CD81 association with SAMHD1 enhances HIV-1 reverse transcription by increasing dNTP levels

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In this study, we report that the tetraspanin CD81 enhances human immunodeficiency virus (HIV-1) reverse transcription in HIV-1-infected cells. This is enabled by the direct interaction of CD81 with the deoxynucleoside triphosphate phosphorydrolase SAMHD1. This interaction prevents endosomal accumulation and favours the proteasome-dependent degradation of SAMHD1. Consequently, CD81 depletion results in SAMHD1 increased expression, decreasing the availability of deoxynucleoside triphosphates (dNTP) and thus HIV-1 reverse transcription. Conversely, CD81 overexpression, but not the expression of a CD81 carboxy (C)-terminal deletion mutant, increases cellular dNTP content and HIV-1 reverse transcription. Our results demonstrate that the interaction of CD81 with SAMHD1 controls the metabolic rate of HIV-1 replication by tuning the availability of building blocks for reverse transcription, namely dNTPs. Together with its role in HIV-1 entry and budding into host cells, the data herein indicate that HIV-1 uses CD81 as a rheostat that controls different stages of the infection.

In this study, we show that SAMHD1 is a molecular partner of tetraspanin CD81, and that CD81 regulates RT by controlling SAMHD1 expression, thereby modulating the intracellular dNTP content.

Results

CD81 associates with SAMHD1. A putative interaction between CD81 and SAMHD1 was suggested by high-throughput mass...
spectrometry studies in primary human T lymphoblasts, not being validated at that time\(^3\). The association between CD81 and SAMHD1 was first probed by pull-down experiments using biotinylated tetraspanin C-terminal peptides. SAMHD1 specifically bound to CD81 C-terminal peptides, whereas no association was observed with tetraspanins CD9 and CD151 (Fig. 1a). The association between endogenous molecules was then assessed by co-immunoprecipitation, and SAMHD1 was detected in CD81 immunoprecipitates and vice versa (Fig. 1b). These results indicate that SAMHD1 and CD81 directly associate through the CD81 C-terminal domain.

Since SAMHD1 is not expressed in T cell lines, like Jurkat or CEM (Supplementary Fig. 1), we have used primary T lymphoblasts and Hela cells to investigate its cellular distribution. Different permeabilizing conditions were used to co-stain CD81, which is a transmembrane protein expressed at the plasma membrane and in intracellular vesicular compartments, and SAMHD1, which has been mainly characterized as a nuclear protein, although also described at the cytoplasm of T lymphocytes\(^2\). Increasing concentrations of the permeabilizing agent allowed the detection of nuclear SAMHD1, but diminished the tetraspanin signal (Supplementary Fig. 2a–d). Cytoplasmic localization of the enzyme could be observed with these permeabilizing conditions, as well as in Jurkat cells transfected with SAMHD1–GFP (green fluorescent protein) (Supplementary Fig. 2a–e). Using mild permeabilizing conditions (0.5% Triton X-100 for 5 min), intracellular CD81 was co-stained with cytoplasmic and nuclear SAMHD1 in primary T lymphoblasts and Hela–R5 cells (Fig. 1c and Supplementary Fig. 2c). However, the absence of clear co-localization in resting conditions indicates a transient interaction.

Therefore, we assessed SAMHD1 localization upon tetraspanin crosslinking in Hela–R5 cells stably expressing CD4 and CCR5. Cells plated onto poly-l-lysine (PLL), as a control, or onto anti-CD9 antibody showed weak SAMHD1 staining (Fig. 1a). Interestingly, the hydrolase accumulated in patches at the cellular basal layer

![Fig. 1](image-url)
upon CD81 or CD4 (a CD81 partner) crosslinking (Fig. 1d). This clustering was abrogated by siRNA depletion of CD81 (siCD81) (Fig. 1d). Moreover, in situ proximity ligation assays revealed SAMHD1–CD81 interactions in a significant number of primary T lymphoblasts (Fig. 1e). We used SAMHD1/CD147 as a negative control, since CD147 is a membrane receptor with higher expression than CD81, and CD81/ERM (ezrin, radixin and moesin) as a positive control, corroborating that proximity ligation signal could be attained between a membrane-bound molecule and an intracellular connector that were previously shown to interact at the uropod of T-lymphoblasts22.

**CD81 positively regulates HIV-1 reverse transcription.** Since SAMHD1 regulates HIV-1 RT, we assessed the role of CD81 in this step of the viral cycle. Hela–R5 cells were transfected with GFP, GFP-tagged CD81 (CD81GFP) or a CD81 mutant lacking the C-terminal cytoplasmic region (CD81ΔcytGFP)38, and infected with wild-type R5-tropic HIV-1 (Bal strain). Early and late viral RT products were measured by quantitative polymerase chain reaction (qPCR) analysis. CD81GFP expression increased HIV-1 RT, and this effect was dependent on the CD81 C-terminal domain (Fig. 2a, HIV-1 Bal). CD81GFP overexpression also highly increased RT of VSV-G-pseudotyped HIV-1 (HIV-VSV-G) (Fig. 2a, HIV-VSV-G), which enters the cells via attachment of the VSV glycoprotein G, ruling out any effect of CD81 in HIV-1 entry, assembly or release40,42. Moreover, CD81 positively regulated HIV-1 replication of single-cycle luciferase (Luc) reporter viruses (pseudotyped with HIV-1 or VSV-G envelopes), through a process mediated by its C-terminal domain (Fig. 2b).

Conversely, CD81 knockdown using siRNA (Supplementary Fig. 3a), or full knockout using the CRISPR–Cas9 technology (CRISPR–Cas9–CD81; Fig. 2c) in Hela–R5 cells reduced RT of both HIV-1 wild type and HIV-VSV-G (Supplementary Fig. 3a–b). CD81 deficiency also diminished the luciferase activity after infection with single-cycle HIV–1–R5–Luc or HIV-VSV-G–Luc reporter viruses (Fig. 2c). The expression levels of CD4, CCR5 or tetraspanins CD82, CD9, CD151 or CD36 were not affected by CD81 knockdown or knockout (Supplementary Fig. 3c–d). In addition, pre-treatment of Hela–R5 cells with fluorescently labelled cytopermeable peptides corresponding to the sequence of the CD81 C-terminal region (CD81pept), which functionally mimics the effects of CD81 knockdown in different models38,39, significantly reduced RT of wild-type HIV-1 and HIV-VSV-G (Fig. 2d), further corroborating the involvement of CD81 C-terminal domain in the regulation of viral RT.

In a more physiological setting, pre-treatment of primary T lymphoblasts with CD81pept clearly impaired RT of wild-type HIV-1 (NL4-3 strain) or HIV-VSV-G (Fig. 3a). Importantly, CD81 knockdown with siRNA in primary T lymphoblasts also specifically decreased RT (Fig. 3b). Together, our results suggest that CD81 positively regulates HIV-1 RT of both R5- and X4-tropic viruses.

**CD81 regulates intracellular dNTP levels via SAMHD1.** To determine whether the function of CD81 in RT relied on the regulation of SAMHD1, we analysed the effect of CD81 knockdown in Jurkat cells, which do not express SAMHD1 (Supplementary Fig. 1). In these cells, CD81 knockdown or treatment with CD81pept did not alter the levels of RT products in comparison with control cells (Fig. 4a,b), indicating that CD81 does not affect HIV-1 RT in the absence of SAMHD1.

SAMHD1 dNTPase activity was then directly analysed by quantification of the intracellular dNTP content in cell lysates after CD81 overexpression or depletion. In parallel to the effects observed on viral RT, the dNTP pool was significantly reduced in both Hela–R5 cells and primary T lymphoblasts depleted for CD81 (Fig. 4c) or treated with CD81pept (Supplementary Fig. 3e). Conversely, CD81GFP overexpression in Hela–R5 cells increased the dNTP content, which remained unaffected by CD81ΔcytGFP expression (Fig. 4d). These results indicate that CD81 regulates the intracellular dNTP content by associating with SAMHD1 through its C-terminal domain.

**CD81 controls SAMHD1 degradation by the proteasome and its subcellular localization.** We next investigated the mechanism by which CD81 could regulate SAMHD1. Interestingly, CD81 deficiency increased the expression of SAMHD1, both in primary T lymphoblasts transfected with CD81 siRNA and in Hela–R5 CRISPR–Cas9–CD81 (Fig. 5a,b). However, no differences were observed in the levels of SAMHD1 phosphorylation (Fig. 5a). Although SAMHD1 has a predicted molecular weight of ∼70 kDa, additional bands with different molecular weights could be detected, suggesting cleavage, splicing variants40 or post-translational modifications of the enzyme37,38,41. Specificity of the antibody was confirmed using whole cell lysates from cells expressing SAMHD1–GFP or transfected with SAMHD1 siRNA (Supplementary Fig. 4).

To assess whether the increase in SAMHD1 expression was related to altered protein degradation, cells were treated with MG132, which prevents proteasome function, or with ammonium chloride (NH4Cl), which blocks the acidification of lysosomes. SAMHD1 expression was twofold higher in Hela–R5 control cells upon treatment with MG132 (Fig. 5c), but it was only slightly affected by increasing concentrations of NH4Cl (Fig. 5d), indicating that the proteasome is the main route for SAMHD1 turnover. In CRISPR–Cas9–CD81 cells, despite the expected higher SAMHD1 basal levels in cells treated with the vehicle, the increase in the expression of the enzyme after MG132 treatment was completely abolished (Fig. 5c), suggesting that CD81 is essential for SAMHD1 degradation by the proteasome. Treatment of CRISPR–Cas9–CD81 cells with NH4Cl slightly increased SAMHD1 expression, suggesting that lysosomal degradation was unaffected or slightly favoured on proteasomal blockade by CD81 (Fig. 5d).

Since CD81 deletion increases SAMHD1 expression by protecting the enzyme from proteasomal degradation (Fig. 5) and CD81 crosslinking triggers SAMHD1 accumulation beneath the plasma membrane (Fig. 1d), we investigated whether CD81 could regulate the subcellular distribution of SAMHD1. In the absence of CD81, SAMHD1 accumulated in cytoplasmic speckles, which displayed increased number and size with respect to control cells (Fig. 6a and Supplementary Fig. 5a,b). Similar results were obtained when cells were treated with CD81pept (Fig. 6b and Supplementary Fig. 5a,b). Accordingly, the presence and area of these SAMHD1 circular cytoplasmic structures was reversibly reduced in CD81GFP-expressing cells, while no differences were observed between cells expressing CD81ΔcytGFP or GFP (Fig. 6c and Supplementary Fig. 5c).

To characterize these intracellular structures, Hela–R5 cells were co-stained with antibodies against SAMHD1 and markers of different intracellular compartments. We could not observe any co-localization between SAMHD1 cytoplasmic speckles and markers of late endosomes (HGS–HR5), multivesicular bodies (CD63) or lysosomes (LAMP-1) (Fig. 6d and Supplementary Fig. 6). Interestingly, the circular intracellular structures that accumulated SAMHD1 partially co-localized with EEA1, a marker of early endosomes. SAMHD1–EEA1 co-localization was increased in CD81 knocked down cells, as quantified by Pearson’s coefficient and the frequency of SAMHD1 co-localizing with EEA1 when compared with the total SAMHD1 signal (Fig. 6d).

Altogether, our results suggest that CD81 deletion controls SAMHD1 expression by protecting the enzyme from proteasomal degradation via its subcellular compartmentalization in early endosomes.

**Discussion**

In this study, we show that tetraspanin CD81 regulates HIV-1 RT through its molecular association with SAMHD1, and the control of the expression and subcellular localization of the hydrolase. We...
provide strong evidence of CD81 association with SAMHD1 in primary T lymphoblasts by (1) pull-down with synthetic peptides containing the CD81 C-terminal sequence but not of other tetraspanins; (2) co-immunoprecipitation of endogenous CD81 and SAMHD1 using detergent lysis conditions (0.5% NP-40) that mostly disrupt TEMs, thus impairing indirect connections; and (3) in situ proximity ligation assay, which provides strong evidence that the CD81–SAMHD1 molecular association occurs in vivo. Moreover, crosslinking with monoclonal antibodies against CD81 and CD4 triggers SAMHD1 juxta-membrane clustering in a CD81-dependent manner.
SAMHD1 is a cellular inhibitor of HIV-1 RT in resting cells. Although widely studied in past years, the mechanisms that control this enzyme are not yet fully understood. SAMHD1 phosphoroylation at T592 was shown to be important for the control of SAMHD1 RNase and dNTPase activities. However, it was recently suggested that this phosphorylation cannot fully explain the restriction effect. The SAMHD1 exonuclease activity is also controversial, with recent studies indicating that viral restriction is not related to this enzymatic activity, which could even derive from contaminants in the sample. In the absence of CD81, SAMHD1 expression is induced and its dNTPase activity is increased, without noticeable differences in its phosphorylation levels.

Our results clearly indicate that CD81 is an important player in the regulation of SAMHD1-dependent restriction of HIV-1 replication, through a mechanism dependent on its C-terminal domain. When CD81 is depleted, SAMHD1 expression is increased and the subsequent reduction in the intracellular pool of dNTPs impairs viral RT and replication. The effects of CD81 depletion on RT are observed only 48 h post infection, probably because during the first 24 h, despite increased SAMHD1 activity, the remaining intracellular pool of dNTPs would be sufficient to allow initial viral replication until exhaustion observed at 48 h. Accordingly, HIV-1 reverse transcription continues until exhaustion observed at 48 h. Accordingly, HIV-1 reverse transcription continues until exhaustion observed at 48 h.
transcriptase can efficiently synthesize viral DNA in the presence of low dNTP concentrations. On the contrary, when CD81 is overexpressed, SAMHD1 expression is reduced and the cellular dNTP content is higher, allowing a huge increase in HIV-1 RT even at 24 h. The observed increase in dNTP levels in these cells was not as impressive as the increase in RT, further indicating that the viral reverse transcriptase is very sensitive to slight changes in the intracellular availability of dNTPs. Thus, even a small increase in the levels of these nucleotides is sufficient for the huge increase in viral genome replication.

The precise subcellular site where viral RT takes place remains to be determined. Although SAMHD1 has been mainly described as a nuclear protein, we and others could detect this enzyme in the cytoplasm. In this regard, it has been recently shown that oxidized SAMHD1 is specifically located at the cytoplasm. Tetraspanins, which are transmembrane proteins present in both the plasma membrane and intracellular compartments, can either promote or inhibit HIV-1 transmission, playing negative or positive roles in different steps of the viral cycle. The involvement of tetraspanins in RT would imply that the virus hijacks intracellular membranes...
Fig. 6 | SAMHD1 is partially enriched at early endosomes. a, Hela–R5 cells were transfected with control or CD81 siRNA, adhered for 4 h onto fibronectin (FN), fixed, permeabilized in PBS 0.1% Triton X-100 for 5 min and immunolabelled for SAMHD1. The images show one single confocal plane; the nuclei are blue. The arrows indicate SAMHD1 accumulation in circular intracellular structures. Scale bars, 10 μm. The graphs show the means ± s.e.m. of the number (counts per cell) and area (μm² per cell) of the cytoplasmic structures observed (n=230 cells, 4 independent experiments analysed by Student’s t-test, ***P=0.0005 (upper) and ***P=0.0006 (bottom)). b, Hela–R5 cells treated with 2 μM scramble or CD81pept were analysed as in a (n=400 cells, 4 independent experiments analysed by Student’s t-test; **P=0.0063 (upper) and **P=0.0054 (bottom)). c, Hela–R5 cells transfected with GFP, CD81GFP or CD81ΔcytGFP were analysed as in a (n=20 cells, 2 independent experiments analysed by one-way ANOVA with Tukey’s post-test).

d, Hela–R5 transfected with control or CD81 siRNA were treated as in a. The images show SAMHD1 (green), EEA1 (red), LAMP-1 (magenta), nuclei (blue), DIC, SAMHD1–EEA1 co-localization channel (white) and merged images. One single confocal plane is shown. Scale bars, 10 μm. Graphs represent the quantification of SAMHD1–EEA1 co-localization performed in 3D stack confocal microscopy images, showing means ± s.e.m. of the Pearson coefficient and of the percentage of SAMHD1 signal co-localized with EEA1 signal with respect to the total SAMHD1 signal in the cell (n=200 cells, 3 independent experiments analysed by Student’s t-test, *P=0.0262 (left) and *P=0.0479 (right)).
to support its early replication. Interestingly, CD81 crosslinking triggers SAMHD1 enrichment beneath the plasma membrane, and CD81 knockdown increases SAMHD1 permanence in an early endosomal compartment, with partial SAMHD1 and EEa1 colocalization. Our results indicate that degradation of the hydrolyase can partially occur through lysosomes but it is mostly dependent on the proteasome. CD81 does not affect SAMHD1 degradation by acidic compartments, but it clearly controls the degradation of the hydrolyase by the proteasome. Therefore, CD81 regulation of SAMHD1 subcellular localization seems to be important for the turnover of the enzyme. Other cellular proteins have been described to regulate SAMHD1 degradation by acidic compartments, but it clearly controls the degradation of the hydrolyase by the proteasome. Cyclin L2 interacts with SAMHD1 at the nucleus, driving its proteosomal degradation through a mechanism dependent on the ubiquitin ligase adaptor DCAF1120. Interaction with the eukaryotic elongation factor 1A1 (eEF1A1) at the cytoplasm also targets SAMHD1 for proteosomal turnover, through the association with Cullin4A and Rbx1121. Interestingly, we also detected eEF1A1 as a putative molecular partner of CD81 in our previous proteomic study122.

In summary, CD81 regulates HIV-1 early replication via direct association with SAMHD1, modulating the intracellular dNTP content through the control of SAMHD1 expression and subcellular localization. The evidence that SAMHD1 is included in TEMs highlights the importance of these membrane microdomains during HIV-1 replication, not only in the entry and assembly phases of the viral cycle, but also in RT. More detailed knowledge on how SAMHD1 blocks HIV-1 infection will provide insights to reinforce antiviral strategies. More detailed knowledge on how SAMHD1 blocks HIV-1 infection will provide insights to reinforce antiviral strategies. More detailed knowledge on how SAMHD1 blocks HIV-1 infection will provide insights to reinforce antiviral strategies. More detailed knowledge on how SAMHD1 blocks HIV-1 infection will provide insights to reinforce antiviral strategies.

Flow cytometry. Cells were fixed in 2% paraformaldehyde (PFA); Electron Microscopy Sciences), permeabilized using the BD Cytofix/Cytoperm kit when observing intracellular proteins, and stained with primary antibodies followed by species-matching secondary antibodies (Invitrogen). Primary antibodies were anti-CCR5 (Santa Cruz), anti-SAMHD1 (polyclonal antibody; Sigma), anti-CD81 (TS2b, kindly provided by E. Rubinstein, Villejuif), and antibodies produced in our laboratory (anti-CD9 (VJ1/20), anti-CD51 (LIa1/1), anti-CR3 (Texa/18) and anti-CR2 (H2/22). Data were acquired with a FACScantoll flow cytometer (BD) and analysed with BD FACSdiva (BD) software or FlowJo (FlowJo LLC) software.

Fluorescence confocal microscopy. Cells were adhered onto PLL (Sigma), CD4, CD81 or CD9 monoclonal antibodies (10 µg ml−1) for 2 h, or onto fibronectin (5 µg ml−1; Sigma) for 2 or 4 h at 37 °C. Fixed cells were incubated with 4% PFA, permeabilized with PBS 0.1%, 0.5% or 1% Triton X-100 for 5 min or 30 min, stained with primary antibodies followed by species-matching secondary antibodies coupled to Alexa Fluor fluorochromes (Invitrogen) and mounted in Prolong antifading medium (Invitrogen). Primary antibodies were anti-CD81 (SA6), anti-SAMHD1 (monoclonal or polyclonal antibodies; Sigma), anti-EEa1, CD4 or CD9 (Santa Cruz) and incubated with the secondary anti-IgG-Alexa 488 or 568 (LifeTechnologies) and incubated with the secondary anti-IgG-Alexa 488 or 568 (LifeTechnologies) and incubated with the secondary anti-IgG-Alexa 488 or 568 (LifeTechnologies) and incubated with the secondary anti-IgG-Alexa 488 or 568 (LifeTechnologies). Double or triple staining and immunolabeling were performed with a secondary anti-IgG-Alexa 568 (LifeTechnologies), anti-CD9 (VJ1/20) and anti-LAMP-1 (1B3) antibodies. Data were acquired with a FACScantoll flow cytometer (BD) and analysed with BD FACSdiva (BD) or FlowJo (FlowJo LLC) software.

Reagents and constructs. Tetramethylrhodamine (TAMRA) N-terminal labelled peptides with the sequences RRRRRRCGIRNSSY (CD81) or RRRRRSSYVNICRCCS (Scrambled) were purchased from LifeTein. N-terminally biotinylated peptides containing a SSGS linker sequence connected to the cytoplasmic C-terminal domains of the proteins of interest were purchased from Ray Biotech, and have been previously described12,13. The constructs CD14-GFP and CD81-GFP were previously described14,15 and SAMHD1–GFP was kindly provided by N. Landau (New York). Control siRNA and siRNA for CD81 (CAATTGGTTGCTCCCTCGGCG (siCD81) were purchased from Eurogentec. We have validated three different sequences to knockdown CD81 containing similar phenotypes in other systems15-17, siRNA for SAMHD1 was purchased from Dharmacon (SMARTpool ON-TARGETplus containing four different siRNA sequences).

Pull-down, immunoprecipitation and immunoblot assays. For immuno-precipitation, primary T lymphoblasts (2 × 107) were lysed in PBS 0.5% NP-40 containing protease and phosphatase inhibitors. Lysates were preclarred for 2 h at 4 °C with protein G-Sepharose (Amersham Biosciences), and incubated for 2 h at 4 °C with anti-CD81 5A6 monoclonal (S. Levy, Stanford) or mouse polyclonal anti-SAMHD1 (Sigma) antibodies immobilized on protein G-Sepharose beads. Following with lysis buffer, complexes were eluted in Laemmli buffer, and resolved by SDS–PAGE.

N-terminally biotinylated peptides (30 nmol) were conjugated to 40 µl of streptavidin sepharose (GE Healthcare). Pull-down assays were carried out as previously described18. Briefly, cells were washed once with ice-cold PBS and lysed in PBS 1% NP-40 containing protease and phosphatase inhibitors (Complete, PhosSTOP; Roche). Lysates were preclarred for 2 h at 4 °C with streptavidin sepharose (GE Healthcare) and incubated for 2 h at 4 °C with biotinylated peptides immobilized on streptavidin sepharose beads.

For immunoblotting, untreated cells or cells incubated with different concentrations of ammonium chloride (NH4Cl, Sigma) or MG132 (Sigma) for 6 h were lysed in PBS 1% NP-40 containing protease and phosphatase inhibitors. All blots were revealed with FUJIFilm LAS-4000 after membrane incubation with specific antibodies and peroxidase-conjugated secondary antibodies (Pierce). Primary antibodies were against: SAMHD1 (polyclonal antibody; Sigma), CD81 (SA6), α-tubulin (clone DM1A, Sigma), p190glued (BD Biosciences), coflin (Abcam), and β-actin (Abcam, and Sigma). Band intensities were quantified using ImageJ (FUJIFILM) and results normalized with respect to the intensity signal of the loading controls.

HIV-1 viral preparation and infection. Preparation of wild-type HIV-1 NL4-3 (X4-tropic) or BaL (R5-tropic), and VSV-G-pseudotyped HIV recombinant virus (HIV-VSV-G), which allows the analysis of replication independently of HIV entry, was performed as described19. Pseudotyped viruses with a luciferase reporter gene were produced by co-transfection of HEK293T cells with an equal mixture of the pNL4-3.LucR+Rpr-Luc reporter plasmid (N. Landau, NIH AIDS Reagent Program), and HIV-1 envelope expression plasmid (NIH AIDS Reagent Program) or pcDNA3-VSV plasmid (encoding the vesicular stomatitis virus G-protein) using calcium phosphate transfection in OPTIMEM (Gibco, Invitrogen) at 240 V and 34 ms (Gene Pulser II, Bio-Rad).

Viral titres were quantified using a Gag p24 ELISA kit (INNOTEST HIV-1 Antigen Multiplex; Biokit). For analysis of HIV-1 RT, Hela–R5 cells were infected with 100 ng per 106 cells with HIV-1 BaL or with 50 ng per 24 h of HIV-VSV-G, and primary T lymphoblasts or Jurkat cells were infected with 100 ng per 106 cells with HIV-1 NL4-3 or 200 ng per 106 cells with HIV-1 VSV-G. After 2 h of infection, cells were extensively washed and incubated at 37 °C for 24 or 48 h and lysed in 0.2% NP-40, total genomic DNA was extracted using a Qiagen Mini kit (Qiagen). qPCR analysis for the measurement of HIV reverse transcription was performed by amplifying genomic DNA using a Power SYBR Green PCR master mixture (Applied Biosystems):
forward primer 5'-CAGGATTCTTGCCTAGAGCTG-3' and reverse primer 5'-GGAGCAGGAAGAAGACATGT-3' for early reverse transcription products, and forward primer 5'-TGGTGCACCTGCTTGTTG-3' and reverse primer 5'-CAGGCTTCCGGTCGAGAGAT-3' for late reverse transcription products. The β-actin gene was amplified to measure DNA concentration and used for normalization. Each reaction was performed in triplicates.

For single-cycle infection assay, Hela-R5 cells were plated on a 24 well plate and infected with 50 ng HIV-1 p24Ag/10^5 cells. After 2 h of infection, cells were extensively washed and incubated at 37 °C for 48 h. Then cells were washed with PBS, and lysed with a Steady Glo luciferase assay buffer (Promega Corporation, WI, USA). The light intensity of each sample was measured on a luminometer at 1400 Microbe Luminescence Counter.

Intracellular dNTP measurement. For dNTP analysis and quantification, cells were harvested, lysed in ice cold 65% methanol and vigorously vortexed for 2 min. Extracts were incubated at 95 °C for 3 min, then supernatants were collected and dried in a speed vacuum. Samples were processed in a blinded manner for the single nucleotide incorporation assay as described47. Each dNTP (dATP, dCTP, dGTP and dTTP) was detected separately, and for the analysis their levels in cells overexpressing or depleted for CD81 were normalized to the levels in control cells.

Statistical analysis. All statistical analyses were performed with GraphPad Prism (GraphPad Software Inc.). P values were calculated using two-tailed Student's t-test, one-way ANOVA with Tukey's or two-way ANOVA with Bonferroni's post hoc multiple comparison tests. When indicated, one-way ANOVA with Dunn's post-test was used. Statistical significance was assigned at *P < 0.05, **P < 0.01 and ***P < 0.001.

Data availability. The data that support the findings of this study are available from the corresponding author upon request. Complete blots for all the figures and supplementary figures are shown in Supplementary Figs. 7 and 8.

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**Author contributions**

V.R.P., F.S.M. and M.Y.M. conceived and designed research. V.R.P., H.S., S.A., S.L.M., G.L. and M.Y.M. performed experimental work. S.A., F.V.C., S.L., B.K., M.A.M.F., F.S.M. and M.Y.M. provided reagents. V.R.P. and M.Y.M. analysed the data. V.R.P. wrote the paper.

**Competing interests**

The authors declare no competing financial interests.

**Additional information**

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1. Sample size
   Describe how sample size was determined.

   For quantification of HIV-1 reverse transcription by qPCR, each experiment was performed in triplicate (biologicals) and qPCRs were done in triplicates (technical). Methods, page 18; Legends of Figures 2, 3 and 4, pages 25-26; Legend of Supplementary Figure 3.

   For the quantification of the luciferase activity after infection with single-cycle luciferase pseudotyped reporter virus, three independent experiments were performed in triplicates (biologicals). Methods, page 19; Legend of Figure 2, page 25.

   For quantification of number and size of fluorescent SAMHD1+ spots in Hela/RS cells, or SAMHD1/EEA1 co-localization, two or three independent experiments were performed, and the number of cells analyzed in confocal microscopy images ranged from 20 to 400, depending on the experiment. In each independent experiment the number of cells analyzed for each condition was similar. Methods, page 17. Legends of Figure 1, page 24; and Figure 6, page 27; Legend of Supplementary Figure 5.

   For the quantification of the dNTP intracellular content, three independent experiments were performed. Methods, page 19. Legend of Figure 4, page 26; Legend of Supplementary Figure 3.

   For quantification of immunoblot signals, two or three independent experiments were performed. In each experiment, results were normalized with respect to the loading control. Methods, page 16; Legend of Figure 5, page 26.

2. Data exclusions
   Describe any data exclusions.

   No data were excluded from the analyses.

3. Replication
   Describe whether the experimental findings were reliably reproduced.

   Each experiment was performed two or three independent times, as stated in the Figure Legends, and data from all the experiments were pooled. All attempts of replication were successful.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.

   Not applicable. Our experimental groups were: 1- control cells compared with cells overexpressing CD81GFP or the mutant CD81dcytGFP; 2- control cells compared with cells knocked-down for CD81 by siRNA or CD81 knock-out with the CRISPR/Cas9 technology; 3- control cells compared with cells treated with cell-permeable peptides with the sequence of the C-terminal region of CD81.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Measurement of cellular dNTP content was performed on blinded samples.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

- **n/a**
- Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars

*See the web collection on statistics for biologists for further resources and guidance.*

### Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

All statistical analyses were performed with GraphPad Prism (GraphPad Software Inc). Statistical significance was assessed by two-tailed Student’s t test, one-way ANOVA with Tukey’s or Dunn’s multiple comparison post-test, or two-way ANOVA with Bonferroni’s multiple comparison post-test, as required. Methods, page 19; and Legends of Figures and Supplementary Figures.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

### Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All unique materials used are readily available from the authors, or from standard commercial sources, as indicated in the Methods section, page 20.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

- alpha-tubulin (DM1A) mAb - Sigma T6199; p150 glued mAb - BD Biosciences 610473; coflin mAb - Abcam 42824; EEA1 (N-19) Ab - Santa Cruz sc-6415; CD63 (H-193) Ab - Santa Cruz sc-15363; HGS/HRS Ab - Abcam ab72053; LAMP1-647 (H4A3) mAb - Biolegend 328612; LaminA/C (N-18) - Santa Cruz sc-6215; SAMHD1 mAb - Sigma SAB4100235;

- CD81 mAb (SA6) - provided by Dr. S. Levy (Methods, page 15). Described in Takahashi et al, J Immunol, 1990; ERM 90:3 - provided by Dr. H Furthmayr (Methods, page 16). Described in Amieva & Furthmayr; Exp Cell Res, 1995; CD82 (T582b) mAb - provided by Dr. E. Rubinstein (Methods, page 16). Described in Charrin et al, J Biol Chem, 2001; CD9 (VJ1/20), CD151 (LIA1/1), CD63 (Tea3/18), and CD4 (HP2/6) - references 17, 49, 50 (Methods, page 16); CD147 (VJ1/9) - reference 17 (Methods, page 17);

- SAMHD1 (366-380) polyclonal Ab - Sigma SAB1101454. This antibody was further validated in this study by immunoblot of Hela/R5 cell lysates (Supplementary Figure 4), in which it recognizes SAMHD1-GFP, and knock-down of SAMHD1 by siRNA.
### 10. Eukaryotic cell lines

**a. State the source of each eukaryotic cell line used.**

Source of cells are stated in Methods, page 14. Hela P4.r5 MAGi cells (Hela/R5) were obtained through the NIH AIDS Reagent Program; human kidney cell line HEK293T, Vb8 Jurkat T cells (J77 Cl20), CEM T cells and Raji B cells were obtained from ATCC.

**b. Describe the method of cell line authentication used.**

Since cell lines were obtained from the NIH AIDS Reagent Program and ATCC they were not further authenticated, as stated in Methods, page 14.

**c. Report whether the cell lines were tested for mycoplasma contamination.**

All cell lines are routinely tested for mycoplasma contamination and tested negative, as stated in Methods page 14.

**d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.**

No commonly misidentified cell lines were used.

### Animals and human research participants

Policy information about **studies involving animals**; when reporting animal research, follow the **ARRIVE guidelines**

#### 11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

**No animals were used.**

Policy information about **studies involving human research participants**

#### 12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

**This study did not involve human research participants.**