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SAMHD1: a new contributor to HIV-1 restriction in resting CD4⁺ T-cells

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Abstract

Resting CD4⁺ T-cells are critical for establishing HIV-1 reservoirs. It has been known for over two decades that resting CD4⁺ T-cells are refractory to HIV-1 infection, but the underlying mechanisms are not fully understood. Compared with activated CD4⁺ T-cells that support HIV-1 infection, resting CD4⁺ T-cells have lower levels of dNTPs, which limit HIV-1 reverse transcription. The dNTPase SAMHD1 has been identified as an HIV-1 restriction factor in non-cycling myeloid cells. Two recent studies revealed that SAMHD1 restricts HIV-1 infection in resting CD4⁺ T-cells, suggesting a common mechanism of HIV-1 restriction in non-cycling cells that may contribute to viral immunopathogenesis.

Introduction

HIV-1-induced CD4⁺ T-cell depletion and establishment of viral reservoir are two hallmarks during AIDS development. Resting CD4⁺ T-cells are critical for the establishment and maintenance of HIV-1 reservoirs through multifaceted mechanisms [1]. It has been known for over two decades that quiescent/resting CD4⁺ T-cells are highly refractory to HIV-1 infection [2,3], particularly to post-entry infection of R5-tropic, but not to X4-tropic viruses since resting CD4⁺ T-cells express the HIV-1 co-receptor CXCR4. However, the underlying mechanisms of HIV-1 restriction in resting CD4⁺ T-cells are not fully understood.

Compared with activated CD4⁺ T-cells that support productive HIV-1 infection, resting CD4⁺ T-cells have lower levels of dNTPs, which limit efficient HIV-1 reverse transcription and thereby block HIV-1 infection [3]. Addition of exogenous deoxynucleotides as dNTP precursors in quiescent CD4⁺ T-cells only partially rescues HIV-1 reverse transcription. The dNTP concentrations in quiescent CD4⁺ T-cells (0.3–5 μM) are approximately 6–10-fold lower relative to activated CD4⁺ T-cells (3–30 μM) and are around 6–100-fold higher compare with those in non-cycling macrophages (~0.05 μM) [3,4]. However, the molecular basis of maintaining low dNTP

levels in non-cycling cells remained unclear until the recent discovery of a host protein named SAMHD1.

SAMHD1 was initially identified as an HIV-1 restriction factor in non-cycling myeloid cells, including primary monocytes, monocyte-derived dendritic cells and macrophages [5-7]. SAMHD1 has been proposed as a cellular regulator of the intrinsic antiviral response [8], while its physiological role remains largely unknown. As a dGTP-dependent deoxynucleotide triphosphohydrolase, SAMHD1 hydrolyzes intracellular dNTPs and reduces their concentrations to below the levels required for HIV-1 reverse transcription, thereby blocking viral infection in non-cycling myeloid cells [4]. These studies raised a significant question whether SAMHD1 contributes to HIV-1 restriction in resting CD4⁺ T-cells. If so, what are the implications of SAMHD1-mediated HIV-1 restriction in CD4⁺ T-cell depletion, establishment of the viral reservoir, and immune evasion?

New findings and implications

Two recent studies revealed that SAMHD1 restricts HIV-1 infection in resting CD4⁺ T-cells [9,10], providing new insights into the mechanisms of HIV-1 restriction in non-cycling cells. SAMHD1-mediated HIV-1 restriction in myeloid cells has been proposed as an immune evasion strategy for HIV-1 to avoid antiviral innate and adaptive immune responses [5-7]. This proposed immune evasion mechanism likely also exists in resting CD4⁺ T-cells. In addition, HIV-1 restriction mediated by SAMHD1 in resting CD4⁺ T-cells may contribute

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to viral immunopathogenesis, such as T-cell depletion and establishment of the viral reservoir and latency (Figure 1).

Both studies by Baldauf *et al.* and Descours *et al.* demonstrated that resting CD4⁺ T-cells isolated from the peripheral blood of healthy donors express high levels of endogenous SAMHD1 protein, which are similar to those detected in primary myeloid cells or the monocytic THP-1 cell line [9,10]. Baldauf *et al.* showed that SAMHD1 is highly expressed in explants of human tonsil and likely localizes to non-proliferating macrophages, dendritic cells

and CD4⁺ T-cells [9], which are the major target cell types when HIV-1 encounters lymphoid or mucosal tissues *in vivo*. Descours *et al.* further demonstrated that three circulating CD4⁺ T-cells subsets, including naïve (CD45RA⁺, CCR7⁺), central memory (CD45RA⁻, CCR7⁺) and effector memory cells (CD45RA⁻, CCR7⁻), express similarly high levels of SAMHD1 [10]. Moreover, circulating CD8⁺ T-cells and CD19⁺ B-cells also express SAMHD1 [10], although the physiologically relevant function of SAMHD1 and its regulation of dNTP levels in these cells remain to be examined. These results expanded

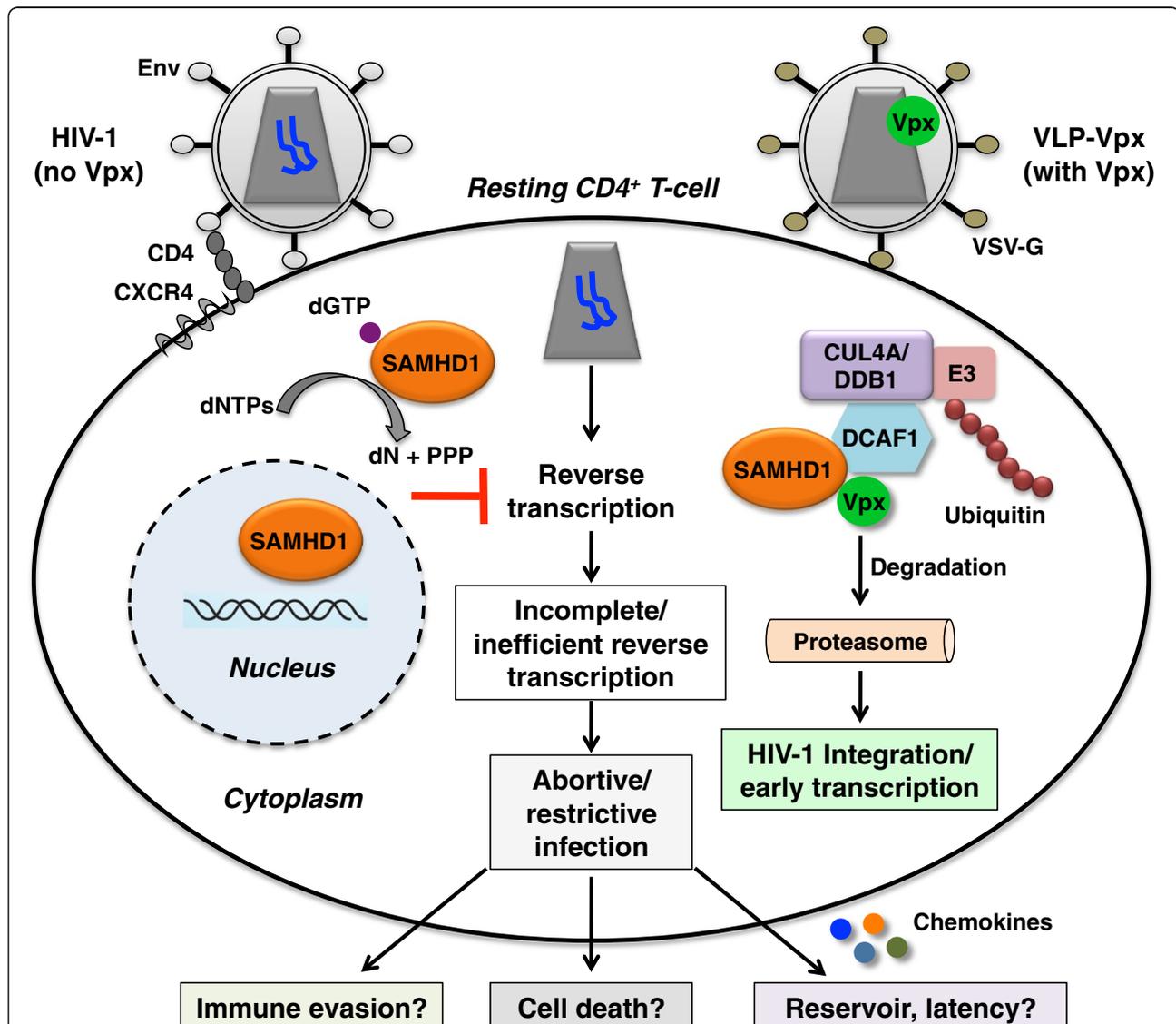


Figure 1 SAMHD1 restricts HIV-1 infection in resting CD4⁺ T-cells by limiting viral reverse transcription. SAMHD1 is a dNTP triphosphohydrolase that converts intracellular dNTPs to deoxynucleosides (dN) and inorganic triphosphates (PPP), thereby limiting HIV-1 reverse transcription in resting CD4⁺ T-cells. Treatment of resting CD4⁺ T-cells with SIVsm/HIV-2 Vpx-containing VLPs or expression Vpx in resting CD4⁺ T-cells results in proteasomal degradation of SAMHD1 and leads to HIV-1 integration and early gene transcription. SAMHD1-mediated HIV-1 restriction in resting CD4⁺ T-cells might contribute to different aspects of viral immunopathogenesis, including CD4⁺ T-cell depletion and establishment of viral reservoirs and latency. Establishing HIV-1 latency in resting CD4⁺ T-cells requires chemokine-induced modifications in the actin cytoskeleton. Env, HIV-1 envelope protein.

our knowledge of SAMHD1 expression profile in major immune cell types that interact with HIV-1.

It has been shown that the lentiviral protein Vpx from HIV-2 and sooty mangabey-lineage SIV (SIVsm) counteracts SAMHD1-mediated HIV-1 restriction in myeloid cells through proteasomal degradation of SAMHD1 [5-7]. Similarly, Vpx from HIV-2 or SIVsm degrades SAMHD1 in resting CD4⁺ T-cells and efficiently increases single-cycle and replication-competent HIV-1 infection, which is correlated with increased products of full-length viral cDNA and 2-LTR circles in infected cells [9,10]. However, the degradation of SAMHD1 in resting CD4⁺ T-cells by treatment of Vpx-containing virus-like particles (VLP-Vpx) derived from SIV appears much less efficient compared with that in primary monocytes and activated CD4⁺ T-cells [10]. This might be due to distinct efficiencies of nuclear and cytoplasmic exchange among different cell types, given that Vpx-mediated SAMHD1 degradation is dependent on its nuclear export [11]. Furthermore, Vpx increases HIV-1 infection of all resting CD4⁺ T-cell subsets (naïve, central memory and effector memory cells) by approximately 8- to 15-fold [10]. Importantly, VLP-Vpx treatment neither induces CD4⁺ T-cell activation and proliferation nor affects HIV-1 infection of activated CD4⁺ T-cells [10], indicating that SAMHD1 specifically restricts HIV-1 infection in resting CD4⁺ T-cells, but not in activated CD4⁺ T-cells despite comparable expression levels of endogenous SAMHD1 in these cells.

Vpx-mediated SAMHD1 degradation in resting CD4⁺ T-cells can only enable early steps of HIV-1 life cycle, such as reverse transcription, but not later stages of HIV-1 replication [9,10]. Descours *et al.* found that, when SAMHD1 is degraded by Vpx in resting CD4⁺ T-cells, cytomegalovirus promoter-driven HIV-1 gene expression is significantly enhanced, while HIV-1 LTR promoter-driven viral gene expression remains blocked despite efficient reverse transcription [10]. These results suggest that HIV-1 transcriptional restriction in resting CD4⁺ T-cells is independent of SAMHD1. By contrast, Baldauf *et al.* showed that infection of resting CD4⁺ T-cells with a replication-competent HIV-1 carrying SIV Vpx significantly enhances viral gene expression, which is under the control of the HIV-1 LTR promoter [9]. Notably, Descours *et al.* used VSV-G-pseudotyped HIV-1 vectors in their infection assays [10], while Baldauf *et al.* used a CXCR4-tropic replication-competent HIV-1 [9]. Distinct envelope proteins of HIV-1 used in these studies and/or other different experimental conditions might result in the discrepancy of HIV-1 transcriptional gene expression, which remains to be confirmed. The use of VSV-G-pseudotyped HIV-1 vectors expands the host-cell range to beyond human CD4⁺ T-cells, suggesting that Descours *et al.* might examine SAMHD1

function in cell types that are not necessarily relevant to HIV-1 infection. Although Vpx treatment of resting CD4⁺ T-cells enables early steps of the HIV-1 and HIV-2 life cycle, it cannot enhance the production of HIV-1 p24 or HIV-2 p27 capsid proteins in the supernatants from the infected cells [9], suggesting that additional mechanisms are involved in HIV-1 and HIV-2 restriction in resting CD4⁺ T-cells.

Of note, T-cell activation does not affect SAMHD1 expression levels in CD4⁺ T-cells [9,10]. Consistent results were observed when CD4⁺ T-cell activation was achieved either with phytohemagglutinin treatment [9,10] or through T-cell receptor stimulation with anti-CD3/anti-CD28 in the presence of interleukin-2 [10]. Although activated CD4⁺ T-cells have 3- to 8-fold higher dATP/dTTP concentrations relative to resting CD4⁺ T-cells, SAMHD1 expression remains the same in resting and activated CD4⁺ T-cells [9]. It is likely that intracellular dNTP levels can be significantly increased when activated CD4⁺ T-cells become dividing cells. How activated CD4⁺ T-cells upregulate dNTP levels without down-regulating SAMHD1 expression remains to be investigated. Cellular ribonucleotide reductase increases the dNTP pool in cells through the *de novo* dNTP synthesis pathway. Thus, it is important to analyze expression levels and the activity of ribonucleotide reductases in resting and activated CD4⁺ T-cells to further understand the mechanisms by which SAMHD1 regulates the dNTP pool and HIV-1 infection efficiency in these cells.

SAMHD1 is primarily a nuclear protein [5,8] that has a nuclear localization signal in the N-terminus [11]. Using nuclear-cytoplasmic fractionation and three-dimensional reconstructions of confocal images, Baldauf *et al.* observed that large amounts of SAMHD1 in both the nucleus and cytoplasm of resting CD4⁺ T-cells, activated CD4⁺ T-cells, and macrophages [9], suggesting that SAMHD1 can be a cytoplasm-nucleus shuttling protein that depletes the dNTP pool in the cytoplasm of resting CD4⁺ T-cells and macrophages where HIV-1 reverse transcription happens. Further microscopy studies of SAMHD1 intracellular trafficking and its interactions with HIV-1 particles in resting CD4⁺ T-cells and myeloid cells will help dissect the molecular details of SAMHD1-mediated HIV-1 restriction.

Baldauf *et al.* showed that addition of exogenous deoxynucleotides in resting CD4⁺ T-cells or infection of resting CD4⁺ T-cells with Vpx-carrying HIV-1 increases the concentrations of intracellular dATP and dTTP, which correlates with enhanced HIV-1 infection [9]. These results suggest that the intracellular dNTP pools in resting CD4⁺ T-cells are rate limiting for HIV-1 reverse transcription, and SAMHD1 plays a regulatory role in this process. Furthermore, silencing SAMHD1 expression in post-activated resting CD4⁺ T-cells using specific

siRNA or shRNA significantly increases HIV-1 infection, further suggesting that SAMHD1 suppresses HIV-1 infection in post-activated resting CD4⁺ T-cells [9].

SAMHD1 gene mutations are associated with Aicardi-Goutières syndrome (AGS), an inherited autoimmune disease mimicking congenital viral infection with elevated type I interferon production [8]. Berger *et al.* have reported that, within peripheral blood mononuclear cells (PBMCs) from SAMHD1-deficient AGS patients, CD14⁺ monocytes are more susceptible to HIV-1 infection compared with cells from healthy donors [7]. Baldauf *et al.* and Descours *et al.* further demonstrated that HIV-1 infection of SAMHD1-deficient resting CD4⁺ T-cells in PBMCs from AGS patients is significantly increased compared with that of SAMHD1-expressing normal cells [9,10]. Moreover, VLP-Vpx treatment of SAMHD1-deficient resting CD4⁺ T-cells cannot further enhance HIV-1 infection [10], indicating that SAMHD1 is necessary for Vpx to relieve HIV-1 restriction in resting CD4⁺ T-cells. These *ex vivo* studies using SAMHD1-deficient cells provided further evidence that SAMHD1 functions as an HIV-1 restriction factor.

It is conceivable that SAMHD1-mediated HIV-1 restriction in non-cycling cells may play an important role in viral immunopathogenesis. Doitsh *et al.* found that abortive HIV-1 reverse transcription in resting tonsil CD4⁺ T-cells results in cell death through proapoptotic and proinflammatory response, which is triggered by accumulation of incomplete HIV-1 reverse transcripts [12]. However, it is unclear whether and how SAMHD1-mediated restriction of HIV-1 in resting CD4⁺ T-cells may trigger innate immune recognition of viral cDNA and result in cell death.

Conclusions and future directions

The studies by Baldauf *et al.* and Descours *et al.* revealed that SAMHD1 restricts HIV-1 infection in resting CD4⁺ T-cells by limiting viral cDNA synthesis via depleting the intracellular dNTP pool, at least in part [9,10]. SAMHD1-mediated HIV-1 restriction in myeloid cells and resting CD4⁺ T-cells is likely a general mechanism to protect host cells from productive viral infection, which can also be a potential strategy of HIV-1 immune evasion to avoid efficient anti-viral innate immunity [13].

Because addition of deoxynucleotides in resting CD4⁺ T-cells only partially restores HIV-1 reverse transcription, other mechanisms may also contribute to HIV-1 restriction imposed by SAMHD1 in non-cycling cells. It is possible that SAMHD1 requires a cellular cofactor in non-cycling cells for its HIV-1 restriction function. On the contrary, it is also plausible that T-cell activation or myeloid cell differentiation diminishes the expression and/or function of a SAMHD1 suppressor, thereby rendering HIV-1 restriction activity of SAMHD1. Further

studies are needed to delineate the cellular mechanism by which SAMHD1 inhibits HIV-1 infection in non-cycling cells.

We have reported that polymorphisms of the *SAMHD1* gene are not associated with HIV-1 infection and natural control in Europeans and African-Americans [14]. It would be important to examine the role of SAMHD1 in HIV-2 infection given that HIV-1 has not evolved a viral antagonist to SAMHD1. It is also important to further study the effect of Vpx on SIV infection and viral immunopathogenesis using SIV and non-human primate models. *SAMHD1* gene knockout mice are currently unavailable. It would be intriguing to examine whether *SAMHD1* knockout mice mirror the AGS symptoms in human patients and become more susceptible to the infection with murine retroviruses or other RNA or DNA viruses. The *SAMHD1* knockout mice will also be valuable to understand the role SAMHD1 in innate and adaptive immunity.

Post-transcriptional regulation and/or post-translational modifications of SAMHD1 might be important for its function in restricting HIV-1 infection in non-cycling cells. For example, a new study by Welbourn *et al.* identified naturally occurring splice variants of SAMHD1 in a variety of cell types [15]. These splice variants do not have the dNTPase activity and they are metabolically unstable and therefore can be rapidly degraded in the absence of Vpx [15], suggesting a post-transcriptional mechanism regulating the hydrolase activity of SAMHD1.

Further elucidating the mechanisms of SAMHD1-mediated HIV-1 restriction in non-cycling myeloid cells and resting CD4⁺ T-cells may help develop new therapeutic approaches against HIV-1 infection. For instance, one can use pharmacologic induction of low dNTP pools in HIV-1 target cells to inhibit viral infection or to block SAMHD1 activity to elicit protective anti-HIV-1 immune responses. However, these strategies likely will be used in combination with other available anti-HIV-1 interventions and need to be carefully designed and evaluated to avoid potential detrimental effects on the host.

Abbreviations

SAMHD1: Sterile alpha motif domain- and HD domain-containing protein 1; HIV-1: Human immunodeficiency virus type 1; HIV-2: Human immunodeficiency virus type 2; SIV: Simian immunodeficiency virus; dNTP: Deoxynucleoside triphosphate; dNTPase: dNTP triphosphohydrolase; VSV-G: Vesicular stomatitis virus G protein; LTR: Long terminal repeat; siRNA: Small interfering RNA; shRNA: Short hairpin RNA.

Competing interests

The author declares that he has no competing interests.

Authors' contributions

LW conceived the topic and wrote the manuscript.

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References

1. Eisele E, Siliciano RF: **Redefining the Viral Reservoirs that Prevent HIV-1 Eradication.** *Immunity* 2012, **37**(3):377–388.
2. Zack JA, Arrigo SJ, Weitsman SR, Go AS, Haislip A, Chen IS: **HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure.** *Cell* 1990, **61**(2):213–222.
3. Gao WY, Cara A, Gallo RC, Lori F: **Low levels of deoxynucleotides in peripheral blood lymphocytes: a strategy to inhibit human immunodeficiency virus type 1 replication.** *Proc Natl Acad Sci U S A* 1993, **90**(19):8925–8928.
4. Lahouassa H, Daddacha W, Hofmann H, Ayinde D, Logue EC, Dragin L, Bloch N, Maudet C, Bertrand M, Gramberg T, et al: **SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates.** *Nat Immunol* 2012, **13**(3):223–228.
5. Laguette N, Sobhian B, Casartelli N, Ringeard M, Chable-Bessia C, Segaler E, Yatim A, Emiliani S, Schwartz O, Benkirane M: **SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx.** *Nature* 2011, **474**(7353):654–657.
6. Hrecka K, Hao C, Gierszewska M, Swanson SK, Kesik-Brodacka M, Srivastava S, Florens L, Washburn MP, Skowronski J: **Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein.** *Nature* 2011, **474**(7353):658–661.
7. Berger A, Sommer AF, Zwarg J, Hamdorf M, Welzel K, Eslly N, Panitz S, Reuter A, Ramos I, Jatiani A, et al: **SAMHD1-deficient CD14+ cells from individuals with Aicardi-Goutieres syndrome are highly susceptible to HIV-1 infection.** *PLoS Pathog* 2011, **7**(12):e1002425.
8. Rice GI, Bond J, Asipu A, Brunette RL, Manfield IW, Carr IM, Fuller JC, Jackson RM, Lamb T, Briggs TA, et al: **Mutations involved in Aicardi-Goutieres syndrome implicate SAMHD1 as regulator of the innate immune response.** *Nat Genet* 2009, **41**(7):829–832.
9. Baldauf HM, Pan X, Erikson E, Schmidt S, Daddacha W, Burggraf M, Schenkova K, Ambiel I, Wabnitz G, Gramberg T, et al: **SAMHD1 restricts HIV-1 infection in resting CD4(+) T cells.** *Nat Med* 2012, doi:10.1038/nm.2964. Epub ahead of print.
10. Descours B, Cribier A, Chable-Bessia C, Ayinde D, Rice G, Crow Y, Yatim A, Schawartz O, Laguette N, Benkirane M: **SAMHD1 restricts HIV-1 reverse transcription in quiescent CD4+ T-cells.** *Retrovirology* 2012, **9**(1):87.
11. Brandariz-Nunez A, Valle-Casuso JC, White TE, Laguette N, Benkirane M, Brojatsch J, Diaz-Griffero F: **Role of SAMHD1 nuclear localization in restriction of HIV-1 and SIVmac.** *Retrovirology* 2012, **9**:49.
12. Doitsh G, Cavrois M, Lassen KG, Zepeda O, Yang Z, Santiago ML, Hebbeler AM, Greene WC: **Abortive HIV infection mediates CD4 T cell depletion and inflammation in human lymphoid tissue.** *Cell* 2010, **143**(5):789–801.
13. St Gelais C, Wu L: **SAMHD1: a new insight into HIV-1 restriction in myeloid cells.** *Retrovirology* 2011, **8**(1):55.
14. Coon S, Wang D, Wu L: **Polymorphisms of the SAMHD1 Gene Are Not Associated with the Infection and Natural Control of HIV Type 1 in Europeans and African-Americans.** *AIDS Res Hum Retroviruses* 2012, Epub on April 26.
15. Welbourn S, Miyagi E, White TE, Diaz-Griffero F, Strebel K: **Identification and characterization of naturally occurring splice variants of SAMHD1.** *Retrovirology* 2012, **9**(1):86.

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