PIM kinases facilitate lentiviral evasion from SAMHD1 restriction via Vpx phosphorylation

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Lentiviruses have evolved to acquire an auxiliary protein Vpx to counteract the intrinsic host restriction factor SAMHD1. Although Vpx is phosphorylated, it remains unclear whether such phosphorylation indeed regulates its activity toward SAMHD1. Here we identify the PIM family of serine/threonine protein kinases as the factors responsible for the phosphorylation of Vpx and the promotion of Vpx-mediated SAMHD1 counteraction. Integrated proteomics and subsequent functional analysis reveal that PIM family kinases, PIM1 and PIM3, phosphorylate HIV-2 Vpx at Ser13 and stabilize the interaction of Vpx with SAMHD1 thereby promoting ubiquitin-mediated proteolysis of SAMHD1. Inhibition of the PIM kinases promotes the antiviral activity of SAMHD1, ultimately reducing viral replication. Our results highlight a new mode of virus–host cell interaction in which host PIM kinases facilitate promotion of viral infectivity by counteracting the host antiviral system, and suggest a novel therapeutic strategy involving restoration of SAMHD1-mediated antiviral response.
R
ciprocals interplay between virus and host proteins plays
important roles in both promoting and suppressing viral
replication. Several host proteins exert significant anti-
viral effects at multiple steps of the viral life cycle, but viruses
have evolved to counteract such host defenses and achieve effi-
cient replication. Moreover, by hijacking host cell machinery,
viruses take advantage of cellular regulatory systems to promote
successful progeny production and spread.

Sterile alpha motif and histidine-aspartate domain-containing
protein 1 (SAMHD1) was identified as an inhibitor of several
tetiviruses, including human immunodeficiency virus (HIV).
SAMHD1 acts as a deoxynucleotide triphosphate (dNTP) phos-
phohydrolase, and thus lowers the concentration of dNTPs
required for viral reverse transcription in infected non-dividing
myeloid cells and resting T cells. On the other hand, viruses
can overcome SAMHD1-mediated viral restriction through utilizing
viral protein X (Vpx), an accessory protein encoded by human
immunodeficiency virus type 2 (HIV-2) and some strains of
simian immunodeficiency virus (SIV). In fact, Vpx hijacks a Cullin-RING
ubiquitin ligase (CRL) complex that associates
with the host E3 ubiquitin ligase components, i.e., DCAF1, DDB1, and CUL4, to promote the ubiquitin-mediated proteolysis
of SAMHD1. These events, by suppressing the host innate immune system, ultimately result in efficient viral replication.

Host protein kinases directly phosphorylate viral proteins
during infection and control the efficiency of viral replication. In
HIV infection, the human protein kinases ERK2 and atypical PKC
phosphorylate the HIV-1 Gag protein to regulate viral
assembly, release, and infectivity. Moreover, phosphorylation of the
HIV-1 capsid by maternal embryonic leucine zipper kinase
(MELK) promotes viral uncoating and cDNA synthesis. Previous reports demonstrated that Vpx is also phosphorylated
during infection. Although phosphorylation of Vpx seems to
influence its nuclear import or packaging into virions, it
remains uncertain whether Vpx phosphorylation contributes to its
crucial function in the degradation of SAMHD1. Moreover, it
is not clear which host kinases are responsible for the functional
phosphorylation of Vpx.

In this study, we perform a comprehensive proteomic analysis
for the molecular interactions between human protein kinases
and HIV-2 Vpx, and identify PIM kinases as regulatory factors
for Vpx-mediated SAMHD1 degradation. Our findings thus
reveal a regulatory mechanism of virus–host interaction that
governs viral escape from an intrinsic cellular immune defense via
the post-translational modification of viral protein.

Results
Identification of host kinases that phosphorylate Vpx. To
comprehensively survey host kinase(s) that might bind to HIV-2
Vpx and influence its function, we performed an in vitro
protein–protein interaction screen using the amplified lumines-
cent proximity homogenous assay (AlphaScreen). Full-length
Vpx and 412 human protein kinases were separately synthesized
using the wheat cell-free protein production system, and each
kinase was screened for interaction with Vpx (Supplementary
Fig. 1). When a relative light unit per cutoff (RLU/Co) ratio of
≥1.5 was set as the threshold, 50 host kinases were identified as
potentially interacting with Vpx in vitro (Fig. 1a). To search for
biologically significant kinases, we next performed a cell-based interaction analysis using the recently developed NanoLuc bio-
luminescence resonance energy transfer (NanoBRET)
assay (Supplementary Fig. 2). Among the 50 candidates selected in the
first screening, we identified three host kinases, namely, PIM1, PIM3, and PDK1 (Fig. 1b), that interacted intimately with Vpx in
living cells, as revealed by the fact that these kinases yielded the
highest BRET signal (RLU/Co ratio of ≥20). Since two of these
proteins, PIM1 and PIM3, are members of PIM (Proviral Integration site for Moloney murine leukemia virus) family of
kinases, we decided to focus on PIM kinase family. An immuno-
precipitation analysis further revealed that PIM1 and PIM3,
but not another PIM kinase family member, PIM2, stably inter-
acted with Vpx (Fig. 1c). To functionally analyze the PIM family
kinases in relation to Vpx, we investigated whether the PIM
kinases could directly phosphorylate Vpx in vitro. To this end,
biotinylated Vpx was incubated with recombinant kinases, and
this mixture was then processed for an in vitro kinase assay using
radioactive adenosine 5′-triphosphates. The result revealed that
phosphorylation of Vpx could be performed by PIM3, and to a
lesser extent by PIM1, while PIM2 phosphorylated much less Vpx
protein than PIM1 and PIM3 in vitro (Fig. 1d). We also detected
Vpx phosphorylation by FYN kinase, as reported previously (Fig. 1d). Together, these results indicate that the PIM family
kinases, namely, PIM1 and PIM3, are previously uncharacterized
host mediators of Vpx phosphorylation.

PIM kinases phosphorylate the Ser13 residue of Vpx. To
determine the sites on Vpx phosphorylated by PIM kinases, we
performed a proteomic analysis. For these experiments, cells were
transfected with Vpx with or without PIM3, and immunoprecip-
itated Vpx was subjected to liquid chromatography tandem-
mass spectrometry analysis. Subsequent in-depth data analysis
revealed that PIM3 could phosphorylate three amino acid resi-
dues (Ser13, Thr88, and Ser101) of Vpx (Fig. 2a, b and Supple-
mental Fig. 3). Multiple sequence alignment revealed that the
amino acid sequence N-terminal to the Ser13 phospho-acceptor
site is highly conserved (98.7%) among HIV-2 and SIV isolates
(SIVmac and SIVsmm) (Fig. 2c). To further investigate the roles of
PIM kinases in Vpx function, we created a phospho-specific
antibody that specifically recognizes site-specific phosphorylation
of Vpx Ser13. Using this antibody, we confirmed that PIM1 and
PIM3, but not PIM2, could phosphorylate Vpx Ser13 in vitro
(Fig. 2d). Ectopic expression of wild-type PIM3, but not its
kinase-dead mutant (K69M), significantly enhanced the phos-
phorylation of Vpx Ser13 in cells (Fig. 2e). We confirmed that
this phosphorylation was detected in wild-type Vpx, but not its S13A
mutant (Supplementary Fig. 4a). Moreover, we found that
another candidate, PDK1, selected by initial screening, failed to
phosphorylate this site of Vpx (Supplementary Fig. 4b). Interest-
ingly, PIM3 could phosphorylate SIVmac Vpx (Fig. 2f), sug-
uggesting that the PIM kinase-mediated Vpx phosphorylation at
Ser13 is a conserved post-translational modification across the
HIV-2 and SIV lineages.

Low infectivity of HIV-2 bearing Vpx S13A. To investigate the
functional impact of Vpx Ser13 phosphorylation, we next performed a single-cycle viral infection assay using HIV-2
encoding a luciferase reporter gene (HIV-2-Luc) (Fig. 3a). In
this assay, we used wild-type HIV-2-Luc and viruses encoding
either Vpx-S13A or Vpx-Q76A (lacking the ability to interact
with the CRL4 E3 complex); the Vpx-null (ΔVpx) derivative
was used as a control. The amounts of viral production and Vpx
incorporation into virions were almost equivalent in all of these
viruses (Supplementary Fig. 5). Notably, the infectivity of Vpx-
S13A virus was significantly lower than that of the wild-type
virus in SAMHD1-positive (Monomac6-derived) macrophages
(Fig. 3b). However, this was not the case in SAMHD1-negative
(U937-derived) macrophages (Fig. 3c). Immunoblot analysis
revealed that the levels of SAMHD1 were reduced in macro-
phages infected with wild-type HIV-2, but this reduction was
less prominent in cells infected with the Vpx-S13A virus
We also found that Vpx-S13A virus exhibited much lower infectivity than wild-type virus with sustained expression of SAMHD1, in primary human monocyte–derived macrophages (Fig. 3d). We performed parallel experiments using a virus carrying phosphomimetic Vpx-S13E. Both infectivity and SAMHD1 degradation of the Vpx-S13E virus were comparable to those of the wild-type virus (Supplementary Fig. 6), demonstrating the functional significance of Ser13 phosphorylation. We next performed a multi-cycle HIV-2 replication analysis using SAMHD1-positive (THP1-derived) macrophages (Fig. 3e). Consistent with previous reports, HIV-2 virus bearing Vpx-Q76A or ΔVpx exhibited significantly lower replication competency than the wild-type virus in THP1-derived macrophages (Fig. 3f). In line with the results of single-cycle infection, HIV-2 carrying Vpx-S13A had lower replication capacity (Fig. 3f), presumably due to the persistent SAMHD1 expression (Fig. 3g). These results were also confirmed by a flow cytometry analysis in which infected cells were gated (Supplementary Fig. 7a). Our results revealed that S13A virus exhibited lower viral infectivity and less potently decreased SAMHD1 levels than the wild-type virus (Supplementary Fig. 7b, c). Together, these data indicate that the Ser13 residue of Vpx is functionally significant in SAMHD1 degradation and lentiviral replication in human macrophages.
Vpx Ser13 phosphorylation promotes Vpx–SAMHD1 interaction. The Ser13 residue of Vpx was located at the vicinity of the SAMHD1-binding site (Fig. 4a), as well as in part of the DCAF1-binding site (Fig. 4b). To further delineate the molecular mechanism underlying Ser13 phosphorylation, we performed a molecular dynamics simulation based on the previously solved structure of the Vpx–DCAF1–SAMHD1 complex to predict phosphorylation-induced conformational changes. The models constructed in this study included either unphosphorylated or phosphorylated Vpx, and revealed that phosphorylation status does not cause an obvious difference in the structure of Vpx (Fig. 4b). However, our molecular simulation suggested that the phosphorylation of Vpx Ser13 may create an additional hydrogen bond with SAMHD1 Ser616, leading to stabilization of the Vpx–SAMHD1 interaction (Fig. 4c).

To confirm the results of the molecular dynamics simulation, we investigated the interaction between Vpx and SAMHD1 in living cells using NanoBRET. We observed a constantly high BRET signal, reflecting Vpx–SAMHD1 interaction, in the case of wild-type Vpx, whereas the signal was significantly reduced in the Vpx-S13A mutant (Fig. 4d). On the other hand, the Vpx-S13A mutation had no prominent effect on the interaction between Vpx and DCAF1 (Fig. 4e). These trends were also observed in an immunoprecipitation analysis in which the Vpx-S13A mutant bound SAMHD1 to a lesser extent than wild-type Vpx (Fig. 4f). Moreover, Vpx-S13A was less capable of poly-ubiquitinating SAMHD1 than wild-type Vpx (Fig. 4i). Consistent with this, we found that Vpx-S13A was less able than wild-type Vpx to degrade SAMHD1, despite similar expression levels (Supplementary Information).
infection of the Vpx-S13A virus was comparable to that of the wild-type virus, albeit at a relatively low level, in PIM1/3-depleted macrophages (Fig. 5b, c). Moreover, when PIM1 and PIM3 kinases were depleted in producer cells, viral infectivity was not significantly affected (Fig. 5d–f). These results together indicate that PIM1 and PIM3 have a profound effect upon Vpx Ser13 in target cells, but not in producer cells, implying their action on the early stages of viral life cycle.

Currently, several potential pharmacological inhibitors of PIM kinase are under development, and a few are in clinical trials in anti-cancer treatments. To determine whether PIM kinase inhibitors could block HIV-2 infection, we treated primary human monocyte–derived macrophages with the pan-PIM kinase inhibitor AZD1208 prior to HIV-2-Luc infection (Fig. 6a). As expected, this compound inhibited HIV-2 infection in a dose-dependent manner while preventing SAMHD1 degradation (Fig. 6b, c). However, this effect was not observed in cells infected with the Vpx-S13A virus, which has low infectivity in nature (Fig. 6b, c). Moreover, we found that the inhibitory effects of AZD1208 were significantly attenuated when SAMHD1 was depleted in target macrophages (Fig. 6d, e). AZD1208 was not cytotoxic at effective concentrations (~1 µM), but interestingly, this drug exhibited a greater cytotoxic effect in Monomac6-derived cells than in primary macrophages (Supplementary...
Another PIM inhibitor, SGI1776, also exhibited inhibitory effects on HIV-2 infection similar to those of AZD1208 (Fig. 6f). Together, our data clearly indicate that selective PIM kinase inhibitors potentiate the antiviral activity of SAMHD1 by suppressing Vpx phosphorylation and function.

**PIM kinase inhibitors block lentiviral replication.** To clarify the effect of PIM kinase inhibitors on multi-cycle viral replication in primary cells, we treated human primary macrophages with HIV-2 in the presence or absence of PIM kinase inhibitors for 7 days, and then calculated the yield of progeny virus (Fig. 7a). Notably, our results demonstrated that treatment with only 1 µM of either AZD1208 or SGI1776 could block the HIV-2 replication (Fig. 7b). Notably, these compounds exerted no observable effect on the replication of HIV-1 that does not encode Vpx (Fig. 7b).

The results described above indicated that the Ser13 residue of Vpx is highly conserved within the HIV-2/SIVmac/SIVsmm lineage (Fig. 2c); consistent with this, SIVmac Vpx was also phosphorylated by PIM3 (Fig. 2f). Accordingly, we investigated whether PIM kinase inhibitors could block SIVmac replication.
ex vivo. To this end, we prepared virally infected mononuclear cell mixtures from the lymph nodes of SIVmac-infected rhesus macaques. Prior to brief stimulation, only macrophages adhered to the bottoms of dishes were recovered. The cells were then treated with PIM kinase inhibitors for 7 days (Fig. 7c). Notably, treatment with either AZD1208 or SGI1776 could significantly block viral replication (Fig. 7d). Taken together, these results confirm that PIM kinases are potent therapeutic targets for lentiviral infection.

Discussion
In recent years, a great deal of effort has been made to discover a new mode of virus–host protein interactions to understand the molecular basis underlying virus infection1,2. During viral replication, viruses take advantage of cellular enzymes to optimize viral protein function in infected cells. Phosphorylation of viral proteins by host kinases is critical for efficient viral replication and pathogenesis23,24. Several viruses encode their own protein kinases for these modifications (e.g., herpes simplex virus-thymidine kinase), but lentiviruses have no such virally encoded kinase. Instead, these viruses have evolved to hijack host protein kinases for viral protein phosphorylation in order to ensure efficient viral replication. Indeed, a previous study predicted a major role for host kinases in viral replication25. Their functional modification could facilitate the development of new antiviral strategies24. In our current study, by screening for human protein kinases that target
lentiviral Vpx, we identified PIM family kinases (PIM1 and PIM3) as previously unrecognized positive regulators of lentiviral replication that enable viral evasion from SAMHD1-mediated restriction. Our current data indicate that these PIM family kinases target a highly conserved residue of Vpx, Ser13, and that this phosphorylation stabilizes the binding of Vpx to SAMHD1, resulting in efficient SAMHD1 degradation and viral replication (Fig. 7c). These findings demonstrate a previously undescribed paradigm in virus-host interaction ensuring the efficient lentiviral replication despite the existence of host restriction system.

The antiviral activity of SAMHD1 is limited to non-dividing cells such as macrophages and resting T cells, partially due to its role in limiting the supply of cytoplasmic dNTP. To overcome this potent antiviral factor, Vpx expropriates the host CRL4 E3 ubiquitin ligase complex and induces degradation of SAMHD1\(^5,6,9\). Although the mechanistic action of Vpx on SAMHD1 is well characterized, it is largely unknown how the antagonizing activity of Vpx is regulated. Previous studies have reported that host kinases FYN and ERK2 can phosphorylate tyrosine residues (Tyr66, 69, 71) of Vpx, thereby regulating its nuclear export and viral infectivity\(^14,15\). However, no previous study had reported evidence that Vpx phosphorylation is functionally relevant to viral escape from SAMHD1-mediated restriction. Our current results strongly suggest that the PIM family kinases PIM1 and PIM3 target a highly conserved residue of Vpx, Ser13, and that this phosphorylation affects the binding of Vpx to SAMHD1, and thus its activity. Moreover, we showed here that PIM kinase inhibitors selectively block lentiviral replication by preventing Vpx-mediated SAMHD1 proteolysis. Notably, the pan-PIM kinase inhibitor AZD1208 has undergone clinical trials for hematological cancers\(^25\). Because PIM kinase promotes replication of Hepatitis C virus\(^26\), AZD1208 might provide new therapeutic options against a series of virus infection.

PIM family kinases were originally identified as a target for proviral activation in T-cell lymphomas induced by murine leukemia virus\(^27\). Accordingly, PIM kinases are highly expressed in patients with lymphoma, leukemia, and prostate cancers\(^28,29\). Because elevated expression of PIM kinases prevents apoptosis, thereby increasing oncogenic activity, PIM kinases have been suggested as attractive drug targets in cancer. We found that AZD1208 has relatively high cytotoxicity in Monomac6-derived macrophages (Supplementary Fig. 11), presumably because this cell line was derived from human monoblastic leukemia, a known therapeutic target of AZD1208\(^20,30\). Importantly, however, this drug is much less toxic in primary macrophages, warranting its use in antiviral therapy. Although PIM family kinases are highly homologous to one another and share similar substrate specificity\(^31\), our results showed that both PIM1 and PIM3 could phosphorylate Vpx Ser13. At present, we cannot exclude the possibility that PIM2 phosphorylates other residue(s) of Vpx. Additionally, our in vitro kinase assays revealed that PIM1 may more selectively phosphorylate Ser13, whereas PIM3 broadly phosphorylates other residues in Vpx as well (Figs. 1d and 2d). Detailed experiments should be needed to reveal the substrate specificity and functional significance of the Vpx phosphorylation in HIV-2/SIV replication.

Reporting summary

For additional information on the reporting overview, the nature and type of primary data, and further guidance, see our Reporting Summary (https://doi.org/10.1038/s42003-022-01676-5).

Source data

Source data are provided as a Source Data file.
The amino acid residue Ser13 in Vpx is highly conserved among clinically isolated HIV-2 strains, whose Vpx proteins are capable of degrading SAMHD1. A mutagenesis study revealed that alanine substitution of Vpx Ser13 results in lower viral infectivity than the wild-type virus, even though the mutant protein is as stable as WT Vpx. Our molecular dynamics simulation and subsequent biomolecular analyses clearly demonstrated that Vpx Ser13 phosphorylation could enhance SAMHD1 binding, presumably by forming an additional hydrogen bond between phospho-Vpx and SAMHD1. Consequently, phosphorylation of Vpx enhances ubiquitin-mediated proteolysis of SAMHD1. Consistent with this, phosphorylation of the conserved amino acid of HIV proteins seems to be associated with viral escape from host antiviral immunity. For example, Vif-Ser144 and Vpu-Ser52,56 are highly conserved phospho-acceptor sites whose phosphorylation regulates antagonizing activity against the restriction factors APOBEC3G and Tetherin, respectively. These post-translational modifications thus represent attractive targets for the development of novel anti-HIV agents that potentiate the host innate immune system.
Methods

AlphaScreen-based protein-protein interaction assays. For the initial screen, we used a total of 412 complementary DNA library encoding human protein kinases. DNA templates containing a biotin-ligating sequence were amplified by split-PCR with cDNAs and corresponding primers, and then used with the GenDecorder protein production system (CellFree Science)35–39. For synthesis of Vpx protein, cDNA sequences derived from the pG-LAN proviral plasmid40 were generated by split-PCR and used as DNA templates. Flag-tagged Vpx proteins were mixed with biotinylated kinases in 15 µl of reaction buffer (20 mM Tris–HCl pH 7.6, 5 mM MgCl2, 1 mM DTT) in 384-well OptiPlates (PerkinElmer) and incubated at 26 °C for 1 h. Each sample was then mixed with AlphaScreen buffer containing anti-antiimmunoglobulin G (proteintype A) acceptor beads and streptavidincoated donor beads (0.1 µl each; PerkinElmer) and the anti-FLAG M2 antibody (5 µg ml−1; Sigma-Aldrich), and further incubated at 26 °C. One hour later, AlphaScreen signals from the mixture were detected on an EnVision device (PerkinElmer) using the AlphaScreen signal detection program.

NanoBRET-based protein-protein interaction assays. Expression vectors encoding N-terminally HaloTag-conjugated host proteins (kinases, DCAF1 and SAMHD1) were prepared by Kusaza Genome Technologies (Chiba, Japan) and purchased from Promega. For NanoBRET analysis45, HEK293 cells in white 96-well plates were transfected with vectors encoding HaloTag-fused protein (100 ng) and NanoLuc-fused Vpx (1 ng). At 48 h post-transfection, NanoBRET activity was measured using the NanoBRET Nano-Glo Detection System (Promega).

In vitro kinase assays. Recombinant Vpx proteins were incubated with GST-tagged or Histagged PIM kinases for 1 h at 37 °C in reaction buffer (20 mM Tris–HCl pH 7.5, 1 mM EDTA, 1 mM DTT, 150 mM NaCl, 5 mM MgCl2, 0.05% Tween-20, 100 µM ATP, and 2 µC [γ-32P] ATP). The reaction mixture was subjected to electrophoresis on 10% SDS-polyacrylamide gels, and the proteins were visualized by a BioImager analyser (Fujifilm, Japan). Alternatively, the proteins were subjected to immunoblotting using a phospho-specific polyclonal antibody against Vpx phosphorylated at Ser13 (produced by Scrip Inc., Japan).

Liquid chromatography tandem-mass spectrometry analysis. HEK293 cells expressing FLAG-Vpx and HA-PIM3, grown in 10-cm dishes, were immunoprecipitated with EZview Red Flag M2 Affinity Gel (Sigma-Aldrich), and bound proteins were subjected to liquid chromatography tandem-mass spectrometry analysis. Bead-bound proteins were denatured with 8 M urea and 50 mM ammonium bicarbonate, and subsequently digested with trypsin for 16 h at 37 °C after reduction and alklylation. The resulting peptides were desalted using C18 Stage Tips42 filled with C18 and SDB Empore disc membranes (3 M) and evaporated in a vacuum concentrator. Phosphopeptides were then enriched using Titrasure TiO2 bulk beads (GL Sciences, Tokyo, Japan). After re-desalting with C18 stage Tips, phosphopeptides were analysed on an LTQ Orbitrap Velos (Thermo Fisher Scientific) equipped with an UltiMate 3000 LC system (Thermo Fisher Scientific). Protein identification was performed using the MASCOT search engine, version 2.5.1 (Matrix Science) with the Swiss-Prot database (as of July 2014) with the following parameters: enzyme, trypsin; peptide mass tolerance, ± 5 ppm; fragment mass tolerance, ± 0.5 Da; maximum missed cleavage sites, 2; variable modifications: carboxymethylation of cysteine, phosphorylation of serine or threonine, oxidation of methionine. The protein false discovery rate was < 0.05 as a cut-off to export results from the analysis by MASCOT. In addition, phosphopeptides that yielded a peptide ion score > 30 were considered positive identifications.

Cells and virus preparation. HEK293 cells (ATCC, #CRL-1573) were maintained in DMEM supplemented with 10% fetal bovine serum (FBS). THP-1 (JCRB, #0112) and U937 (JCRB, #0921) were cultured in RPMI (Wako) supplemented with 10% FBS, Monomac cells, a human monocye-derived cell line exhibiting a mature monocye phenotype, were kindly provided by Dr. Akinori Takaoka (Hokkaido University). The animal study was conducted under the guidelines provided by both Primate Research Institute (PRI) and Institute for Frontier Life and Medical Sciences (INFRONT), Kyoto University after the approval of Animal Welfare and Care Committee in the University. Research permission numbers were 2016–081 and 2017–116 for PRI and B15-3 and B15-3-2 for INFRONT, respectively. After removal of suspension cells, the remaining cells were stimulated with TNF-α (50 ng ml−1) for 24 h, and then treated with either SGI1776, AZD1208, or darunavir (1 µM) for 7 days. The levels of progeny virions in the culture supernatants were determined using the SIV p27 ELISA kit (ZeptoMetrix).

Multi-cycle viral replication assay. Differentiated THP-1 cells in 24-well plates were infected with equal amounts of viruses (105 reverse transcriptase units), and replication kinetics were calculated by reverse transcriptase activity of virus containing culture supernatants. For the experiment, Pimaskin inhibitors, macrophages in 24-well plates were infected with replication-competent HIV-2_cGL (5 ng of Gag p27 antigen) or HIV-1m8 (20 ng of Gag p24 antigen) using the ViroMag Transfection Reagent (OZ Biosciences). Replication kinetics were calculated based on the level of extracellular viral capsid antigens using the SIV p27 or HIV p24 ELISA kit (ZeptoMetrix). For the experiment, Pimaskin inhibitors, we used frozen cells derived from the lymph node of a macaque monkey infected with SIVmac251. The animal study was conducted under the guidelines provided by both Primate Research Institute (PRI) and Institute for Frontier Life and Medical Sciences (INFRONT), Kyoto University after the approval of Animal Welfare and Care Committee in the University. Research permission numbers were 2016–081 and 2017–116 for PRI and B15-3 and B15-3-2 for INFRONT, respectively. After removal of suspension cells, the remaining cells were stimulated with TNF-α (50 ng ml−1) for 24 h, and then treated with either SGI1776, AZD1208, or darunavir (1 µM) for 7 days. The levels of progeny virions in the culture supernatants were determined using the SIV p27 ELISA kit (ZeptoMetrix).

Molecular dynamics (MD) simulation. The X-ray crystal structure of SAMHD1-Vpx-DCAF1 (Protein Data Bank code: 4CC9) was used as the template structure. On the basis of the predicted structural model of SAMHD1 with wild-type Vpx, three-dimensional structures of SAMHD1 with either unphosphorylated or phosphorylated Vpx were constructed using Molecular Builder in the Molecular Operating Environment software. MD simulations were performed by the pmemd module in the Amber 11 program package47 with the AMBER ff99SB-ILDN force field48 and the TIP3P water model for simulations of aqueous solutions49. A non-periodic box with 30Å cut-off was used for all MD simulations. The interaction of Vpx with CD4 was monitored during the last 50 ns of the simulation (Fig. 1). The X-ray crystal structure of SAMHD1–Vpx-DCAF1 (Protein Data Bank code: 4CC9) was used as the template structure. On the basis of the predicted structural model of SAMHD1 with wild-type Vpx, three-dimensional structures of SAMHD1 with either unphosphorylated or phosphorylated Vpx were constructed using Molecular Builder in the Molecular Operating Environment software. MD simulations were performed by the pmemd module in the Amber 11 program package47 with the AMBER ff99SB-ILDN force field48 and the TIP3P water model for simulations of aqueous solutions49. A non-periodic box with 30Å cut-off was used for all MD simulations. The interaction of Vpx with CD4 was monitored during the last 50 ns of the simulation (Fig. 1).

Data availability

The MS raw data have been deposited to the JOPTREpo (Japan Proteome Standard Repository) with the dataset identifier PXD013154. The source data underlying Figs. 1d, 2d–f, 3b–g, 4d–i, 5b–d, 6b–f, 7b and d, and Supplementary Figs. 4a, 5, 6a, 6b, 8a, 9, 10b, c and 11 are provided as a Source Data file.
References

1. Guarinoud, T., Delmonte, S. & Navratil, V. VirHostNet 2.0: surfing on the web of virus-host molecular interactions data. Nucleic Acids Res. 43, D583–D587 (2015).

2. Brito, A. F. & Pinney, J. W. Protein-protein interactions in virus-host systems. Front. Microbiol. 8, 1557 (2017).

3. Kirchhoff, F. Immune evasion and counteraction of restriction factors by HIV-1 and other primate lentiviruses. Cell. Host. Microbe 8, 55–67 (2010).

4. Goldstone, D. C. et al. HIV-1 restriction factor SAMHD1 is a deoxyribonucleoside triphosphate triphosphohydrolase. Nature 480, 379–382 (2011).

5. Lahouassa, H. et al. SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxyribonucleotides triphosphates. Nat. Immunol. 13, 223–228 (2012).

6. Baldauf, H. M. et al. SAMHD1 restricts HIV-1 infection in resting CD4+ T cells. Nat. Med. 18, 1682–1687 (2012).

7. Hreka, K. et al. Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. Nature 474, 658–661 (2011).

8. Laguette, N. et al. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature 474, 654–657 (2011).

9. Altor, J. et al. Human 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 (2012).

10. Hennonot, B. et al. The host cell MAP kinase ERK-2 regulates viral assembly and release by phosphorylating the p6gag protein of HIV-1. J. Biol. Chem. 279, 32426–32434 (2004).

11. Kodoh, A. et al. The phosphorylation of HIV-1 Gag by atypical protein kinase C facilitates viral infectivity by promoting Vpr incorporation into virions. Retrovirology 11, 9 (2014).

12. Takeuchi, H. et al. Phosphorylation of the HIV-1 capsid by MEK5 triggers uncoating to promote viral cDNA synthesis. PLoS Pathog. 13, e1006441 (2017).

13. Nandi, J. & Banerjee, K. Tyrosine phosphorylation as a possible regulatory mechanism in the expression of human immunodeficiency virus genes. Med. Hypotheses 45, 476–480 (1995).

14. Rajendra Kumar, P., Singh, P. K., Subba Rao, M. R. & Mahalingam, S. Phosphorylation by MAPK regulates simian immunodeficiency virus Vpx protein nuclear import and virus infectivity. J. Biol. Chem. 280, 8553–8563 (2005).

15. Singhal, P. K., Rajendra Kumar, P., Subba Rao, M. R. & Mahalingam, S. Nuclear export of simian immunodeficiency virus Vpx protein. J. Virol. 80, 12271–12282 (2006).

16. Deneen, B. et al. PIM3 proto-oncogene kinase is a common transcriptional target of divergent EWS/ETS oncoproteins. Mol. Cell. Biol. 23, 3897–3908 (2003).

17. Srivastava, S. et al. Lentiviral Vpx accessory factor targets VprBP/DCAF1 substrate adaptor for cullin 4 E3 ubiquitin ligase to enable macrophage infection. PLoS Pathog. 4, e1000599 (2008).

18. Nomaguchi, M., Doi, N. & Adachi, A. Viral localization of DCAF1 substrate adaptor for cullin 4 E3 ubiquitin ligase to enable APOBEC3G degradation. Genes Dev. 28, 21840–21852 (2015).

19. Miyakawa, K. et al. Molecular disruption of HIV evasion from restriction factor tethers: a new perspective for antiviral cell therapy. Oncotarget 6, 21840–21852 (2015).

20. Case, D. et al. AMBER 11, 2010. Vol. 5 (University of California, San Francisco, 2010).

21. Lindorff-Larsen, K. et al. Improved side-chain torsion potentials for the AMBER99SB force field. Proteins 78, 1950–1958 (2010).

22. Collman, R. et al. An infectious molecular clone of an unusual macrophage-tropic and highly cytopathic strain of human immunodeficiency virus type 1. J. Virol. 66, 7517–7521 (1992).

23. Miyakawa, K. et al. Molecular disruption of HIV-1 Vif-mediated counteraction. Nat. Commun. 6, 6945 (2015).

24. Collman, R. et al. An infectious molecular clone of an unusual macrophage-tropic and highly cytopathic strain of human immunodeficiency virus type 1. J. Virol. 66, 7517–7521 (1992).

25. Miyakawa, K. et al. Molecular disruption of HIV-1 Vif-mediated counteraction. Nat. Commun. 6, 6945 (2015).

26. Miyakawa, K. et al. Development of a cell-based assay to identify hepatitis B virus entry inhibitors targeting the sodium taurocholate cotransporting polypeptide. Oncotarget 9, 23681–23694 (2018).

27. Case, D. et al. AMBER 11, 2010. Vol. 5 (University of California, San Francisco, 2010).

28. Lindorff-Larsen, K. et al. Improved side-chain torsion potentials for the AMBER99SB force field. Proteins 78, 1950–1958 (2010).

29. Jorgensen, W. L., Chandrasekhar, J., Madura, D. J., Impey, R. W. & Klein, M. L. Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. 79, 926–935 (1983).

30. Ryckaert, J.-F., Ciccotti, G. & Berendsen, H. J. Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. J. Comput. Phys. 33, 327–341 (1979).

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Author contributions

K.M. designed and performed the research, analyzed the data, and wrote the manuscript; S.M., M.T., M.Nomaguchi and M.Nishii performed the research and analyzed the data; Y.K., H.H., T.T. and K.M. provided reagents and analyzed the data; H.A., T.M. and A.A. contributed reagents and analyzed the data; T.S. developed the screening system; A.R. directed the research, analyzed the data, and wrote the manuscript.
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