# SAM domain-containing N-terminal region of SAMHD1 plays a crucial role in its stabilization and restriction of HIV-1 infection

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SAMHD1 restricts human immunodeficiency virus type 1 (HIV-1) infection in a cell-type specific manner. Other than primary monocyte derived cells and resting CD4+ T cells, the SAMHD1-mediated HIV-1 block was reported only in phorbol 12-myristate 13-acetate (PMA)-differentiated THP-1 and U937 monocyte cell lines. We previously reported that SAMHD1 restricted HIV-1 infection in TE671 rhabdomyosarcoma cells in addition to these cell lines. In this study, we compared the amounts of the full-length SAMHD1 and its deletion mutants, SAM domain containing N-terminal fragment (residues 1-119, SAMHD1n) and HD domain containing C-terminal fragment (120-626, SAMHD1c) in U937, TE671, and HeLa cells. The results showed that the full-length SAMHD1 and SAMHD1n proteins were significantly more abundant than the SAMHD1c protein in TE671 and differentiated U937 cells. The proteasome inhibitor MG132 increased the amount of the SAMHD1c and the SAMHD1c-fused GFP proteins. In contrast, the fusion of the SAMHD1n to the APOBEC3G protein inhibited Vif-induced proteasomal degradation in TE671 and in differentiated U937 cells. These results indicated that the SAMHD1 C-terminal HD domain-containing region leads the SAMHD1 to proteasomal degradation, and the SAMHD1 N-terminal SAM domain-containing region stabilizes the protein. Our study showed that the SAMHD1 protein expression is post-translationally regulated and the significance of SAM and HD domains for the full-length SAMHD1 protein stability. Further, we suggest that the SAM domain-containing N-terminal region participate in the cell-type specific restrictive function of SAMHD1 against HIV-1 infection, by protein stabilization.

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## Introduction

SAMHD1 (1-626 aa), composed of a tandemly linked-SAM domain (45-110 aa) and HD domain (164-319 aa), has recently been identified as a restriction factor against HIV-1. SAMHD1 was originally identified as a human homolog of mouse *Mg11* isolated from IFNγ-stimulated mouse dendritic cells [1,2], suggesting that SAMHD1 is an IFN-stimulated gene [3,4] though it depends on cell-type [3,5].

In parallel to the hosts obtaining restriction factors, viruses have developed mechanisms to overcome these restriction factors. Vpx, encoded by HIV-2 and simian immunodeficiency viruses (SIVs) but not by HIV-1 [6,7,8,9,10], is a potent counterpart of SAMHD1. These Vpx proteins bind to the SAMHD1 C-terminal motif (606-626 aa) and deliver the complex to the CRL4DCAF1 ubiquitin E3 ligase [11] for subsequent proteasome-dependent degradation.

HD domain contains a catalytic core for hydrolysis activity and the several reports showed that the HD domain-containing C-terminal region of SAMHD1 (120-626 aa) inhibits HIV-1 reverse transcription (RT) by its dGTP dependent deoxynucleoside triphosphate-triphosphatase (dGTP triphosphatase)

activity [12,13]. In addition, recombinant SAMHD1 protein possesses 3' to 5' exonuclease activities against DNAs and RNAs, as well as nucleic acids and viral genome binding abilities in which the SAM domain containing N-terminal region (1-118 aa) plays a crucial role [14].

Interestingly, various cell lines endogenously express the SAMHD1 [1,6,7], but SAMHD1 can inhibit HIV-1 infection only in terminally differentiated myeloid cells, such as PMA-differentiated THP-1 [6,9,15], monocyte-derived macrophages (MDMs) [13] and monocyte-derived dendritic cells (MD-DCs) [6,7,13], and resting CD4$^+$ T cells [8,16] but not in undifferentiated THP-1 and HEK293T cells [7]. Exogenously over-expressed SAMHD1 restricted the infection in PMA-differentiated U937 macrophage cells but not in undifferentiated U937 and HeLa cells [3]. Thus, there are unknown mechanism(s) contributing to the cell type-specific HIV-1 restriction by SAMHD1.

Meanwhile, the protection of innate immunity-associated proteins from ubiquitination and degradation, *i.e.,* protein stabilization, is one of the mechanisms to induce innate immune signaling. For example, Lee *et al.* [17] recently showed that the TBK1 protein, a key mediator in type I IFN expression, is stabilized by CDC37 in IFN-stimulated DNA and retrovirus sensing. Korczeniewska *et al.* [18] showed that the COP9 signalosome stabilizes the IFN regulatory factor 5 (IRF5) protein, an important role player in the induction of type I IFNs and proinflammatory cytokines. Thus, protein stabilization is a critical event in innate immunity.

To understand the involvement of SAMHD1 stabilization in the SAMHD1-induced cell-type specific HIV-1 infection restriction, we analyzed the expression levels and stability of the full-length SAMHD1 protein and its deletion mutants in U937, TE671, and HeLa cells. We describe supporting evidences for SAMHD1 is post-translationally regulated and the significance of SAM domain-containing and HD domain-containing region for the SAMHD1 protein stability.

## Materials and Methods

**Cells.** THP-1 and U937 cells were grown in RPMI medium (Wako) at 37 ℃ in a 5% CO$_2$ incubator. HeLa and TE671 cells were grown in Dulbecco's modified Eagle medium (D-MEM) (Wako). Both media were supplemented with 8% heat-inactivated fetal bovine serum (FBS) (PAA Laboratories GmbH) and 1 % penicillin-streptomycin (Sigma-Aldrich). Unless indicated, all cells were seeded at 1x10$^6$ cells per 6 cm culture-dish. At 24 hrs after transfection, U937 cells were stimulated by phorbol 12-myristate 13-ac-

etate (PMA) at various concentrations, to promote differentiation into macrophages.

## Real-time PCR

THP-1 cells were seeded in 24-well dishes (1x105/well), and total RNA samples were prepared by Trizol Reagent (Ambicon) after differentiation with 30, 50, and 500 ng/ml PMA for 48 hrs. Semi-quantitative RT-PCR was performed with a high fidelity RT-PCR kit (TAKARA). Real-time PCR was performed with M-MLV reverse transcriptase (NEB). A SYBR Green PCR kit (TOYOBO) was used to quantitate the PCR products on the ABI PRISM 7900HT real-time PCR detection system. The amounts of the SAMHD1 PCR products were normalized by those of the GAPDH PCR products. The nucleotide sequences of the primers for the SAMHD1 mRNA were 5'-AGCGATTGGTTCAAATCCAC-3' and 5'-TCGATTGTGTGAAGCTCCTG-3', and for the GAPDH mRNA were 5'-GAGTCAACGGATTTGGTCGT-3' and 5'-TTGATTTTGGAGGGATCTCG-3'.

**Plasmids.** The C-terminally HA epitope-tagged full-length SAMHD1 (1-626 aa), SAMHD1n (1-119 aa), and SAMHD1c (120-626 aa) expression plasmids were constructed by amplifying each sequence from the full-length SAMHD1 expression plasmid (OriGene Technologies), as a template. The pcDNA3.1 vector was purchased from Invitrogen.

The PCR primers for amplifying the full-length HA-tagged SAMHD1 sequence were a forward primer 5'-GTGTAGC-CATGCAGCGA-3' (SAMHD1 F) and a reverse primer, 5'-TCATGCGTAATCCGGAACATCGTACGGGTACATT-GGGTCATCTTTAAAAAGCTG-3' (SAMHD1-HA-R); for HA-tagged SAMHD1n sequence, the SAMHD1 F primer and a reverse primer, 5'-TCATGCGTAATCCGGAACATCG-TACGGGTAATTAATTACCTTCATTGTATC-3'; and for HA-tagged SAMHD1c sequence, a forward primer, 5'-GTAGCCA-TGGATCCTATCCATGGCCAC-3', and the SAMHD1-HA-R primer. Each PCR product was inserted into the pcDNA3.3-TOPO vector (Invitrogen).

An enhanced green fluorescent protein (EGFP) expression plasmid was generated in our laboratory. The EGFP stop codon was replaced by an *Eco*RI sequence in the EGFP expression plasmid (EGFP-EcoRI). To construct the C-terminally SAMHD1c-fused EGFP expression plasmid (GFP-HD), the HD region was amplified by PCR, using the SAMHD1c-HA expression plasmid as the template, with the *Eco*RI sequence-containing forward primer, 5'-TTATT-AGAATTCGATCCTATCCATCATCAC-3', and the *Xho*I

sequence-containing reverse primer, 5'-TTATTACTCGAGT-CACATTGGGTCATCTTT-3'. The PCR product was digested with the *Eco*RI and *Xho*I restriction enzymes and inserted into the EGFP-EcoRI plasmid at the *Eco*RI and *Xho*I sites.

The expression plasmid for C-terminally Myc-tagged APOBEC3G was a kind gift from Dr. Yasumasa Iwatani (National Hospital Organization Nagoya Medical Center, Nagoya, Japan) [19]. The Vif expression plasmid was obtained from Dr. Klaus Strebel (National Institutes of Health, Bethesda, MD, United States of America) [20], through Dr. Yasumasa Iwatani. The N-terminally SAMHD1n-fused APOBEC3G expression plasmid (SA3G) was generated as follows. The SAMHD1n-*Sal*I sequence was produced by PCR with the SAMHD1 F primer and the *Sal*I sequence-containing reverse primer 5'-CCCGTCGACATTAATTAC-CTTCAT-3', and cloned into the pCR 2.1 TOPO-vector (Invitrogen). The SalI-APOBEC3G-Myc plasmid was generated by introducing a *Sal*I sequence in frame at the 5' side of the start codon of the APOBEC3G-Myc expression plasmid with the *Sal*I sequence-containing forward primer, 5'-CTG-CAGAATGTCGACATGAAGCCTCACTT -3', and a reverse primer, 5'-AGGCTTCATGTCGACATTCTGCAGATATCC-3'. The SAMHD1n-SalI plasmid was digested with the *Eco*RI and *Sal*I restriction enzymes. The SalI-APOBEC3G-Myc plasmid was digested with the *Sal*I and *Hind*III restriction enzymes. The Myc-tagged APOBEC3G expression plasmid was digested with the *Eco*RI and *Hind*III restriction enzymes and used as the vector. The *Eco*RI/*Sal*I-digested SAMHD1n fragment, the *Sal*I/*Hind*III-digested APOBEC3G fragment, and the *Eco*RI/*Hind*III-digested vector fragment were ligated.

All plasmids described above are driven by the CMV promoter. The nucleotide sequences of the plasmid DNAs constructed in this study were confirmed by sequencing (Applied Biosystems).

**Transfection.** $1 \times 10^6$ cells per 6cm culture-dish was seeded, and incubated at 37 °C in a 5% CO$_2$ incubator for 24 hrs. Two $\mu$g of plasmid DNA were mixed with 10 $\mu$l of FuGENE HD transfection reagent (Promega), in 100 $\mu$l of D-MEM medium without FBS and antibiotics. The transfection mixture was added to the cell supernatant.

**SDS-PAGE and western blotting analysis.** A 10 mM MG132 (Sigma) stock solution was prepared in dimethyl sulfoxide. At 24 hrs after transfection, the cell culture medium was replaced with fresh medium, and MG132 was added to a 5 $\mu$M final concentration. Cell lysates were pre-

pared 24 hrs after with 1x sample buffer (10% glycerol, 1.5% SDS, 0.005% bromophenol blue, 50 mM Tris/HCl, pH 6.8), and equal quantities of total protein from each sample were fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

For western blot (WB) analyses, the proteins were transferred to a PVDF membrane (Millipore) in a wet blotter (Bio-Rad). The membranes were incubated with the corresponding primary antibodies overnight at 4 °C, and then incubated with HRP-conjugated anti-mouse IgG (Bio-Rad) or with protein G (Bio-Rad) for 1 hr at 4 °C. The primary antibody-bound proteins were visualized by enhanced chemiluminescence reagents (Bio-Rad). Immunoblotting images were captured using Fluor Chem Imaging System (IS-8800, Alpha Innotech). The relative intensity levels of each protein compared to $\beta$-actin were calculated with the equation, protein intensity/$\beta$-actin protein intensity by using Alpha Ease software.

**Primary antibodies.** Monoclonal antibodies against $\beta$-actin (Santa Cruz Biotechnology), HA (COVANCE), GFP (Nacalai Tesque), and Myc (Cell Signaling) were used. The anti-Vif antibody was obtained through the AIDS Research and Reference Reagent Program, NIAID, NIH, from Dr. Michael H. Malim [21,22]. Polyclonal antibody against human SAMHD1 (residues 88-337) was purchased from Proteintech (12586-1-AP).

## Results

**The SAMHD1 C-terminal HD domain-containing region is degraded in the proteasome, and the N-terminal SAM domain-containing region inhibits the degradation.** THP-1 monocyte and U937 monocyte cells express and do not express endogenous SAMHD1 protein, respectively. However, the transduction of U937 cells with SAMHD1, followed by the PMA-induced differentiation dramatically restricts the HIV-1 infection similar extent to the PMA-differentiated THP-1 cells [6,9,15,23]. Why SAMHD1 becomes functional as an anti-HIV-1 factor after the PMA-induced differentiation in monocyte cells? To address this query, Lahouassa *et al.* showed that the expression of exogenous SAMHD1 protein was elevated after the PMA-induced differentiation in U937 cells [13]. This led us to speculate that the SAMHD1 protein level may be regulated at certain stage. Thus, we compared the endogenous SAMHD1 protein expression in THP-1 cells treated or untreated with the PMA stimulation. As shown in Figure 1A, the

SAMHD1 protein level was significantly enhanced by PMA stimulation in THP-1 cells. To know if these protein expression levels reflect a quantitative difference of SAMHD1 mRNA before and after the PMA stimulation, we performed the real-time PCR by using specific primers for SAMHD1 on total RNAs extracted from THP-1 cells treated with different PMA quantity (0, 10, 50, and 500 ng/mL). To our surprise, there was no difference in SAMHD1 mRNA level before and after the stimulation, regardless of increased quantity of the PMA (Fig. 1B). There are two possible ways to regulate protein abundance: post-transcriptional control of transcripts and post-translational control of protein. However, in many cases, protein abundance is post-translationally regulated by proteasomal degradation. In fact, the HD region contains many putative ubiquitination sites, as determined with the CKSAAP_UbSite program (data not shown). Thus, it is highly speculated that endogenous SAMHD1 protein expression was post-translationally regulated in THP-1 cells.
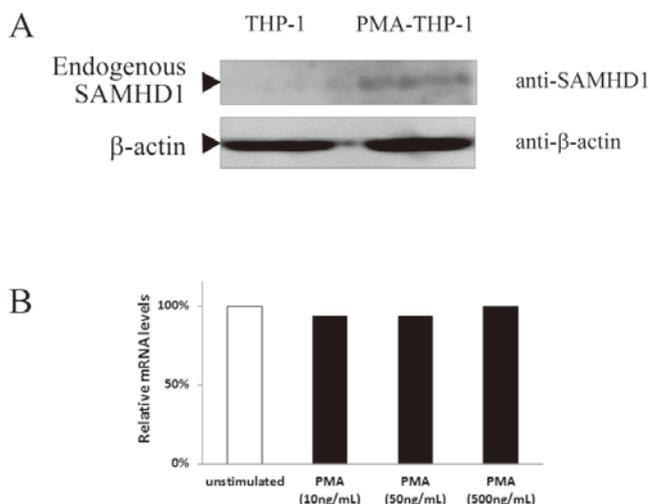


**Figure 1. The SAMHD1 protein is post-translationally-regulated.** (A) The endogenous SAMHD1 protein expression was analyzed in undifferentiated THP-1 and PMA-differentiated THP-1 cells. THP-1 cells were stimulated with 500ng/mL PMA for overnight. A WB analysis with a SAMHD1-specific antibody was performed. $\beta$-actin was detected as a loading control. (B) Real-time PCR for quantitation of SAMHD1 mRNA with specific primers were performed on THP-1 cells stimulated with different quantity of PMA. Relative quantities of SAMHD1 mRNA to GAPDH mRNA are indicated. Data are representative of similar results from two independent experiments.

We next wanted to identify which domain is responsible for the post-translational regulation of SAMHD1. We generated the C-terminally HA-tagged full-length SAMHD1 (1-626 aa), the N-terminal SAM domain-containing aa 1-119

region (SAMHD1n), and the C-terminal HD domain-containing aa 120-626 region (SAMHD1c) expression plasmids (Fig. 2A). Each plasmid was transfected into U937 cells and the cells were PMA-differentiated. As in undifferentiated THP-1 cells, none of the proteins were detected in undifferentiated U937 cells (Fig. 2B lane 1, 3, and 5). In contrary, all the proteins were expressed upon the PMA-induced differentiation, and the expression levels of the full-length SAMHD1 and SAMHD1n proteins were much higher than that of the SAMHD1c protein in PMA-differentiated U937 cells (Fig.2B, lane 2, 4, and 6), leading us to speculate that the SAMHD1n stabilizes the SAMHD1 protein in differentiated U937 cells. However, it may simply reflect the PMA stimulation-induced protein expression from CMV promoter [24]. We had previously searched for various cell lines in which SAMHD1 restricts HIV-1 infection without the PMA stimulation and, as a result, we found TE671 rhabdomyosarcoma cells moderately restricted the infection by transiently expressed SAMHD1 (manuscript in press). Of note, transiently expressed SAMHD1-mediated HIV-1 infection restriction was not observed in HeLa, H292, C33A, and NP2 (manuscript in press). Thus, in this study, we transfected each plasmid into TE671 cells and HeLa cells, the cells in which SAMHD1 does and does not restrict the HIV-1 vector infection, respectively, and the cells were treated with the proteasome inhibitor, MG132. As in PMA-differentiated U937 macrophage cells, the SAMHD1c protein was barely detected in TE671 cells and in HeLa cells, but the MG132 treatment significantly enhanced the protein abundances (Fig. 2C, lane 5 versus lane 6, and Fig. 3, lane 5 versus lane 6). These results indicated that the SAMHD1c protein is degraded by the proteasome. On the other hand, the amounts of the full-length SAMHD1 and SAMHD1n proteins were much higher than that of the SAMHD1c protein in TE671 cells (Fig.2C lane 1, 3, and 5), indicating that the SAMHD1n protects the full-length SAMHD1 protein from proteasomal degradation induced by SAMHD1c in TE671 cells. These former two protein expression levels are not significantly affected by the MG132 treatment (Fig. 2C lane 1 versus 2, and lane 3 versus 4), In contrary to that observed in TE671 cells, the amount of full-length protein was much lower than SAMHD1n protein in HeLa cells (Fig. 3 lane 1 versus 3), indicating that the SAMHD1n-mediated full-length SAMHD1 protein stabilization occurs more efficiently in TE671 cells than in HeLa cells. Moreover, the expression of full-length SAMHD1 was much higher than the SAMHD1n protein in U937 macrophage cells compare to that observed in TE671 cells (Fig. 2B lane 2 versus 4 and Fig.2C lane 1 versus 3). Thus, we speculate that the full-length SAMHD1
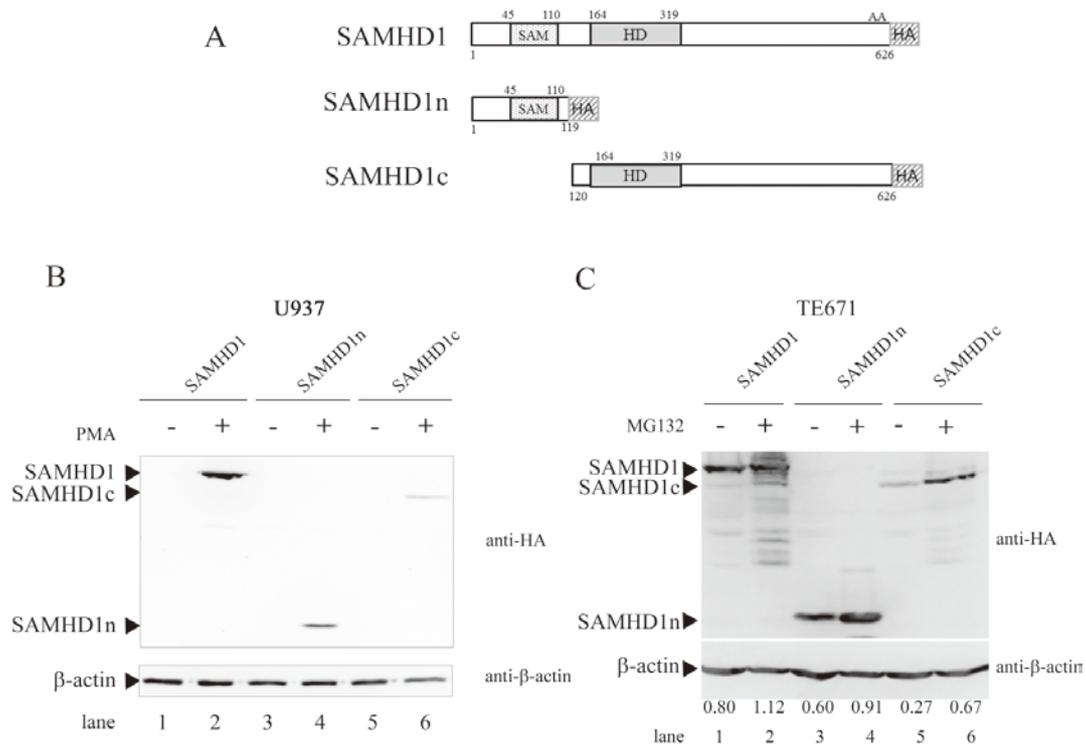
**Figure 2. The SAMHD1c protein is degraded in the proteasome, and the SAMHD1n inhibits the degradation.** (A) Schematic representations of SAMHD1 and its truncated mutants are indicated. SAMHD1 consists of the sterile-$\alpha$ motif (SAM) domain (aa 45-110) and the hydrolysis (HD) domain (aa 164-319). The SAMHD1n deletion mutant contains amino acid residues 1-119. The SAMHD1c mutant contains amino acid residues 120-626. Each plasmid encodes a C-terminally HA-tagged protein. Vectors encoding SAMHD1(1-626 aa), SAMHD1n (1-119 aa), and SAMHD1c (120-626 aa) were transfected into (B) U937 cells, followed by PMA untreatment or treatment for overnight, and into (C) TE671 cells, followed by MG132 untreatement or treatement for overnight. The samples were analyzed by WB, using anti-HA and anti-$\beta$-actin antibodies, in which $\beta$-actin was used as a loading control (B and C). Relative levels of each protein compared to $\beta$-actin are shown above the lane numbers (C). Data are representative of similar results from two independent experiments.
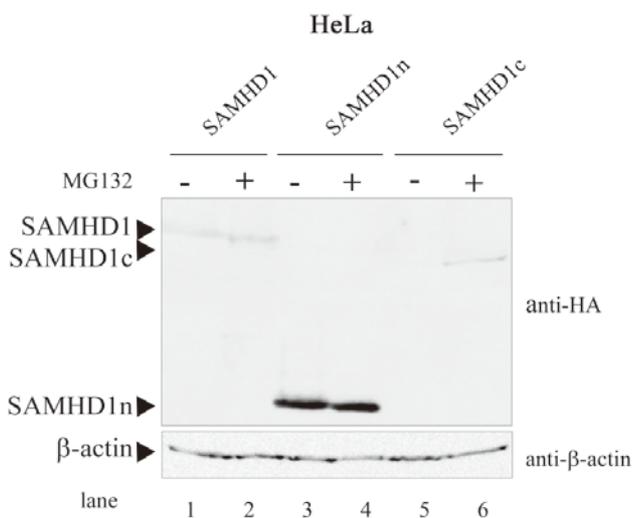


**Figure 3.** HeLa cells were untreated or treated with MG132 for overnight, followed by SDS-PAGE and WB. Each sample was analyzed with anti-HA and anti-$\beta$-actin antibodies. Data is a representative of similar results from two independent experiments.

protein stability may correlate with SAMHD1-mediated HIV-1 vector infection restriction. Overall, these results suggested that the SAMHD1n protects the SAMHD1 protein from the SAMHD1c -mediated proteasome degradation.

**The SAMHD1 C-terminal region leads to the proteasome-dependent degradation of a heterologous protein.** To assess the involvement of the SAMHD1c (120-626 aa) in the protein degradation, we constructed an expression plasmid encoding the C-terminally SAMHD1c-fused EGFP protein (GFP-HD) (Fig. 4A), and compared its expression with that of the control EGFP protein (GFP) in TE671 cells. As shown in Fig. 4B, the amount of the GFP-HD protein was increased by MG132 treatment, while the MG132 treatment did not significantly affect the GFP protein abundance. Our results clearly demonstrated that the SAMHD1c region has the ability to lead not only SAMHD1 but also a heterologous protein into proteasome-dependent degradation.
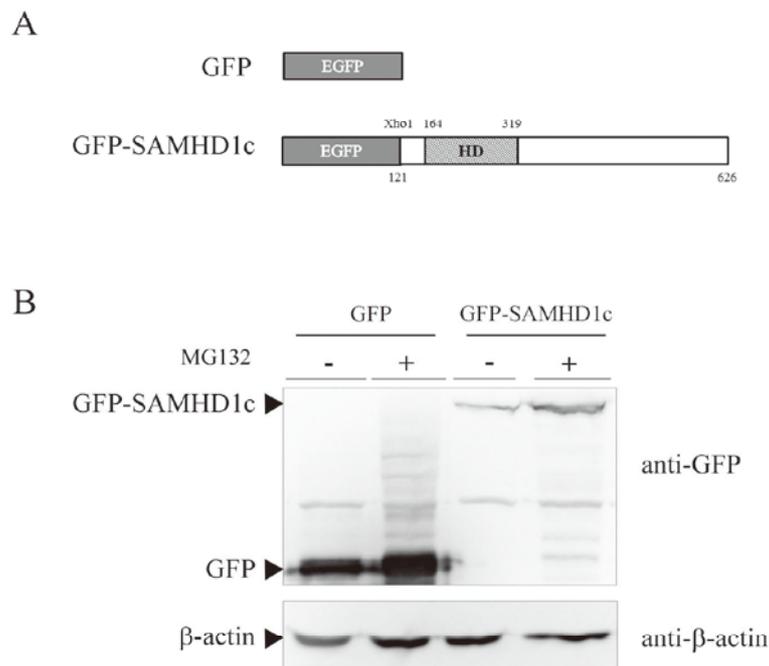
**Figure 4. The SAMHD1c leads to proteasome-dependent degradation of a heterologous protein.** (A) Schematic representations of GFP and C-terminally SAMHD1c fused GFP proteins are indicated. GFP and SAMHD1c were fused at the *Xho*I site. (B) GFP and GFP-SAMHD1c were transfected into TE671 cells. After 24 hrs of culture, the transfected cells were treated with MG132, and then further cultured for 24 hrs. Whole cell lysates were collected, fractionated by SDS-PAGE and analyzed by WB. The expression of the GFP and GFP-SAMHD1c proteins was analyzed with an anti-GFP antibody. *β*-actin was used as a loading control. Data are representative of similar results from two independent experiments.

**The SAMHD1 N-terminal region protects full-length SAMHD1 from protesome-dependent degradation.** To assess the role of the SAMHD1n in protein stabilization, we constructed an expression plasmid encoding an N-terminally SAMHD1n-fused, C-terminally Myc-tagged APOBEC3G protein (SA3G) (Fig. 5A). Human APOBEC3G (A3G) is degraded in the proteasome by the SIV or HIV-1 accessory protein, Vif [25,26]. The SA3G and A3G expression plasmids were co-transfected together with the Vif or control pcDNA3.1 expression plasmid into TE671 cells. As shown in Fig. 5B, similar levels of the SA3G and A3G proteins were detected without Vif. The Vif co-expression decreased the A3G protein expression (28-fold), while, the SA3G protein was slightly but steadily resistant to the Vif-mediated protesomal degradation (8-fold), indicating that the SAMHD1n can protect the heterologous A3G protein from proteasomal degradation in TE671 cells.

**The SAMHD1 N-terminal region is critical for SAMHD1 stabilization in differentiated U937 cells.** To know if the SAMHD1n-mediated protein stabilization is correlated to cell-type specific HIV-1 infection restriction ability of

SAMHD1, the same experiment was performed on U937 cells, followed by PMA treatment. As in TE671 cells, the addition of Vif dramatically reduced the amount of the A3G protein (70-fold), while that of the SA3G protein was hardly affected (3-fold), indicating that SA3G was highly resistant to Vif-mediated proteasomal degradation in U937 cells (Fig. 5C). Although the SAMHD1n protected the A3G protein from Vif-mediated proteasomal degradation in TE671 cells, Vif still reduced the amount of the SA3G protein significantly in this cell line. Thus, these results suggested that the ability of the SAMHD1n to confer resistance to proteasome-dependent degradation was more prominent in U937 cells than in TE671 cells.

**Trans-expression of SAM mutant protein does not affect SAMHD1 protein stability.**

In addition, we tested if the *trans*-expressed SAMHD1n can rescue protein from the degradation. SAMHD1n expression plasmid was transfected into TE671 cells, together with the full-length SAMHD1 or SAMHD1c expression plasmid. As a result, neither the SAMHD1 nor SAMHD1c protein abundance was elevated by the trans-expression of
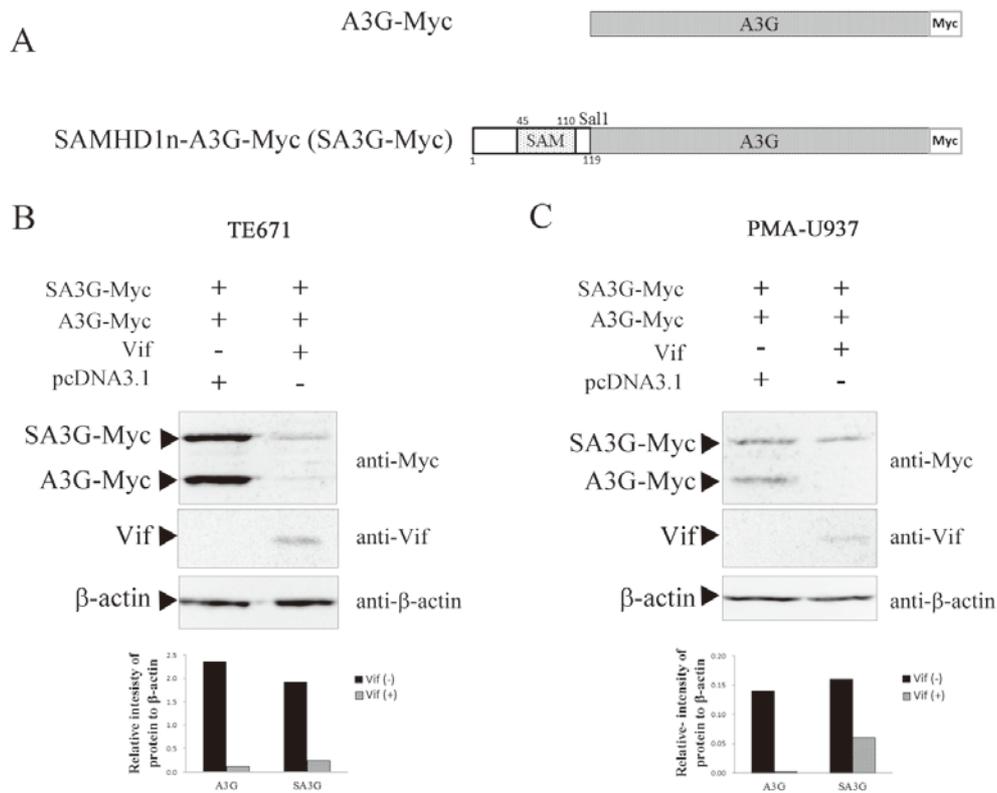
**Figure 5. The SAMHD1n can protect a heterologous protein from proteasome-dependent degradation in TE671 and PMA-differentiated U937 macrophage cells.** (A) Schematic representations of the A3G and N-terminally SAMHD1n-fused A3G (SA3G) proteins are indicated. SAMHD1n and A3G were fused at the *Sal*I site. Each plasmid encodes a C-terminally Myc-tagged protein. (B) TE671 cells and (C) U937 cells were co-transfected with A3G and SA3G, together with pcDNA3.1 or Vif. U937 cells were treated with PMA after the transfection. The expression of the A3G and SA3G proteins was analyzed by WB, using the anti-Myc antibody. *β*-actin was used as a loading control and relative levels of each protein compared to *β*-actin are shown. Data are representative of similar results from two independent experiments.

SAMHD1n protein (Fig. 6), indicating that only the *cis*-linked SAMHD1n can protect the full-length SAMHD1 from degradation.

## Discussion

Our study is the first report showing that the HD domain-containing C-terminal region of SAMHD1 is responsible for the proteasome-dependent degradation of SAMHD1 in the absence of Vpx, whereas the SAM domain-containing N-terminal region is critical for the stability of the full-length SAMHD1 protein. SAMHD1 can restrict HIV-1 vector infection in PMA-differentiated U937 macrophage and TE671 rhabdomyosarcoma cells, but not in HeLa cells. The SAMHD1n-mediated stabilization of the SAMHD1 protein occurs more efficiently in differentiated U937 cells than in TE671 cells, correlated to the degree of restriction of these cells. Interestingly, while we were preparing this manuscript, White *et al.* and Cribier *et al.* showed that the phos-
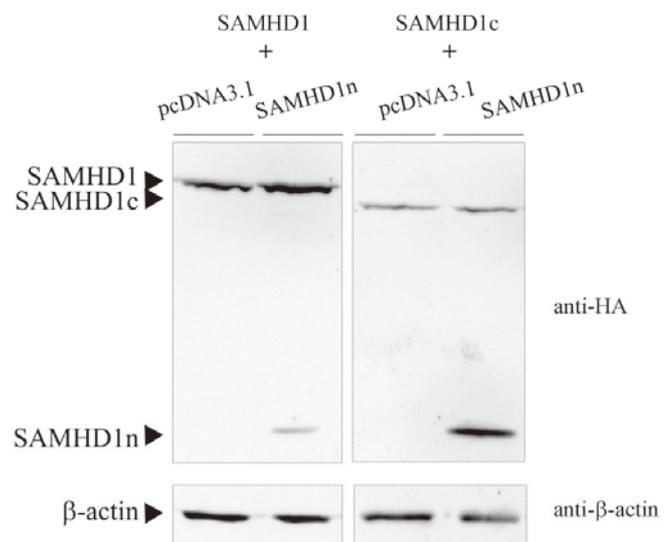


**Figure 6. Trans expression of SAMHD1n mutant protein does not affect SAMHD1 protein stability.** TE671 cells were transfected with a vector encoding the full-length SAMHD1 (left panel) or the SAMHD1c mutant (right panel) protein, together with pcDNA3.1 or the SAMHD1n mutant. Cell lysates from the transfected cells were analyzed by WB. Data are representative of similar results from two independent experiments.

phorylation of SAMHD1 determines the cell-type specific restriction of HIV-1 infection by SAMHD1 [27,28]. They proposed that the phosphorylation of SAMHD1 C-terminal at T592 aa regulates the retroviral restriction ability of SAMHD1. The close relationship between phosphorylation and ubiquitination has been well understood [29]. It is highly speculated that the ubiquitination of the SAMHD1c is also regulated by the phosphorylation of this region. Nevertheless, our study suggests that the stabilization of SAMHD1 by the SAMHD1n contributes to its restriction of HIV-1 infection.

Previous report has shown that N-terminal region-deleted SAMHD1 protein (112-626 aa) could still restrict HIV-1 vector infection in PMA-differentiated U937 cells [15], suggesting that the SAM domain containing N-terminal region is dispensable for the HIV-1 restrictive function of SAMHD1. However, it is still possible that the SAM domain participates in the infection restriction, since a SAM domain (45-110)-deleted SAMHD1 mutant protein reportedly fails to restrict HIV-1 infection in PMA-differentiated U937 cells, and its abundance is lower than that of the wild type SAMHD1 [15]. Several reports have shown that the amounts of N-terminally truncated SAMHD1 proteins are lower than that of the full-length protein [9,10,11,15,23]. In addition, recent report revealed that the SAMHD1 N-terminal region (1-118) is necessary to maximize the dGTP triphosphatase and nuclease activities, and indispensable for nucleic acid and viral genome binding activities of SAMHD1 [14]. Together, these reports suggested that the SAM domain is necessary for the SAMHD1-mediated restriction of HIV-1 infection and protein stability.

Notably, the SA3G protein was remarkably resistant to the Vif-induced degradation in differentiated U937 cells. The tertiary structure of SA3G is considered to have minor or no effect to the A3G interaction with Vif by the SAMHD1n fusion, because the amount of the SA3G protein was significantly reduced by Vif in TE671 cells. To further support the involvement of the SAM domain in the protein protection from proteasome-dependent degradation, Park *et al.* [30] reported an interesting feature about the SAM domain of the tyrosine kinase-type A2 receptor (EPHA2) protein: the group showed that the SAM domain of the EPHA2 protein plays a critical role in its stability, by modulating the proteasome-dependent process. The SAMHD1 SAM domain shares 40% similarity and 16% identity with that of

EPHA2 (Phyre2 program). The SAM domain is responsible for protein-protein or protein-RNA interactions [31]. Therefore, an interaction between the SAMHD1n and an unknown cellular factor may stabilize the SAMHD1 protein.

Vpx at its N-terminal region forms a complex with SAMHD1 C-terminal region (606-626 aa) and loads it to the CRL4$^{DCAF1}$ ubiquitin E3 ligase, followed by proteasomal degradation[11]. However, we have shown that the SAMHD1c contains a motif that leads SAMHD1 into proteasome-dependent degradation, in the absence of Vpx. The SAMHD1 protein may be stabilized by an intramolecular interaction between the SAMHD1n and SAMHD1c regions. Vpx may compete with the SAMHD1n, and induce the degradation of the full-length SAMHD1 protein. Identifying the relationship between these core domain containing regions and the Vpx is our next target of interest in parallel with disclosing the contribution of SAMHD1c phosphorylation to the full-length SAMHD1 stability.

In summary, the HD domain-containing C-terminal region leads the SAMHD1 protein to proteasomal degradation. The SAM domain-containing N-terminal region stabilizes the SAMHD1 protein efficiently in differentiated U937 cells and in TE671 cells, in which SAMHD1-mediated HIV-1 infection restrictions were observed, suggesting that the SAM-mediated stabilization of the SAMHD1 protein is important for its anti-virus activity. Further studies are required to understand the mechanism by which the SAMHD1n stabilizes the SAMHD1 protein.

## Acknowledgements

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