Cyclophilin A Prevents HIV-1 Restriction in Lymphocytes by Blocking Human TRIM5α Binding to the viral Core.

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ABSTRACT

Disruption of cyclophilin A (CypA)-capsid interactions negatively affects human immunodeficiency virus-1 (HIV-1) replication in human lymphocytes. To investigate the molecular events that lead to a decrease in HIV-1 infection upon disruption of CypA-capsid interactions in lymphocytes, we used Jurkat cells, primary human peripheral blood mononuclear cells (PBMCs), and primary human $CD4^+$ T cells as experimental models. Our results showed that the inhibition of HIV-1 infection caused by the disruption of CypA-capsid interactions was dependent on human TRIM5α (TRIM5α_{hu}) expression, suggesting that TRIM5α_{hu} restricts HIV-1 infection. Accordingly, we found that TRIM5α_{hu} binds to the HIV-1 core. Disruption of CypA-capsid interactions failed to affect HIV-1-A92E infection of human lymphocytes, correlating with the loss of TRIM5α_{hu} binding to HIV-1-A92E mutant cores. These results suggested that CypA protects the HIV-1 core from TRIM5α_{hu}. We found that the disruption of CypA-capsid interactions in PBMCs and $CD4^+$ T cells had a greater inhibitory effect on HIV-1 infection compared with that in Jurkat cells. In accordance with our hypothesis that CypA protects the core from TRIM5α_{hu}, HIV-1-A92E infection of PBMCs and $CD4^+$ T cells was unaffected by the disruption of CypA-capsid interactions. Consistent with the restriction activity of TRIM5α proteins, disruption of CypA-capsid interactions in $CD4^+$ T cells dramatically decreased the efficiency of reverse transcription. Overall, our results showed that the binding of CypA to the HIV-1 core protects it from the restriction factor TRIM5α_{hu}.

Keywords: HIV-1; CypA; TRIM5$\alpha_{\eta\upsilon}$; core; capsid; restriction; $CD4^+$ T cells

INTRODUCTION

The early steps of human immunodeficiency virus (HIV-1) replication involve delivery of the viral core into the host cytoplasm. The viral core is a conical structure composed of ~1,500 monomers of the HIV-1 capsid protein, which protects the viral RNA genome (Pornillos et al., 2009; Sundquist and Hill, 2007). The capsid protein plays a role in uncoating, reverse transcription, and nuclear import. To perform these functions, the capsid protein has been shown to functionally interact with several host dependency factors, including cyclophilin A (CypA) (Luban et al., 1993), Nup153 (Buffone et al., 2018; Di Nunzio et al., 2013) (Brass et al., 2008; Konig et al., 2008; Lee et al., 2010; Matreyek and Engelman, 2011; Matreyek et al., 2013; Zhou et al., 2008), RANBP2 (Di Nunzio et al., 2012; Meehan et al., 2014; Ocwieja et al., 2011), and TNPO3 (Brass et al., 2008; Christ et al., 2008; Konig et al., 2008; Krishnan et al., 2010; Levin et al., 2010; Ocwieja et al., 2011; Thys et al., 2011; Valle-Casuso et al., 2012; Zhou et al., 2008). Effective HIV-1 infection may occur only when these factors interact with the HIV-1 core at the appropriate time and in the appropriate order, thus raising the possibility that the initial binding of particular factors may modulate the subsequent binding of other factors.

The interaction of CypA with monomeric HIV-1 capsid was originally reported about 30 years ago (Luban et al., 1993), and it has been well-established that this interaction modulates HIV-1 infection (Braaten et al., 1996; Braaten and Luban, 2001; Li et al., 2009; Schaller et al., 2011; Sokolskaja et al.,

2004; Thali et al., 1994; Towers et al., 2003). Although the ability of CypA to modulate HIV-1 infection is cell-type dependent, it is clear that the disruption of CypA-capsid interactions negatively affects HIV-1 infection of lymphocytes (Braaten and Luban, 2001; Li et al., 2009; Ptak et al., 2008; Sokolskaja et al., 2004; Towers et al., 2003; Wainberg et al., 1988; Yin et al., 1998); however, the underlying mechanism remains elusive.

To investigate the molecular events that result in a decrease in HIV-1 infection upon disruption of CypA-capsid interactions in lymphocytes, we used Jurkat cells, primary human peripheral blood mononuclear cells **(**PBMCs), and primary human $CD4^+$ T cells as experimental models. Our experiments revealed that inhibition of HIV-1 infection caused by disrupting CypA-capsid interactions is dependent on the expression TRIM5α_{hu}. These results suggested that TRIM5$\alpha_{\eta\upsilon}$ is restricting HIV-1 infection upon disruption of CypA-capsid interactions. In agreement, we found that TRIM5α_{hu} binds to the HIV-1 core. Interestingly, TRIM5α_{hu} does not bind to HIV-1 cores bearing the capsid mutant A92E (HIV-1-A92E). Infection of HIV-1-A92E viruses was not affected by disruption of CypA-capsid interactions, which correlates with the loss of TRIM5α_{hu} binding to HIV-1 cores bearing the A92E mutation. These experiments suggested that CypA protects the HIV-1 core from TRIM5$\alpha_{\eta\upsilon.}$ Although the infectivity defect observed in Jurkat cells is small, disruption of CypA-interactions in PBMCs and $CD4^+$ T cells causes a major defect in infectivity. In agreement with our hypothesis that CypA is protecting the core from TRIM5$\alpha_{\eta\upsilon}$, infection of PBMCs and $CD4^+$ T cells by HIV-1-A92E viruses was insensitive to the disruption of CypA-capsid

interactions. Consistently with a block imposed by TRIM5α proteins, disruption of CypA-capsid interactions in $CD4^+$ T cells dramatically decreased the efficiency of reverse transcription. Overall, our investigations suggested a model in which CypA binding to the HIV-1 core protects the core from the restriction factor TRIM5α_{hu}.

RESULTS

HIV-1 infection of Jurkat cells is sensitive to Cyclosporin A treatment.

To understand the role of CypA in HIV-1 infection of $CD4^+$ T cells, we used the human T-cell line Jurkat as an experimental model. Cyclosporin A (CsA) is a molecule that binds to CypA, and thus blocks cellular CypA activity. To measure the effect of CsA treatment on HIV-1 infectivity, we infected Jurkat cells with HIV-1 tagged with green fluorescent protein (HIV-1-GFP) in the presence of 10 µM CsA. Consistent with results from previous studies (Braaten and Luban, 2001; Hatziioannou et al., 2005; Li et al., 2009; Ptak et al., 2008; Sokolskaja et al., 2004; Towers et al., 2003; Wainberg et al., 1988; Yin et al., 1998), HIV-1-GFP infection of human Jurkat T cells was sensitive to CsA treatment (Figure 1A, upper panel). Experiments were repeated multiple times and the fraction of GFP-positive cells normalized to the dimethyl sulfoxide (DMSO)-treated control is shown (Figure 1A, lower panel). To further confirm the importance of CypA-capsid interactions in HIV-1 infection, we challenged Jurkat T cells with p24-normalized amounts of HIV-1-P90A and HIV-1-G89V, which are mutant versions of HIV-1 that disrupt CypA-capsid interactions (Gamble et al., 1996; Thali et al., 1994). Infection was determined by measuring the percentage of GFP-positive cells at 48 h post-infection. We found that the mutant HIV-1 strains bearing capsid changes that disrupted CypA-capsid interactions were less infectious than wild-type HIV-1 (Figure 1B, upper panel). Experiments were repeated multiple times and the fraction of GFP-positive cells normalized to control is shown

(Figure 1B, lower panel). Although Jurkat cells failed to show a dramatic decrease in HIV-1 infectivity as a result of disrupted CypA-capsid interactions, we still used this cell line as a model to mechanistically understand the contribution of CypA to HIV-1 infection.

Human TRIM5α (TRIM5α_{hu}) binds to the HIV-1 capsid.

Previous studies have shown that CypA modulates the ability of rhesus tripartite motif (TRIM) protein, TRIM5α (TRIM5α_{rh}), to restrict HIV-1 infection (Berthoux et al., 2005; Burse et al., 2017; Keckesova et al., 2006; Lin and Emerman, 2008; Stremlau et al., 2006b), which is not surprising because both proteins interact with the HIV-1 core. It is well known that TRIM5α_{rh} cages the HIV-1 core using cooperative binding during infection (Diaz-Griffero et al., 2009; Ganser-Pornillos et al., 2011; Stremlau et al., 2006a). Because both CypA and TRIM5α_{rh} bind to the HIV-1 core, it is possible that CypA modulates the binding of TRIM5α_{rh} to the HIV-1 core and vice versa.

More recent studies have suggested that TRIM5α_{hu} blocks HIV-1 infection (Jimenez-Guardeno et al., 2019; OhAinle et al., 2018), possibly by binding to the HIV-1 core. Therefore, we tested the ability of TRIM5α_{hu} to bind to the HIV-1 core using our capsid binding assay (Selyutina et al., 2018). We found that TRIM5α_{hu} binds to the HIV-1 core (Figure 2). TRIM5α_{hu} showed a stronger interaction with the HIV-1 core compared with TRIM5α_{rh} (Figure 2). Our results showed for the first time that TRIM5α_{hu} interacted with the HIV-1 core. One

possibility is that CypA modulates the ability of TRIM5α TRIM5α_{hu} to interact with the HIV-1 core.

TRIM5α_{hu} expression is necessary for the inhibition of HIV-1 infection mediated by the disruption of CypA-capsid interactions.

Because of the ability of TRIM5α_{hu} to bind to the HIV-1 core, we tested whether TRIM5α_{hu} is involved in the blocking of HIV-1 infection mediated by the disruption of CypA-capsid interactions. For this purpose, we achieved knockdown (KD) of TRIM5α_{hu} expression in Jurkat cells using a specific short hairpin RNA (shRNA) against TRIM5α_{hu} (Figure 3), as described in Materials and Methods. To test for TRIM5α_{hu} expression, we challenged different sets of TRIM5α_{hu} KD cells with increasing amounts of N-tropic murine leukemia virus GFP (N-MLV-GFP), which is restricted by TRIM5α_{hu}. We found that Jurkat TRIM5α_{hu} KD cells were susceptible to N-MLV infection (Figure 3A), whereas in shRNA control cells, N-MLV-GFP infection was blocked. As a control, we also showed that TRIM5α_{hu} KD cells were susceptible to B-tropic murine leukemia virus GFP (B-MLV-GFP), which is not restricted by TRIM5α_{hu} (Figure 3A). These results confirmed that KD of TRIM5α_{hu} expression was successfully achieved.

Next, we assessed the infection phenotype observed in Jurkat TRIM5α_{hu} KD cells when CypA-capsid interactions are disrupted (Figure 3B). Surprisingly, TRIM5α_{hu} KD cells treated with CsA did not show marked inhibition of HIV-1 infection compared with control cells (Figure 3B), suggesting that TRIM5α_{hu} is involved in the inhibition of viral infection observed in CsA-treated cells that have disrupted CypA-capsid interactions.

HIV-1-P90A is a mutant form of HIV-1 in which CypA-capsid interactions are disrupted during infection. We tested whether HIV-1-P90A infection was inhibited in TRIM5α_{hu} KD Jurkat cells. As shown in Figure 3C, we found that HIV-1-P90A infection was not inhibited in TRIM5α_{hu} KD cells compared with control cells. This result supports our hypothesis that expression of TRIM5α_{hu} is important for the infection phenotype observed when CypA-capsid interactions are disrupted. It is possible that CypA prevents the binding of TRIM5α_{hu} to the HIV-1 core via steric hindrance, thus protecting the core from the restriction factor during the early steps of infection.

CypA prevents the interaction of TRIM5α_{hu} with the HIV-1 core.

To test whether CypA modulates the binding of TRIM5α_{hu} to the HIV-1 core, we analyzed the binding of TRIM5α_{hu} to the HIV-1 core in the presence of CsA, which binds to endogenously expressed CypA in cellular extracts and prevents CypA binding to the HIV-1 core. As shown in Figure 4A, TRIM5α_{hu} binding to the HIV-1 core increased 3- to 4-fold in the presence of CsA. We also found that the binding of TRIM5α_{hu} to mutant P90A HIV-1 cores was 5- to 6-fold higher than that of wild-type HIV-1 cores (Figure 4A) in the absence or presence of CsA. These results suggested that CypA prevents the binding of TRIM5α_{hu} to the HIV-1 core during infection. It is possible that CypA and TRIM5α_{hu} compete with each other to interact with the incoming core. To test this hypothesis, we investigated whether the addition of exogenous CypA decreased TRIM5α_{hu} binding to the HIV-1 core. For this purpose, we assessed the ability of TRIM5α_{hu} from cellular

extracts to bind to the HIV-1 core in the presence of 10 µg of recombinant CypA protein. We found that the addition of recombinant CypA significantly decreased the binding of TRIM5α_{hu} to the HIV-1 core (Figure 4B). As expected, recombinant CypA did not affect the binding of TRIM5α_{hu} to HIV-1 cores bearing the P90A mutation. Overall, these results suggested that CypA protects the core from the restriction factor TRIM5α_{hu}.

Mutant HIV-1 strains that are impaired for capsid-TRIM5α_{hu} interactions are insensitive to CsA treatment.

Previous studies have shown that infection of Jurkat cells by the HIV-1 strain bearing the capsid mutation A92E (HIV-1-A92E-GFP) was less sensitive to CsA treatment when compared with wild-type HIV-1 (Hatziioannou et al., 2005; Sokolskaja et al., 2004). Consistent with these findings, we observed that HIV-1-A92E infection of Jurkat cells was insensitive to CsA treatment (Figure 5A). This insensitivity may occur due to one or both of the following scenarios: 1) HIV-1-A92E may have a higher affinity for CypA, which may better protect the virus from TRIM5α_{hu}, and 2) HIV-1-A92E may have a lower affinity for TRIM5α_{hu}, which may prevent inhibition of infection. To explore these possibilities, we tested the ability of CypA to interact with HIV-1 cores containing the A92E capsid mutation. We found that CypA did not bind to the mutant A92E core, which suggested no or poor interaction between HIV-1-A92E and CypA during infection (Figure 5B). As a control, we showed that the binding of CypA to wild-type HIV-1 cores was CsA-sensitive (Figure 5B). Because CypA does not bind to HIV-1-

261 A92E, a stronger binding between HIV-1-A92E and TRIM5α_{hu} may result in
262 inhibition of infection. However, we found that the infection of Jurkat cells by HIV-
263 1-A92E was not affected when compared with that of wild-type HIV-1 (Figure
264 5A), suggesting that HIV-1-A92E may be unable to interact with TRIM5α_{hu}.
265 Consistent with our hypothesis, TRIM5α_{hu} did not interact with mutant HIV-1
266 cores bearing the capsid change A92E (Figure 5C). Overall, these results
267 indicated that mutant HIV-1 A92E cores bind neither CypA nor TRIM5$\alpha_{hu,}$ which
268 may explain the reason that CsA treatment does not affect HIV-1-A92E infection
269 of Jurkat cells.
270
271 **CypA-capsid interactions are required for HIV-1 infection of human PBMCs**
272 **and CD4$^+$ T cells.**
273 To understand the role of CypA in HIV-1 infection of human primary
274 PBMCs, we challenged PBMCs and CD4$^+$ T cells from different donors with HIV-
275 1 in the presence of increasing concentrations of CsA, which prevents the
276 binding of CypA to HIV-1 capsid (Diaz-Griffero et al., 2006; Luban et al., 1993).
277 As shown in Figure S1, CsA treatment inhibited HIV-1 infection in PBMCs and
278 CD4$^+$ T cells. This result suggested that CypA-capsid interactions are required
279 for HIV-1 infection of human PBMCs and CD4$^+$ T cells. To further corroborate
280 these findings, we challenged human PBMCs and CD4$^+$ T cells with increasing
281 amounts of HIV-1 particles bearing the capsid mutations P90A and G89V, both of
282 which prevent CypA-capsid interactions (Gamble et al., 1996; Thali et al., 1994).
283 Infection was determined by measuring the percentage of GFP-positive cells at

48 h post-infection. Wild-type and mutant HIV-1-GFP viruses were normalized by quantifying the levels of p24. We found that HIV-1-P90A and HIV-1-G89V were unable to infect human PBMCs and $CD4^+$ T cells (Figure 6A and B). Although this experiment was performed in PBMCs and $CD4^+$ T cells obtained from six different donors, all of which showed the same results, we have only shown sample results obtained from three donors. Our results suggested that CypA-capsid interactions are required for HIV-1 infection in human primary PBMCs and $CD4^+$ T cells. Importantly, the lack of CypA appeared to have a stronger effect on HIV-1 infection in primary human lymphocytes compared with that in Jurkat cells (Figures 1B and 6A&B), thus indicating that CypA was crucial for HIV-1 infection of human primary cells. Overall, these results suggested that the disruption of CypA-capsid interaction is detrimental for HIV-1 infection of PBMCs and $CD4^+$ T cells.

Disruption of HIV-1 binding to TRIM5α_{hu} does not affect viral infection in human PBMCs and $CD4^+$ T cells.

Mutant HIV-1 bearing the capsid change A92E lacks the ability to interact with TRIM5α_{hu}. We examined the ability of HIV-1-A92E to infect PBMCs and $CD4^+$ T cells to study the role of TRIM5α_{hu} during viral infection in these cells. Consistent with the results observed in Jurkat cells, HIV-1-A92E infection levels were comparable to that of wild-type HIV-1 in human PBMCs (Figure 7A). As a control, we also challenged PBMCs with increasing concentrations of HIV-1-P90A, an HIV-1 mutant that is completely inhibited in PBMCs (Figure 7A). We

observed similar results when we challenged $CD4^+$ T cells using HIV-1-A92E (Figure 7B). Consistent with our previous binding experiments, we found that HIV-1-A92E infection of PBMCs and $CD4^+$ T cells was not affected by TRIM5α_{hu}, which correlated with the inability of TRIM5α_{hu} to bind to mutant HIV-1-A92E cores.

Depletion of CypA expression in human $CD4^+$ T cells inhibits HIV-1 infection.

We have previously shown that CypA-capsid interactions are important for HIV-1 infection. To directly test the role of CypA in viral infection, we challenged CypA knockout $CD4^+$ T cells with HIV-1_{NL4-3}-GFP. To create CypA knockouts, $CD4^+$ T cells were separately electroporated with CRISPR/Cas9 complexes containing five different guides against CypA (CypA-KO#1–5). Expression of CypA was analyzed by western blotting using anti-CypA antibodies at 3 days post-electroporation. As shown in Figure 8A, all five guides were able to eliminate the expression of CypA. The CypA knockout cells were then challenged with HIV-1_{NL4-3}-GFP. Consistent with our previous results, human $CD4^+$ T cells depleted for CypA expression were resistant to HIV-1 infection (the results from two donors are shown in Figure 8B). As a control, we used a CRISPR/Cas9 complex containing an RNA guide for CXCR4, which completely inhibits infection of HIV-1_{NL4-3}-GFP. We also used a CRISPR/Cas9 complex containing a non-targeting RNA guide. These results demonstrated that CypA expression is essential for HIV-1 infection in human primary $CD4^+$ T cells.

CypA-capsid interactions are required for efficient HIV-1 reverse transcription in human CD4$^+$ T cells.

Our analysis showed that HIV-1 strains bearing capsid mutations that disrupt CypA-capsid interactions are unable to infect human CD4$^+$ T cells. To identify the step in the HIV-1 life cycle at which infection is disrupted, we challenged human CD4$^+$ T cells with p24-normalized levels of GFP expressed by wild-type and capsid mutant HIV-1 particles, and measured reverse transcription, nuclear import, and integration using real-time polymerase chain reaction (PCR) (Figure 9). Infection was measured by determining the percentage of GFP-positive cells at 72 h post-infection (Figure 9A). Consistent with our previous results, HIV-1-P90A and HIV-1-G89V strains showed weak infection in CD4$^+$ T cells (Figure 9A). Reverse transcription was measured by determining the levels of late reverse transcripts (LRT) at 10 h post-infection (Figure 9B). Interestingly, reverse transcription was reduced in HIV-1-P90A and HIV-1-G89V, suggesting that CypA-capsid interactions were important for HIV-1 reverse transcription in CD4$^+$ T cells. As a control, we used Nevirapine (Nev), which inhibits reverse transcription (Figure 9B). For nuclear import, we measured the levels of 2-long terminal repeat (2-LTR) circles at 24 h post-infection (Figure 9C). Consistent with a reverse transcription block, our results showed that both viruses were blocked for nuclear import. Viral integration was determined using Alu-PCR at 48 h post-infection (Figure 9D). As a control, the integration inhibitor Raltegravir (Ral) was also used. As expected, the integration of HIV-1-P90A and HIV-1-G89V was

blocked. Overall, these results suggested that HIV-1 requires interaction with CypA in order to undergo efficient reverse transcription in human $CD4^+$ T cells. These results also suggested that in the absence of CypA, HIV-1 particles are exposed to TRIM5$\alpha_{\eta\upsilon}$, which inhibits reverse transcription.

DISCUSSION

A few years after the discovery of HIV-1 capsid interaction with CypA (Luban et al., 1993), it was established that CsA treatment disrupted this interaction, which then affected HIV-1 infectivity. It was soon realized that CsA exerted varying effects on HIV-1 infection depending upon the human cell lines used. Although CypA-capsid interactions have been studied for over 25 years, the mechanism by which CsA affects HIV-1 infection remains elusive. Here, we explored the role of CypA-capsid interactions in human lymphocytes.

Several recent studies have suggested that TRIM5α_{hu} may function as an HIV-1 restriction factor (Jimenez-Guardeno et al., 2019; OhAinle et al., 2018). This led us to hypothesize that TRIM5α_{hu} binds to the HIV-1 core, which was proven by our results. Remarkably, we observed that TRIM5α_{hu} showed stronger binding to the HIV-1 core than TRIM5α_{rh}. Because TRIM5α_{hu} and CypA both bind to the HIV-1 core, it is possible that these two factors compete for the HIV-1 core during infection—CypA may modulate the binding of TRIM5α_{hu} to the HIV-1 core or vice versa. We showed that the depletion of TRIM5α_{hu} expression rescued the HIV-1 infectivity phenotype in Jurkat cells caused by the disruption of CypA-capsid interactions. In cellular extracts, we also observed that CsA treatment increased binding of TRIM5α_{hu} to the HIV-1 core. These results were consistent with our hypothesis that CypA modulates the binding of TRIM5α_{hu} to the HIV-1 core. The results of the binding experiments suggested that CypA sterically hinders the binding of TRIM5α_{hu} to the HIV-1 core, which could explain the

observed infectivity phenotype when CypA-capsid interactions were disrupted in lymphocytes. Conversely, we also demonstrated that increasing the amounts of CypA prevented the binding of TRIM5α_{hu} to the HIV-1 core. Based on these results, we propose that the prevention of CypA binding allows TRIM5α_{hu} to bind to the HIV-1 core, consequently inhibiting infection.

Our results indicated that the interaction of TRIM5α_{hu} with the HIV-1 core leads to restriction of infection. However, several previous studies have shown that overexpression of TRIM5α_{hu} has little or no effect on HIV-1 infection (Yap et al., 2005)(Li et al., 2006; Pham et al., 2010). One possibility is that restriction of HIV-1 infection by TRIM5α_{hu} requires a specific cellular cofactor expressed only in Jurkat cells and human primary lymphocytes. This cofactor may have been absent or present only in insufficient amounts in other human cell lines that were used for TRIM5α_{hu} overexpression experiments. Alternatively, TRIM5α_{hu} might be recruited to the HIV-1 core by other TRIM proteins through the formation of higher-order complexes (Diaz-Griffero et al., 2009). TRIM5α dimers are known to associate with other dimers and form higher-order self-association complexes. For example, TRIM5α_{rh} forms higher-order complexes with human TRIM34 and TRIM6 (Li et al., 2011). Alternatively, TRIM5α_{hu} might recruit other human TRIM orthologs that are important for restriction. Future studies will explore the existence of other human TRIM orthologs that may form higher-order complexes with TRIM5α_{hu}.

Mutations in the CypA-binding loop of the capsid, such as P90A and G89V, cause an infectivity defect similar to that caused by CsA treatment during

422 wild-type HIV-1 infection of Jurkat cells; however, infection of HIV-1 viruses
423 bearing the capsid change A92E are insensitive to CsA treatment in Jurkat cells
424 (Hatziioannou et al., 2005; Sokolskaja et al., 2004). Here, we showed that this
425 insensitivity was due to the lack of TRIM5α_{hu} binding to HIV-1-A92E mutant
426 cores. The loss of viral infectivity correlated with the inability of the HIV-1-A92E
427 core to bind to TRIM5α_{hu}. Additionally, the HIV-1-A92E mutant core also did not
428 interact with CypA, indicating that the A92E mutation abolishes interaction of the
429 HIV-1 core with both TRIM5α_{hu} and CypA. Based on these results, it is possible
430 that TRIM5α_{hu} binds to the CypA-binding loop or in its proximity.
431 The disruption of CypA-capsid interactions had a stronger effect on viral
432 infectivity in human primary cells compared with Jurkat cells. Mutant HIV-1 (P90A
433 or G89V) infection of PBMCs or $CD4^+$ T cells was 10–20 fold weaker than that of
434 wild-type HIV-1. Similarly, KD of CypA expression in human primary $CD4^+$ T cells
435 potently blocked HIV-1 infection. In contrast, HIV-1-A92E infection of primary
436 lymphocytes was not affected when compared to that of wild-type HIV-1. Taken
437 together, these results suggested that TRIM5α_{hu} blocks HIV-1 infection of human
438 primary lymphocytes when CypA-capsid interactions are disrupted. Consistent
439 with this idea, we observed that the inhibition of infection caused by the
440 disruption of CypA-capsid interactions in $CD4^+$ T cells occurred prior to HIV-1
441 reverse transcription.
442 Our work has shown that CypA protects the core from TRIM5$\alpha_{hu.}$
443 Intriguingly, new- and old-world monkeys express a protein in which the TRIM
444 domain of TRIM5α and CypA are fused (Newman et al., 2008; Sayah et al.,

2004) (Brennan et al., 2008). Based on our results, it is possible that evolution may have taken advantage of a positive factor, in this case CypA, and fused to the TRIM domain to develop a restriction factor.

Overall, this work showed the mechanism by which disruption of CypA-capsid interactions affect HIV-1 infection of human primary lymphocytes. Future experiments will explore whether a similar mechanism operates in macrophages or dendritic cells.

MATERIALS AND METHODS

Cell lines and drugs

Human Jurkat lymphocytes cells obtained from the American type culture collection (ATCC) were grown at 37°C in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Human 293T (ATCC) cells were grown at 37°C in Dulbecco's modified eagle medium (DMEM) supplemented with 10% FBS and 1% penicillin-streptomycin. Cyclosporin A (CsA) was dissolved in dimethyl sulfoxide (DMSO) to create a stock of 10 mM.

Infection using HIV-1-GFP reporter viruses

Recombinant human immunodeficiency virus-1 (HIV-1) strains such as HIV-1-G89V, HIV-1-P90A, and HIV-1-A92E expressing GFP were prepared as previously described (Diaz-Griffero et al., 2008). Viral challenges were performed in 24-well plates by infecting 50,000 cells (Jurkat, PBMCs, or $CD4^+$ T cells) per well. Infection was determined by measuring the percentage of GFP-positive cells using flow cytometry (Becton Dickinson).

Capsid expression and purification

pET-11a vectors were used to express the HIV-1 capsid protein containing the A14C and E45C mutations. Point mutations P90A and A92E were introduced using the QuikChange II site-directed mutagenesis kit (Stratagene) according to

the manufacturer's instructions. All proteins were expressed in *Escherichia coli* one-shoot BL21star (DE3) cells (Invitrogen), as previously described (Selyutina et al., 2018). Briefly, cells were inoculated in Luria-Bertani (LB) medium and cultured at 30°C until mid-log phase (A_{600}, 0.6–0.8). Protein expression was induced with 1 mM isopropyl-β-d-thiogalactopyranoside (IPTG) overnight at 18°C. Cells were harvested by centrifugation at 5,000 × g for 10 min at 4°C, and pellets were stored at −80°C until purification. Purification of capsids was carried out as follows: pellets from 2 L of Luria containing bacteria were lysed by sonication (Qsonica microtip: 4420; A = 45; 2 min; 2 sec on; 2 sec off for 12 cycles), in 40 ml of lysis buffer (50 mM Tris pH = 8, 50 mM NaCl, 100 mM β-mercaptoethanol, and Complete EDTA-free protease inhibitor tablets). Cell debris was removed by centrifugation at 40,000 × g for 20 min at 4°C. Proteins from the supernatant were precipitated by incubation with one-third the volume of saturated ammonium sulfate containing 100 mM β-mercaptoethanol for 20 min at 4°C, and centrifugation at 8,000 × g for 20 min at 4°C. Precipitated proteins were resuspended in 30 ml of buffer A (25 mM 2-(N-morpholino) ethanesulfonic acid (MES) pH 6.5 and 100 mM β-mercaptoethanol) and sonicated 2–3 times (Qsonica microtip: 4420; A = 45; 2 min; 1 sec on; 2 sec off). The protein sample was dialyzed three times in buffer A (2 h, overnight, 2 h), sonicated, diluted in 500 ml of buffer A, and was chromatographed sequentially on a 5-ml HiTrap Q HP column and on a 5-ml HiTrap SP FF column (GE Healthcare), which were both pre-equilibrated with buffer A. The capsid protein was eluted from the HiTrap SP FF column using a linear gradient of concentrations ranging from 0–2

M NaCl. The eluted fraction that had highest protein levels was selected based on absorbance at 280 nm. Pooled fractions were dialyzed three times (2 h, overnight, 2 h) in storage buffer (25 mM MES, 2 M NaCl, 20 mM β-mercaptoethanol). The sample was reduced to a concentration of 20 mg/ml using Centricon and stored at □80°C.

Assembly of stabilized HIV-1 capsid tubes

One milliliter of monomeric capsid (3 mg/ml or 1 mg/ml) was dialyzed in SnakeSkin dialysis tubing 10K MWCO (Thermo Scientific) using a buffer that is high in salt and contains a reducing agent (buffer 1: 50 mM Tris, pH 8, 1 M NaCl, 100 mM β-mercaptoethanol) at 4°C for 8 h. Subsequently the protein was dialyzed using buffer 1 without the reducing agent β-mercaptoethanol (buffer 2: 50 mM Tris, pH 8, 1 M NaCl) at 4°C for 8 h. The absence of β-mercaptoethanol in the second dialysis allows the formation of disulfide bonds between Cysteine 14 and 45 inter-capsid monomers in the hexamer. Finally, the protein was dialyzed using buffer 3 (20 mM Tris, pH 8,0, 40 mM NaCl) at 4°C for 8 h. Assembled complexes were kept at 4°C for up to 1 month.

Capsid binding assay protocol. Human HEK293T cells were transfected for 24 h with a plasmid expressing the protein of interest (TRIM5$\alpha_{\eta\upsilon}$,TRIM5α_{rh}, or CypA). The cell medium was completely removed and cells were scraped off the plate and lysed in 300 µl of capsid binding buffer (CBB: 10 mM Tris, pH 8,0, 1,5 mM $MgCl_2$, 10 mM KCl). Cells were rotated at 4°C for 15 min and then

537 centrifuged to remove cellular debris (21,000 × g ,15 min, 4°C). Cell lysates were
538 incubated with stabilized HIV-1 capsid tubes for 1 h at 25°C. Subsequently, the
539 stabilized HIV-1 capsid tubes were centrifuged at 21,000 × g for 2 min. Pellets
540 were washed 2-3 times by resuspension and centrifugation in CBB or phosphate-
541 buffered saline (PBS). Pellets were resuspended in Laemmli buffer 1× and
542 analyzed by western blot using anti-p24 and the indicated antibodies.
543
544 **Preparation of PBMCs and $CD4^+$ T cells**
545 PBMCs from healthy donor whole blood were isolated by density gradient
546 centrifugation using Ficoll-Paque Plus (GE Health Care, #17-1440-02). 40 ml of
547 whole blood was centrifuged at 300 × g for 10 min, and the plasma layer was
548 removed and replaced with Hank's Balanced Salt solution (HBSS; Sigma Aldrich,
549 #H6648). The whole blood was then diluted to a ratio of 1:2 with HBSS with 20
550 ml of the diluted blood layered on top of 20 ml Ficoll-Paque Plus and centrifuged
551 at 300 × g for 30 min. The resulting whitish buffy coat layer was collected,
552 washed twice with HBSS, and resuspended in 10 RPMI medium containing 10%
553 (vol/vol) FBS and 1% (vol/vol) penicillin-streptomycin, and activated with IL-2
554 (100 U/ml) (Human IL-2; Cell Signaling Technology, #8907SF) and
555 phytohemagglutinin (1 µg/ml) for 3 days. $CD4^+$ T cells were obtained via negative
556 selection from PBMCs using a human $CD4^+$ T-cell isolation kit (MACS Miltenyi
557 Biotec, #130-096-533). Per 1×10^7 total cells, PBMCs were resuspended in 40 µl
558 $CD4^+$ T-cell isolation buffer (PBS, pH 7.2, 0.5% bovine serum albumin, and 2 mM
559 EDTA). Ten microliters of $CD4^+$ T-cell biotin-antibody cocktail was then added

560 and incubated at 4°C for 5 min. Thirty microliters of $CD4^+$ T-cell isolation buffer
561 and 20 µl of $CD4^+$ T-cell microbead cocktail were then added and further
562 incubated for 10 min at 4°C. Depending on the amount of PBMCs isolated, LS
563 Column (MACS Miltenyi Biotec, #130-042-401) or MS Column (MACS Miltenyi
564 Biotec, #130-042-201) attached to a MACS Separator was pre-washed with 3 ml
565 or 6 ml of ice-cold $CD4^+$ T-cell isolation buffer, respectively. PBMC suspension
566 was added to the column and the flow-through was collected in a 15-ml tube. The
567 LS or MS Columns were then washed with 3 ml or 6 ml of ice-cold $CD4^+$ T-cell
568 isolation buffer, respectively, and the flow-through was collected. The newly
569 isolated $CD4^+$ T cells were then centrifuged at 800 *x g* for 5 min and resuspended
570 in RPMI medium supplemented with IL-2 (100 U/ml).
571
572 **Knockdown of TRIM5$\alpha_{\eta\upsilon}$ expression in Jurkat cells**
573 Lentiviral particles were produced in human 293T cells by co-transfection
574 of 1 µg TAT, 1 µg REV, 1.5 µg VSVG, 1 µg HDM(codon optimized gag-pol), and
575 7.5 µg of the lentiviral vector pLKO1, or 7.5 µg the pLKO1 vector containing an
576 shRNA that targets TRIM5$\alpha_{\eta\upsilon}$ (pLKO1-shRNA-TRIM5$\alpha_{\eta\upsilon}$). Viruses were
577 harvested at 48 h post-transfection and used to transduce Jurkat cells for 5 days
578 at a 1:1 virus to complete media ratio. Transduced cells were selected in 0.15
579 µg/ml of puromycin. Cells that stably contained pLKO1 or pLKO1-shRNA-
580 TRIM5$\alpha_{\eta\upsilon}$ were maintained in puromycin. Expression of TRIM5$\alpha_{\eta\upsilon}$ was evaluated
581 by challenging the cells with N-tropic murine leukemia virus (N-MLV) and B-tropic
582 murine leukemia virus (B-MLV).

CRISPR-Cas9 RNP Production, CD4+ T Cell Isolation, and Electroporation

Detailed protocols for RNP production and primary CD4+ T cell editing have been previously published (Hultquist *et al. Nature Protocols* 2018). Briefly, lyophilized crRNA and tracrRNA (Dharmacon) were suspended at a concentration of 160 µM in 10 mM Tris-HCL (7.4 pH) with 150 mM KCl. 5µL of 160µM crRNA was mixed with 5µL of 160µM tracrRNA and incubated for 30 min at 37°C. The gRNA:tracrRNA complexes were then mixed gently with 10µL of 40µM Cas9 (UC-Berkeley Macrolab) to form Cas9 ribonucleoproteins (RNPs). Five 3.5µL aliquots were frozen in Lo-Bind 96-well V-bottom plates (E&K Scientific) at -80°C until use. All crRNA guide sequences used in this study were derived from the Dharmacon pre-designed Edit-R library for gene knock-out, including the non-targeting (U-007502, U-007501) and CYPA-targeting guides (CM-004979-01 through CM-004979-05).

Primary human CD4+ T cells from healthy donors were isolated from donated leukoreduction chambers after Trima Apheresis (Blood Centers of the Pacific). Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll centrifugation using SepMate tubes (STEMCELL, per manufacturer's instructions). Bulk CD4+ T cells were subsequently isolated from PBMCs by magnetic negative selection using an EasySep Human CD4+ T Cell Isolation Kit (STEMCELL, per manufacturer's instructions). Isolated CD4+ T cells were suspended in complete Roswell Park Memorial Institute (RPMI), consisting of RPMI-1640 (Sigma) supplemented with 5mM 4-(2-hydroxyethyl)-1-

piperazineethanesulfonic acid (HEPES, Corning), 2mM Glutamine (UCSF Cell Culture Facility), 50µg/mL penicillin/streptomycin (P/S, Corning), 5mM sodium pyruvate (Corning), and 10% fetal bovine serum (FBS, Gibco). Media was supplemented with 20 IU/mL IL-2 (Miltenyi) immediately before use. For activation, bulk CD4+ T cells were immediately plated on anti-CD3 coated plates [coated for 2 hours at 37°C with 20µg/mL anti-CD3 (UCHT1, Tonbo Biosciences)] in the presence of 5µg/mL soluble anti-CD28 (CD28.2, Tonbo Biosciences). Cells were stimulated for 72 hours at 37°C / 5% CO2 prior to electroporation.

Each electroporation reaction consisted of 4x10^5 T cells, 3.5 µL RNP, and 20 µL electroporation buffer. After three days of stimulation, cells were suspended and counted. RNPs were thawed and allowed to come to room-temperature. Immediately prior to electroporation, cells were centrifuged at 400xg for 5 minutes, supernatant was removed by aspiration, and the pellet was resuspended in 20 µL of room-temperature P3 electroporation buffer (Lonza) per reaction. 20 µL of cell suspension was then gently mixed with each RNP and aliquoted into a 96-well electroporation cuvette for nucleofection with the 4D 96-well shuttle unit (Lonza) using pulse code EH-115. Immediately after electroporation, 80 µL of pre-warmed media without IL-2 was added to each well and cells were allowed to rest for at least one hour in a 37°C cell culture incubator. Cells were subsequently moved to 96-well flat-bottomed culture plates pre-filled with 100 µL warm complete media with IL-2 at 40 IU/mL (for a final concentration of 20 IU/mL) and anti-CD3/anti-CD2/anti-CD28 beads (T cell Activation and Stimulation Kit, Miltenyi) at a 1:1 bead:cell ratio. Cells were

629 cultured at 37°C / 5% CO_2 in a dark, humidified cell culture incubator for 4 days
630 to allow for gene knock-out and protein clearance, with additional media added
631 on day 2. To check CYPA knock-out efficiency, 50 µL of mixed culture was
632 removed to a centrifuge tube. Cells were pelleted, supernatant was removed,
633 and pellets were resuspended in 100 µL 2.5x Laemmli Sample Buffer. Protein
634 lysates were heated to 98°C for 20 min before storage at -20°C until Western
635 blotting (below).
636
637 **Detection of Late Reverse Transcripts, 2-LTR circles, and integration in**
638 **$CD4^+$ T cells**
639 Total DNA was extracted from 1×10^6 infected cells. Samples were processed at
640 12, 36, and 66 h post-infection using QIAmp DNA micro kit (QIAGEN). Viral DNA
641 forms of wild-type and mutant HIV-1 were amplified using real-time PCR (Butler
642 et al., 2001; Valle-Casuso et al., 2012). β-actin amplification was used for
643 normalization. LRT, which refers to the whole synthesis of DNA by HIV-1, were
644 measured at 12 h post-infection. 2-LTR circles were measured at 36 h post-
645 infection to assess nuclear import. Provirus integration in cellular genome was
646 measured at 66 h post-infection using Alu-PCR. Reactions were performed in 1X
647 FastStart Universal Probe Master Mix (Rox) 2× (Roche) in 20 µl volume. The
648 PCR reaction consisted of the following steps: initial annealing (50°C 2 min),
649 denaturation step (95°C 15 min), 40 cycles of amplification (95°C 15 sec, 58°C
650 30 sec, 72°C 30 sec). Primer or probe sequences are as follows: LRT MH531: 5'-
651 TGTGTGCCCGTCTGTTGTGT-3'; MH532: 5'-GAGTCCTGCGTCGAGAGAGC-

652 3'; LRT-Probe: 5′-(FAM)-CAGTGGCGCCCGAACAGGGA-(TAMRA)-3'. 2-LTR
653 circles: MH535: 5'-AACTAGGGAACCCACTGCTTAAG-3'; MH536: 5′-
654 TCCACAGATCAAGGATATCTTGTC-3′; 2-LTR probe : MH603: 5'-(FAM)-
655 ACACTACTTGAAGCACTCAAG-GCAAGCTTT-(TAMRA)-3'. Alu-PCR 1st round :
656 Alu 1: 5'-TCCCAGCTACTCGGGAGGCTGAGG-3'; Alu 2: 5'-
657 GCCTCCCAAAGTGCTGGGATTACAG-3'; Lambda U3: 5′-
658 ATGCCACGTAAGCGAAACTTTCCGCTGGGGACTTTCCAGGG-3′. Alu
659 quantitative PCR 2nd round: Lambda: (5'-ATGCCACGTAAGCGAAACT-3'); U5:
660 (5'-CTGACTAAAAGGGTCTGAGG-3'; Probe: 5'-(FAM)-
661 TTAAGCCTCAATAAAGCTTGCCTTGAGTGC-(TAMRA). β-actin: FX: 5'-
662 AACACCCCAGCCATGTACGT-3', RX: 5-CGGTGAGGATCTTCATGAGGTAGT,
663 Actin-Probe: (6FAM)-CCAGCCAGGTCCAGACGCAGGA-(BBQ).
664
665 **Western blot analysis**.
666 Cells were lysed in lysis buffer (50 mM Tris [pH 8.0], 280 mM NaCl, 0.5%
667 IGEPAL 40, 10% glycerol, 5 mM $MgCl_2$). Proteins were detected by western blot
668 using anti-FLAG (1:1,000 dilution; Sigma), anti-hemagglutinin (HA) (1:1,000
669 dilution; Sigma), anti-CypA (1:5,000 dilution; catalog number ab58144; Abcam),
670 or anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:5,000; Ambion),
671 or anti-p24 (1:1,000, catalog number 183-H12-5C; NIH) antibodies. Secondary
672 antibodies against rabbit and mouse IgG conjugated to IRDye 680LT or IRDye
673 800CW were obtained from Li-Cor (1:10,000 dilution). Bands were detected by

scanning blots using the Li-Cor Odyssey imaging system in the 700-nm or 800-nm channels.

FIGURE LEGENDS

Figure 1. CypA is important for HIV-1 infection of Jurkat cells. (A) Human Jurkat cells were challenged with increasing amounts of HIV-1 expressing GFP as a reporter of infection (HIV-1-GFP) in the presence of 10 µM cyclosporin A (CsA) (upper panel). DMSO was used as control. Infection was determined at 48 h post-challenge by measuring the percentage of GFP-positive cells using a flow cytometer. Similar results were obtained from three independent experiments and a representative experiment is shown. The result of three independent experiments normalized to wild-type HIV-1-GFP infection with standard deviation is shown (lower panel). * indicates P-value < 0.005 as determined by the unpaired t-test. **(B)** Jurkat cells were challenged with increasing amounts of p24-normalized HIV-1, HIV-1-P90A, or HIV-1-G89V expressing GFP (upper panel). Infection was determined at 48 h post-challenge by measuring the percentage of GFP-positive cells using a flow cytometer. Similar results were obtained from three independent experiments and a representative experiment is shown. The results of three independent experiments normalized to wild-type HIV-1-GFP infection with standard deviation is shown (lower panel). * indicates P-value < 0.005, ns indicates not significant as determined by the Two-way ANOVA Tukey's multiple comparisons test.

Figure 2. TRIM5α$_{hu}$ binds to the HIV-1 core. Human 293T cells were transfected with plasmids expressing the wild-type human TRIM5α tagged with an HA epitope (TRIM5α$_{hu}$-HA). At 24 h post-transfection, cells were lysed in capsid binding buffer (CBB), as described in Materials and Methods. Inputs containing different amounts of TRIM5α$_{hu}$ were prepared (INPUT). Cell extracts containing different amounts of TRIM5α$_{hu}$-HA were mixed with 20 µl of stabilized HIV-1 capsid tubes (5 mg/ml) and incubated for 1 h at room temperature. Stabilized HIV-1 capsid tubes were collected by centrifugation and washed twice using CBB. Pellets were resuspended in 1× Laemmli buffer (BOUND). INPUT and BOUND fractions were analyzed by western blotting using anti-HA and anti-p24 antibodies. As control, similar experiments were performed using the capsid binder, rhesus TRIM5α tagged with an HA epitope (TRIM5α$_{rh}$-HA). Each experiment was repeated at least six times and a representative experiment is shown.

Figure 3. Expression of TRIM5α$_{hu}$ is necessary for the inhibition of HIV-1 infection caused by the disruption of CypA-capsid interactions. (A) Human Jurkat cells stably expressing a specific shRNA against TRIM5α$_{hu}$ (Jurkat-TRIM5α$_{hu}$-KD #1–4) or the empty vector (Jurkat-Control #4 and 5) were challenged using increasing amounts of N-MLV-GFP, which are restricted by TRIM5α$_{hu}$. Infection was determined at 48 h post-challenge by measuring the

percentage of GFP-positive cells using a flow cytometer. As a control, similar infections were performed using B-MLV-GFP viruses, which are not restricted by TRIM5α_{hu}. **(B)** Jurkat-TRIM5α_{hu}-knockdown (KD) and Jurkat-control cells were challenged with increasing amounts of HIV-1-GFP in the presence of cyclosporin A (CsA). Infection was determined at 48 h post-challenge by measuring the percentage of GFP-positive cells using a flow cytometer. As a control, similar experiments were performed using DMSO. **(C)** Jurkat-TRIM5α_{hu}-KD and Jurkat-control cells were challenged with increasing amounts of p24-normalized HIV-1-GFP or HIV-1-P90A-GFP. Infection was determined at 48 h post-challenge by measuring the percentage of GFP-positive cells using a flow cytometer. Each experiment was repeated at least three times and a representative experiment is shown.

Figure 4. CypA prevents the binding of TRIM5$\alpha_{\eta\upsilon}$ to the HIV-1 core. (A) Human 293T cells were transfected with plasmids expressing TRIM5α_{hu}-HA. At 24 h post-transfection, cells were lysed in capsid binding buffer (CBB) (INPUT). Cell extracts containing TRIM5α_{hu}-HA were mixed with 20 µl of stabilized wild-type or P90A HIV-1 capsid tubes (5 mg/ml) in the presence of 10 µM CsA. Mixtures were incubated for 1 h at room temperature. Stabilized HIV-1 capsid tubes were collected by centrifugation and washed twice using CBB. Pellets were resuspended in 1X Laemmli buffer (BOUND). INPUT and BOUND fractions were analyzed by western blotting using anti-HA, anti-CypA, and anti-p24 antibodies. Experiments were repeated three times and a representative experiment is

743 shown. The bound fraction relative to input for three independent experiments
744 with standard deviation is shown. * indicates P-value < 0.005, ns indicates not
745 significant as determined by the Two-way ANOVA Tukey's multiple comparisons
746 test. **(B)** Cell extracts containing TRIM5α_{hu}-HA were mixed with 20 µl of stabilized
747 wild-type or P90A HIV-1 capsid tubes (5 mg/ml) in the presence of 10 µg
748 recombinant CypA. Mixtures were incubated for 1 h at room temperature.
749 Stabilized HIV-1 capsid tubes were collected by centrifugation and washed twice
750 using CBB. Pellets were resuspended in 1× Laemmli buffer (BOUND). INPUT
751 and BOUND fractions were analyzed by western blotting using anti-HA, anti-
752 CypA, and anti-p24 antibodies. Experiments were repeated three times and a
753 representative experiment is shown. The bound fraction relative to input for three
754 independent experiments with standard deviation is shown. * indicates P-value <
755 0.005, ns indicates not significant as determined by the Two-way ANOVA
756 Tukey's multiple comparisons test.
757
758 **Figure 5. HIV-1 viruses bearing a capsid mutant that does not interact with**
759 **TRIM5α□u are insensitive to CsA. (A)** HIV-1-A92E infection of Jurkat cells.
760 Jurkat cells were challenged with increasing amounts of p24-normalized HIV-1-
761 GFP, HIV-1-P90A-GFP (left panel), or HIV-1-A92E-GFP (right panel) in the
762 presence of 10 µM Cyclosporin A (CsA). DMSO was used as a negative control.
763 Infection was determined at 48 h post-challenge by measuring the percentage of
764 GFP-positive cells using a flow cytometer. Experiments were repeated three
765 times and a representative experiment is shown. **(B)** Binding of CypA to HIV-1

766 cores bearing the capsid change A92E. Cell extracts from human 293T cells
767 containing CypA-V5 were mixed with 20 µl of stabilized wild-type or A92E HIV-1
768 capsid tubes (5 mg/ml) in the presence of 10 µM CsA. Mixtures were incubated
769 for 1 h at room temperature. Stabilized HIV-1 capsid tubes were collected by
770 centrifugation and washed twice using capsid binding buffer (CBB). Pellets were
771 resuspended in 1× Laemmli buffer (BOUND). INPUT and BOUND fractions were
772 analyzed by western blotting using anti-V5 and anti-p24 antibodies. Experiments
773 were repeated three times and a representative experiment is shown. The bound
774 fraction relative to input for three independent experiments with standard
775 deviation is shown. **(C)** Binding of TRIM5α_{hu} to HIV-1 cores bearing the capsid
776 change A92E. Similarly, cell extracts containing TRIM5α_{hu}-HA were mixed with
777 20 µl of stabilized wild-type or A92E HIV-1 capsid tubes (5 mg/ml) in the
778 presence of 10 µM CsA. Mixtures were incubated for 1 h at room temperature.
779 Stabilized HIV-1 capsid tubes were collected by centrifugation and washed twice
780 using CBB. Pellets were resuspended in 1× Laemmli buffer (BOUND). INPUT
781 and BOUND fractions were analyzed by western blot using anti-HA and anti-p24
782 antibodies. Experiments were repeated three times and a representative
783 experiment is shown. The bound fraction relative to input for three independent
784 experiments with standard deviation is shown.
785
786 **Figure 6. CypA-capsid Interactions are required for HIV-1 infection of**
787 **human peripheral blood mononuclear cells (PBMCs) and CD4$^+$ T cells. (A)**
788 PBMCs from two different donors were challenged with increasing amounts of

789 HIV-1-GFP, HIV-1-G89V-GFP, or HIV-1-P90A-GFP. Infection was determined at
790 72 h post-challenge by measuring the percentage of GFP-positive cells.
791 Experiments were repeated three times per donor and a representative
792 experiment is shown. **(B)** Purified $CD4^+$ T cells from two different donors were
793 challenged with increasing amounts of HIV-1-GFP, HIV-1-G89V-GFP, or HIV-1-
794 P90A-GFP. Infection was determined at 72 h post-challenge by measuring the
795 percentage of GFP-positive cells. Experiments were repeated three times per
796 donor and a representative experiment is shown.
797
798 **Figure 7. Infection of HIV-1 bearing the capsid change A92E, which does**
799 **not bind TRIM5$\alpha_{\eta\upsilon}$, is not affected in human PBMCs and $CD4^+$ T cells. (A)**
800 PBMCs from three different donors were challenged with increasing amounts of
801 HIV-1-GFP, HIV-1-P90A-GFP, or HIV-1-P92E-GFP. Infection was determined at
802 72 h post-challenge by measuring the percentage of GFP-positive cells.
803 Experiments were repeated three times per donor and a representative
804 experiment is shown. **(B)** $CD4^+$ T cells from three donors were challenged with
805 increasing amounts of HIV-1-GFP, HIV-1-P90A-GFP, or HIV-1-P92E-GFP.
806 Infection was determined at 72 h post-challenge by measuring the percentage of
807 GFP-positive cells. Experiments were repeated three times per donor and a
808 representative experiment is shown.
809
810 **Figure 8. Depletion of CypA expression in human $CD4^+$ T cells inhibits HIV-**
811 **1 infection. (A)** Human primary $CD4^+$ T cells from two different donors were

knocked-out for CypA expression using the CRISPR-Cas9 system, as described in methods. Briefly, $CD4^+$ T cells were electroporated using five different guide RNAs (gRNAs) against CypA (gRNA#1-5) together with the Cas9 protein. At 72 h post-electroporation, endogenous expression of CypA in $CD4^+$ T was analyzed by western blotting using antibodies against CypA. As controls, we electroporated a gRNA against CXCR4 and a non-targeting gRNA. Expression of GAPDH was used as a loading control. Similar results were obtained using two different donors and a representative blot is shown. **(B)** Human primary $CD4^+$ T cells depleted for CypA expression were challenged with a replication-competent HIV-1 expressing GFP as a reporter of infection (HIV-1_{NL4-3}-GFP). Infection was determined at 72 h post-infection by measuring the percentage of GFP-positive cells. Experiments were performed in triplicates, and results for two different donors with standard deviation are shown.

Figure 9. CypA-capsid interactions are required for HIV-1 reverse transcription in human $CD4^+$ T cells. Human primary $CD4^+$ T cells were challenged with DNase-pretreated HIV-1-GFP, HIV-1-P90A-GFP, or HIV-1-G89V-GFP at a multiplicity of infection (MOI) < 1.0. Infection was determined by measuring the percentage of GFP-positive cells using flow cytometry at 72 h post-challenge **(A)**. In parallel, total DNA was extracted from similar infections at 12, 36, and 72 h post-infection. The DNA samples collected at 12 h post-infection were used to determine the levels of late reverse transcripts (LRTs) using real-time PCR **(B)**, as described in methods. As a control, we inhibited reverse

transcription by using 10 µm of Nevirapine (Nev). HIV-1 2-LTR circles, which is an indirect measure of nuclear import, were quantified using real-time PCR in DNA samples collected 36 h post-infection **(C)**, as described in methods. Integrated proviruses were measured by determining the amount of Alu-PCR (Alu) products using real-time PCR in DNA samples collected at 72 h post-infection **(D)**, as described in methods. As a control, we inhibited proviral integration by using 10 µM Raltegravir (Ral). LRT, 2-LTR, and Alu products were normalized to actin. Results were analyzed using the unpaired t-test. Differences were considered statistically significant at $P < 0.01$ (**), $P < 0.001$ (***), $P < 0.0001$ (****).

Figure S1. Inhibition of CypA-capsid interactions with CsA restricts HIV-1 infection in PBMCs and $CD4^+$ T cells. (A) PBMCs from three different donors were challenged with HIV-1-GFP in the presence of increasing concentrations of CsA. DMSO was used as a control. Infection was determined at 72 h post-challenge by measuring the percentage of GFP-positive cells. **(B)** $CD4^+$ T cells from two different donors were challenged with HIV-1-GFP in the presence of 10 µM CsA. DMSO was used as a control. Infection was determined at 72 h post-challenge by measuring the percentage of GFP-positive cells.

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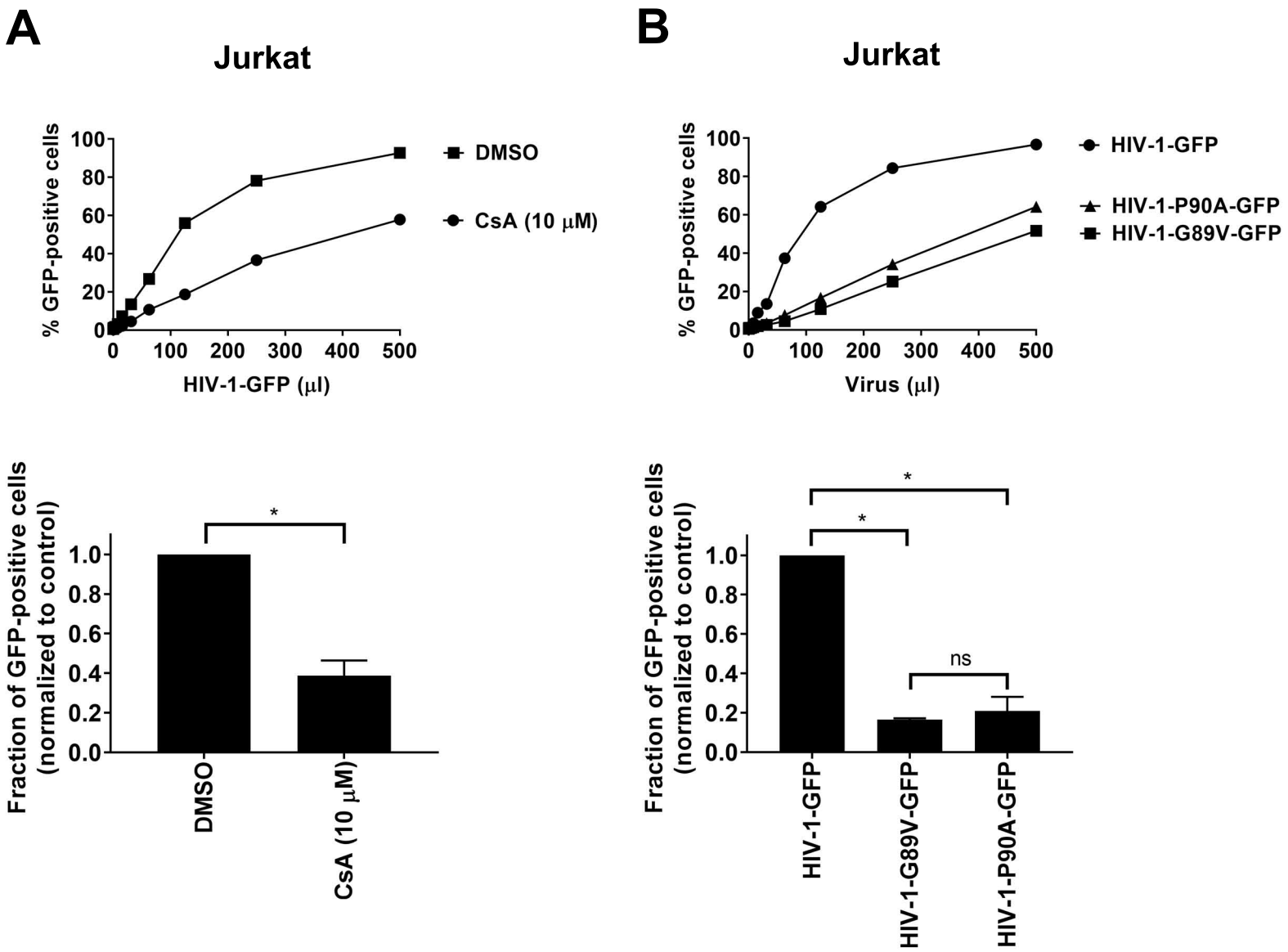

A
Jurkat
% GFP-positive cells
HIV-1-GFP (μl)
DMSO
CsA (10 μM)
Fraction of GFP-positive cells (normalized to control)
*
DMSO
CsA (10 μM)
B
Jurkat
% GFP-positive cells
Virus (μl)
HIV-1-GFP
HIV-1-P90A-GFP
HIV-1-G89V-GFP
Fraction of GFP-positive cells (normalized to control)
*
*
ns
HIV-1-GFP
HIV-1-G89V-GFP
HIV-1-P90A-GFP

Figure 1

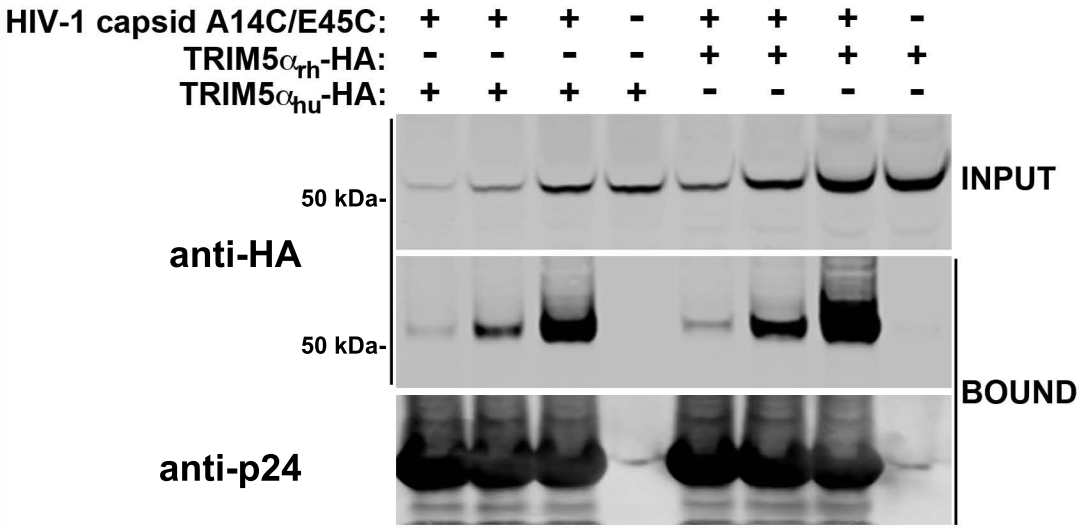

HIV-1 capsid A14C/E45C: + + + - + + + -
TRIM5αrh-HA: - - - - + + + +
TRIM5αhu-HA: + + + + - - - -
50 kDa-
INPUT
anti-HA
50 kDa-
BOUND
anti-p24

Figure 2

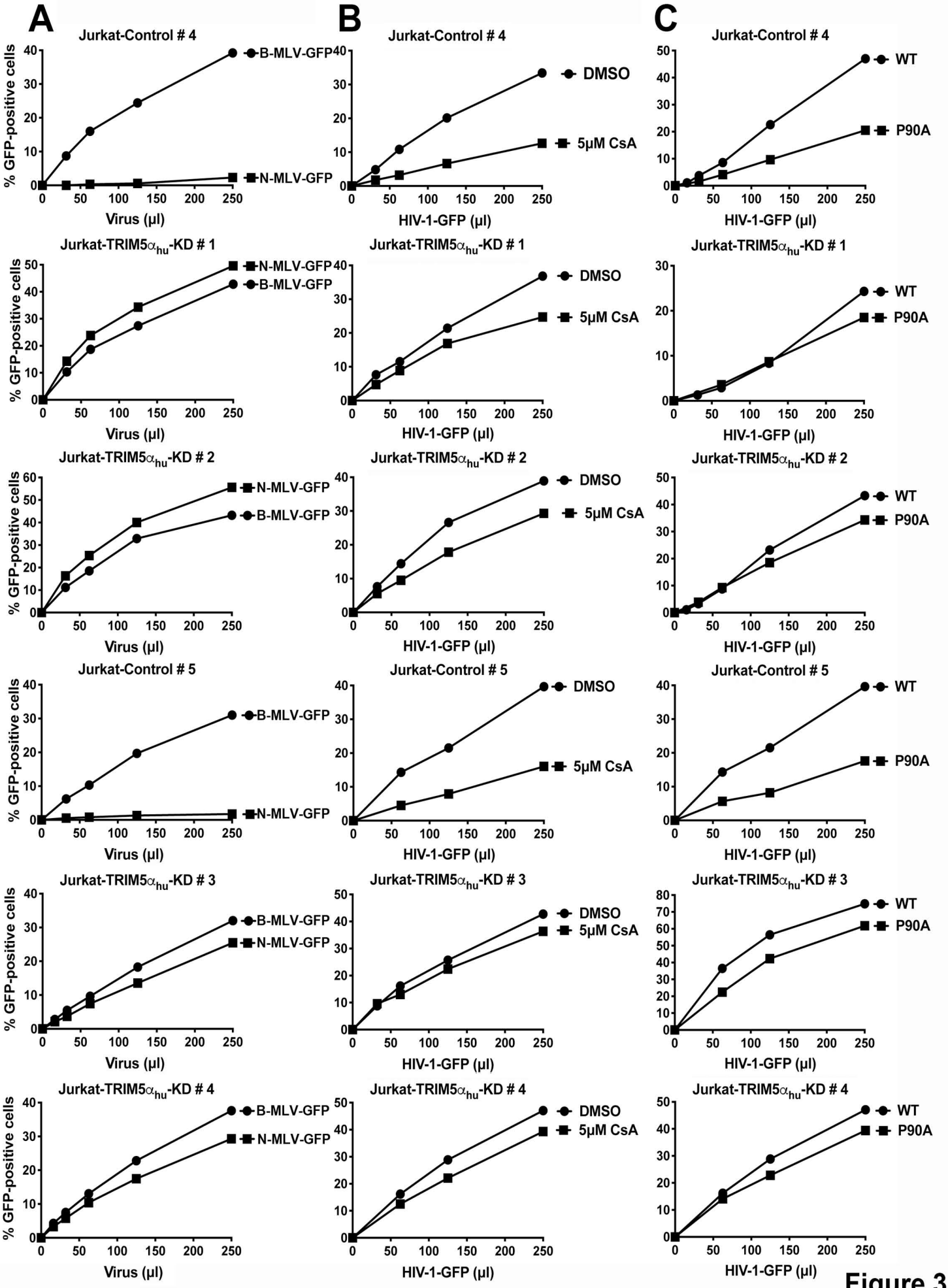

A
B
C
Jurkat-Control # 4
Jurkat-TRIM5αhu-KD # 1
Jurkat-TRIM5αhu-KD # 2
Jurkat-Control # 5
Jurkat-TRIM5αhu-KD # 3
Jurkat-TRIM5αhu-KD # 4
% GFP-positive cells
Virus (µl)
HIV-1-GFP (µl)
B-MLV-GFP
N-MLV-GFP
DMSO
5µM CsA
WT
P90A

Figure 3

A

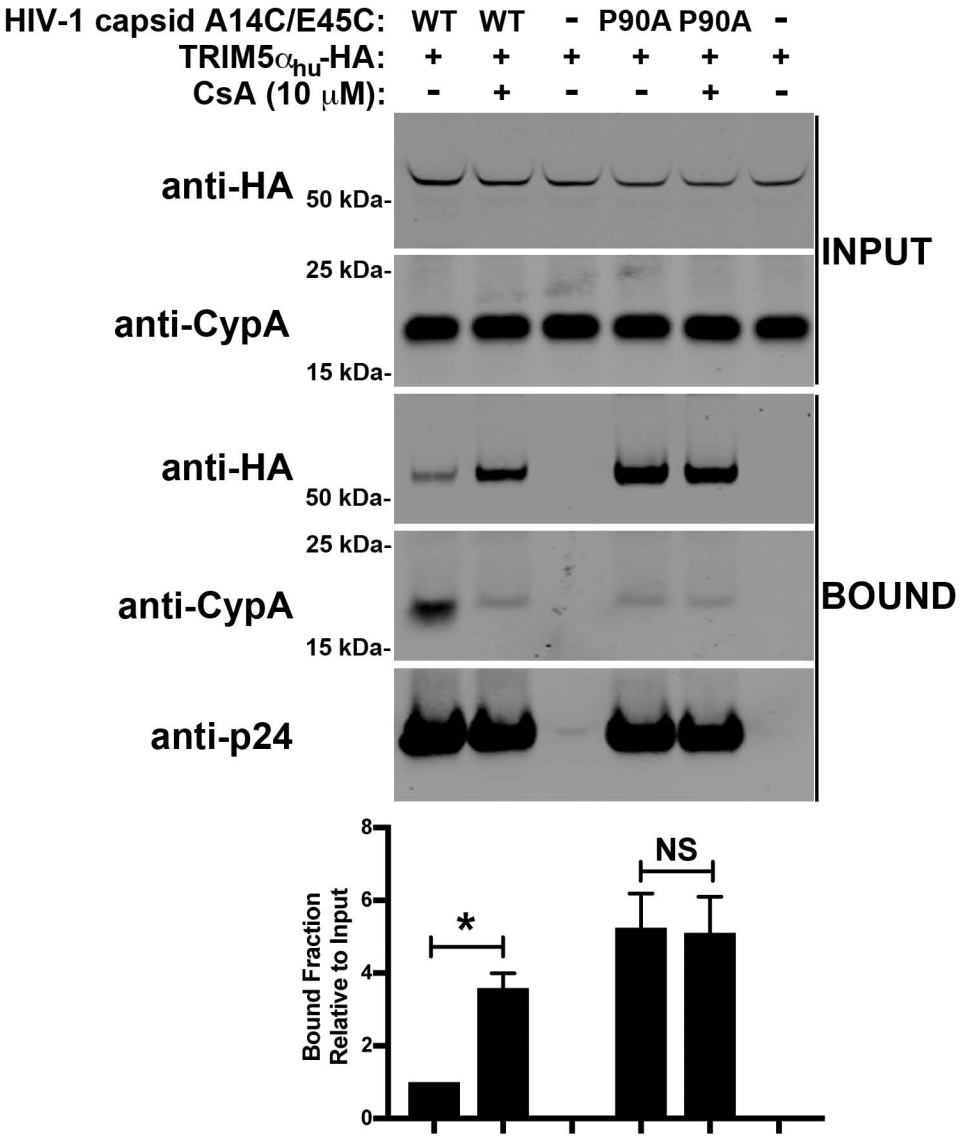

HIV-1 capsid A14C/E45C: WT WT - P90A P90A -
TRIM5αhu-HA: + + + + + +
CsA (10 μM): - + - - + -
anti-HA
50 kDa-
25 kDa-
anti-CypA
15 kDa-
INPUT
anti-HA
50 kDa-
25 kDa-
anti-CypA
15 kDa-
BOUND
anti-p24
Bound Fraction Relative to Input
0
2
4
6
8
*
NS

Figure 4

B

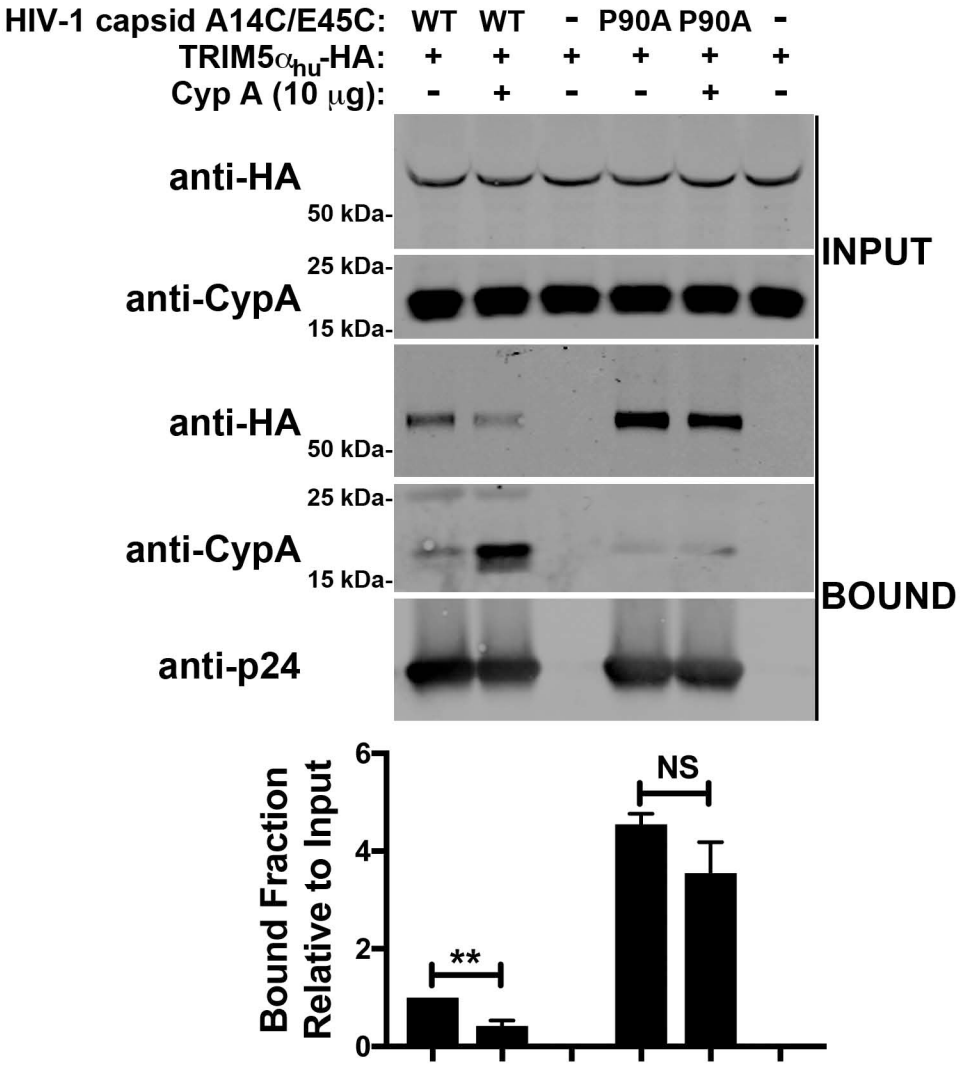

HIV-1 capsid A14C/E45C: WT WT - P90A P90A -
TRIM5αhu-HA: + + + + + +
Cyp A (10 μg): - + - - + -
anti-HA
50 kDa-
25 kDa-
anti-CypA
15 kDa-
INPUT
anti-HA
50 kDa-
25 kDa-
anti-CypA
15 kDa-
BOUND
anti-p24
Bound Fraction Relative to Input
6
4
2
0
**
NS

Figure 4

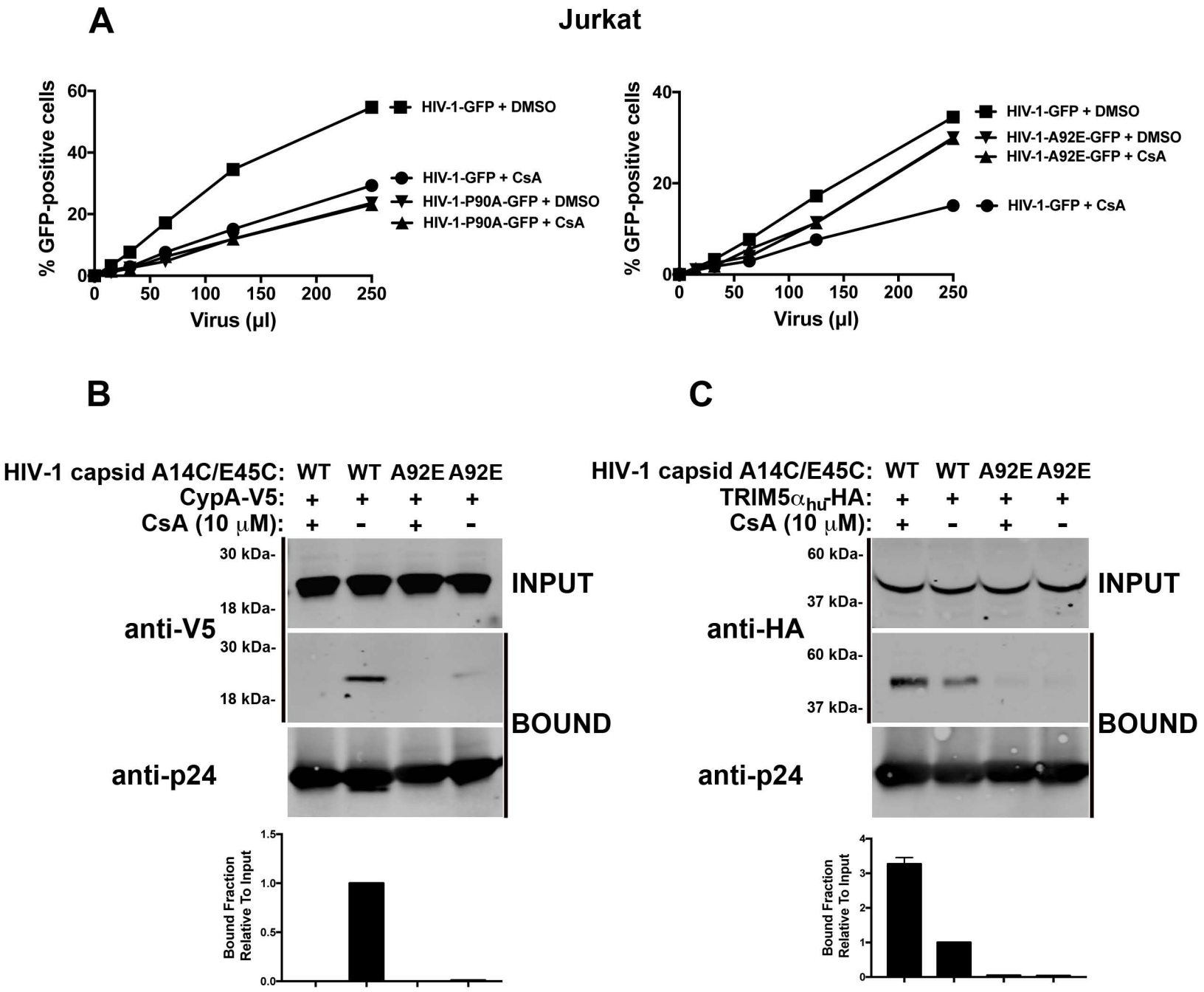

A
Jurkat
% GFP-positive cells
Virus (µl)
HIV-1-GFP + DMSO
HIV-1-GFP + CsA
HIV-1-P90A-GFP + DMSO
HIV-1-P90A-GFP + CsA
HIV-1-A92E-GFP + DMSO
HIV-1-A92E-GFP + CsA
B
C
HIV-1 capsid A14C/E45C: WT WT A92E A92E
CypA-V5: + + + +
CsA (10 µM): + - + -
TRIM5αhu-HA: + + + +
anti-V5
anti-HA
anti-p24
INPUT
BOUND
30 kDa-
18 kDa-
60 kDa-
37 kDa-
Bound Fraction Relative To Input

Figure 5

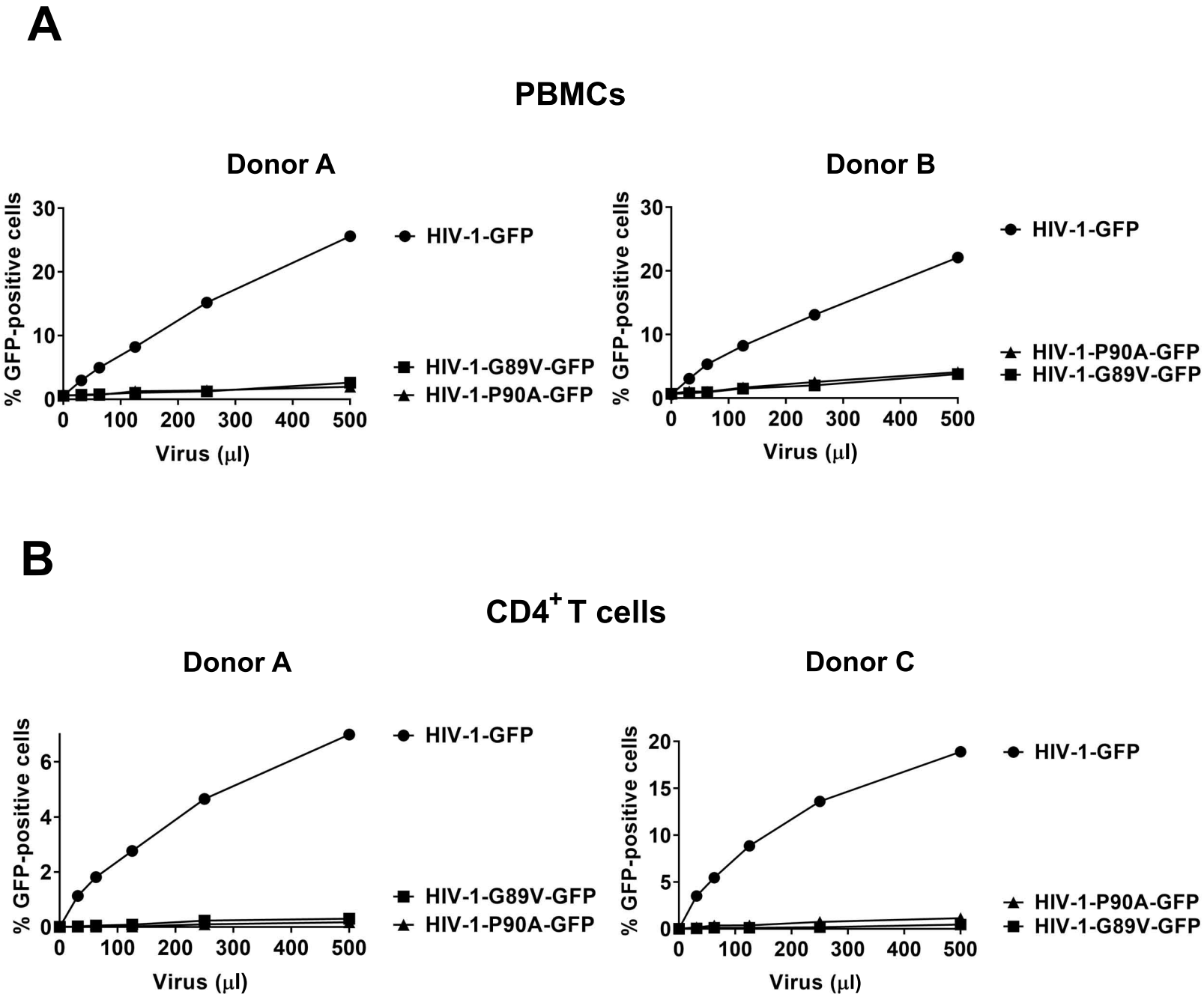

A
PBMCs
Donor A
Donor B
% GFP-positive cells
Virus (μl)
HIV-1-GFP
HIV-1-G89V-GFP
HIV-1-P90A-GFP
B
CD4+ T cells
Donor A
Donor C
% GFP-positive cells
Virus (μl)
HIV-1-GFP
HIV-1-G89V-GFP
HIV-1-P90A-GFP

Figure 6

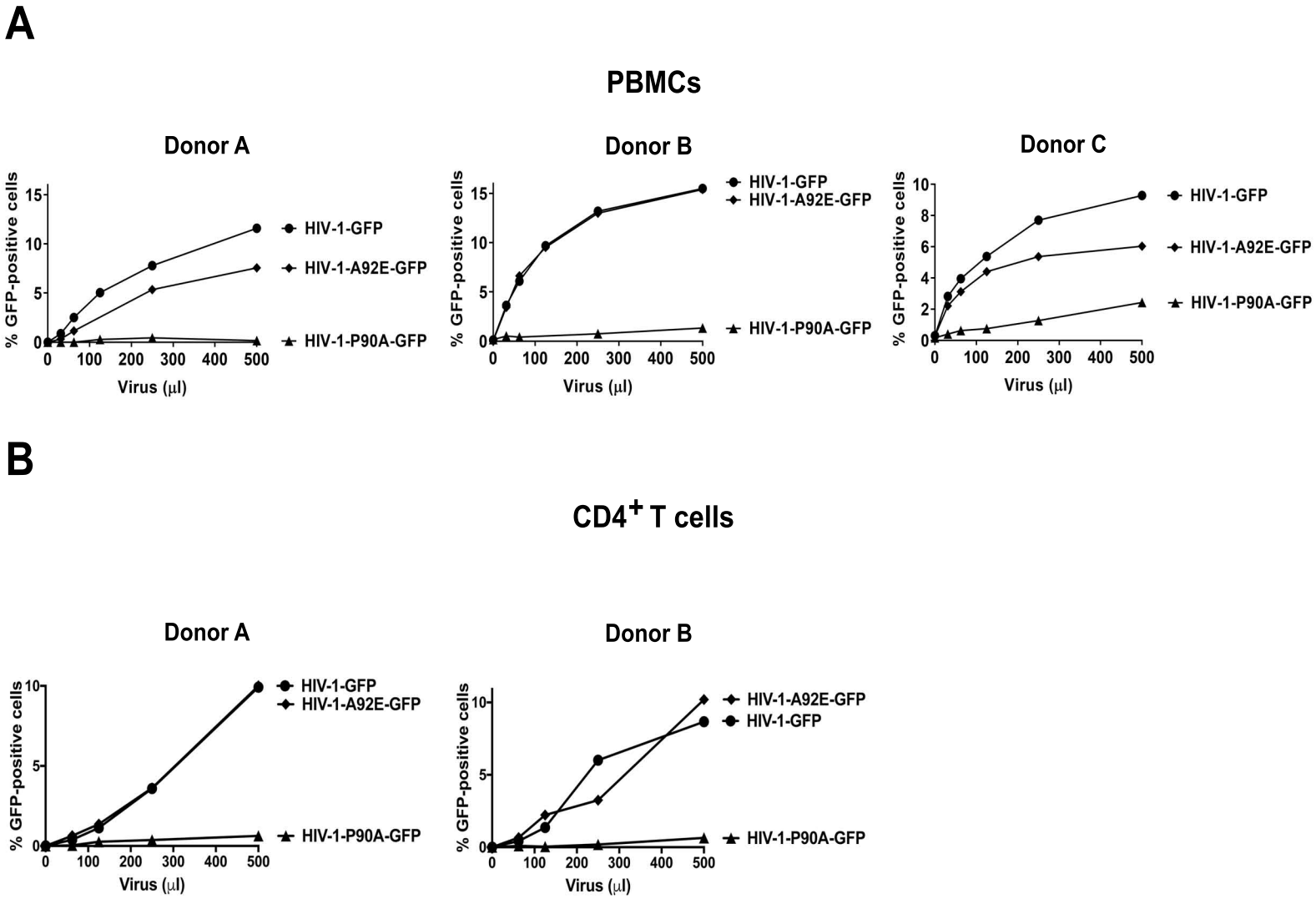
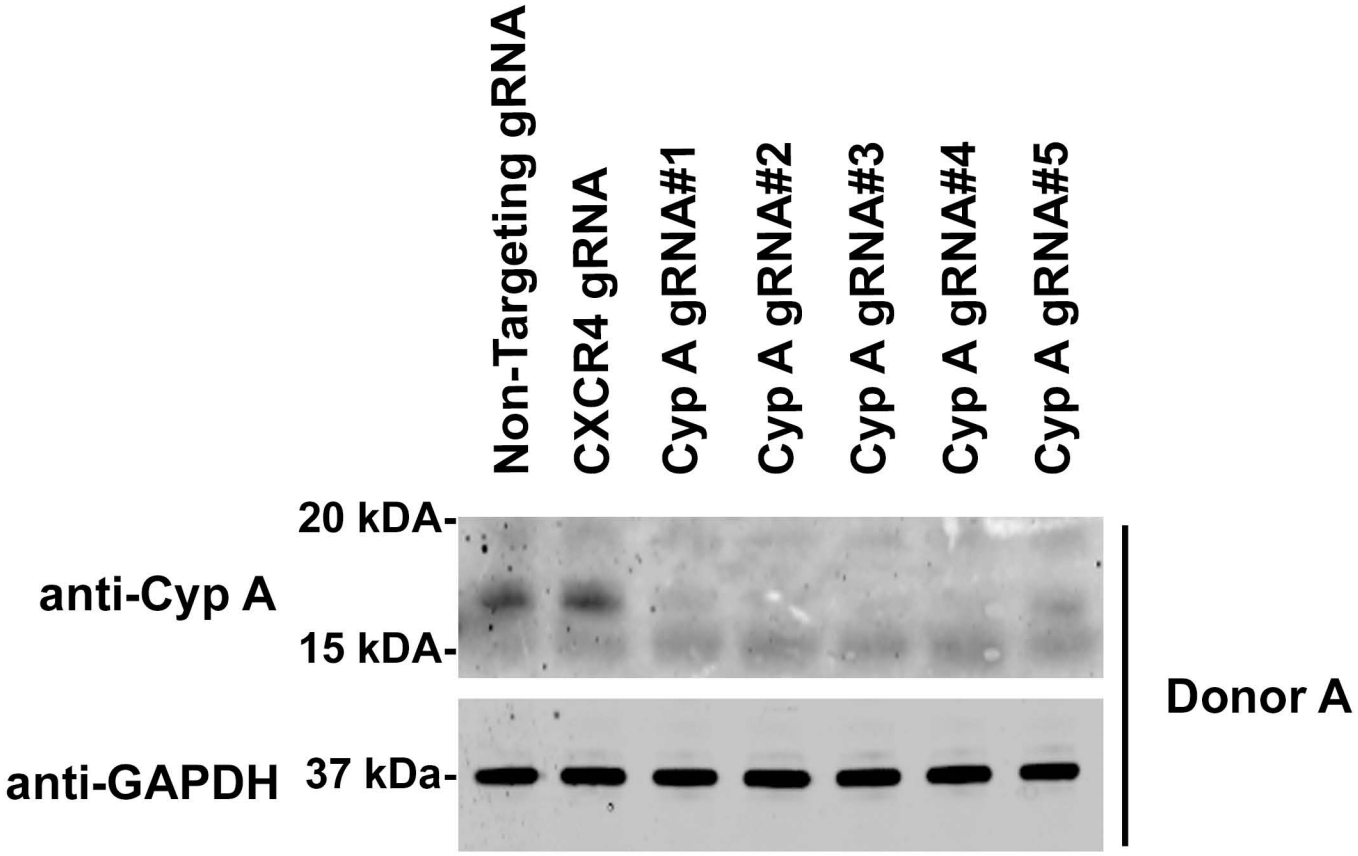

A
PBMCs
Donor A
Donor B
Donor C
% GFP-positive cells
Virus (μl)
HIV-1-GFP
HIV-1-A92E-GFP
HIV-1-P90A-GFP
B
CD4+ T cells
Donor A
Donor B

Figure 7

A


Non-Targeting gRNA
CXCR4 gRNA
Cyp A gRNA#1
Cyp A gRNA#2
Cyp A gRNA#3
Cyp A gRNA#4
Cyp A gRNA#5
20 kDA-
anti-Cyp A
15 kDA-
anti-GAPDH
37 kDa-
Donor A

B

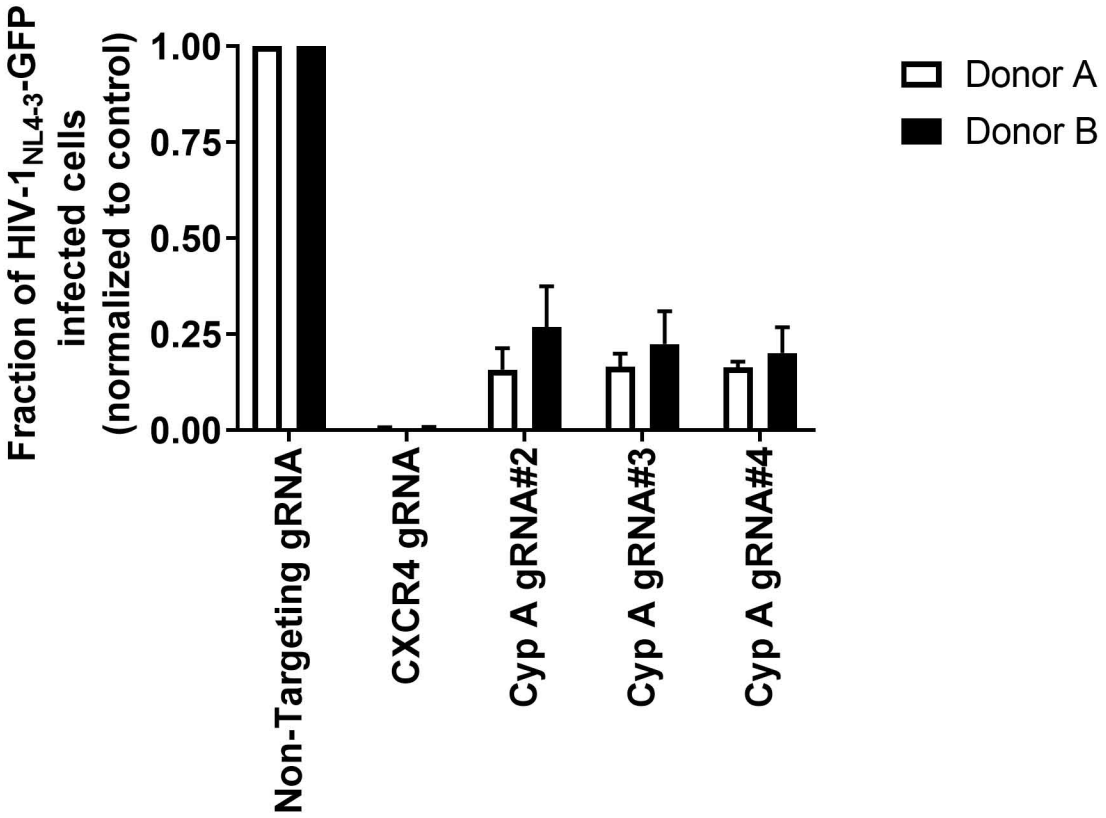

Fraction of HIV-1NL4-3-GFP infected cells (normalized to control)
1.00
0.75
0.50
0.25
0.00
Donor A
Donor B
Non-Targeting gRNA
CXCR4 gRNA
Cyp A gRNA#2
Cyp A gRNA#3
Cyp A gRNA#4

Figure 8

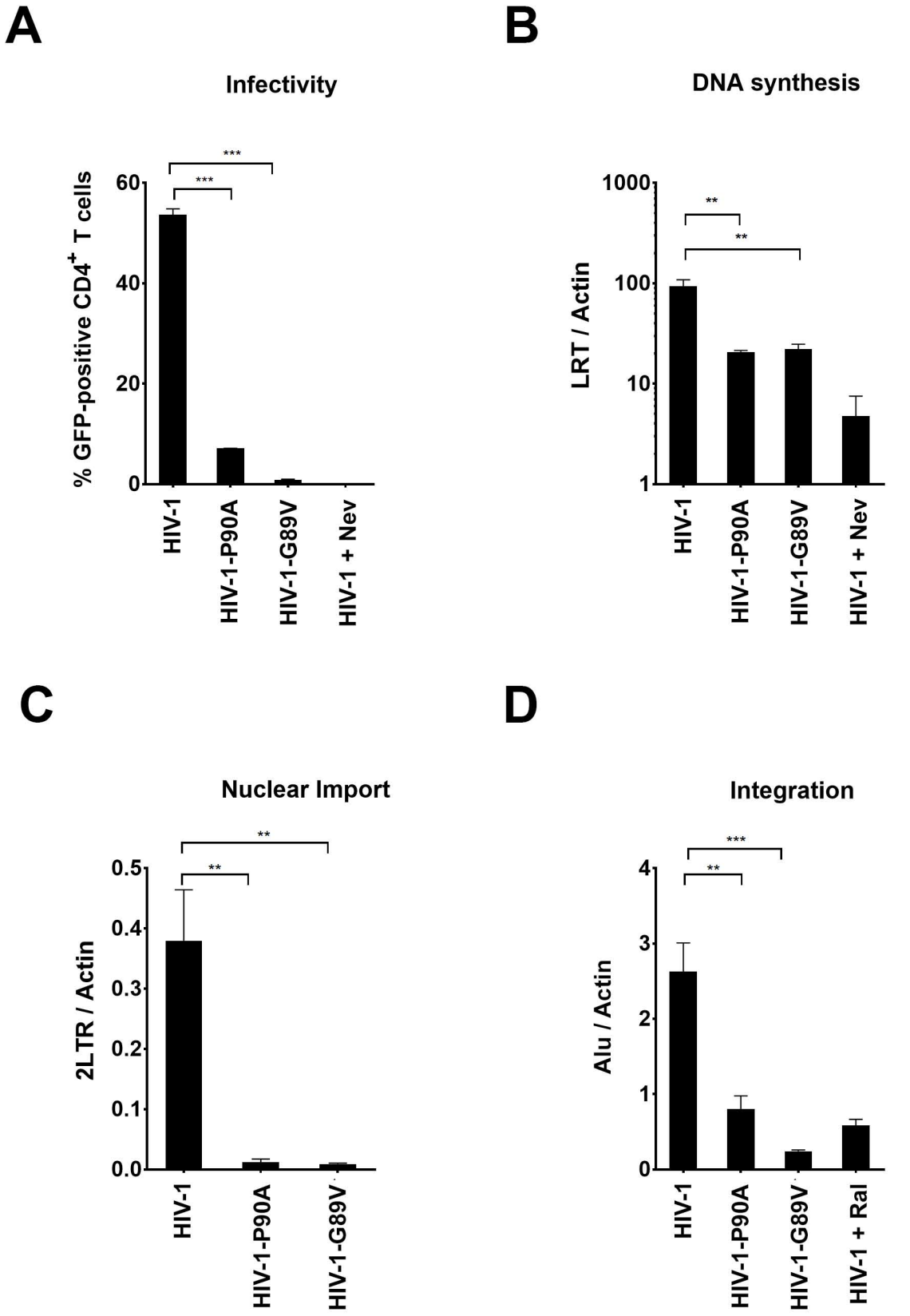

A
Infectivity
% GFP-positive CD4+ T cells
60
40
20
0
***
***
HIV-1
HIV-1-P90A
HIV-1-G89V
HIV-1 + Nev
B
DNA synthesis
LRT / Actin
1000
100
10
1
**
**
HIV-1
HIV-1-P90A
HIV-1-G89V
HIV-1 + Nev
C
Nuclear Import
2LTR / Actin
0.5
0.4
0.3
0.2
0.1
0.0
**
**
HIV-1
HIV-1-P90A
HIV-1-G89V
D
Integration
Alu / Actin
4
3
2
1
0
***
**
HIV-1
HIV-1-P90A
HIV-1-G89V
HIV-1 + Ral

Figure 9