-cell extracts of proliferating and PMA-treated THP1eSAMHD1 were prepared and separated using the Phos-tag acrylamide technology, which provides a phosphate affinity SDS-PAGE for mobility detection of phosphorylated proteins (Kinoshita et al., 2006, 2009). Western blot analysis using SAMHD1-specific antibody revealed multiple phosphorylated forms of SAMHD1 (Figure 2A). Interestingly, PMA treatment of the cells results in reduction of the intensity of phosphorylated forms (Figure 2A compare lane 2 to
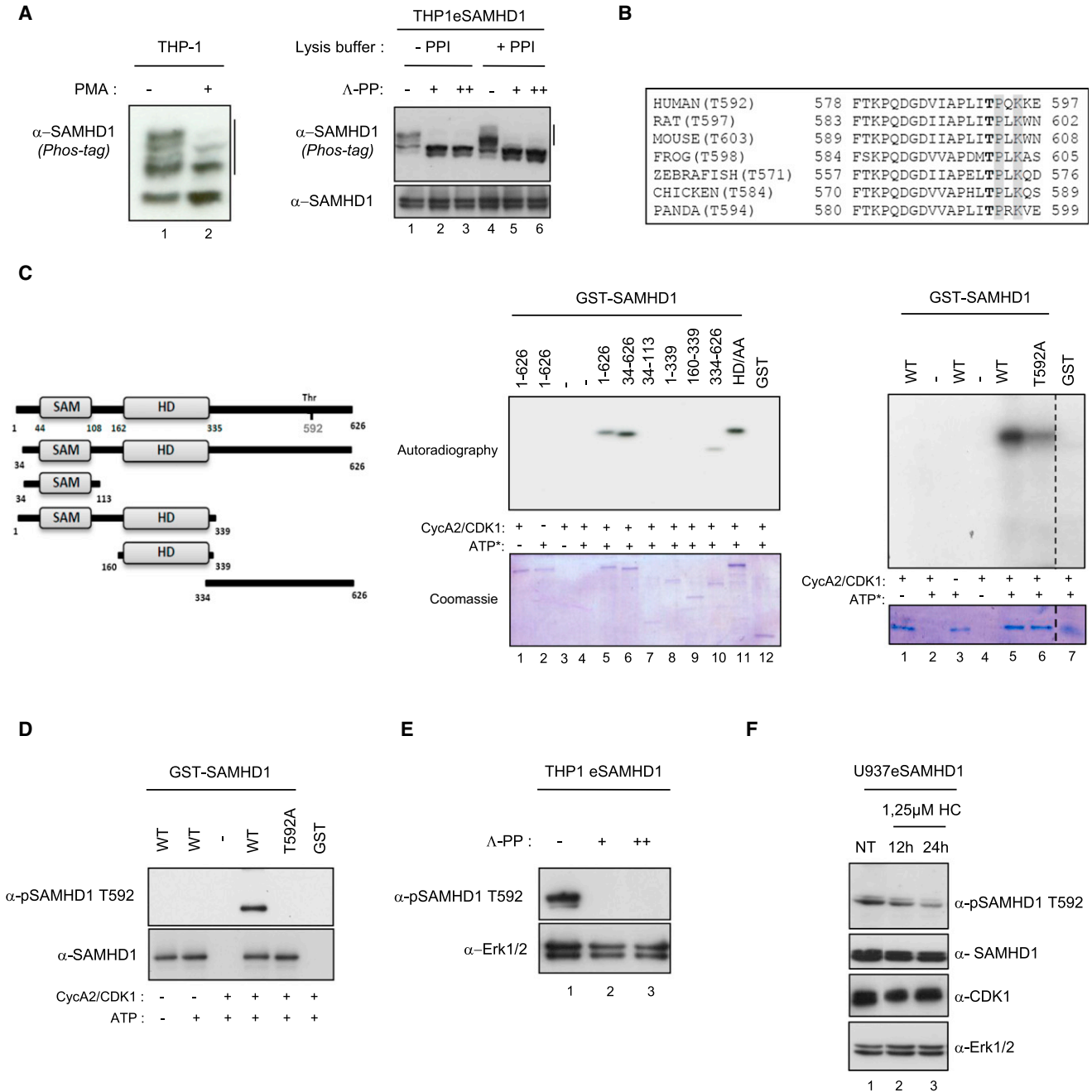
Figure 2. SAMHD1 T592 Is Phosphorylated by Cyclin A2/CDK1

(A) SAMHD1 is phosphorylated in proliferating THP1. Whole-cell extracts (WCE) from mock- or PMA-treated THP1 were separated on a 6% Phos-Tag acrylamide gel and analyzed by western blot using anti-SAMHD1 antibody (left panel). WCE were prepared from THP1eSAMHD1 in absence (−PPI) or in presence (+PPI) of phosphatase inhibitor. Extracts were treated with 0U (−), 200 U (+), or 400 U (++) of Lambda phosphatase before separation on a 6% Phos-Tag acrylamide or on a classical 10% acrylamide gels. SAMHD1 was revealed by western blot using anti-SAMHD1 antibody (right panel).

(B) Sequence alignment of the SAMHD1 region containing the threonine 592 residue in vertebrates. The conserved threonine is represented in bold and CDK1 consensus sequence, i.e., (S/T)PX(K/R), is highlighted in gray.

(C) Cyclin A2/CDK1 phosphorylates SAMHD1 in vitro at threonine 592. Left panel: Schematic representation of GST-SAMHD1 constructs (GST is not pictured) used for in vitro kinase assay. The sterile alpha motif (SAM) and phospho-hydrolase (HD) domains are shown as well as the threonine 592 (Rice et al., 2009). Middle and right panels: In vitro kinase assays using GST-SAMHD1 recombinant proteins as indicated together with recombinant cyclin A2/CDK1 and 1 μCi [γ-^{32}P] ATP (ATP*). Reactions were separated on SDS-PAGE and visualized by autoradiography (upper panels) or Coomassie stained (lower panels).

(D) Samples corresponding to the in vitro kinase assay shown in (C) (right panel) were separated on SDS-PAGE and immunoprobed with phospho-specific Thr592 antibody (upper panel) and anti-SAMHD1 antibody (lower panel).

(legend continued on next page)

lane 1). To confirm that the high-molecular-weight bands correspond to phosphorylated forms of SAMHD1, whole-cell extracts from proliferating THP1eSAMHD1 cells were mock- or lambda-phosphatase-treated and separated on Phos-tag acrylamide gel. Immunodetection of SAMHD1 revealed that phosphatase treatment leads to reduction of slow migration bands and accumulation of fast-migrating bands. Furthermore, when cell extracts were prepared in the presence of phosphatase inhibitors, the intensity of SAMHD1 phosphorylated forms was enhanced (Figure 2A, compare 4 to lane 1). None of the treatments affected the expression levels of SAMHD1 (Figure 2A, lower panel). Taken together, these experiments show that SAMHD1 is modified by phosphorylation. Alignment of SAMHD1 amino acid sequence from different species revealed that the threonine 592 (T592) is well conserved in many vertebrate species including human, mouse, and rat, and the surrounding sequence matches the consensus target site (S/TPxK/R) of CDK1 (Figure 2B). Thus, we first investigated the ability of CDK1 to phosphorylate SAMHD1 in vitro using wild-type recombinant GST-SAMHD1 or truncation mutants (Figure 2C, left panel) and recombinant cyclin A2/CDK1 in the presence or absence of [γ-^{32}P]ATP. While cyclin A2/CDK1 was able to phosphorylate wild-type SAMHD1, SAMHD1 HD domain mutant (HD/AA), and the C-terminal region (334–626 aa) (Figure 2C, middle panel lanes 5, 6, 10, and 11), no phosphorylation was observed when C-terminal deletion mutants were used in the reactions (lanes 7–9). This result demonstrates that in vitro cyclin A2/CDK1 phosphorylates SAMHD1 at the C-terminal domain containing the CDK1 consensus sequence. To test whether the threonine 592 is the residue phosphorylated by CDK1, GST-SAMHD1 WT and GST-SAMHD1T592A (T592 substituted by alanine) were used in in vitro kinase assay. While CDK1 efficiently phosphorylated wild-type SAMHD1, it only marginally phosphorylated the SAMHD1T592A mutant (Figure 2C, right panel, compare lane 5 to lane 6). These results demonstrate that cyclin A2/CDK1 phosphorylates SAMHD1 at the T592 in vitro. To further confirm in vivo SAMHD1 phosphorylation at the T592 residue, we generated phospho-peptide-specific antibody against the phospho-peptide sequence surrounding the T592 residue. Specificity of the phospho-antibody was validated using in vitro phosphorylation of GST-SAMHD1 by the cyclinA2/CDK1 complex in the presence of cold ATP. The phospho-specific antibody against the SAMHD1 phospho-T592 residue detected SAMHD1 protein in the presence of both the cyclin A2/CDK1 complex and cold ATP, but could not detect the GST-SAMHD1T592A mutant (Figure 2D, upper panel). Western blot analysis using anti-SAMHD1 antibody revealed equal amount of both wild-type and T592 SAMHD1 proteins (Figure 2D, lower panel). Next, in vivo detection of SAMHD1 phosphorylation at the T592 residue was tested using cell lysates of U937 cells expressing eSAMHD1 or eSAMHD1T592A mutant. The phospho-specific antibody recognized wild-type SAMHD1 but not the SAMHD1T592A mutant (data not shown). Both wild-type and SAMHD1T592A mutant

show similar nuclear localization (data not shown). Endogenous SAMHD1 proteins were also recognized by the phospho-specific antibody, whereas phosphatase-treated proteins were not (Figure 2E, compare lanes 2 and 3 to lane 1). Furthermore, treatment of U937eSAMHD1 cells with selective CDK1 inhibitor (CGP-74514A hydrochloride, HC) reduced SAMHD1 phosphorylation at T592 residue as witnessed by western blot using anti-phosphoT592 antibody (Figure 2F). Taken together, these data show that the generated antibody is specific for the phosphorylated T592 residue of SAMHD1 and that this residue is a target phosphorylation site of the cyclin A2/CDK1 complex.

SAMHD1 Phosphorylation at T592 Is Regulated upon T Cell Activation and Interferon Treatment and Is a Negative Regulator of Its Antiviral Activity

The antiviral activity of SAMHD1 has been observed only in differentiated noncycling cells such as dendritic cells, macrophages, monocytes, and quiescent CD4+ T cells. Indeed, despite a similar level of SAMHD1 expression in THP1 cells, its restriction of HIV-1 replication is witnessed only when the cells are differentiated through PMA treatment (Goujon et al., 2008). Similarly, resting and activated CD4+ T cells express equal amount of SAMHD1, while the restriction of HIV-1 is observed only in the former. Data presented in Figure 2A show that SAMHD1 phosphorylation is reduced upon PMA treatment of THP1 cells. Taken together, these results suggest that SAMHD1 phosphorylation inhibits its antiviral activity. We first asked whether Vpx-mediated degradation of SAMHD1 is affected by Thr592 phosphorylation. Figure 3A (left panel) shows that Vpx induces degradation of both unphosphorylated and phosphorylated forms of SAMHD1. To assess whether SAMHD1 phosphorylation at T592 residue inversely correlates with its antiviral activity, THP1 cells were mock- or PMA-treated. The levels of SAMHD1 and its T592 phosphorylated form were determined by western blot. Figure 3A (right panel) shows that PMA treatment of THP1 cells results in loss of SAMHD1 phosphorylation at T592 without affecting the level of SAMHD1. Furthermore, TCR stimulation of peripheral blood mononuclear cells (PBMCs) and purified CD4+ T cells obtained from four healthy donors resulted in dramatic increase of T592 phosphorylation (Figure 3B, upper panels), while SAMHD1 expression level was unchanged (Figure 3B, lower panels). These results show that T592 phosphorylation of SAMHD1 is highly regulated and correlates with the permissiveness of the cells to HIV-1. In support of this hypothesis, treatment of THP1 cells with type 1 interferon resulted in reduced amount of SAMHD1 phosphorylated on Thr592 residue without affecting the levels of SAMHD1 (Figure 3C, left panel). Consistently, we observed that IFN treatment of activated CD4+ T cells, monocyte-derived macrophage (MDM), and monocyte-derived dendritic cells (MDDCs) also resulted in reduced amounts of SAMHD1 phosphorylated on Thr592 residue without affecting the overall levels of SAMHD1

(E) SAMHD1 is phosphorylated in vivo on Thr592 residue. Samples (4–6) corresponding to experiment shown in the middle panel of (A) were separated on SDS-PAGE and immunoprobed with phospho-specific Thr592 antibody and with ERK1/2 antibody.

(F) CDK1 phosphorylates SAMHD1 Thr592 in vivo. U937eSAMHD1 were mock-treated (NT) or treated with 1.25 μM of CDK1 inhibitor (CGP-74514A hydrochloride) for 12 or 24 hr. Cell extracts were prepared and separated on SDS-PAGE. Western blot analysis was performed using the indicated antibodies.
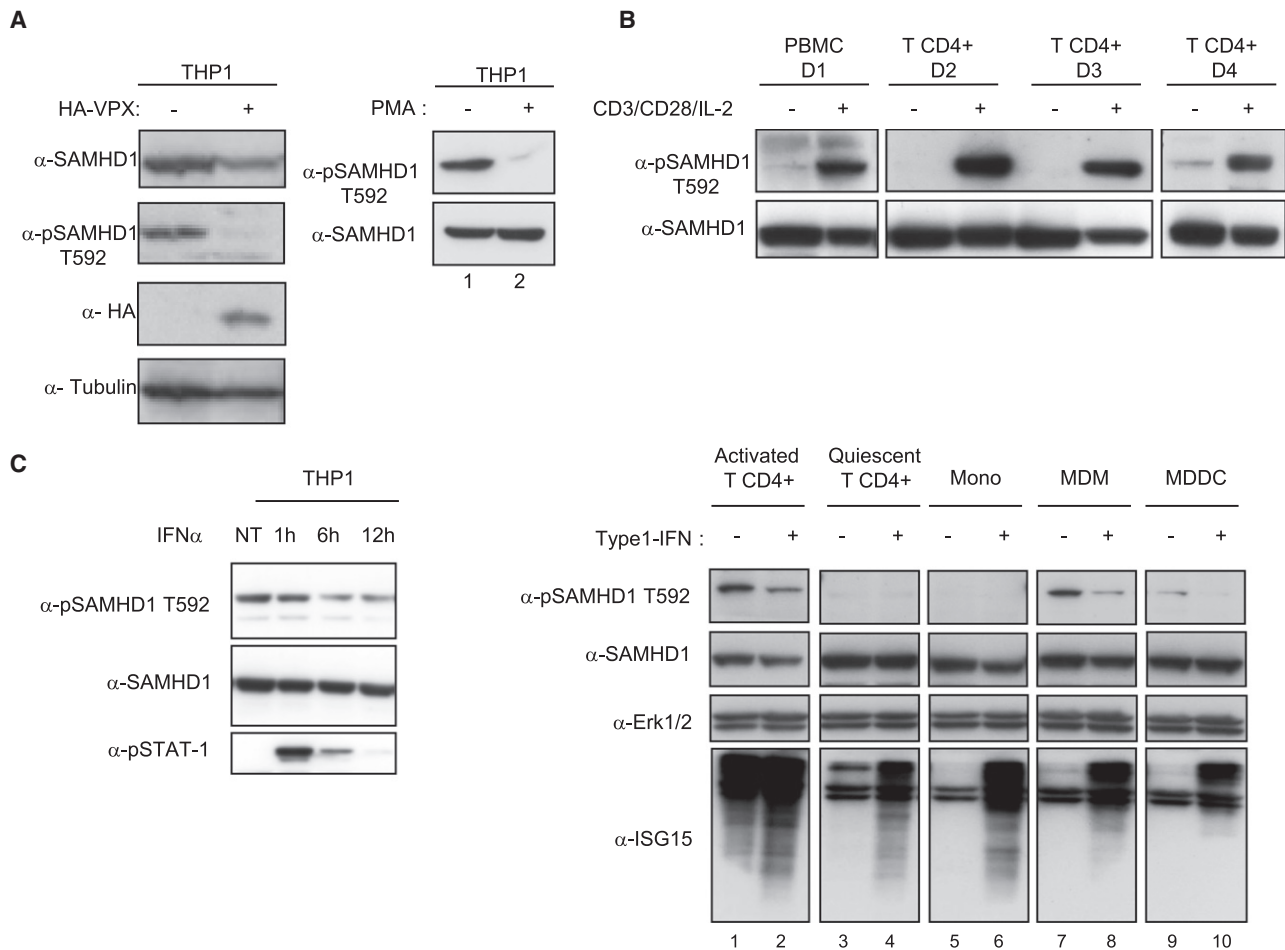
Figure 3. SAMDH1 Phosphorylation on Thr592 Residue Is Increased in HIV-1-Permissive Cells and Is Regulated by Type 1 IFN

SAMHD1 is phosphorylated at T592 in HIV-1-permissive THP1, stimulated PBMCs, and CD4+ T lymphocytes.

(A) Western blot analysis of cell extracts from mock- or Vpx-expressing THP1 cells (left panel) and mock- or PMA-treated THP1 (right panel) using the phospho-specific SAMHD1, anti-HA, and tubulin antibodies as indicated.
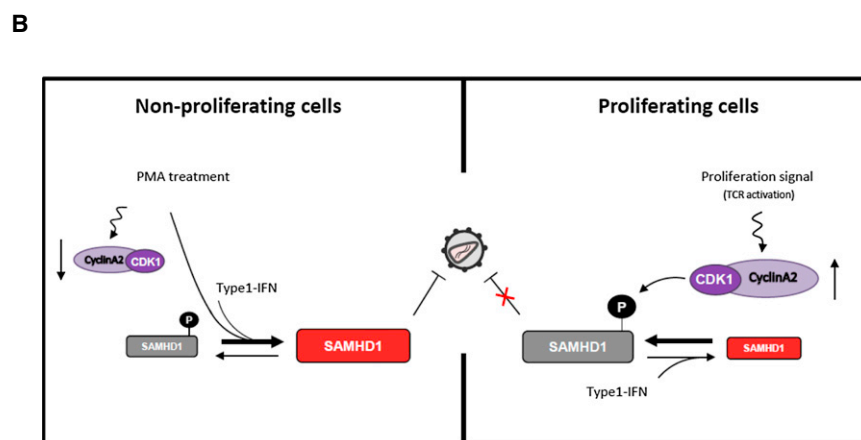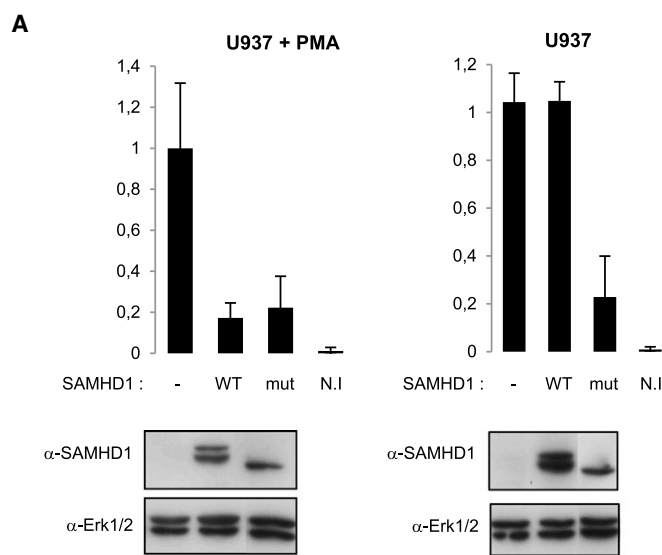
(B) PBMCs and purified CD4+ T cells isolated from four healthy donors (D1 to D4) were mock- or TCR-stimulated (CD3/CD28/IL-2). Whole-cell extracts were separated on SDS-PAGE and analyzed by western blot using the indicated antibodies.

(C) Type 1 IFN treatment downregulates SAMHD1 phosphorylation on Thr592 residue. Left panel: THP1 cells were untreated (NT) or treated with IFN-α (1,000 U/ml) for 1, 6, and 12 hr. Whole-cell extracts were separated on SDS-PAGE and analyzed by western blot using the indicated antibodies. Right panel: TCR-activated CD4+ T cells, quiescent CD4+ T cells, monocytes (Mono), macrophages (MDM), and dendritic cells (MDDC) isolated from healthy donor were treated with type1-IFN (100 U/ml) for 48 hr. Whole-cell extracts were analyzed by western blot using the indicated antibodies.

(Figure 3C, right panel). No phosphorylation of Thr592 was observed in quiescent CD4+ T cells and monocytes, which are highly refractory to HIV-1 (Figure 3C, right panel). To explore whether T952 phosphorylation of SAMHD1 inhibits its antiviral activity, we compared the antiviral activity of wild-type SAMHD1 and the phosphorylation-defective mutant in mock- and PMA-treated U937 cells. Consistent with results obtained in Figure 1A, wild-type SAMHD1 restriction activity toward HIV-1 was witnessed only in PMA-treated U937 cells (Figure 4A). Importantly, SAMHD1 phosphorylation-defective mutant not only restricted HIV-1 replication in PMA-treated cells, but could also restrict HIV-1 in untreated U937 cells (Figure 4A). These results show that phosphorylation of SAMHD1 T592 residue is a negative regulator of its restriction activity toward HIV-1.

DISCUSSION

The observation that the sole expression of SAMHD1 in proliferating cells is not sufficient to confer resistance to HIV-1 led us to hypothesize that SAMHD1 antiviral activity might be regulated by posttranslational modification and/or requires a partner expressed only in the nonproliferating cells where its antiviral activity is witnessed. Purification of SAMHD1 and its partners from proliferating and nonproliferating cells led to the recovery of cyclin A2/CDK1 only in the former. Using in vitro kinase assay and phospho-specific antibody, we showed that cyclin A2/CDK1 phosphorylates SAMHD1 at the Thr592 residue, which is highly conserved in vertebrate species and present within a consensus target site of CDK1. In support for a role of SAMHD1 Thr592 in

A

B

phorylation on Thr592, highlighting a pathway of regulation of restriction factors by IFN. It has been reported that type 1 IFN induces the expression of the CDK inhibitor p21$^{Waf1/Cip1}$ (Mandal et al., 1998; Katayama et al., 2007), which might explain the reduced phosphorylation of Thr592 residue by CDK1. Alternatively, IFN might induce a phosphatase activity responsible for SAMHD1 dephosphorylation. It will be also important to explore whether the reduced phosphorylation of Thr592 residue by IFN might contribute to the antiviral state induced by IFN in MDDCs, which is counteracted by Vpx (Pertel et al., 2011). In conclusion, Thr592 phosphorylated and nonphosphorylated forms of SAMHD1 exist in an equilibrium, which is in favor of one form or the other depending on the cells proliferation status and type 1 interferon treatment (Figure 4B). Finally, unlike wild-type SAMHD1, the sole expression of SAMHD1 phosphorylation-defective

regulating its antiviral activity, we observed that Thr592 was phosphorylated in HIV-1-permissive cells. Treatment of proliferating THP1 by PMA, which renders them nonpermissive, resulted in dramatic reduction of Thr592 phosphorylation. While Thr592 phosphorylation of SAMHD1 is low in resting CD4$^+$ T cells, which are nonpermissive to HIV-1, TCR activation of primary CD4$^+$ T cells, which confers high susceptibility to HIV-1, induced Thr592 phosphorylation. Interestingly, SAMHD1 phosphorylation at Thr592 is also regulated by type 1 interferon. Indeed, treatment of THP1 cells, activated CD4$^+$ T cells, MDMs and MDDC by IFN reduced Thr592 phosphorylation without affecting SAMHD1 levels. This observation is in accordance with the recent report showing that, unlike the other restriction factors, SAMHD1 expression is not regulated by IFN in activated CD4$^+$ T cells and in MDDCs (St Gelais et al., 2012) and extends this observation to resting CD4$^+$ T cells, monocytes, and MDMs. Importantly, we show that IFN regulates SAMHD1 antiviral activity in these primary cells and in THP1 cells by reducing its phos-

mutant is able to confer resistance to HIV-1. Taken together, our experiments demonstrate that phosphorylation of SAMHD1 at the Thr592 by cyclin A2/CDK1 regulates its restriction activity toward HIV-1. SAMHD1 is a dGTP-dependent deoxynucleotide triphosphohydrolase that reduces the cellular pool of dNTPs in differentiated, noncycling myeloid cells to levels below those required to support HIV-1 DNA synthesis. Additionally, it has recently been shown that SAMHD1 is a nucleic acid binding protein that displays a preference for RNA over DNA (Goncalves et al., 2012). SAMHD1 also possesses metal-dependent 3′ to 5′ exonuclease activity against single-stranded DNAs and preferentially cleaves 3′ overhangs and RNA in the blunt-ended RNA/DNA duplexes in vitro. It will be important to determine whether Thr592 phosphorylation of SAMHD1 might affect its enzymatic activities. The discovery of SAMHD1 interaction with and phosphorylation by cyclinA2/CDK1 is not only important for our understanding of its antiviral activity but will also help deciphering the cellular function for SAMHD1. Indeed, cyclin

A2/CDK1 is known to inhibit both DNA replication and histone mRNA accumulation, marking the end of S phase and entry into G2 phase. Cyclin A2 is highly expressed during S phase and degraded for completion of mitosis (Koseoglu et al., 2010). The fact that SAMHD1 is substrate for this mitotic cyclin suggests that SAMHD1 might also function in regulating cell-cycle progression.

EXPERIMENTAL PROCEDURES

Detailed experimental procedures can be found in Extended Experimental Procedures.

Cell Lines
Adherent and suspension cells were grown in Dulbecco's modified Eagle's medium (DMEM) or in Roswell Park Memorial Institute medium (RPMI-1640) respectively. Medium were supplemented with 10% of fetal calf serum, antibiotics (100 units/ml penicillin G, 100 mg/ml streptomycin), and ultraglutamine (0.2 mM). All cell-culture reagents were purchased from Lonza. Cell lines expressing Flag- and HA-tagged proteins were obtained using the previously described MMLV-based retroviral constructs (Nakatani and Ogryzko, 2003; Kumar et al., 2009) that contain a selectable marker (puromycin resistance gene [pOZ-puro]). Puromycin-selected cell lines were grown in appropriate media supplemented with 1 μg ml^{-1} puromycin. U937 and THP-1 cells were differentiated overnight with 30 ng ml^{-1} of phorbol 12-myristate 13-acetate (PMA) (Sigma).

Lambda Phosphatase Treatment
Four millions of nondifferentiated THP-1-SAMHD1-F/H were lysed in TETN-150 without EDTA containing or not phosphatase inhibitor (PPI cocktail, Sigma, P2850). Whole-cell extracts (40 μg) were treated with 0, 0.5, or 1 μl of lambda protein phosphatase (400 U/μl, New England Biolabs, P0753S) for 1 hr 30 at 30°C. After adding 10 μl of 6× SDS sample buffer, samples were boiled for 5 min at 95°C.

In Vitro Phosphorylation Assay
Same amount of GST fusion proteins (400 ng) were incubated for 30 min at 30°C with recombinant cyclin A2/CDK1 (Sigma) in 20 μl kinase buffer (25 mM Tris [pH 7.4], 50 mM NaCl, 10 mM MgCl$_2$, supplemented with 1 mM dithiothreitol [DTT] prior to use) including 1 μCi (γ-^{32}P) ATP or 1 mM cold ATP. Reactions were stopped by the addition of 6× sample buffer and heating for 5 min at 95°C. Proteins were separated by SDS-PAGE, followed by autoradiography or analyzed by western blot with a SAMHD1 antibody and p-SAMHD1 T592.
Infection
After transduction of U937 cells with lentiviral vectors allowing the expression of SAMHD1-WT or mutants, cells were mock- or PMA-treated for at least 16 hr. Cells were then infected with doses corresponding to 50 ng or 150 ng of HIV-1 CAp24 with HIV-LUC-G viruses, depending on the experiments. After 24 hr of infection, infected cells were harvest and viral infection was measured by quantification of the luciferase activity using the luciferase assay system according to the manufacturer's recommendations (Luciferase assay, Promega) and a Mithras luminometer (Berthold Technologies).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.03.017.

LICENSING INFORMATION

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