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Article

The Retroviral Restriction Ability of SAMHD1, but Not Its Deoxynucleotide Triphosphohydrolase Activity, Is Regulated by Phosphorylation

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SUMMARY

SAMHD1 is a cellular enzyme that depletes intracellular deoxynucleoside triphosphates (dNTPs) and inhibits the ability of retroviruses, notably HIV-1, to infect myeloid cells. Although SAMHD1 is expressed in both cycling and noncycling cells, the antiviral activity of SAMHD1 is limited to noncycling cells. We determined that SAMHD1 is phosphorylated on residue T592 in cycling cells but that this phosphorylation is lost when cells are in a noncycling state. Reverse genetic experiments revealed that SAMHD1 phosphorylated on residue T592 is unable to block retroviral infection, but this modification does not affect the ability of SAMHD1 to decrease cellular dNTP levels. SAMHD1 contains a target motif for cyclin-dependent kinase 1 (cdk1) (^{592}TPQK^{595}), and cdk1 activity is required for SAMHD1 phosphorylation. Collectively, these findings indicate that phosphorylation modulates the ability of SAMHD1 to block retroviral infection without affecting its ability to decrease cellular dNTP levels.

INTRODUCTION

Efficient infection of human primary macrophages, dendritic cells, and resting CD4$^+$ T cells by macaque simian immunodeficiency virus (SIV$_{mac}$) requires the accessory protein Vpx (Arfi et al., 2008; Baldauf et al., 2012; Descours et al., 2012; Goujon et al., 2003, 2007, 2008). Vpx is essential for both SIV infection of primary macrophages and viral pathogenesis in vivo (Belshan et al., 2006; Fletcher et al., 1996; Gibbs et al., 1995; Hirsch et al., 1998). Vpx is incorporated into viral particles, suggesting that it might act immediately after viral fusion (Jin et al., 2001; Kappes et al., 1993; Park and Sodroski, 1995; Selig et al., 1999). Viral reverse transcription is prevented in primary macrophages when cells are infected with either Vpx-deficient SIV$_{mac}$ or HIV-2 (Bergamaschi et al., 2009; Fujita et al., 2008; Goujon et al., 2007; Kaushik et al., 2009; Srivastava et al., 2008). Interestingly, Vpx also increases the ability of HIV-1 to efficiently infect macrophages, dendritic cells, and resting CD4$^+$ T cells when Vpx is incorporated into HIV-1 particles or supplied in *trans* (Baldauf et al., 2012; Descours et al., 2012; Goujon et al., 2008; Sunseri et al., 2011). Recent work identified SAMHD1 as the protein that blocks infection of SIVΔVpx, HIV-2ΔVpx, and HIV-1 before reverse transcription in macrophages, dendritic cells, and resting CD4$^+$ T cells (Baldauf et al., 2012; Berger et al., 2011; Descours et al., 2012; Hrecka et al., 2011; Laguette et al., 2011). Mechanistic studies have suggested that Vpx induces the proteasomal degradation of SAMHD1 (Berger et al., 2011; Hrecka et al., 2011; Laguette et al., 2011). In agreement with this, the C-terminal region of SAMHD1 contains a Vpx binding motif, which is important for the ability of Vpx to degrade SAMHD1 (Ahn et al., 2012; Laguette et al., 2012; Lim et al., 2012; Zhang et al., 2012). SAMHD1 is a deoxyguanosine triphosphate (dGTP)-regulated deoxynucleotide triphosphohydrolase (dNTPase) that decreases the overall cellular levels of dNTPs (Goldstone et al., 2011; Kim et al., 2012; Lahouassa et al., 2012; Powell et al., 2011). Taken together, these results suggested that an overall decrease on the level of dNTPs is responsible for the block imposed on lentiviral reverse transcription.

SAMHD1 comprises the sterile alpha motif (SAM) and histidine-aspartic (HD) domains. The HD domain of SAMHD1 is a dGTP-regulated dNTPase that decreases the cellular levels of dNTPs (Goldstone et al., 2011; Kim et al., 2012; Lahouassa et al., 2012; Powell et al., 2011). In agreement with this, the sole HD domain is sufficient to potently restrict infection by different viruses (White et al., 2013). The HD domain is also necessary for the ability of SAMHD1 to oligomerize and to bind RNA (White et al., 2013). The decrease in dNTP levels in myeloid cells correlates with the inability of lentiviruses to undergo reverse transcription. Even though it is known that SAMHD1 blocks lentiviral infection by depleting the pool of dNTPs, the regulation of the antiviral activity of SAMHD1 is not understood.

Remarkably, cycling and noncycling cells can express SAMHD1; however, SAMHD1's antiviral activity is only observed in noncycling cells. Several examples illustrate the fact that SAMHD1 is antivirally active in noncycling cells. Human monocytic THP-1 cells that endogenously express SAMHD1 restrict HIV-1 when cells are differentiated to a noncycling state by treatment with phorbol-12-myristate-13-acetate (PMA) (Schwende et al., 1996). Similarly, the human monocytic U937 cells stably expressing an exogenous FLAG-tagged SAMHD1 protein only

A

Cell Type	Cellular State	Residue	Phosphorylated/Unphosphorylated	HIV-1 Restriction
THP-1	cycling	S33	1.1	No
		T592	5.5	
	noncycling	S33	0.16	Yes
		T592	0.16	
U937 SAMHD1-FLAG	cycling	S33	0.39	No
		T592	10.31	
	noncycling	S33	0.39	Yes
		T592	0.56	
MDM	noncycling	S33	No Phosphorylation Detected*	Yes
		T592	No Phosphorylation Detected*	

*No observable phosphorylation above signal-to-noise detection limit

B

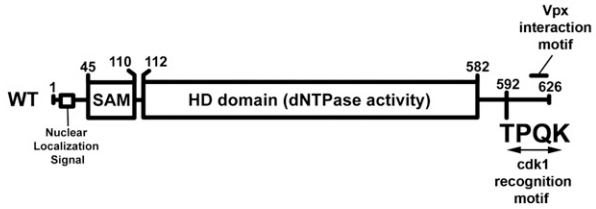

Figure 1. Phosphorylation State of SAMHD1 in Cycling and Noncycling Cells

(A) Endogenously expressed SAMHD1 was immunoprecipitated using anti-SAMHD1 antibodies from PMA-treated (noncycling) or untreated (cycling) human monocytic THP-1 cells. Proteins were separated by SDS-PAGE, and a Coomassie-blue-stained band containing the SAMHD1 protein was subjected to phosphopeptide mapping by mass spectrometry. The ratios of phosphorylated to unphosphorylated peptides are shown. The ability of the different cells to restrict HIV-1 is presented. Similar analysis was performed for determining the phosphorylation state of the endogenously expressed SAMHD1 in human MDMs. In parallel, PMA-treated or untreated human monocytic U937 cells stably expressing SAMHD1-FLAG were used to immunoprecipitate and determine the phosphorylation state of SAMHD1, as described in Experimental Procedures. Mass-spectrometry analysis was performed three times, and a representative experiment is shown. See also Figure S1.

(B) Wild-type (WT) human SAMHD1 protein is depicted showing the numbers of the amino acid residues at the boundaries of each domain. The residue T592 is depicted in the consensus motif ^{592}TPQK^{595} for recognition and phosphorylation by cdk1.

restrict HIV-1 when cells are differentiated with PMA to a noncycling state. In addition, endogenous expression of SAMHD1 by noncycling cells such as macrophages and dendritic cells shows potent restriction of HIV-1 infection. More recently, it was discovered that noncycling resting CD4$^+$ T cells potently block HIV-1 infection (Baldauf et al., 2012; Descours et al., 2012). By contrast, the cycling human HeLa and 293T cells that endogenously express SAMHD1 did not show activity against HIV-1 even when SAMHD1 was overexpressed.

This work explores the molecular basis by which SAMHD1 blocks HIV-1 infection only when expressed in noncycling cells. Our mass-spectrometry findings revealed that SAMHD1 is unphosphorylated at position T592 in noncycling cells. By contrast, SAMHD1 is phosphorylated at position T592 in all the cycling cells studied here. We studied the role of T592 phosphorylation in the ability of SAMHD1 to block retroviral infection by replacing T592 with phosphomimetic or unphosphorytable residues. SAMHD1 variants with a phosphomimetic residue at position 592 revealed that a phosphorylated SAMHD1 is unable to block retroviral infection without affecting the ability of SAMHD1 to decrease the cellular levels of dNTPs or the intrinsic enzymatic dNTPase activity of the protein. We also showed that the ability of phosphorylation to regulate restriction requires the HD domain and the C-terminal residues 583–626 of SAMHD1. Finally, we explored the nature of the kinase that might be phosphorylating SAMHD1 in cycling cells.

RESULTS

SAMHD1 Exhibits Differential Phosphorylation when Comparing Cycling and Noncycling Cells

SAMHD1 is expressed in cycling and noncycling cells; however, the ability of SAMHD1 to block infection by different retroviruses is only observed in noncycling cells (Baldauf et al., 2012; Berger et al., 2011; Descours et al., 2012; Hrecka et al., 2011; Laguette et al., 2011; White et al., 2013). To find the molecular basis for this phenotype, we explored the state of SAMHD1 phosphorylation in cycling and noncycling cells by using mass spectrometry. Immunoprecipitated SAMHD1 proteins from cycling and noncycling cells were separated by SDS-PAGE and analyzed by mass spectrometry (Figure S1 available online). Interestingly, we observed that SAMHD1 is phosphorylated at residue T592 when endogenously expressed in cycling THP-1 cells (Figure 1A). Similarly, cycling human monocytic U937 cells, which do not exhibit detectable levels of endogenous SAMHD1, engineered to stably express SAMHD1-FLAG (U937-SAMHD1-FLAG) revealed a phosphorylated SAMHD1-FLAG protein at residue T592 (Figure 1A). By contrast, noncycling cells, such as THP-1 and U937-SAMHD1-FLAG cells differentiated to a noncycling state by PMA, exhibited a SAMHD1 protein unphosphorylated at position T592 (Figure 1A). These results correlated the phosphorylation state of SAMHD1 with its antiviral activity (Figure 1A). Because these experiments suggested that unphosphorylated SAMHD1 is antivirally active, we tested the state of SAMHD1 phosphorylation in human primary monocyte-derived macrophages (MDMs). Similarly, we analyzed immunoprecipitated SAMHD1 from human primary MDM via mass spectrometry (Figure S1). In agreement with our findings, human primary MDMs contained an unphosphorylated SAMHD1 in position T592 (Figure 1A). We also found that a small fraction of SAMHD1 was phosphorylated in position S33; however, this phosphorylation did not change when comparing cycling with noncycling cells (Figure 1).

Our results suggest that unphosphorylated SAMHD1 on T592 in noncycling cells is antivirally active and that phosphorylated SAMHD1 on T592 in cycling cells is antivirally inactive (Figure 1B).

Phosphorylation Analysis of SAMHD1 in Different Human Cell Lines and Primary Cells

To analyze the phosphorylation state of SAMHD1 in different human cell lines and primary cells, we studied the phosphorylation

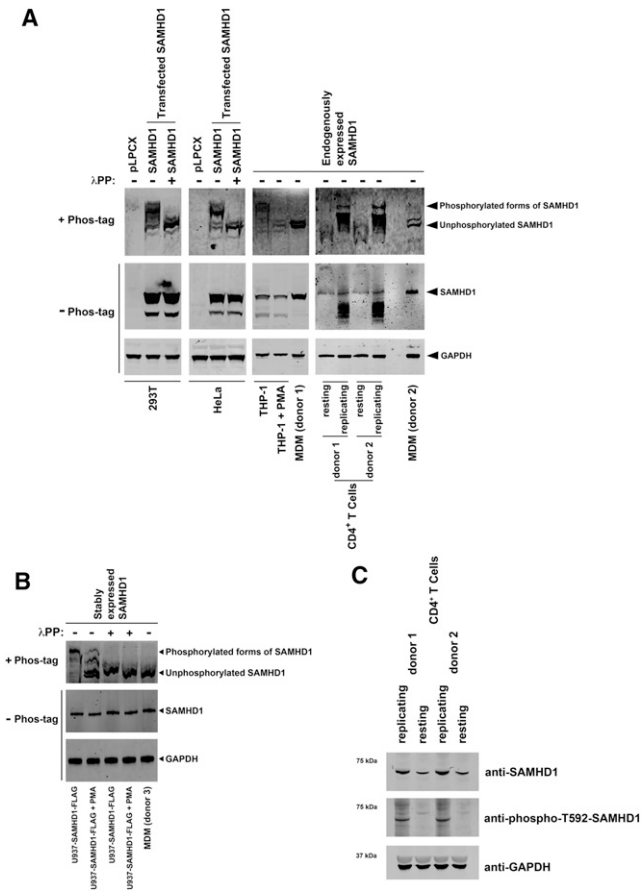

Figure 2. Phosphorylation State of SAMHD1 in Different Human Cell Lines and Primary Cells

(A) 293T or HeLa cells transfected with a plasmid expressing SAMHD1-FLAG or pLPCX were lysed 24 hr after transfection and treated or not with λPP, which is a protein phosphatase with activity toward phosphorylated serine, threonine, and tyrosine residues. Protein samples were separated by SDS-PAGE containing Phos-tag (+Phos-tag), a ligand that shifts the mobility of phosphorylated proteins, and analyzed by western blotting using FLAG antibodies. We also analyzed the phosphorylation state of endogenously expressed SAMHD1 proteins using anti-SAMHD1 antibodies in different cells: human THP-1 cells, human primary MDMs, human primary resting $CD4^+$ T cells, and replicating $CD4^+$ T cells. Protein samples were also separated in SDS-PAGE gels without Phos-tag (−Phos-tag).

(B) Similarly, we analyzed the phosphorylation state of SAMHD1-FLAG stably expressed in U937 cells in the presence or absence of PMA. As a loading control, cell lysates were western blotted using GAPDH antibodies.

(C) The phosphorylation state of SAMHD1 in resting and replicating $CD4^+$ T cells from two donors was analyzed by using a specific antibody that recognizes a phosphorylated SAMHD1 at position T592 (anti-phospho-T592-SAMHD1). As a control, we analyzed the samples by western blotting using anti-SAMHD1 and anti-GAPDH antibodies. Similar results were obtained in three independent experiments, and a representative experiment is shown.

of SAMHD1 by western blotting using SDS-PAGE gels containing Phos-tag, a ligand that shifts the mobility of phosphorylated proteins (Kinoshita et al., 2006). Initially, we analyzed transfected SAMHD1-FLAG in human 293T cells. As shown in Figure 2A, SAMHD1-FLAG transfected in 293T cells showed a broad migration pattern, reflecting the different phosphorylated forms of SAMHD1-FLAG. By contrast, SAMHD1 treated with λprotein phosphatase (λPP) showed a faster-migrating, more compacted band revealing the unphosphorylated form of SAMHD1. This experiment showed that SAMHD1 is phosphorylated in human 293T cells, which is in agreement with the fact that 293T cells stably expressing SAMHD1-FLAG do not restrict retroviral infection.

Next, we analyzed the phosphorylation of SAMHD1 in HeLa cells (Figure 2A). Similarly, SAMHD1-FLAG transfected or stably expressed in HeLa cells is phosphorylated, which is in agreement with the fact that HeLa cells by themselves or stably expressing SAMHD1 do not restrict retroviral infection (data not shown).

Because endogenously expressed SAMHD1 in THP-1 cells only restricts when THP-1 cells are differentiated to a noncycling state by using PMA, we compared the phosphorylation state of SAMHD1 between cycling and noncycling THP-1 cells (Figure 2A). In agreement with our mass-spectrometry analysis, we found that noncycling cells contained an endogenously expressed, unphosphorylated SAMHD1 protein. On the contrary, SAMHD1 proteins from cycling THP-1 cells were phosphorylated. This evidence suggests that unphosphorylated SAMHD1 in noncycling THP-1 cells blocks retroviral infection.

Recent evidence demonstrated that SAMHD1 is responsible for the block imposed by resting $CD4^+$ T cells to HIV-1 infection (Baldauf et al., 2012; Descours et al., 2012); therefore, we tested the phosphorylation state of SAMHD1 in primary human resting and replicating $CD4^+$ T cells (Figure 2A). In agreement with the hypothesis that unphosphorylated SAMHD1 is responsible for retroviral restriction, we found that resting $CD4^+$ T cells exhibit an unphosphorylated SAMHD1 protein, whereas replicating $CD4^+$ T cells exhibit a phosphorylated SAMHD1 protein (Figure 2A). To further test the phosphorylation state of SAMHD1 in resting $CD4^+$ T cells, we developed a rabbit polyclonal antibody for a peptide derived from SAMHD1 containing a phosphorylated T592 (anti-phospho-T592-SAMHD1). In agreement with the Phos-tag results, we found that anti-phospho-T592-SAMHD1 only recognized the SAMHD1 protein from replicating $CD4^+$ T cells (Figure 2C). These results are in agreement with our hypothesis that the unphosphorylated form of SAMHD1 is responsible for restriction.

We also analyzed the phosphorylation state of SAMHD1 in human primary MDMs. As suggested by our mass-spectrometry results, endogenously expressed SAMHD1 in human primary MDMs is unphosphorylated (Figures 2A and 2B).

Finally, we analyzed the phosphorylation state of SAMHD1 in cycling and noncycling human monocytic U937 cells stably expressing SAMHD1-FLAG (Figure 2B). Similarly, noncycling U937 cells stably express SAMHD1-FLAG, which blocks retroviral replication and is unphosphorylated. Overall, our analysis revealed that in all studied noncycling cells that restrict retroviral infection, SAMHD1 is unphosphorylated, whereas cycling cells contain a phosphorylated SAMHD1.

Regulation of SAMHD1 Restriction by Phosphorylation

Our biochemical analysis suggested that phosphorylated SAMHD1 in cycling cells does not restrict retroviral infection. To test the regulation of SAMHD1 restriction by phosphorylation, we generated a series of SAMHD1 phosphorylation variants

Table 1. SAMHD1 Phosphorylation Variants

SAMHD1 Variant	HIV-1 Restriction[a]	Oligomerization[b]	RNA Binding[c]	Localization[d]	Cellular dATP Level[e]	Enzymatic Activity[f]
WT	+	+	+	N	low	+
T592A	+	+	+	N	low	+
T592D	–	+	+	N	low	+
T592E	–	+	+	N	low	+
T592V	+	+	+	N	low	+
P593A	+	+	+	N	low	+
S33A	+	ND	ND	N	ND	ND
S33D	+	ND	ND	N	ND	ND
112-626	+	+	+	C	low	+
112-626-T592D	–	+	+	C	low	+
S18A	+	ND	ND	N	ND	ND
S18D	+	ND	ND	N	ND	ND
T21A	+	ND	ND	N	ND	ND
T21D	+	ND	ND	N	ND	ND
T25A	+	ND	ND	N	ND	ND
T25D	+	ND	ND	N	ND	ND

WT, wild-type; ND, not determined.

[a]HIV-1 restriction was measured by infecting U937 cells stably expressing the indicated SAMHD1 variants with HIV-1-GFP. After 48 hr, the percentage of GFP-positive cells (infected cells) was determined by flow cytometry.

[b]Oligomerization of the different SAMHD1 variants was determined by measuring the ability of the SAMHD1-FLAG variant to interact with the corresponding SAMHD1-HA variant, as described in Experimental Procedures. "+ " indicates 100% oligomerization, which corresponds to the amount of wild-type SAMHD1-HA that interacts with wild-type SAMHD1-FLAG.

[c]SAMHD1-FLAG variants were assayed for their ability to bind the dsRNA analog ISD-PS, as described in Experimental Procedures. "+" indicates the RNA binding achieved by wild-type SAMHD1.

[d]Subcellular localization of the different SAMHD1 variants in HeLa cells was performed as described in Experimental Procedures. "N" indicates nuclear localization; "C" indicates cytoplasmic localization.

[e]The cellular dATP levels of PMA-treated U937 cells stably expressing the different SAMHD1 variants were determined by primer extension as described in Experimental Procedures. "Low" indicates similar to the dATP levels observed in PMA-treated U937 cells stably expressing wild-type SAMHD1.

[f]The enzymatic activity of the different SAMHD1 variants was measured by a dTTP hydrolysis reaction, as described in Experimental Procedures. "+" indicates wild-type levels of activity.

wherein T592 was replaced by a phosmimetic (D and E) or unphosphorytable (V and A) residue (Table 1). We initially analyzed the phosphorylation state of the different variants via western blotting using SDS-PAGE gels containing Phos-tag in human 293T cells (Figure 3A). Interestingly, SAMHD1 variants, wherein T592 was replaced by a phosphomimetic or an unphosphorytable residue, are not phosphorylated when compared to the wild-type SAMHD1, suggesting that T592 is the residue phosphorylated in human cells (Figure 3A). To confirm that these mutants were no longer phosphorylated in 293T cells, we tested whether these proteins are recognized by the anti-phospho-T592-SAMHD1 antibody. As shown in Figure 3B, the wild-type SAMHD1 is recognized by the anti-phospho-T592-SAMHD1 antibody. By contrast, all variants wherein T592 was replaced by a different residue were not recognized by anti-phospho-T592-SAMHD1.

To test the ability of SAMHD1 variants to restrict HIV-1 infection in noncycling cells, we stably expressed SAMHD1 variants in U937 cells (Figure 3C) and tested the ability of these cell lines to restrict increasing amounts of HIV-1 expressing GFP as a reporter for infection (HIV-1-GFP), as previously described (Brandariz-Nuñez et al., 2012; White et al., 2013). As shown in Figure 3D and Table 1, SAMHD1 containing a phosphomimetic residue such as D or E lost the ability to block retroviral restriction in noncycling U937 cells, suggesting that SAMHD1 phosphorylated at position T592 is unable to block retroviral infection. By contrast, SAMHD1 variants wherein T592 was replaced by an unphosphorytable residue such as V or A were not affected in their ability to block infection by HIV-1-GFP (Figure 3D and Table 1). Similar restriction patterns were observed when using HIV-2, SIVΔVpx, feline immunodeficiency virus, equine infectious anemia virus, bovine immunodeficiency virus, and N-tropic and B-tropic murine leukemia viruses (data not shown), as described in White et al. (2013). These results suggest that a phosphorylated SAMHD1 in position T592 is unable to restrict infection by different retroviruses.

Our initial mass-spectrometry analysis of endogenously expressed SAMHD1 in THP-1 cells revealed that a small fraction of SAMHD1 was phosphorylated in position S33, and this pattern did not change when comparing cycling with noncycling cells (Figure 1A). To understand the contribution of S33 phosphorylation in the ability of SAMHD1 to restrict HIV-1 infection, we replaced S33 with a phosphomimetic (D) or an unphosphorytable (A) residue. SAMHD1 variants were stably expressed in U937 cells (Figure S2A), and the ability of these variants to restrict HIV-1-GFP was measured (Figure S2B). As shown in Figure 3E,

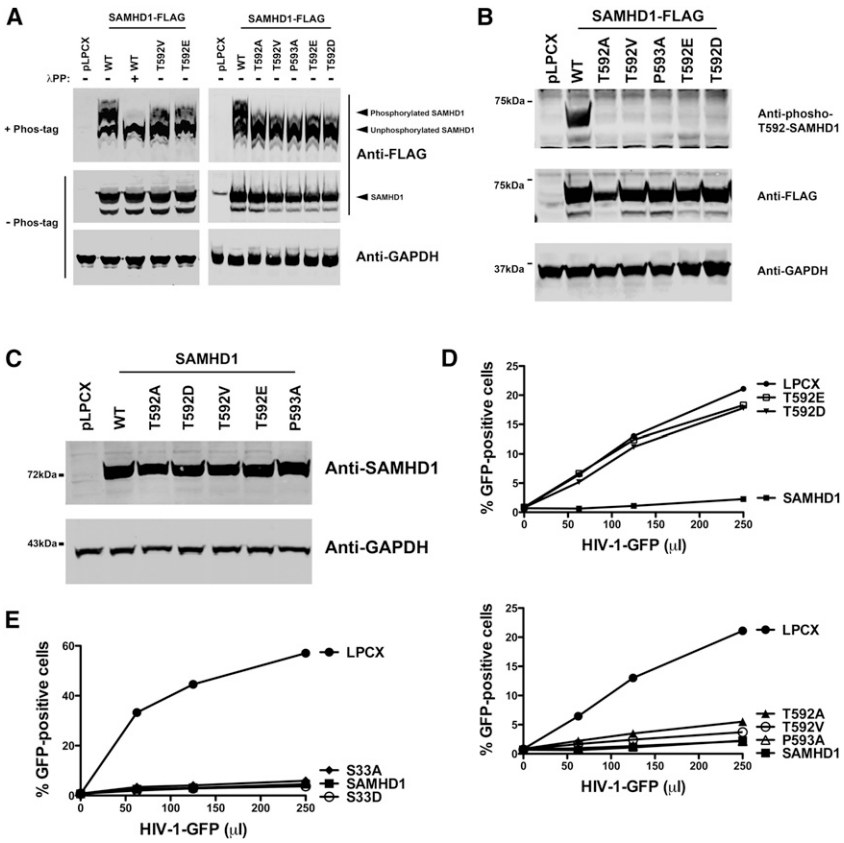

Figure 3. Ability of SAMHD1 Phosphorylation Variants to Restrict HIV-1

(A) Human 293T cells were transfected with wild-type or the indicated SAMHD1 variant. Cells were lysed 24 hr after transfection, and protein samples were separated using SDS-PAGE gels containing Phos-tag (+Phos-tag). SAMHD1 variants were detected by western blotting using anti-FLAG antibodies. The indicated sample was treated with λPP for detecting the migration of the unphosphorylated SAMHD1. In parallel, we performed a similar analysis using the empty vector pLPCX. Protein samples were also separated in SDS-PAGE gels without Phos-tag (−Phos-tag). As a loading control, cell lysates were western blotted using GAPDH antibodies.

(B) The different SAMHD1 variants expressed in 293T cells were also analyzed by western blotting using anti-phospho-T592-SAMHD1 antibodies, which only recognize a phosphorylated SAMHD1 protein at residues T592.

(C) Human monocytic U937 cells were stably transduced with wild-type SAMHD1 and the indicated SAMHD1 variant. PMA-treated stable cells were analyzed for SAMHD1 expression by western blotting using anti-FLAG antibodies. Similarly, western blot analysis of GAPDH was used as loading control.

(D and E) PMA-treated human monocytic U937 cells stably expressing the indicated SAMHD1 variants were challenged with increasing amounts of HIV-1-GFP. Infection is shown as the percentage of GFP-positive cells 48 hr postinfection measured by flow cytometry. As a control, U937 cells stably transduced with the empty vector pLPCX were challenged with increasing amounts of HIV-1-GFP. See also Figure S2. Experiments were performed in triplicate, and a representative result is shown. WT, Wild-type.

changes on S33 did not affect the ability of SAMHD1 to block HIV-1 infection.

Because our results indicated that the unphosphorylated form of SAMHD1 blocks HIV-1 infection in noncycling cells, we tested the ability of SAMHD1-T592V, which could not be phosphorylated, to restrict HIV-1 in cycling cells. As shown in Figures S2C and S2D, expression of SAMHD1-T592V in HeLa cells did not block HIV-1 infection. However, when SAMHD1-T592V was tested in cycling U937 cells, it showed a mild effect on HIV-1 infection (Figure S2E).

Finally, we tested the ability of Vpx from the ROD strain of HIV-2 (Vpx_{ROD}) to degrade the different SAMHD1 phosphorylation variants. As shown in Figure S2F, Vpx_{ROD} degraded the different SAMHD1 phosphorylation variants equally, suggesting that the ability of Vpx_{ROD} to degrade SAMHD1 is independent of the phosphorylation state of SAMHD1.

Oligomerization, RNA Binding, and Localization of SAMHD1 Phosphorylation Variants

Allosteric regulation of the enzymatic activity of SAMHD1 occurs in the dimerization interface of the HD domain (Goldstone et al., 2011). In agreement with this, we found that the SAMHD1 variant HD206AA is partially affected in its ability to oligomerize, which suggests that the defect in the ability of HD206AA to restrict HIV-1 might also be due to an oligomerization deficiency (White et al., 2013). To evaluate the possibility that the phosphorylation variants of SAMHD1 lose restriction due to an oligomerization defect, we tested the ability of the different SAMHD1-FLAG variants to oligomerize with the SAMHD1-hemagglutinin (HA) corresponding variant (Figure S2G). Interestingly, we found that SAMHD1 variants that lost the ability to block retroviral infection did not lose oligomerization ability, suggesting that the inability of SAMHD1 variants to restrict retroviral infection is not due to a defect in oligomerization.

We and others have previously demonstrated the ability of SAMHD1 to bind RNA (Goncalves et al., 2012; White et al., 2013); therefore, we tested the ability of the different SAMHD1 phosphorylation variants produced in human 293T cells to bind RNA (Figure S2H), as described previously (White et al., 2013). As shown in Figure S2H, all the studied SAMHD1 phosphorylation variants showed the ability to bind the double-stranded RNA (dsRNA) analog phosphorothioate-containing interferon-stimulatory DNA (ISD-PS) as strongly as the wild-type SAMHD1 protein.

Finally, we tested the cellular localization of the different SAMHD1 phosphorylation variants in human HeLa cells (Figure S2I), as described previously (Brandariz-Nuñez et al., 2012; White et al., 2013). These results showed that all the studied SAMHD1 phosphorylation variants localized to the nuclear compartment, similarly to the wild-type SAMHD1 protein (Figure S2I). Overall, these results demonstrated that the SAMHD1 phosphorylation variants used in these studies do not exhibit a major defect in the different properties of SAMHD1.

The Role of SAMHD1 N-Terminal Residues 1–112 in the Regulation of Retroviral Restriction by Phosphorylation of T592

Because our observations suggested that the phosphorylation state of T592 regulates the ability of SAMHD1 to block retroviral restriction, we tested the role of the N-terminal residues (1–112) of SAMHD1 (Figure 1B) in the ability of T592 phosphorylation to regulate restriction. Our previous observations suggested that a construct containing SAMHD1 residues 112–626 is sufficient for potent restriction of HIV-1 (White et al., 2013). To test the role of residues 1–112 in SAMHD1 regulation by phosphorylation, we created a construct containing the residues 112–626 of SAMHD1 wherein T592 was replaced by the phosphomimetic residue D (112-626-T592D). The phosphorylation levels of 112–626 and 112-626-T592D were measured in human 293T cells via western blotting using SDS-PAGE gels containing Phos-tag (Figure 4A). In agreement with our findings in the full-length SAMHD1, the protein composed of residues 112–626 was phosphorylated (Figure 4A). Interestingly, 112-626-T592D revealed an unphosphorylated protein similar to the full-length SAMHD1-T592D in noncycling cells (Figure 4A). To test the ability of 112-626-T592D to block HIV-1 infection, we stably expressed it in U937 cells (Figure 4B) and challenged cells with increasing amounts of HIV-1-GFP (Figure 4C). Interestingly, 112-626-T592D completely lost its ability to block HIV-1 infection when compared to 112–626 or full-length SAMHD1 (Figure 4C). Our results indicate that the SAMHD1 N-terminal residues 1–112 are dispensable for the ability of the T592 phosphorylation to regulate retroviral restriction.

Level of Cellular dNTPs in U937 Cells Stably Expressing the Different SAMHD1 Phosphorylation Variants

Previous observations have suggested that SAMHD1 blocks HIV-1 replication by decreasing the intracellular pool of dNTPs (Goldstone et al., 2011; Kim et al., 2012; Lahouassa et al., 2012; Powell et al., 2011; White et al., 2013). A decrease in cellular dNTPs will prevent the occurrence of retroviral reverse transcription, a process that requires dNTPs. Given that SAMHD1 phosphorylation variants did not show an obvious defect of the known properties of SAMHD1, we decided to test whether the loss of restriction by SAMHD1 phosphorylation variants correlated with a gain in cellular dNTP levels. For this purpose, we measured the intracellular levels of dNTPs in differentiated U937 cells stably expressing the different SAMHD1 phosphorylarion variants (Figure 5A), as described previously (White et al., 2013). Remarkably, U937 cells stably expressing SAMHD1 variants wherein T592 was replaced by a phosphomimetic residue (D or E) showed dNTP levels comparable to those of U937 cells stably expressing wild-type SAMHD1. In agreement with this, the cellular dNTP levels of U937 cells stably expressing the SAMHD1 construct 112-626-T592D, which loses the ability to block HIV-1 infection, were similar to the levels found in U937 cells stably expressing wild-type SAMHD1 (Figure S3 and Table 1). These results indicate that the inability of SAMHD1-T592D, SAMHD1-T592E, and 112-626-T592D to block HIV-1 infection is not due to a defect in the dNTPase activity of the protein. These findings indirectly suggest that the dNTPase activity of SAMHD1-T592D, SAMHD1-T592E, and 112-626-T592D is intact.

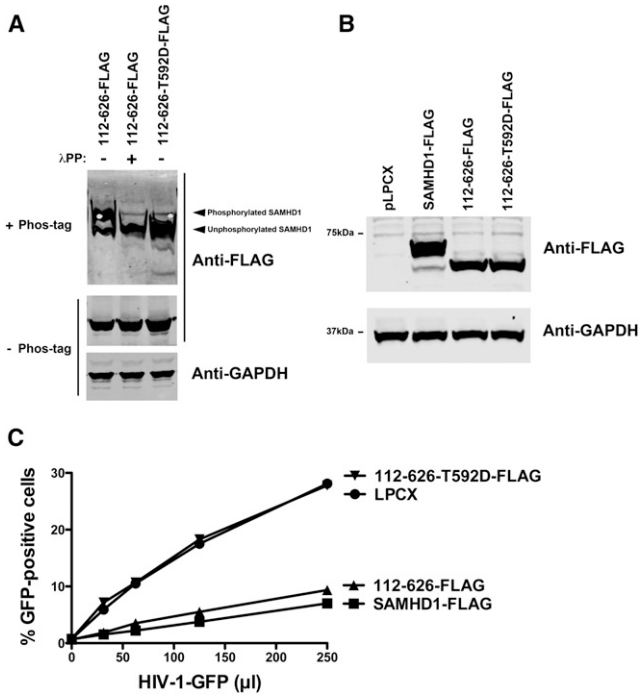

Figure 4. Role of the N-Terminal Residues 1–112 in the Ability of Phosphorylation to Regulate Retroviral Restriction

(A) Human 293T cells were transfected with the indicated SAMHD1 variant. Cells were lysed 24 hr after transfection, and protein samples were separated using SDS-PAGE gels containing Phos-tag (+Phos-tag). SAMHD1 variants were detected by western blotting using anti-FLAG antibodies. Protein samples were also separated in SDS-PAGE gels without Phos-tag (−Phos-tag). As a loading control, cell lysates were western blotted using GAPDH antibodies.

(B) Human monocytic U937 cells were stably transduced with wild-type, and the indicated SAMHD1 variants were analyzed for SAMHD1 expression by western blotting using anti-FLAG antibodies. Western blot analysis of GAPDH was used as a loading control.

(C) Human monocytic U937 cells stably expressing the different SAMHD1 variants were challenged with increasing amounts of HIV-1-GFP. Infection is shown as the percentage of GFP-positive cells 48 hr postinfection as measured by flow cytometry. As a control, U937 cells stably transduced with the empty vector pLPCX were challenged with increasing amounts of HIV-1-GFP. See also Figure S3. Experiments were performed in triplicate, and a representative result is shown.

Enzymatic Activity of the Different SAMHD1 Phosphorylation Variants

To directly analyze the enzymatic activity of SAMHD1 phosphorylation variants, we tested the ability of immunoprecipitated SAMHD1 variants (Figure 5B) to hydrolyze α-^{32}P-labeled thymidine triphosphate (α-[^{32}P]TTP) to deoxythymidine (dT) and α-[^{32}P]PP, in the presence of the allosteric activator dGTP (Figure 5C). For this purpose, we incubated the indicated SAMHD1 variant in the presence of radio-labeled α-[^{32}P]TTP. Reaction products were separated using thin-layer chromatography in order to determine the amount of hydrolyzed α-[^{32}P]PP (Figure 5C), as previously shown (White et al., 2013). In agreement, immunoprecipitated SAMHD1-T592D and SAMHD1-T592E exhibited similar enzymatic activity when compared to wild-type SAMHD1 (Figure 5C). As a control, we measured the enzymatic activity of the different SAMHD1 variants in the presence of an

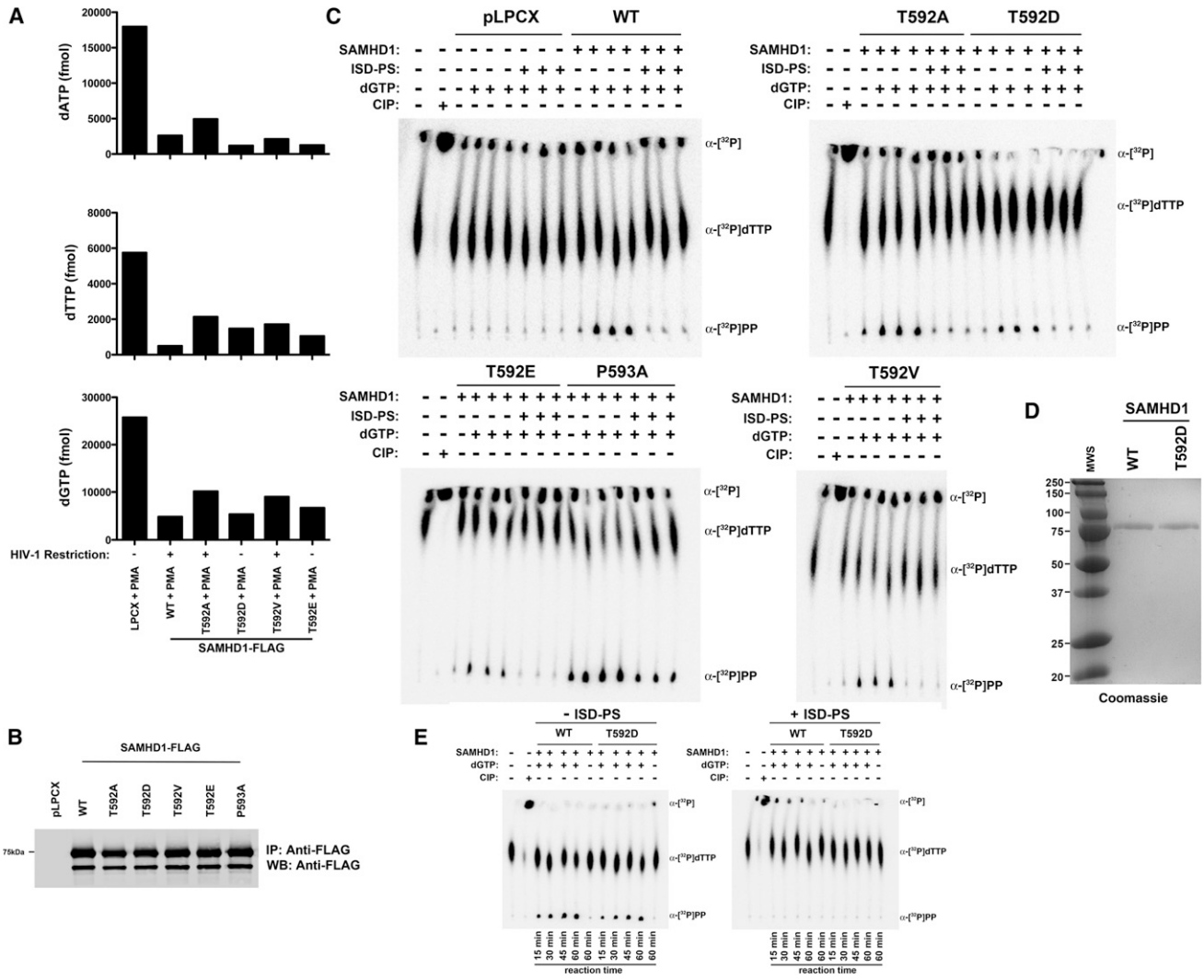

Figure 5. Analysis of Enzymatic Activity of the Different SAMHD1 Variants

(A) Quantification of dATP, dTTP, and dGTP levels from PMA-treated U937 cells expressing the indicated SAMHD1 variants was performed with a primer-extension assay, as described in Experimental Procedures. Similar results were obtained in three separate experiments, and a representative experiment is shown.

(B) Immunoprecipitated SAMHD1 variants from human 293T cells were normalized by western blotting using anti-FLAG antibodies. WT, wild-type; WB, western blot; IP, immunoprecipitation.

(C) Thin-layer chromatography analysis of the dTTP-triphosphohydrolase activity of the different immunoprecipitated SAMHD1 variants. For this purpose, we incubated radio-labeled α-[^{32}P]dTTP in the presence of the indicated SAMHD1 variants. Products from the α-[^{32}P]dTTP hydrolysis were separated by thin-layer chromatography using polyethyleneimine cellulose. Hydrolysis of α-[^{32}P]dTTP yields dT and α-[^{32}P]PP, which are visualized by using a phosphoimager. As a control, we also measured the dTTP-triphosphohydrolase activity in the presence of the dsRNA analog ISD-PS. The results of three independent enzymatic reactions per treatment are shown.

(D) Recombinant SAMHD1 and SAMHD1-T592D proteins were purified from baculovirus-infected Sf9 insect cells. Proteins were separated by SDS-PAGE gels and stained with Coomassie blue.

(E) Similarly, the dTTPase activity of baculovirus recombinant SAMHD1 and SAMHD1-T592D proteins was determined by measuring the hydrolysis of radio-labeled α-[^{32}P]dTTP of the indicated recombinant SAMHD1 protein. Reactions were stopped at the indicated times and separated by thin-layer chromatography using polyethyleneimine cellulose, as described in Experimental Procedures. See also Figure S4. A representative result of three independent experiments is shown.

analog for dsRNA, which inhibits the enzymatic activity of SAMHD1 (White et al., 2013). These experiments showed that the enzymatic activity of SAMHD1 phosphorylation variants is not affected. We also confirmed these results by measuring nucleotide hydrolysis via high-pressure liquid chromatography (HPLC) (Figure S4A), as described in Experimental Procedures. Overall, these findings suggest that the enzymatic activity of SAMHD1 is not sufficient for restriction of HIV-1. Similarly, we measured the ability of SAMHD1-T592D purified from insect cells to hydrolyze α-[^{32}P]TTP in the presence of the allosteric

activator dGTP (Figures 5D, 5E S4B, and S4C), as previously shown (White et al., 2013). In agreement, purified SAMHD1-T592D showed similar α-[^{32}P]TTP hydrolyse activity when compared to the wild-type purified protein (Figures 5D, 5E, and S4C). In the same way, we confirmed these results by measuring nucleotide hydrolysis via HPLC (Figure S4B).

Involvement of Cyclin-Dependent Kinase 1 in the Phosphorylation of SAMHD1

Covalent capture of kinase-specific phosphopeptides from total HeLa extracts revealed that a peptide derived from SAMHD1 containing T592 is the substrate for the human cdk1 complex (Blethrow et al., 2008). These experiments suggested that human cdk1 might be the cellular kinase of SAMHD1. In agreement, analysis of the SAMHD1 protein sequence with the software Scansite (http://scansite.mit.edu) revealed that residues ^{592}TPQK^{595} constitute a consensus sequence motif for recognition and phosphorylation by cdk1 (Errico et al., 2010). To test the role of cdk1 in phosphorylation of SAMHD1, we assayed the level of SAMHD1 phosphorylation in the presence of the human dominant-negative cdk1 mutant cdk1-D146N, which is a cdk1-defective variant on its active site (van den Heuvel and Harlow, 1993). As shown in Figure 6A, the presence of Cdk1-D146N decreased the amount of phosphorylated SAMHD1, suggesting that human cdk1 is phosphorylating SAMHD1 in vivo. To directly test the ability of human cdk1 to phosphorylate SAMHD1, we incubated bacterially expressed recombinant glutathione S-transferase (GST)-SAMHD1 with an active cdk1 complex in an in vitro kinase assay (Figure 6B). These experiments showed that cdk1 phosphorylates GST-SAMHD1, but not the GST control protein. We performed a similar in vitro kinase assay using the human histone H1, which is a specififc substrate for the cdk1 complex, as a positive control. These results, together with the evidence that SAMHD1 variants on T592 are not phosphorylated in vivo (Figures 3A and 3B), suggest that SAMHD1 is a substrate for the kinase complex cdk1. The results are also in agreement with the observation that mutating P593A in the cdk1 recognition motif ^{592}TPQK^{595} abrogates SAMHD1 phosphorylation (Figure 3). The SAMHD1-P593A protein behaved similarly to SAMHD1-T592A/V, suggesting that this mutant lost its ability to be regulated by phosphorylation (Figures 3 and 5).

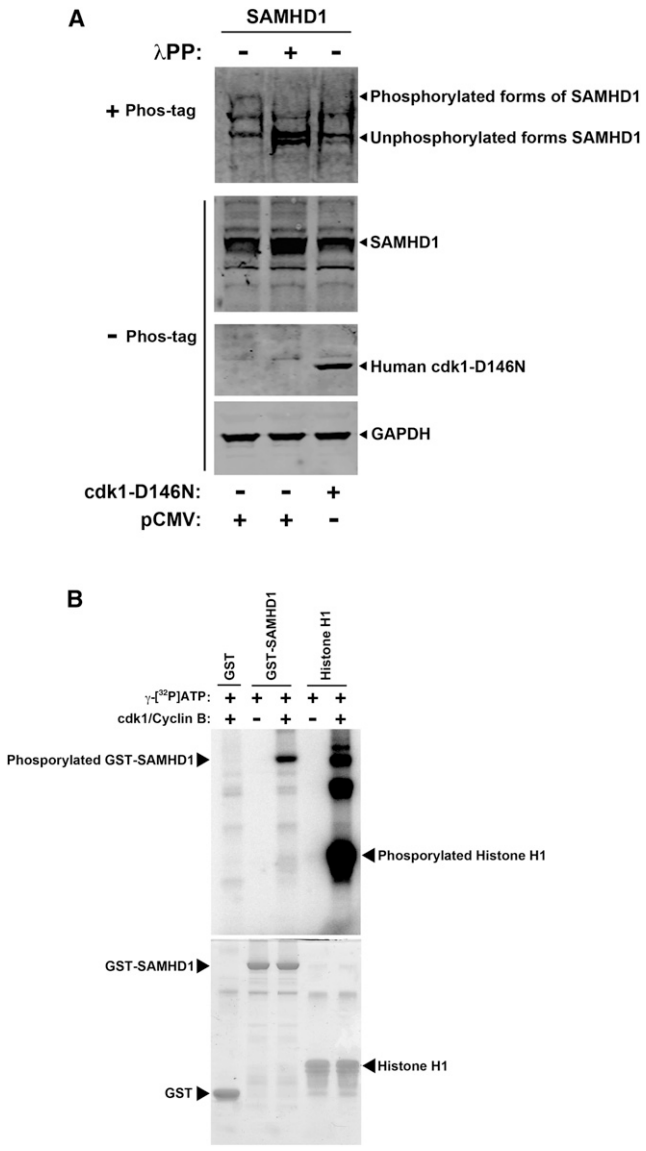

Figure 6. Involvement of cdk1 in the Phosphorylation of SAMHD1

(A) Effect of the Cdk1 dominant-negative mutant D146N (Cdk1-D146N) on the phosphorylation levels of SAMHD1. Cell lysates from human 293T cells transfected with plasmids expressing the SAMHD1-FLAG and Cdk1-D146N-HA proteins were separated by SDS-PAGE containing Phos-tag (+Phos-tag). SAMHD1-FLAG and Cdk1-D146N-HA were detected by western blotting using anti-FLAG and anti-HA antibodies, respectively. As a control, the levels of SAMHD1 phosphorylation were determined in the presence of the empty vector pCMV. Protein samples were also separated in SDS-PAGE gels without Phos-tag (−Phos-tag). Loading was normalized by western blotting using GAPDH antibodies. Similar results were obtained in three independent experiments, and a representative experiment is shown.

(B) In vitro phosphorylation of recombinant GST-SAMHD1 purified from bacteria by cdk1 complex, which was purified from baculovirus-infected cells using γ-[^{32}P]ATP as a phosphate donor. As a positive control, we incubated the cdk1 kinase complex with the human histone H1, which is a known substrate for this kinase complex. As a negative control, we incubated a similar amount of purified GST protein with the kinase complex cdk1.

DISCUSSION

Cycling and noncycling cells have the potentiality to express the restriction factor SAMHD1; however, SAMHD1 is only antivirally active in noncycling cells, suggesting that differences exist between cycling and noncycling cells that might be regulating the ability of SAMHD1 to block retroviral infection. The first possible explanation is that SAMHD1 is posttranslationally modified when comparing cycling with noncycling cells. Second, noncycling cells might be expressing a cofactor required for restriction, and a third possibility would be that SAMHD1 post-translational modifications and a cofactor are required for retroviral restriction. The present work revealed that the ability of SAMHD1 to restrict retroviral infection is regulated by phosphorylation of T592. Interestingly, we found that all tested cycling cells expressed a SAMHD1 protein phosphorylated at residue

T592. By contrast, all tested noncycling cells revealed an unphosphorylated SAMHD1 protein at position T592.

The replacement of T592 with phosphomimetic amino acids completely abrogated the ability of SAMHD1 to block retroviral infection. SAMHD1 phosphorylation variants T592D and T592E completely lost their capacity to block retroviral infection without losing RNA binding, dNTPase activity, oligomerization, and localization. The fact that these variants did not lose most of the known SAMHD1 properties suggests that these variants are properly folded. One possibility is that phosphorylation of SAMHD1 at residue T592 induces a conformational change that eliminates the ability of SAMHD1 to block retroviral infection without affecting all the known properties of SAMHD1. A second possibility is that phosphorylation of residue T592 regulates the ability of SAMHD1 to interact with an unknown cofactor required for retroviral restriction.

The ability of phosphorylation to regulate the restriction capacity of SAMHD1 only requires the HD domain and the C-terminal residues 583–626 (Figure 1B). Previous studies showed that a SAMHD1 construct containing only the HD domain and the C-terminal residues 583–626 (112–626) is sufficient for potent restriction of retroviruses (White et al., 2013); this work suggested that the SAM domain is dispensable for restriction. Our investigations revealed that the SAMHD1 construct 112–626 wherein T592 has been replaced by a phosphomimetic residue loses its restriction capacity, suggesting that regulation of restriction by phosphorylation does not require the N-terminal residues 1–112. In agreement with this, 112-626-T592D lost the ability to block retroviral infection without losing dNTPase activity.

Several lines of evidence have correlated the ability of SAMHD1 to decrease the levels of dNTPs with the ability of SAMHD1 to block infection by different retroviruses, suggesting that decreasing the cellular levels of dNTPs is what limits viral replication (Goldstone et al., 2011; Kim et al., 2012; Lahouassa et al., 2012; Powell et al., 2011; White et al., 2013). The present work found SAMHD1 variants that lost their ability to block retroviral infection but preserved the ability to decrease the cellular dNTP levels. In addition, these variants were also proven to be enzymatically active when tested as a purified protein from insect or mammalian cells. These results indicated that the decrease of cellular dNTP levels by SAMHD1 is not sufficient to achieve retroviral restriction. One possible explanation is that both the enzymatic activity of SAMHD1 and an unknown function is required for restriction. A second possibility is that the enzymatic activity of SAMHD1 is not necessary for the ability of SAMHD1 to block retroviral infection. Although the variant HD206AA, which targets the active site of SAMHD1, loses retroviral restriction (Hrecka et al., 2011; Laguette et al., 2011), its oligomerization capacity is also defective, making it difficult to pinpoint which defect causes loss of restriction (White et al., 2013). In addition, these results implied that decreasing the levels of cellular dNTPs to the levels achieved by wild-type SAMHD1 is not sufficient to block infection by different retroviruses.

Recent observations have suggested that SAMHD1 exhibits 3′ to 5′ exonuclease activity against single-stranded DNA and RNA in vitro (Beloglazova et al., 2013). An alternative possibility is that phosphorylated SAMHD1 at position T592 exhibits low exonuclease activity without losing the ability to decrease the cellular levels of dNTPs. By contrast, a dephosphorylated SAMHD1 would exhibit high exonuclease activity. This would suggest that SAMHD1 is stopping viral replication by degrading the genetic material of the virus, which is in agreement with the ability of SAMHD1 to restrict diverse RNA viruses such as lentiviruses and γ-retroviruses (White et al., 2013). Future experiments will attempt to understand whether the enzymatic activity of SAMHD1 is required for restriction.

Previous observations demonstrated that residue T592 is the substrate of the human cdk1 complex in HeLa cells (Blethrow et al., 2008). In agreement with this, residues ^{592}TPQK^{595} are a consensus sequence motif for recognition and phosphorylation by cdk1 (Errico et al., 2010), and replacement of T592 in the ^{592}TPQK^{595} motif by an unphosphorytable residue diminishes the ability of SAMHD1 to become phosphorylated in human cycling cells. Similarly, the replacement of P593 in the same motif by an alanine diminished SAMHD1 phosphorylation. To confirm this evidence, we assayed the level of SAMHD1 phosphorylation in the presence of the human dominant-negative cdk1 mutant (cdk1-D146N) to show that expression of the cdk1 dominant-negative mutant decreases the level of phosphorylated wild-type SAMHD1. We also demonstrated the ability of purified cdk1 complex to phosphorylate purified SAMHD1. In agreement with these findings, several groups have reported that differentiation of cells to a noncycling state involves the downmodulation of cdk1 expression (Hass et al., 1992; Kim et al., 2008; Traore et al., 2005). However, a phosphatase might also be regulating the phosphorylation state of SAMHD1 at position T592. Even though in most of the known cases regulation by phosphorylation is determined by the kinase, there are examples wherein the phosphatase plays an important role in regulating the phosphorylation state of a particular protein (Cheng et al., 2011). Future experiments will attempt to understand whether the phosphorylation of SAMHD1 is regulated by cellular phosphatases. Taken together, this evidence suggests that SAMHD1 is the substrate of cdk1 in human cycling cells, and that the downmodulation of cdk1 in noncycling cells results in the presence of unphosphorylated SAMHD1 protein, which is antivirally active.

EXPERIMENTAL PROCEDURES

Generation of U937 Cells Stably Expressing SAMHD1 Variants

Retroviral vectors encoding wild-type or mutant SAMHD1 proteins were created using the LPCX vector. Recombinant viruses were produced in 293T cells by cotransfecting the LPCX plasmids with the pVPack-GP and pVPack-VSV-G packaging plasmids. Transduced human monocytic U937 cells were selected in 0.4 μg/ml puromycin.

Infection with Retroviruses Expressing GFP

Recombinant retroviruses expressing GFP, pseudotyped with the VSV-G glycoprotein, were prepared as described (Diaz-Griffero et al., 2008). For infections, 6×10^4 cells were seeded in 24-well plates and treated with PMA. Subsequently, cells were incubated with the indicated retrovirus for 48 hr. The percentage of GFP-positive cells was determined by flow cytometry (Becton Dickinson).

SAMHD1 Oligomerization Assay

Human 293T cells were cotransfected with plasmids encoding SAMHD1 variants tagged with FLAG and HA. Precleared lysates containing SAMHD1

proteins were incubated with anti-FLAG-agarose beads. Anti-FLAG-agarose beads were washed three times, and immune complexes were eluted using a FLAG tripeptide. The eluted samples were analyzed by western blotting using either anti-HA or anti-FLAG antibodies.

Nucleic-Acid Binding Assay

The nucleic-acid binding assay was performed as previously described (Goncalves et al., 2012; White et al., 2013). In brief, the synthetic-DNA ISD-PS, which is an RNA analog, was used. Precleared lysates ("Input" in Figure S2) were incubated with immobilized nucleic acids in the presence of calf-thymus DNA as a competitor. Unbound proteins were removed by three consecutive washes. Bound proteins to nucleic acids ("Bound") were eluted and analyzed by western blotting using FLAG antibodies.

Cellular dNTPs Quantification by a Primer Extension Assay

Cells were pelleted, resuspended on ice-cold methanol, and dried using a SpeedVac. The dried samples were resuspended in water, and analyzed for dNTP content, as described previously (Kim et al., 2012; Lahouassa et al., 2012).

Immunoprecipitation and Mass Spectrometry

U937 cells stably expressing SAMHD1-FLAG, THP-1 cells, or primary MDMs were lysed and precleared using protein A-agarose. Precleared lysates were incubated with anti-FLAG-agarose beads or protein A-agarose with the anti-SAMHD1 antibody when appropriate. Beads containing the immunoprecipitates were washed three times. Subsequently, immune complexes were eluted and samples were separated by SDS-PAGE. Bands corresponding to the molecular weight of SAMHD1 were excised and sent to the Taplin Mass Spectrometry Facility (Harvard Medical School) for phosphopeptide mapping.

SDS-PAGE Gels Containing Phos-Tag

Preparation of Phos-tag gels was performed as previously described (Kinoshita et al., 2006). The gel consisted of a separating and a stacking gel containing Zn-Phos-tag. The acrylamide-pendant Phos-tag ligand and two equivalents of $Zn(NO3)_2$ were added to the separating gel before polymerization. After electrophoresis, the gel was transferred to a nitrocellulose membrane using an iBlot Gel Transfer system.

In Vitro cdk1 Complex Kinase Assay

Recombinant GST-SAMHD1 or GST was incubated with recombinant cdk1 complex (Sigma-Aldrich) in phosphorylation buffer suplemented with 5 μCi of γ-[^{32}P]ATP. The phosphorylation reaction was stopped by the addition of sample buffer. Proteins were resolved on 10% polyacrilamide SDS-PAGE. The resultant gel was dryed on a Bio-Rad gel dryer and analyzed using a phosphoimager.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.chom.2013.03.005.

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REFERENCES

Ahn, J., Hao, C., Yan, J., DeLucia, M., Mehrens, J., Wang, C., Gronenborn, A.M., and Skowronski, J. (2012). HIV/simian immunodeficiency virus (SIV) accessory virulence factor Vpx loads the host cell restriction factor SAMHD1 onto the E3 ubiquitin ligase complex CRL4DCAF1. J. Biol. Chem. *287*, 12550–12558.

Arfi, V., Rivière, L., Jarrosson-Wuillème, L., Goujon, C., Rigal, D., Darlix, J.L., and Cimarelli, A. (2008). Characterization of the early steps of infection of primary blood monocytes by human immunodeficiency virus type 1. J. Virol. *82*, 6557–6565.

Baldauf, H.M., Pan, X., Erikson, E., Schmidt, S., Daddacha, W., Burggraf, M., Schenkova, K., Ambiel, I., Wabnitz, G., Gramberg, T., et al. (2012). SAMHD1 restricts HIV-1 infection in resting CD4(+) T cells. Nat. Med. *18*, 1682–1687.

Beloglazova, N., Flick, R., Tchigvintsev, A., Brown, G., Popovic, A., Nocek, B., and Yakunin, A.F. (2013). Nuclease Activity of the Human SAMHD1 Protein Implicated in the Aicardi-Goutieres Syndrome and HIV-1 Restriction. J. Biol. Chem. *288*, 8101–8110.

Belshan, M., Mahnke, L.A., and Ratner, L. (2006). Conserved amino acids of the human immunodeficiency virus type 2 Vpx nuclear localization signal are critical for nuclear targeting of the viral preintegration complex in non-dividing cells. Virology *346*, 118–126.

Bergamaschi, A., Ayinde, D., David, A., Le Rouzic, E., Morel, M., Collin, G., Descamps, D., Damond, F., Brun-Vezinet, F., Nisole, S., et al. (2009). The human immunodeficiency virus type 2 Vpx protein usurps the CUL4A-DDB1 DCAF1 ubiquitin ligase to overcome a postentry block in macrophage infection. J. Virol. *83*, 4854–4860.

Berger, A., Sommer, A.F., Zwarg, J., Hamdorf, M., Welzel, K., Esly, N., Panitz, S., Reuter, A., Ramos, I., Jatiani, A., et al. (2011). SAMHD1-deficient CD14+ cells from individuals with Aicardi-Goutières syndrome are highly susceptible to HIV-1 infection. PLoS Pathog. *7*, e1002425.

Blethrow, J.D., Glavy, J.S., Morgan, D.O., and Shokat, K.M. (2008). Covalent capture of kinase-specific phosphopeptides reveals Cdk1-cyclin B substrates. Proc. Natl. Acad. Sci. USA *105*, 1442–1447.

Brandariz-Nuñez, A., Valle-Casuso, J.C., White, T.E., Laguette, N., Benkirane, M., Brojatsch, J., and Diaz-Griffero, F. (2012). Role of SAMHD1 nuclear localization in restriction of HIV-1 and SIVmac. Retrovirology *9*, 49.

Cheng, H.C., Qi, R.Z., Paudel, H., and Zhu, H.J. (2011). Regulation and function of protein kinases and phosphatases. Enzyme Res. *2011*, 794089.

Descours, B., Cribier, A., Chable-Bessia, C., Ayinde, D., Rice, G., Crow, Y., Yatim, A., Schwartz, O., Laguette, N., and Benkirane, M. (2012). SAMHD1 restricts HIV-1 reverse transcription in quiescent CD4(+) T-cells. Retrovirology *9*, 87.

Diaz-Griffero, F., Perron, M., McGee-Estrada, K., Hanna, R., Maillard, P.V., Trono, D., and Sodroski, J. (2008). A human TRIM5alpha B30.2/SPRY domain mutant gains the ability to restrict and prematurely uncoat B-tropic murine leukemia virus. Virology *378*, 233–242.

Errico, A., Deshmukh, K., Tanaka, Y., Pozniakovsky, A., and Hunt, T. (2010). Identification of substrates for cyclin dependent kinases. Adv. Enzyme Regul. *50*, 375–399.

Fletcher, T.M., 3rd, Brichacek, B., Sharova, N., Newman, M.A., Stivahtis, G., Sharp, P.M., Emerman, M., Hahn, B.H., and Stevenson, M. (1996). Nuclear import and cell cycle arrest functions of the HIV-1 Vpr protein are encoded by two separate genes in HIV-2/SIV(SM). EMBO J. *15*, 6155–6165.

Fujita, M., Otsuka, M., Miyoshi, M., Khamsri, B., Nomaguchi, M., and Adachi, A. (2008). Vpx is critical for reverse transcription of the human immunodeficiency virus type 2 genome in macrophages. J. Virol. *82*, 7752–7756.

Gibbs, J.S., Lackner, A.A., Lang, S.M., Simon, M.A., Sehgal, P.K., Daniel, M.D., and Desrosiers, R.C. (1995). Progression to AIDS in the absence of a gene for vpr or vpx. J. Virol. *69*, 2378–2383.

Goldstone, D.C., Ennis-Adeniran, V., Hedden, J.J., Groom, H.C., Rice, G.I., Christodoulou, E., Walker, P.A., Kelly, G., Haire, L.F., Yap, M.W., et al. (2011). HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. Nature *480*, 379–382.

Goncalves, A., Karayel, E., Rice, G.I., Bennett, K.L., Crow, Y.J., Superti-Furga, G., and Bürckstümmer, T. (2012). SAMHD1 is a nucleic-acid binding protein that is mislocalized due to aicardi-goutières syndrome-associated mutations. Hum. Mutat. *33*, 1116–1122.

Goujon, C., Jarrosson-Wuilleme, L., Bernaud, J., Rigal, D., Darlix, J.L., and Cimarelli, A. (2003). Heterologous human immunodeficiency virus type 1 lentiviral vectors packaging a simian immunodeficiency virus-derived genome display a specific postentry transduction defect in dendritic cells. J. Virol. *77*, 9295–9304.

Goujon, C., Rivière, L., Jarrosson-Wuilleme, L., Bernaud, J., Rigal, D., Darlix, J.L., and Cimarelli, A. (2007). SIVSM/HIV-2 Vpx proteins promote retroviral escape from a proteasome-dependent restriction pathway present in human dendritic cells. Retrovirology *4*, 2.

Goujon, C., Arfi, V., Pertel, T., Luban, J., Lienard, J., Rigal, D., Darlix, J.L., and Cimarelli, A. (2008). Characterization of simian immunodeficiency virus SIVSM/human immunodeficiency virus type 2 Vpx function in human myeloid cells. J. Virol. *82*, 12335–12345.

Hass, R., Gunji, H., Datta, R., Kharbanda, S., Hartmann, A., Weichselbaum, R., and Kufe, D. (1992). Differentiation and retrodifferentiation of human myeloid leukemia cells is associated with reversible induction of cell cycle-regulatory genes. Cancer Res. *52*, 1445–1450.

Hirsch, V.M., Sharkey, M.E., Brown, C.R., Brichacek, B., Goldstein, S., Wakefield, J., Byrum, R., Elkins, W.R., Hahn, B.H., Lifson, J.D., and Stevenson, M. (1998). Vpx is required for dissemination and pathogenesis of SIV(SM) PBj: evidence of macrophage-dependent viral amplification. Nat. Med. *4*, 1401–1408.

Hrecka, K., Hao, C., Gierszewska, M., Swanson, S.K., Kesik-Brodacka, M., Srivastava, S., Florens, L., Washburn, M.P., and Skowronski, J. (2011). Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. Nature *474*, 658–661.

Jin, L., Zhou, Y., and Ratner, L. (2001). HIV type 2 Vpx interaction with Gag and incorporation into virus-like particles. AIDS Res. Hum. Retroviruses *17*, 105–111.

Kappes, J.C., Parkin, J.S., Conway, J.A., Kim, J., Brouillette, C.G., Shaw, G.M., and Hahn, B.H. (1993). Intracellular transport and virion incorporation of vpx requires interaction with other virus type-specific components. Virology *193*, 222–233.

Kaushik, R., Zhu, X., Stranska, R., Wu, Y., and Stevenson, M. (2009). A cellular restriction dictates the permissivity of nondividing monocytes/macrophages to lentivirus and gammaretrovirus infection. Cell Host Microbe *6*, 68–80.

Kim, M.O., Moon, D.O., Choi, Y.H., Lee, J.D., Kim, N.D., and Kim, G.Y. (2008). Platycodin D induces mitotic arrest in vitro, leading to endoreduplication, inhibition of proliferation and apoptosis in leukemia cells. Int. J. Cancer *122*, 2674–2681.

Kim, B., Nguyen, L.A., Daddacha, W., and Hollenbaugh, J.A. (2012). Tight interplay among SAMHD1 protein level, cellular dNTP levels, and HIV-1 proviral DNA synthesis kinetics in human primary monocyte-derived macrophages. J. Biol. Chem. *287*, 21570–21574.

Kinoshita, E., Kinoshita-Kikuta, E., Takiyama, K., and Koike, T. (2006). Phosphate-binding tag, a new tool to visualize phosphorylated proteins. Mol. Cell. Proteomics *5*, 749–757.

Laguette, N., Sobhian, B., Casartelli, N., Ringeard, M., Chable-Bessia, C., Ségéral, E., Yatim, A., Emiliani, S., Schwartz, O., and Benkirane, M. (2011). SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature *474*, 654–657.

Laguette, N., Rahm, N., Sobhian, B., Chable-Bessia, C., Münch, J., Snoeck, J., Sauter, D., Switzer, W.M., Heneine, W., Kirchhoff, F., et al. (2012). Evolutionary and functional analyses of the interaction between the myeloid restriction factor SAMHD1 and the lentiviral Vpx protein. Cell Host Microbe *11*, 205–217.

Lahouassa, H., Daddacha, W., Hofmann, H., Ayinde, D., Logue, E.C., Dragin, L., Bloch, N., Maudet, C., Bertrand, M., Gramberg, T., et al. (2012). SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. Nat. Immunol. *13*, 223–228.

Lim, E.S., Fregoso, O.I., McCoy, C.O., Matsen, F.A., Malik, H.S., and Emerman, M. (2012). The ability of primate lentiviruses to degrade the monocyte restriction factor SAMHD1 preceded the birth of the viral accessory protein Vpx. Cell Host Microbe *11*, 194–204.

Park, I.W., and Sodroski, J. (1995). Amino acid sequence requirements for the incorporation of the Vpx protein of simian immunodeficiency virus into virion particles. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. *10*, 506–510.

Powell, R.D., Holland, P.J., Hollis, T., and Perrino, F.W. (2011). Aicardi-Goutieres syndrome gene and HIV-1 restriction factor SAMHD1 is a dGTP-regulated deoxynucleotide triphosphohydrolase. J. Biol. Chem. *286*, 43596–43600.

Schwende, H., Fitzke, E., Ambs, P., and Dieter, P. (1996). Differences in the state of differentiation of THP-1 cells induced by phorbol ester and 1,25-dihydroxyvitamin D3. J. Leukoc. Biol. *59*, 555–561.

Selig, L., Pages, J.C., Tanchou, V., Prévéral, S., Berlioz-Torrent, C., Liu, L.X., Erdtmann, L., Darlix, J., Benarous, R., and Benichou, S. (1999). Interaction with the p6 domain of the gag precursor mediates incorporation into virions of Vpr and Vpx proteins from primate lentiviruses. J. Virol. *73*, 592–600.

Srivastava, S., Swanson, S.K., Manel, N., Florens, L., Washburn, M.P., and Skowronski, J. (2008). Lentiviral Vpx accessory factor targets VprBP/DCAF1 substrate adaptor for cullin 4 E3 ubiquitin ligase to enable macrophage infection. PLoS Pathog. *4*, e1000059.

Sunseri, N., O'Brien, M., Bhardwaj, N., and Landau, N.R. (2011). Human immunodeficiency virus type 1 modified to package Simian immunodeficiency virus Vpx efficiently infects macrophages and dendritic cells. J. Virol. *85*, 6263–6274.

Traore, K., Trush, M.A., George, M., Jr., Spannhake, E.W., Anderson, W., and Asseffa, A. (2005). Signal transduction of phorbol 12-myristate 13-acetate (PMA)-induced growth inhibition of human monocytic leukemia THP-1 cells is reactive oxygen dependent. Leuk. Res. *29*, 863–879.

van den Heuvel, S., and Harlow, E. (1993). Distinct roles for cyclin-dependent kinases in cell cycle control. Science *262*, 2050–2054.

White, T.E., Brandariz-Nuñez, A., Carlos Valle-Casuso, J., Amie, S., Nguyen, L., Kim, B., Brojatsch, J., and Diaz-Griffero, F. (2013). Contribution of SAM and HD domains to retroviral restriction mediated by human SAMHD1. Virology *436*, 81–90.

Zhang, C., de Silva, S., Wang, J.H., and Wu, L. (2012). Co-evolution of primate SAMHD1 and lentivirus Vpx leads to the loss of the vpx gene in HIV-1 ancestor. PLoS ONE *7*, e37477.