PIAS1 Potentiates the Anti-viral Activity of SAMHD1 through SUMOylation

Farjana Saiada

Virginia Commonwealth University

Kun Zhang

Virginia Commonwealth University

Renfeng Li (✉ rli@vcu.edu)

Virginia Commonwealth University <https://orcid.org/0000-0002-9226-4470>

Research

Keywords: SAMHD1, PIAS1, restriction factor, Epstein-Barr virus, cytomegalovirus, SUMOylation, herpesvirus, deoxynucleotide triphosphohydrolase, phosphorylation

Posted Date: March 17th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-296406/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Cell & Bioscience on July 8th, 2021. See the published version at <https://doi.org/10.1186/s13578-021-00636-y>.

PIAS1 potentiates the anti-viral activity of SAMHD1 through SUMOylation

Farjana Saiada¹, Kun Zhang¹, and Renfeng Li^{1,2,3,4}

¹Philips Institute for Oral Health Research, School of Dentistry, Virginia Commonwealth University, Richmond, Virginia 23298, USA

²Department of Microbiology and Immunology, School of Medicine, Virginia Commonwealth University, Richmond, Virginia 23298, USA

³Massey Cancer Center, Virginia Commonwealth University, Richmond, Virginia 23298, USA

⁴Corresponding author: rli@vcu.edu (RL)

Abstract

Background: Sterile alpha motif and HD domain 1 (SAMHD1) is a host restriction factor that suppresses the infection of a variety of RNA and DNA viruses, including herpesviruses. The anti-viral activity of SAMHD1 is finely tuned by post-translational modifications, including phosphorylation, acetylation and ubiquitination. Our recent studies also demonstrated that the E3 SUMO ligase PIAS1 functions as an Epstein-Barr virus (EBV) restriction factor. However, whether SAMHD1 is regulated by PIAS1 to restrict viral infection remains unknown.

Results: In this study, we showed that PIAS1 interacts with SAMHD1 and promotes its SUMOylation. We identified three lysine residues (K469, K595 and K622) located on the surface of SAMHD1 as the major SUMOylation sites. We demonstrated that phosphorylation of SAMHD1 slightly promotes its SUMOylation by PIAS1 and SUMOylated SAMHD1 can still be phosphorylated by viral protein kinases. We further demonstrated that SUMOylation-deficient SAMHD1 loses its anti-EBV activity.

Conclusion: Our study reveals that PIAS1-mediated SUMOylation of SAMHD1 enhances its anti-viral activity.

Keywords

SAMHD1; PIAS1; restriction factor; Epstein-Barr virus; cytomegalovirus; SUMOylation; herpesvirus; deoxynucleotide triphosphohydrolase; phosphorylation

Background

Restriction factors serve as the first line of defense against viral infection. One recently discovered restriction factor is the Sterile alpha motif and HD domain 1 (SAMHD1) protein, which hydrolyzes deoxyribonucleoside triphosphates (dNTPs) to reduce the cellular dNTP pool required for viral infection and propagation. In addition to limiting the infection of human immunodeficiency virus-1 (HIV-1) (1), SAMHD1 has also been shown to restrict the infection of herpesviruses consisting of Epstein-Barr virus (EBV), human/mouse cytomegalovirus (HCMV/MCMV) and human simplex virus 1 (HSV-1) (2-7), vaccinia virus (2), human T cell leukemia virus type 1 (8), hepatitis B virus (9) and human papillomavirus 16 (10).

The anti-viral activity of SAMHD1 is counterbalanced by a group of viral proteins, including HIV-2/SIV virion-associated Vpx accessory proteins and the conserved herpesvirus protein kinases. Vpx proteins bind to SAMHD1 and E3 ubiquitin ligase complex to promote SAMHD1 ubiquitination and proteasome-dependent degradation (11-13). Phosphorylation of SAMHD1 by the conserved herpesvirus protein kinases (4-7) and cellular kinases CDK1 and CDK2 diminishes its anti-viral activity (3, 14-16). Although earlier studies suggested that SAMHD1's deoxynucleotide triphosphohydrolase (dNTPase) activity is not regulated by phosphorylation, recent studies revealed that phosphorylation suppress the dNTPase activity to elevate the dNTP level for optimal viral DNA synthesis (6, 17-22). SAMHD1 phosphorylation has also been implicated in its re-localization to cytoplasm during viral infection (16). SAMHD1 is acetylated by the acetyltransferase ARD1 to enhance its dNTPase activity (23).

Protein SUMOylation is mediated by a series of enzymes that catalyze the transfer of small ubiquitin-related modifier (SUMO) to a protein substrate. These enzymes include an E1 activating enzyme complex SAE1/UBA2, an E2 conjugating enzyme UBC9 and an E3 protein ligase that controls substrate specificity (24). Protein SUMOylation plays important roles in diverse biological processes, including gene transcription, DNA replication, and viral infection (24, 25). With the advances in mass spectrometry, recent studies identified thousands of SUMOylation sites within the human proteome (26-28).

In contrast to ubiquitination, there are only a few E3 ligases for SUMOylation. The protein inhibitor of STAT (PIAS) family proteins are E3 SUMO ligases with RING domains responsible for the transfer of SUMO proteins from UBC9 to the protein substrates. The PIAS family proteins consist of PIAS1, PIASx/PIAS2, PIAS3 and PIASy/PIAS4 (29). PIAS1 restricts HSV-1 infection when ICP0 of HSV-1 is deleted (30). Our group recently identified PIAS1 as an EBV restriction factor, which is cleaved by caspases upon lytic induction (31). PIAS1 is the SUMO E3 ligase for a group of cellular and viral proteins, including p53 (32-35) and EBV immediate-early protein RTA (36).

In this study, we demonstrated that PIAS family members PIAS1, PIAS2, PIAS3 and PIAS4 interact with SAMHD1 and PIAS1 has the capability to trigger SAMHD1 SUMOylation both *in vitro* and in intact cells. We identified K469, K595 and K622 as the major SUMOylation sites on SAMHD1. We showed that PIAS1 promotes the anti-EBV activity of SAMHD1 in a SUMOylation dependent manner, which expands our understanding of SAMHD1 regulation by post-translational modifications.

Results

PIAS family proteins interact with SAMHD1

Our previous studies have demonstrated that both PIAS1 and SAMHD1 restrict EBV lytic replication (6, 31). PIAS family is consist of 4 members with different substrate specificity (**Figure 1A**). To determine whether PIAS1 and other members of the PIAS family interact with SAMHD1, we co-transfected SAMHD1 with individual PIAS construct into 293T cells and performed co-immunoprecipitation (Co-IP) experiments. We found that SAMHD1 is strongly Co-IPed by PIAS1, PIAS2, PIAS4 (**Figure 1B, lanes 1, 2 and 4**). The weaker Co-IP signal of SAMHD1 by PIAS3 is possibly due to PIAS3 protein expression level (**Figure 1B, lanes 3**). Interestingly, we also observed an extra band when SAMHD1 is Co-IPed by PIAS1 (**Figure 1B, lanes 1**), suggesting a post-translational modification on SAMHD1.

PIAS1 promotes the SUMOylation of SAMHD1

Because PIAS1 could serve as an E3 SUMO ligase, we reasoned that PIAS1 could trigger the SUMOylation of SAMHD1. To test this possibility, we performed *in vitro* SUMOylation assay with recombinant proteins (**Figure 2A**). We first performed Western Blot (WB) using anti-SUMO2/3 antibody (**Figure 2B**). We found that SAMHD1 is SUMOylated when incubated with E1, E2 and wild-type (WT) SUMO2 (**Figure 2B, lane 3**) but not with SUMO2 mutant (**Figure 2B, lane 2**). We observed that PIAS1 further promotes SAMHD1 poly-SUMOylation with WT

SUMO2 but not SUMO2 mutant (**Figure 2B, lane 4 vs 2**). As a positive control, we also found that PIAS1 triggers the SUMOylation of p53 with WT SUMO2 but not SUMO2 mutant (**Figure 2B, lane 6 vs 5**). To further confirm these results, we used anti-SAMHD1 antibody for WB and found that PIAS1 indeed promotes the SUMOylation of both SAMHD1 and p53 (**Figure 2C, lanes 4 and 6**).

PIAS1 synergizes with SAMHD1 to restrict EBV replication

To determine the regions within PIAS1 responsible for binding to SAMHD1, we co-transfected SAMHD1 with our previously created PIAS1 truncation mutants into 293T cells (**Figure 3A and 3B**). We found that all the PIAS1 truncation mutants (aa 1-433, 101-433, 1-415 and 409-651) all bind to SAMHD1 but only full-length PIAS1 can trigger SAMHD1 modification (**Figure 3B, lanes 1-5**). PIAS1 (aa 1-205) showed less binding to SAMHD1 possibly due to the reduced expression level (**Figure 3B, lane 6**).

To demonstrate whether PIAS1 and SAMHD1 association plays a role in EBV lytic replication, we transfected 293 (EBV+) cells with vectors expressing ZTA (a trigger for EBV lytic reactivation), SAMHD1 and different PIAS1 truncation mutants. As expected, transfection of SAMHD1 only reduced EBV lytic replication (**Figure 3C, lane 2 vs 3**). Interestingly, we found that co-transfection of WT PIAS1 and SAMHD1 greatly suppresses EBV DNA replication (**Figure 3C, lane 3 vs 4**). However, PIAS1 truncation mutants or E3 ligase deficient mutant (C351S) failed to synergize with SAMHD1 in blocking viral replication (**Figure 3C, lanes 4 vs 5-10**). Together, these results suggested that PIAS1-mediated SAMHD1 SUMOylation plays a role in limiting EBV replication.

PIAS1 SUMOylates SAMHD1 at K469, K595, and K622

To identify the SUMOylation sites on SAMHD1, we searched the mass spectrometry database on protein SUMOylation and found that three sites (K469, K595, K622) within SAMHD1 protein are SUMOylated in HeLa cells (26-28). These sites are located within the typical KxE/D motif that is required for SUMOylation (**Figure 4A**).

To determine whether these are the SAMHD1 SUMOylation sites, we mutated these sites individually and in combination to generate four SAMHD1 mutants, namely K469R, K595R, K622R and RRR (K469R/K595R/K622R). We then purified these proteins and performed *in vitro* SUMOylation experiments. We found that K469R and K622R, and, to a lesser extent, K595R, affect the SUMOylation pattern (**Figure 4B, lanes 1-6**). The RRR mutant lost all the major SUMOylation bands detected by anti-SAMHD1 antibody (**Figure 4B, lanes 7 and 8**). Anti-SUMO2/3 blot also showed that the mutation of three sites greatly reduces poly-SUMOylation compared to single mutations, suggesting these three sites are the major SUMOylation site on SAMHD1 mediated by PIAS1 (**Figure 4C, lanes 2, 4, 6 vs 8**).

Based on the tetrameric structure of SAMHD1 obtained by X-ray crystallography (37), we found that both K469 and K595 are located on the surface of the protein (**Figure 4D**). Because K622 is located at the very C-terminal of SAMHD1 and is not part of the solved structure, we used I-TASSER algorithm to predict the full-length SAMHD1 structure and found that K622 is also located on the surface of the protein (**Figure 4E**). We reasoned that the surface localization of these lysine residues is favorable for SUMOylation.

Phosphorylation enhances SAMHD1 SUMOylation and SUMOylated SAMHD1 can be phosphorylated by viral protein kinases

SAMHD1 can be phosphorylated by the conserved herpesvirus protein kinases and cellular kinases CDK1 and CDK2 on T592 (6). To determine whether SAMHD1 phosphorylation affects its SUMOylation, we generated phosphorylated SAMHD1 using EBV protein kinase BGLF4 (**Figure 5A**). The phosphorylated and non-phosphorylated SAMHD1 were then subjected to *in vitro* SUMOylation reactions. We found that phospho-SAMHD1 is still SUMOylated by PIAS1 and its SUMOylation level is slightly higher than that of non-phospho-SAMHD1 (**Figure 5B**).

To determine whether SAMHD1 SUMOylation affects its phosphorylation, we generated SUMOylated SAMHD1 using E1, E2, SUMO2 and PIAS1 (**Figure 5C**). The SUMOylated and non-SUMOylated SAMHD1 were then subjected to *in vitro* phosphorylation reactions. We found that SUMOylated SAMHD1 is still phosphorylated by the conserved herpesvirus protein kinases, including EBV BGLF4 and HCMV UL97 (**Figure 5D-5E**).

These results together suggested that phosphorylation promotes SAMHD1 SUMOylation while SUMOylation does not block SAMHD1 phosphorylation by viral protein kinases.

SUMOylation promotes the anti-viral activity of SAMHD1

To determine the function of SAMHD1 SUMOylation in EBV replication, we transfected 293 (EBV+) cells with vectors expressing PIAS1, WT and SUMOylation-deficient SAMHD1 mutants. We found that PIAS1 synergizes with SAMHD1 to restrict EBV replication (**Figure 6A**, lane 4 vs 3), while K469R, K595R, K622R mutants co-transfected with PIAS1 display higher viral replication compared to those with WT SAMHD1 (lanes 5, 6, 7 vs 4).

To further confirm these results in EBV-naturally infected cells, we used Akata EBV (+) cells with SAMHD1 depleted by CRISPR/Cas9 genomic editing (6). We reconstituted the cells with WT and SUMOylation-deficient SAMHD1 (RRR, K469R/K595R/K622R). We then induced EBV replication with anti-IgG mediated cross-linking of B cell receptor. We found that EBV replication is higher in cells expressing SUMOylation-deficient SAMHD1 compared with cells expressing WT SAMHD1 (**Figure 6B**).

Together, our results suggested that PIAS1-mediated SUMOylation promotes the anti-EBV activity of SAMHD1.

Discussion

Post-translational modifications have been implicated in regulating the diverse aspects of protein function. The advancement in mass spectrometry field has greatly contributed to the discovery of many post-translational modifications, including phosphorylation, ubiquitination, acetylation and SUMOylation (26-28, 38-40). Phosphorylation, acetylation, and ubiquitination all have been reported in the regulation of SAMHD1's stability and/or anti-viral activity (6, 15, 17, 19, 21, 23, 41-43). In this study, we demonstrated that SAMHD1 is SUMOylated and its anti-viral activity is enhanced by PIAS1-mediated SUMOylation.

As a cellular dNTPase, SAMHD1 restricts a wide range of RNA and DNA viruses, including HSV and EBV (1-3, 9). The E3 SUMO ligase PIAS1 also restricts the replication of EBV and ICP0-null HSV-1 (30, 31, 44). Since both PIAS1 and SAMHD1 predominantly localize in the nucleolus, we reasoned that these two proteins might coordinate with each other in restricting viral infection.

Indeed, we showed that PIAS1 and other PIAS family proteins interact with SAMHD1 with PIAS1 capable of promoting SAMHD1 SUMOylation (**Figures 1 and 2**). We mapped the binding regions between SAMHD1 and PIAS1 and discovered that only full-length PIAS1 has the capability to promote SAMHD1 SUMOylation. We demonstrated that PIAS1 synergizes with SAMHD1 to restrict EBV DNA replication while PIAS1 truncation or SUMO ligase-deficient mutants failed to do so (**Figure 3**), indicating that PIAS1-mediated SUMOylation enhances the anti-viral activity of SAMHD1.

To identify the SUMOylation sites, we searched the posttranslational modifications database. Using *in vitro* SUMOylation assays, we discovered three major SUMOylation sites located on the surface of SAMHD1 (**Figure 4**). The mutation of these three sites did not completely block SAMHD1 SUMOylation, implying the existence of other minor SUMOylation sites.

Because phosphorylation on T592 was reported to antagonize SAMHD1's anti-viral activity (6, 15, 17, 19, 21, 41), we also tested whether SUMOylation and phosphorylation could affect each other. We found that phosphorylation of SAMHD1 could slightly promote its SUMOylation mediated by PIAS1, suggesting that SUMOylation could have an additional layer of regulation (**Figure 5**). Furthermore, when SAMHD1 is pre-SUMOylated, we found that it can still be phosphorylated by EBV BGLF4 and HCMV UL97. We also observed that in reaction mixture containing non-SUMOylated SAMHD1 and SUMOylated SAMHD1, the phosphorylation of non-SUMOylated SAMHD1 is reduced (**Figure 5**). Because BGLF4 is a SUMO binding protein (25), it may prefer to bind to SUMOylated SAMHD1 when both SUMOylated and non-SUMOylated proteins exist in the same reaction mixture or cell.

To determine whether SAMHD1 SUMOylation plays any role in EBV replication, we created a series of SUMO-deficient SAMHD1 mutants. We demonstrated that while WT SAMHD1 processes the strong anti-viral activity, the K595R and, to a lesser extent, K469R, and K622R mutants have a reduced anti-viral activity (**Figure 6**). The reconstitution of SAMHD1-knockout cells with WT and K469R/K595R/K622R mutant SAMHD1 further demonstrated that SUMOylated SAMHD1 has higher anti-EBV activity.

According to a recent preprint, SAMHD1 was also shown to be SUMOylated on these three sites and SUMOylation also promote SAMHD1's anti-HIV activity (45). The exact role of SUMOylation in SAMHD1's anti-viral activity is not clear. Because the SUMOylation-deficient mutations do not affect the dNTPase activity of SAMHD1 (45), it is possible that SUMOylation may change the sub-cellular localization of SAMHD1 or alter SAMHD1's other functions. For examples, SUMOylated SAMHD1 may be enriched in viral chromatin/genome to reduce the local dNTP pool required for viral DNA replication. SUMOylated SAMHD1 may have additional function in DNA damage response and immune response critical for blocking viral

replication (46, 47). SUMOylation could also block SAMHD1 ubiquitination and its subsequent degradation (43). These possibilities will be examined in the future.

Conclusion

In summary, our data suggest that SAMHD1 is SUMOylated on multiple sites by PIAS1 and PIAS1-mediated SUMOylation promotes SAMHD1's anti-viral activity. Furthermore, phosphorylation and SUMOylation are not mutually exclusive, suggesting that SUMOylation represents an additional layer of regulation for SAMHD1. These studies lay a molecular foundation to further explore the regulation of SAMHD1 by post-translational modifications.

Material and Methods

Table 1. Key reagents and resources.

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Antibodies and reagents		
Anti-phospho-SAMHD1-T592	Cell Signaling Tech	Cat# 15038
Anti-SAMHD1	Cell Signaling Tech	Cat# 12361
Anti-SAMHD1	Bethyl	Cat# A311-354
Anti-HA	Roche	Cat# 11-867-431-001
Anti-HA-HRP	Cell Signaling Tech	Cat# 14031
SUMO2 Conjugation Kit	UBPBio	Cat# J3120
SUMOLink SUMO2/3 Kit	Active motif	Cat# 40220 (discontinued)
Anti-V5 magnetic beads	MBL	Cat# M167-11
Anti-SUMO2/3	MBL	Cat# M114-3
Anti-SUMO2/3	Active motif	Cat# 101898
Anti-V5-HRP	Thermo Fisher	Cat# R961-25
Anti-V5	Thermo Fisher	Cat# R960-25
Anti-p53	Active motif	Cat# 100853
Mouse anti- β -actin antibody	MP Biomedicals	Cat# 691001
Anti-human IgG (for IgG cross-linking)	MP Biomedicals	Cat# 55087
Anti-ZTA(BZ1)	Santa Cruz	Cat# sc-53904
Halo-tag protein purification kit	VWR/Promega	Cat# PAG6790
Glutathione Sepharose 4B	GE Healthcare	Cat# 17-0756-01
Factor Xa	NEB	Cat# P8010
Glutaraldehyde solution	Sigma	Cat# G7776
Constructs		
pLX304-SAMHD1 (with PAM mutated)	Li Lab (6)	pKZ175
pLX304-SAMHD1-K469R/K595R/K622R (with PAM mutated)	This study	pSF029
pLX304	Addgene	Plasmid# 25890
lentiCRISPR v2 vector	Addgene	plasmid# 52961
pMD2.G	Addgene	plasmid# 12259
psPAX2	Addgene	plasmid#12260
pSG5-ZTA	Hayward Lab Collection	NA
Halo-HA-BGLF4	Hayward Lab Collection	pGL772
Halo-HA-UL97	Hayward Lab Collection	pGL798
Halo-V5-PIAS1	Li Lab (31)	pKZ28
pCMV-XL4-SAMHD1	Origene	Cat# SC114650
pCMV-XL4-SAMHD1-K469R	This study	pSF004
pCMV-XL4-SAMHD1-K595R	This study	pSF005
pCMV-XL4-SAMHD1-K622R	This study	pSF006
pGEX-5x-2-SAMHD1	Li lab (6)	pKZ49

pGEX-5x-2-SAMHD1-K469R	This study	pSF017
pGEX-5x-2-SAMHD1-K595R	This study	pSF018
pGEX-5x-2-SAMHD1-K622R	This study	pSF019
pGEX-5x-2-SAMHD1-K469R/K595R/K622R	This study	pSF028
Cell lines		
Akata (EBV+)	Hayward Lab Collection	NA
Akata-4E3 (EBV-)	Hayward Lab Collection	NA
Akata-BX1 (EBV+)	(48)	NA
Hela-(EBV+)	(49)	NA
Akata (EBV+)-SAMHD1-sg1	Li Lab (6)	NA
Akata (EBV+)-SAMHD1-sg1-pLX-SAMHD1	This study	NA
Akata (EBV+)-SAMHD1-sg1-pLX-SAMHD1-K469R/K595R/K622R	This study	NA
Akata (EBV+)-SAMHD1-sg1-pLX-Vector	Li lab (6)	NA
293T cells	Hayward Lab Collection	NA
HEK 293 (EBV+)	(50)	NA

Cell Lines and Cultures

All Akata (EBV+)-derived cells were grown in RPMI 1640 media supplemented with 10% FBS (Cat# 26140079, Thermo Fisher Scientific) in 5% CO₂ at 37 °C (25, 31, 38, 51, 52). HEK293 (EBV+) cells carrying B95.8 EBV genome (50, 53) and 293T cells were grown in DMEM media supplemented with 10% FBS in 5% CO₂ at 37 °C.

Plasmids Construction

Halo-BGLF4 (WT), pGEX-5x-2-SAMHD1, and Halo-UL97 (WT) were described previously (6). Halo-V5-PIAS1, V5-PIAS1 (full length, aa 1-205, aa 1-415, aa 1-433, aa 409-651, aa 101-433, C351S) plasmids were previously described (31). The pCMV-XL4-SAMHD1 and pGEX-5x-2-SAMHD1 were used as template to create K469R, K595R and K622R mutants using the QuikChange II site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions. Similarly, CRISPR-resistant pLX304-SAMHD1 (nucleotide G321A, silent mutation) (6) was used as a template to create pLX304-SAMHD1-K469R/K595R/K622R using QuikChange II site-Directed Mutagenesis Kit step by step. Primers sequences used are: pSF001 (SAMHD1 K469R-F) 5'-catagtcctcccttctaattttattgtcctgttgctgc-3'; pSF002 (SAMHD1 K469R-R) 5'-gcagccaacaggacaaataaagattagaagggaggactatg-3'; pSF003 (SAMHD1 K595R-F) 5'-ccccactcataacacctcaaagaaggaatggaacga-3'; pSF004 (SAMHD1 K595R-R) 5'-tcgtccattcctttctttgaggtgttatgagtgagg-3'; pSF005 (SAMHD1 K622R-F) 5'-cattgggtcatctctaaaaagctggactctgcttttg-3'; pSF006 (SAMHD1 K622R-R) 5'-ccaaaagcagagtccagcttttagatgaccaatg-3'.

Lentiviral Transduction of SAMHD1

To prepare lentiviruses, 293T cells were transfected with lentiviral vector pLX304 containing the gene of WT SAMHD1, or RRR (K469R/K595R/K622R) mutant and the help vectors (pMD2.G and psPAX2; gifts from Didier Trono; Addgene plasmid #s 12259 and 12260) using

Lipofectamine 2000 reagent. The supernatants were collected 48 hrs after transfection and used for infection of SAMHD1-depleted (sg1) Akata (EBV+) cells. Infected cells were selected in medium containing 2 µg/mL puromycin and 10 µg/mL blasticidin. Expression of SAMHD1 was confirmed by WB analysis.

Cell Lysis and Immunoblotting

Cells were harvested and lysed in 2x SDS-PAGE sample buffer and boiled for 5 minutes. The samples were separated on 4-20% TGX gels (Cat# 4561096, Biorad), transferred onto PVDF membranes, and probed with primary and horseradish peroxidase-conjugated secondary antibodies.

Protein Expression and Purification

Halo-tagged PIAS1, BGLF4 and UL97 proteins were expressed and purified as previously described (6, 31, 38). Briefly, Halo-tagged plasmids were transfected into 293T cells. Three T75 flasks of transfected cells were harvested 48 hrs post-transfection at 100% confluence and lysed with 2 ml HaloTag Protein Purification Buffer (50 mM HEPES pH7.5, 150 mM NaCl, 1mM DTT, 1mM EDTA and 0.005% NP40/IGEPAL CA-630) with Protease Inhibitor Cocktail. Halo-proteins were enriched using the Halo-tag resin and purified proteins were eluted from the resin by washing 3 times with 0.5 ml HaloTag Protein Purification Buffer containing 3 µl Halo-TEV protease. The pGEX-5x-2-SAMHD1 and pGEX-5x-2-SAMHD1 mutants (K469R, K595R, K622R) proteins were purified from *E. coli*, and as described previously (6).

Immunoprecipitation Assay

293T cells (50-60% confluence) were transfected with indicated plasmids using Lipofectamine 2000. The cells were harvested at 48 hrs post-transfection and lysed in RIPA lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP40, 1% deoxycholate, 0.1% SDS and 1 mM EDTA) containing protease inhibitors and phosphatase cocktail I and II. The immunoprecipitation was carried out as previously described (31).

***In Vitro* SUMOylation Assay**

SUMO2 Conjugation Kit and SUMOlink SUMO2/3 kit were used to perform *in vitro* SUMOylation assay. The assay was carried out in a buffer containing 40 mM Tris pH 7.1, 40 mM NaCl, 1 mM β-ME, 5 mM MgCl₂. Each protein SUMOylation reaction contained 100 nM SAE1/SAE2 (E1), 2 µM 6xHis-Ube2I/UBC9 (E2), 50 µM SUMO2, 4 mM ATP. PIAS1 was added as E3 ligase. The reaction mixtures were incubated at 37 °C for 3 h and SAMHD1 SUMOylation was analyzed by WB.

***In Vitro* Kinase Assay**

In vitro kinase assay was performed as described previously (6). Briefly, each non-SUMOylated or pre-SUMOylated SAMHD1 was incubated in 40 µl Kinase Buffer containing 0.75 (v/v) magnesium-ATP cocktail buffer (Cat# 20-113; Upstate) and 6 µl of EBV BGLF4 and HCMV UL97 for 30 min at 30°C. Finally, reaction mixtures were separated by gel electrophoresis and SAMHD1 and phospho-SAMHD1 proteins were detected by WB.

To generate phospho-SAMHD1 for *in vitro* SUMOylation assay, SAMHD1 was phosphorylated by EBV BGLF4 *in vitro* for 6 hours at 4°C as described previously (6, 17). The buffer was

included as for non-phospho-SAMHD1. The phosphorylation of SAMHD1 on T592 was confirmed by WB (6, 17).

Lytic Induction and Cell Treatment

To induce the EBV lytic cycle, the in Akata (EBV+) cells were treated with IgG (1:200, Cat# 55087, MP Biomedicals) for 0 and 48 hrs. For lytic induction of EBV in HEK293 (EBV+) cells, the cells were transfected with EBV ZTA plus other plasmids as appropriate using Lipofectamine 2000 reagent for 48 hrs.

EBV DNA Detection

To measure cell associated viral DNA, total genomic DNA was extracted using the Genomic DNA Purification Kit (Cat# A1120, Promega). The relative viral genome copy numbers were determined by quantitative polymerase chain reaction (qPCR) using primers to *BALF5* gene normalized by *β-actin* as we described previously (6).

Structure Analysis for SAMHD1

Full length SAMHD1 sequence was uploaded to the I-TASSER Protein Structure and Function Prediction tool (<https://zhanglab.ccmb.med.umich.edu/I-TASSER/>) (54, 55) (Roy et al., 2010; Yang et al., 2015; Zhang, 2008) using default settings. Molecular graphics and analyses of tetrameric and monomeric SAMHD1 were performed with the UCSF Chimera package (56) (<http://www.rbvi.ucsf.edu/chimera>). Chimera is developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIGMS P41-GM103311).

Quantification and Statistical Analysis

Statistical analyses employed a two-tailed Student's t test. A p value of ≤ 0.05 was considered statistically significant. Values are given as the mean of three biological replicate experiments. Error bars represent the standard error of the mean or standard deviation from two or three biological replicates.

Abbreviations

EBV: Epstein-Barr virus

HCMV: Human cytomegalovirus

HSV-1: Human simplex virus 1

SAMHD1: Sterile alpha motif and HD domain 1

dNTPs: Deoxyribonucleoside triphosphates

dNTPase: Deoxynucleotide triphosphohydrolase

PIAS1: Protein inhibitor of activated STAT1

WT: Wild-type

WB: Western Blot

Co-IP: Co-immunoprecipitation

qPCR: quantitative polymerase chain reaction

HIV-1: Human immunodeficiency virus-1

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article. All of the data and material in this study are available when requested.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was in part supported by grants from the National Institute of Allergy and Infectious Diseases (AI104828 and AI141410; <https://grants.nih.gov/grants/oer.htm>) to RL. The work was also supported by Institutional Research Grant (IRG-14-192-40) and Research Scholar Grant (134703-RSG-20-054-01-MPC) from the American Cancer Society to RL. RL received support from the VCU Philips Institute for Oral Health Research, the VCU NCI Designated Massey Cancer Center (NIH P30 CA016059) (<https://grants.nih.gov/grants/oer.htm>), and the VCU Presidential Quest for Distinction Award. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions

Conceptualization, R.L. and F.S.; Methodology, F.S., K.Z. and R.L.; Investigation, F.S., K.Z. and R.L.; Writing – Original Draft, R.L.; Writing – Review & Editing, F.S., K.Z. and R.L.; Funding Acquisition, R.L.; Visualization, R.L. and F.S.; Supervision, R.L.

Acknowledgements

We thank S. Diane Hayward (Johns Hopkins) for providing reagent and cells lines. We thank Feng Zhang (MIT/Broad) for sharing the lentiCRISPR v2 plasmid (Addgene plasmid # 52961). We thank Didier Trono (EPFL) for providing the pMD2.G and psPAX2 plasmid (Addgene plasmid #s 12259 and 12260). We thank Henri-Jacques Delecluse (German Cancer Research Centre) and Ayman El-Guindy (Yale University) for providing HEK293 cells carrying B95.8 EBV genomes.

Figure Legends

Figure 1. PIAS family proteins interact with SAMHD1

(A) Schematic representation of V5-Tagged PIAS family proteins. SAP (SAF-A/B, Acinus, and PIAS): DNA and protein binding domain; PINIT: nuclear localization motif; RING finger: E3 ligase domain for protein SUMOylation; SIM: SUMO interacting motif; S/T Rich: variable Ser/Thr rich region.

(B) PIAS1, PIAS2, and PIAS4 interact with SAMHD1. WB analysis showing Co-IP of SAMHD1 with PIAS proteins using 293T cells transfected with SAMHD1 and individual PIAS proteins as indicated. Input, 2% whole-cell lysate used for IP. Arrow denotes modified SAMHD1 in the PIAS1 and SAMHD1 co-transfection group.

Figure 2. PIAS1 promotes the SUMOylation of SAMHD1

(A) Schematic representation of protein SUMOylation process. The process involves E1 (PDB# 1Y8Q), E2 (PDB# 1A3S), SUMO2 (PDB# 2N1W), PIAS1 [modelled structure from I-TASSER, see reference (31)] and protein substrate. Mono- and poly-SUMOylation were labeled as indicated.

(B and C) PIAS1 promotes SAMHD1 SUMOylation. *In vitro* SUMOylation assay was performed with the combination of E1, E2, SUMO2, SUMO2 mutant, PIAS1, and substrates (SAMHD1 and p53) as indicated. The reaction was terminated with SDS sample loading buffer and WB was performed using anti-SUMO2/3 (B) or anti-SAMHD1 and anti-P53 (C) antibodies. Brackets denote SUMOylated SAMHD1 and p53. Arrows denote mono-SUMOylated SAMHD1 and p53.

Figure 3. PIAS1 synergizes with SAMHD1 to restrict EBV replication
(A) The schematic representation of full length PIAS1 (1–651) or PIAS1 truncation mutants.
(B) The central and C-terminal part of PIAS1 interacts with SAMHD1. 293T cells were co-transfected with SAMHD1 and V5-tagged full length PIAS1 (aa 1–651) or PIAS1 truncation mutants as indicated. WB analyses showing that SAMHD1 is Co-IPed with the central and C-terminal part of PIAS1. Co-IP, co-immunoprecipitation; β-actin blot was included as loading controls.
(C) Full-length PIAS1 synergizes with SAMHD1 in restricting EBV replication. HEK293 (EBV+) cells were co-transfected with plasmid DNA encoding ZTA (lytic trigger), SAMHD1, and full-length (WT), truncated PIAS1 (1-205, 1–415, 1–433 and 409–651) or E3 ligase-deficient (C351S) mutant as indicated. The relative EBV copy numbers were measured using the qPCR as described in the method. The expression levels of SAMHD1 and PIAS1 were monitored by WB. β-actin blot was included as loading controls. Results from three biological replicates are presented. Error bars indicate the standard deviation. * p < 0.05, ** p < 0.01.

Figure 4. PIAS1 SUMOylates SAMHD1 at K469, K595, and K622

(A) The schematic representation of SAMHD1 protein (1–626). The three SUMOylation consensus motifs were labeled as indicated. NLS: nuclear localization signal; SAM: sterile alpha motif; HD domain: histidine and aspartic acid domain; V; vpx interacting motif; T592: phosphorylation site mediated by cellular CDK1/2 and the conserved herpesvirus protein kinases. (B and C) *In vitro* SUMOylation assay was performed with the combination of E1, E2, SUMO2, PIAS1, and individual SAMHD1 mutant [K469R, K595R, K622R, or RRR(K469R/K595R/K622R)] as indicated. The reaction was terminated with SDS sample loading buffer and WB was performed using anti-SUMO2/3 (B) or anti-SAMHD1 (C) antibodies. Arrows denote mono-SUMOylated SAMHD1 and bracket denotes SUMOylated SAMHD1 and p53. (D) The localization of K469 and K595 in SAMHD1 tetramer. The SAMHD1 tetramer structure (PDB# 4TNZ) was used to locate K469 and K595 using Chimera software. (E) The localization of K469, K595 and K622 in full-length SAMHD1 monomer. The SAMHD1 structure was predicted using I-TASSER algorithm and localization of K469, K595 and K622 was labeled using Chimera software.

Figure 5. Phosphorylation enhances SAMHD1 SUMOylation and SUMOylated SAMHD1 can be phosphorylated by viral protein kinases

(A) Schematic representation of SAMHD1 phosphorylation and subsequent SUMOylation analysis. Step 1: Recombinant SAMHD1 was phosphorylated by EBV protein kinase BGLF4 *in vitro*. Non-phosphorylated SAMHD1 was similarly processed by incubating with Buffer control. Step 2: The phosphorylated and non-phosphorylated SAMHD1 were used to perform *in vitro* SUMOylation reaction using E1, E2, SUMO2 and PIAS1 or buffer control. Step 3: The reaction was terminated by adding 2x SDS sample loading buffer and the samples were analyzed by WB. (B) Phosphorylated and non-phosphorylated SAMHD1 generated in (A) Step 1 were incubated with buffer control or with SUMOylation machinery. The SUMOylation and phosphorylation of

SAMHD1 were analyzed by WB using anti-SAMHD1 and anti-p-SAMHD1 (T592) antibodies as indicated. Arrows denote SUMOylated SAMHD1.

(C) Schematic representation of SAMHD1 SUMOylation and subsequent phosphorylation analysis. Step 1: Recombinant SAMHD1 was SUMOylated by E1, E2, SUMO2 and PIAS1 *in vitro*. Non-SUMOylated SAMHD1 was similarly processed by incubating with buffer control. Step 2: The SUMOylated and non-SUMOylated SAMHD1 were subjected to *in vitro* kinase reaction using viral protein kinases (EBV BGLF4 or HCMV UL97).

(D) WB analysis showing that SUMOylated SAMHD1 was generated from (C) Step 1. Arrows and bracket denote mono- and poly-SUMOylated SAMHD1, respectively.

(E) SUMOylated and non-SUMOylated SAMHD1 generated in (C) Step 1 were incubated with buffer control or with EBV BGLF4 and HCMV UL97 in step 2. The phosphorylation status was analyzed by WB using anti-p-SAMHD1 (T592) antibodies. Arrows denote SUMOylated SAMHD1.

Figure 6. SUMOylation-deficient SAMHD1 impairs its anti-viral activity.

(A) SUMOylation-deficient SAMHD1 impairs its anti-EBV activity. HEK293 (EBV+) cells were co-transfected with plasmid DNA encoding ZTA (lytic trigger), WT PIAS1, and WT or mutant SAMHD1 as indicated. The relative EBV copy numbers were measured using the qPCR as described in the method. The expression levels of ZTA, SAMHD1 and PIAS1 were monitored by WB. β -actin blot was included as loading controls. Results from three biological replicates are presented. Error bars indicate the standard deviation. * $p < 0.05$, ** $p < 0.01$.

(B) Akata (EBV+)-SAMHD1-sg1 cells (endogenous SAMHD1 is depleted by CRISPR/Cas9) were used to create cell lines using vector control (pLX304), WT SAMHD1 (pLX-304-V5-SAMHD1), and SUMOylation-deficient SAMHD1 [pLX-304-V5-SAMHD1-(RRR)]. EBV lytic cycle was induced by anti-IgG-mediated B cell receptor cross-linking. The relative EBV copy numbers were measured using the qPCR as described in the method. The expression levels of SAMHD1 were monitored by WB using anti-V5 antibody. β -actin blot was included as loading controls. Results from three biological replicates are presented. Error bars indicate the standard deviation. * $p < 0.05$, ** $p < 0.01$.

References

1. Laguette N, Sobhian B, Casartelli N, Ringeard M, Chable-Bessia C, Segéral E, et al. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature*. 2011;474(7353):654-7.
2. Hollenbaugh JA, Gee P, Baker J, Daly MB, Amie SM, Tate J, et al. Host factor SAMHD1 restricts DNA viruses in non-dividing myeloid cells. *PLoS pathogens*. 2013;9(6):e1003481.
3. Kim ET, White TE, Brandariz-Nunez A, Diaz-Griffero F, Weitzman MD. SAMHD1 restricts herpes simplex virus 1 in macrophages by limiting DNA replication. *Journal of virology*. 2013;87(23):12949-56.
4. Deutschmann J, Schneider A, Gruska I, Vetter B, Thomas D, Kiessling M, et al. A viral kinase counteracts in vivo restriction of murine cytomegalovirus by SAMHD1. *Nat Microbiol*. 2019;4(12):2273-84.
5. Businger R, Deutschmann J, Gruska I, Milbradt J, Wiebusch L, Gramberg T, et al. Human cytomegalovirus overcomes SAMHD1 restriction in macrophages via pUL97. *Nat Microbiol*. 2019;4(12):2260-72.
6. Zhang K, Lv DW, Li R. Conserved Herpesvirus Protein Kinases Target SAMHD1 to Facilitate Virus Replication. *Cell reports*. 2019;28(2):449-59 e5.
7. Kim ET, Roche KL, Kulej K, Spruce LA, Seeholzer SH, Coen DM, et al. SAMHD1 Modulates Early Steps during Human Cytomegalovirus Infection by Limiting NF-kappaB Activation. *Cell reports*. 2019;28(2):434-48 e6.
8. Sze A, Belgnaoui SM, Olganier D, Lin R, Hiscott J, van Grevenynghe J. Host restriction factor SAMHD1 limits human T cell leukemia virus type 1 infection of monocytes via STING-mediated apoptosis. *Cell host & microbe*. 2013;14(4):422-34.
9. Chen Z, Zhu M, Pan X, Zhu Y, Yan H, Jiang T, et al. Inhibition of Hepatitis B virus replication by SAMHD1. *Biochemical and biophysical research communications*. 2014;450(4):1462-8.
10. James CD, Prabhakar AT, Otoa R, Evans MR, Wang X, Bristol ML, et al. SAMHD1 Regulates Human Papillomavirus 16-Induced Cell Proliferation and Viral Replication during Differentiation of Keratinocytes. *mSphere*. 2019;4(4).
11. Hrecka K, Hao C, Gierszewska M, Swanson SK, Kesik-Brodacka M, Srivastava S, et al. Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. *Nature*. 2011;474(7353):658-61.
12. Schwefel D, Groom HC, Boucherit VC, Christodoulou E, Walker PA, Stoye JP, et al. Structural basis of lentiviral subversion of a cellular protein degradation pathway. *Nature*. 2014;505(7482):234-8.
13. Schwefel D, Boucherit VC, Christodoulou E, Walker PA, Stoye JP, Bishop KN, et al. Molecular determinants for recognition of divergent SAMHD1 proteins by the lentiviral accessory protein Vpx. *Cell host & microbe*. 2015;17(4):489-99.
14. Welbourn S, Dutta SM, Semmes OJ, Strebel K. Restriction of virus infection but not catalytic dNTPase activity is regulated by phosphorylation of SAMHD1. *Journal of virology*. 2013;87(21):11516-24.

15. White TE, Brandariz-Nunez A, Valle-Casuso JC, Amie S, Nguyen LA, Kim B, et al. The retroviral restriction ability of SAMHD1, but not its deoxynucleotide triphosphohydrolase activity, is regulated by phosphorylation. *Cell host & microbe*. 2013;13(4):441-51.
16. De Meo S, Dell'Oste V, Molfetta R, Tassinari V, Lotti LV, Vespa S, et al. SAMHD1 phosphorylation and cytoplasmic relocalization after human cytomegalovirus infection limits its antiviral activity. *PLoS pathogens*. 2020;16(9):e1008855.
17. Arnold LH, Groom HC, Kunzelmann S, Schwefel D, Caswell SJ, Ordonez P, et al. Phospho-dependent Regulation of SAMHD1 Oligomerisation Couples Catalysis and Restriction. *PLoS pathogens*. 2015;11(10):e1005194.
18. Badia R, Angulo G, Riveira-Munoz E, Pujantell M, Puig T, Ramirez C, et al. Inhibition of herpes simplex virus type 1 by the CDK6 inhibitor PD-0332991 (palbociclib) through the control of SAMHD1. *The Journal of antimicrobial chemotherapy*. 2015.
19. Tang C, Ji X, Wu L, Xiong Y. Impaired dNTPase Activity of SAMHD1 by Phosphomimetic Mutation of Thr-592. *The Journal of biological chemistry*. 2015;290(44):26352-9.
20. Wittmann S, Behrendt R, Eissmann K, Volkmann B, Thomas D, Ebert T, et al. Phosphorylation of murine SAMHD1 regulates its antiretroviral activity. *Retrovirology*. 2015;12(1):103.
21. Yan J, Hao C, DeLucia M, Swanson S, Florens L, Washburn MP, et al. CyclinA2-Cyclin-dependent Kinase Regulates SAMHD1 Protein Phosphohydrolase Domain. *The Journal of biological chemistry*. 2015;290(21):13279-92.
22. Ruiz A, Pauls E, Badia R, Torres-Torronteras J, Riveira-Munoz E, Clotet B, et al. Cyclin D3-dependent control of the dNTP pool and HIV-1 replication in human macrophages. *Cell cycle*. 2015;14(11):1657-65.
23. Lee EJ, Seo JH, Park JH, Vo TTL, An S, Bae SJ, et al. SAMHD1 acetylation enhances its deoxynucleotide triphosphohydrolase activity and promotes cancer cell proliferation. *Oncotarget*. 2017;8(40):68517-29.
24. Gareau JR, Lima CD. The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition. *Nat Rev Mol Cell Biol*. 2010;11(12):861-71.
25. Li R, Wang L, Liao G, Guzzo CM, Matunis MJ, Zhu H, et al. SUMO binding by the Epstein-Barr virus protein kinase BGLF4 is crucial for BGLF4 function. *Journal of virology*. 2012;86(10):5412-21.
26. Tammsalu T, Matic I, Jaffray EG, Ibrahim AFM, Tatham MH, Hay RT. Proteome-wide identification of SUMO2 modification sites. *Sci Signal*. 2014;7(323):rs2.
27. Hendriks IA, D'Souza RC, Yang B, Verlaan-de Vries M, Mann M, Vertegaal AC. Uncovering global SUMOylation signaling networks in a site-specific manner. *Nature structural & molecular biology*. 2014;21(10):927-36.
28. Lumpkin RJ, Gu H, Zhu Y, Leonard M, Ahmad AS, Clauser KR, et al. Site-specific identification and quantitation of endogenous SUMO modifications under native conditions. *Nature communications*. 2017;8(1):1171.
29. Shuai K, Liu B. Regulation of gene-activation pathways by PIAS proteins in the immune system. *Nature reviews Immunology*. 2005;5(8):593-605.
30. Brown JR, Conn KL, Wasson P, Charman M, Tong L, Grant K, et al. SUMO Ligase Protein Inhibitor of Activated STAT1 (PIAS1) Is a Constituent Promyelocytic Leukemia Nuclear Body

Protein That Contributes to the Intrinsic Antiviral Immune Response to Herpes Simplex Virus 1. *Journal of virology*. 2016;90(13):5939-52.

31. Zhang K, Lv DW, Li R. B Cell Receptor Activation and Chemical Induction Trigger Caspase-Mediated Cleavage of PIAS1 to Facilitate Epstein-Barr Virus Reactivation. *Cell reports*. 2017;21(12):3445-57.
32. Chanda A, Ikeuchi Y, Karve K, Sarkar A, Chandhoke AS, Deng L, et al. PIAS1 and TIF1gamma collaborate to promote SnoN SUMOylation and suppression of epithelial-mesenchymal transition. *Cell Death Differ*. 2021;28(1):267-82.
33. Kahyo T, Nishida T, Yasuda H. Involvement of PIAS1 in the sumoylation of tumor suppressor p53. *Mol Cell*. 2001;8(3):713-8.
34. Li C, McManus FP, Plutoni C, Pascariu CM, Nelson T, Alberici Delsin LE, et al. Quantitative SUMO proteomics identifies PIAS1 substrates involved in cell migration and motility. *Nature communications*. 2020;11(1):834.
35. Yang N, Liu S, Qin T, Liu X, Watanabe N, Mayo KH, et al. SUMO3 modification by PIAS1 modulates androgen receptor cellular distribution and stability. *Cell Commun Signal*. 2019;17(1):153.
36. Chang LK, Lee YH, Cheng TS, Hong YR, Lu PJ, Wang JJ, et al. Post-translational modification of Rta of Epstein-Barr virus by SUMO-1. *The Journal of biological chemistry*. 2004;279(37):38803-12.
37. Ji X, Tang C, Zhao Q, Wang W, Xiong Y. Structural basis of cellular dNTP regulation by SAMHD1. *Proceedings of the National Academy of Sciences of the United States of America*. 2014;111(41):E4305-14.
38. Li R, Liao G, Nirujogi RS, Pinto SM, Shaw PG, Huang TC, et al. Phosphoproteomic Profiling Reveals Epstein-Barr Virus Protein Kinase Integration of DNA Damage Response and Mitotic Signaling. *PLoS pathogens*. 2015;11(12):e1005346.
39. Lv DW, Zhong J, Zhang K, Pandey A, Li R. Understanding Epstein-Barr Virus Life Cycle with Proteomics: A Temporal Analysis of Ubiquitination During Virus Reactivation. *OMICS*. 2017;21(1):27-37.
40. Zhou Q, Chaerkady R, Shaw PG, Kensler TW, Pandey A, Davidson NE. Screening for therapeutic targets of vorinostat by SILAC-based proteomic analysis in human breast cancer cells. *Proteomics*. 2010;10(5):1029-39.
41. Cribier A, Descours B, Valadao AL, Laguette N, Benkirane M. Phosphorylation of SAMHD1 by cyclin A2/CDK1 regulates its restriction activity toward HIV-1. *Cell reports*. 2013;3(4):1036-43.
42. Hofmann H, Logue EC, Bloch N, Daddacha W, Polsky SB, Schultz ML, et al. The Vpx lentiviral accessory protein targets SAMHD1 for degradation in the nucleus. *Journal of virology*. 2012;86(23):12552-60.
43. Li Z, Huan C, Wang H, Liu Y, Liu X, Su X, et al. TRIM21-mediated proteasomal degradation of SAMHD1 regulates its antiviral activity. *EMBO Rep*. 2020;21(1):e47528.
44. Zhang K, Lv DW, Li R. Protein inhibitor of activated STAT1 (PIAS1) inhibits IRF8 activation of Epstein-Barr virus lytic gene expression. *Virology*. 2020;540:75-87.
45. Martinat C, Cormier A, Tobaly-Tapiero J, Palmic N, Casartelli N, Coggins SAA, et al. SUMOylation of SAMHD1 at Lysine 595 is required for HIV-1 restriction in non-cycling cells. *bioRxiv*. 2020:2020.06.04.133439.

46. Daddacha W, Koyen AE, Bastien AJ, Head PE, Dhere VR, Nabeta GN, et al. SAMHD1 Promotes DNA End Resection to Facilitate DNA Repair by Homologous Recombination. *Cell reports*. 2017;20(8):1921-35.
47. Coquel F, Silva MJ, Techer H, Zadorozhny K, Sharma S, Nieminuszczy J, et al. SAMHD1 acts at stalled replication forks to prevent interferon induction. *Nature*. 2018;557(7703):57-61.
48. Molesworth SJ, Lake CM, Borza CM, Turk SM, Hutt-Fletcher LM. Epstein-Barr virus gH is essential for penetration of B cells but also plays a role in attachment of virus to epithelial cells. *J Virol*. 2000;74(14):6324-32.
49. Feng WH, Kraus RJ, Dickerson SJ, Lim HJ, Jones RJ, Yu X, et al. ZEB1 and c-Jun levels contribute to the establishment of highly lytic Epstein-Barr virus infection in gastric AGS cells. *Journal of virology*. 2007;81(18):10113-22.
50. Feederle R, Mehl-Lautscham AM, Bannert H, Delecluse HJ. The Epstein-Barr virus protein kinase BGLF4 and the exonuclease BGLF5 have opposite effects on the regulation of viral protein production. *Journal of virology*. 2009;83(21):10877-91.
51. Li R, Zhu J, Xie Z, Liao G, Liu J, Chen MR, et al. Conserved herpesvirus kinases target the DNA damage response pathway and TIP60 histone acetyltransferase to promote virus replication. *Cell host & microbe*. 2011;10(4):390-400.
52. Lv DW, Zhang K, Li R. Interferon regulatory factor 8 regulates caspase-1 expression to facilitate Epstein-Barr virus reactivation in response to B cell receptor stimulation and chemical induction. *PLoS pathogens*. 2018;14(1):e1006868.
53. Li J, Walsh A, Lam TT, Delecluse HJ, El-Guindy A. A single phosphoacceptor residue in BGLF3 is essential for transcription of Epstein-Barr virus late genes. *PLoS pathogens*. 2019;15(8):e1007980.
54. Roy A, Kucukural A, Zhang Y. I-TASSER: a unified platform for automated protein structure and function prediction. *Nat Protoc*. 2010;5(4):725-38.
55. Yang J, Yan R, Roy A, Xu D, Poisson J, Zhang Y. The I-TASSER Suite: protein structure and function prediction. *Nat Methods*. 2015;12(1):7-8.
56. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem*. 2004;25(13):1605-12.

Figures

Figure 1

PIAS family proteins interact with SAMHD1 (A) Schematic representation of V5-Tagged PIAS family proteins. SAP (SAF-A/B, Acinus, and PIAS): DNA and protein binding domain; PINIT: nuclear localization motif; RING finger: E3 ligase domain for protein SUMOylation; SIM: SUMO interacting motif; S/T Rich: variable Ser/Thr rich region. (B) PIAS1, PIAS2, and PIAS4 interact with SAMHD1. WB analysis showing

Co-IP of SAMHD1 with PIAS proteins using 293T cells transfected with SAMHD1 and individual PIAS proteins as indicated. Input, 2% whole-cell lysate used for IP. Arrow denotes modified SAMHD1 in the PIAS1 and SAMHD1 co-transfection group.

Figure 2

PIAS1 promotes the SUMOylation of SAMHD1 (A) Schematic representation of protein SUMOylation process. The process involves E1 (PDB# 1Y8Q), E2 (PDB# 1A3S), SUMO2 (PDB# 2N1W), PIAS1 [modelled structure from I-TASSER, see reference (31)] and protein substrate. Mono- and poly-SUMOylation were

labeled as indicated. (B and C) PIAS1 promotes SAMHD1 SUMOylation. In vitro SUMOylation assay was performed with the combination of E1, E2, SUMO2, SUMO2 mutant, PIAS1, and substrates (SAMHD1 and p53) as indicated. The reaction was terminated with SDS sample loading buffer and WB was performed using anti-SUMO2/3 (B) or anti-SAMHD1 and anti-P53 (C) antibodies. Brackets denote SUMOylated SAMHD1 and p53. Arrows denote mono-SUMOylated SAMHD1 and p53.

Figure 3

PIAS1 synergizes with SAMHD1 to restrict EBV replication (A) The schematic representation of full length PIAS1 (1–651) or PIAS1 truncation mutants. (B) The central and C-terminal part of PIAS1 interacts with SAMHD1. 293T cells were co- transfected with SAMHD1 and V5-tagged full length PIAS1 (aa 1–651) or PIAS1 truncation mutants as indicated. WB analyses showing that SAMHD1 is Co-IPed with the central and C- terminal part of PIAS1. Co-IP, co-immunoprecipitation; β-actin blot was included as loading controls. (C) Full-length PIAS1 synergizes with SAMHD1 in restricting EBV replication. HEK293 (EBV+) cells were co-transfected with plasmid DNA encoding ZTA (lytic trigger), SAMHD1, and full-length (WT), truncated PIAS1 (1-205, 1–415, 1–433 and 409–651) or E3 ligase-deficient (C351S) mutant as indicated.

The relative EBV copy numbers were measured using the qPCR as described in the method. The expression levels of SAMHD1 and PIAS1 were monitored by WB. β -actin blot was included as loading controls. Results from three biological replicates are presented. Error bars indicate the standard deviation. * $p < 0.05$, ** $p < 0.01$.

Figure 4

PIAS1 SUMOylates SAMHD1 at K469, K595, and K622 (A) The schematic representation of SAMHD1 protein (1–626). The three SUMOylation consensus motifs were labeled as indicated. NLS: nuclear localization signal; SAM: sterile alpha motif; HD domain: histidine and aspartic acid domain; V; vpx interacting motif; T592: phosphorylation site mediated by cellular CDK1/2 and the conserved herpesvirus protein kinases. (B and C) In vitro SUMOylation assay was performed with the combination of E1, E2, SUMO2, PIAS1, and individual SAMHD1 mutant [K469R, K595R, K622R, or RRR(K469R/K595R/K622R)] as indicated. The reaction was terminated with SDS sample loading buffer and WB was performed using anti-SUMO2/3 (B) or anti-SAMHD1 (C) antibodies. Arrows denote mono-SUMOylated SAMHD1 and bracket denotes SUMOylated SAMHD1 and p53. (D) The localization of K469 and K595 in SAMHD1 tetramer. The SAMHD1 tetramer structure (PDB# 4TNZ) was used to locate K469 and K595 using Chimera software. (E) The localization of K469, K595 and K622 in full-length SAMHD1 monomer. The SAMHD1 structure was predicted using I-TASSER algorithm and localization of K469, K595 and K622 was labeled using Chimera software

Figure 5

Phosphorylation enhances SAMHD1 SUMOylation and SUMOylated SAMHD1 can be phosphorylated by viral protein kinases (A) Schematic representation of SAMHD1 phosphorylation and subsequent SUMOylation analysis. Step 1: Recombinant SAMHD1 was phosphorylated by EBV protein kinase BGLF4 in vitro. Non-phosphorylated SAMHD1 was similarly processed by incubating with Buffer control. Step 2: The phosphorylated and non-phosphorylated SAMHD1 were used to perform in vitro SUMOylation reaction using E1, E2, SUMO2 and PIAS1 or buffer control. Step 3: The reaction was terminated by adding

2x SDS sample loading buffer and the samples were analyzed by WB. B) Phosphorylated and non-phosphorylated SAMHD1 generated in (A) Step 1 were incubated with buffer control or with SUMOylation machinery. The SUMOylation and phosphorylation of 19 SAMHD1 were analyzed by WB using anti-SAMHD1 and anti-p-SAMHD1 (T592) antibodies as indicated. Arrows denote SUMOylated SAMHD1. (C) Schematic representation of SAMHD1 SUMOylation and subsequent phosphorylation analysis. Step 1: Recombinant SAMHD1 was SUMOylated by E1, E2, SUMO2 and PIAS1 in vitro. Non-SUMOylated SAMHD1 was similarly processed by incubating with buffer control. Step 2: The SUMOylated and non-SUMOylated SAMHD1 were subjected to in vitro kinase reaction using viral protein kinases (EBV BGLF4 or HCMV UL97). (D) WB analysis showing that SUMOylated SAMHD1 was generated from (C) Step 1. Arrows and bracket denote mono- and poly-SUMOylated SAMHD1, respectively. (E) SUMOylated and non-SUMOylated SAMHD1 generated in (C) Step 1 were incubated with buffer control or with EBV BGLF4 and HCMV UL97 in step 2. The phosphorylation status was analyzed by WB using anti-p-SAMHD1 (T592) antibodies. Arrows denote SUMOylated SAMHD1.

Figure 6

SUMOylation-deficient SAMHD1 impairs its anti-viral activity. (A) SUMOylation-deficient SAMHD1 impairs its anti-EBV activity. HEK293 (EBV+) cells were co-transfected with plasmid DNA encoding ZTA (lytic trigger), WT PIAS1, and WT or mutant SAMHD1 as indicated. The relative EBV copy numbers were measured using the qPCR as described in the method. The expression levels of ZTA, SAMHD1 and PIAS1 were monitored by WB. β-actin blot was included as loading controls. Results from three biological replicates are presented. Error bars indicate the standard deviation. * $p < 0.05$, ** $p < 0.01$. (B) Akata (EBV+)-SAMHD1-sg1 cells (endogenous SAMHD1 is depleted by CRISPR/Cas9) were used to create cell lines using vector control (pLX304), WT SAMHD1 (pLX-304-V5-SAMHD1), and SUMOylation-deficient

SAMHD1 [pLX-304-V5-SAMHD1-(RRR)]. EBV lytic cycle was induced by anti-IgG-mediated B cell receptor cross-linking. The relative EBV copy numbers were measured using the qPCR as described in the method. The expression levels of SAMHD1 were monitored by WB using anti-V5 antibody. β -actin blot was included as loading controls. Results from three biological replicates are presented. Error bars indicate the standard deviation. * $p < 0.05$, ** $p < 0.01$.