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

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SUMMARY

Disruption of cyclophilin A (CypA)-capsid interactions affects HIV-1 replication in human lymphocytes. To understand this mechanism, we utilize human Jurkat cells, peripheral blood mononuclear cells (PBMCs), and CD4+ T cells. Our results show that inhibition of HIV-1 infection caused by disrupting CypA-capsid interactions is dependent on human tripartite motif 5a (TRIM5α$_{hu}$), showing that TRIM5α$_{hu}$ restricts HIV-1 in CD4+ T cells. Accordingly, depletion of TRIM5α$_{hu}$ in CD4+ T cells rescues HIV-1 that fail to interact with CypA, such as HIV-1-P90A. We found that TRIM5α$_{hu}$ binds to the HIV-1 core. Disruption of CypA-capsid interactions fail to

affect HIV-1-A92E/G94D infection, correlating with the loss of TRIM5α_{hu} binding to HIV-1-A92E/G94D cores. Disruption of CypA-capsid interactions in primary cells has a greater inhibitory effect on HIV-1 when compared to Jurkat cells. Consistent with TRIM5α restriction, disruption of CypA-capsid interactions in CD4$^+$ T cells inhibits reverse transcription. Overall, our results reveal that CypA binding to the core protects HIV-1 from TRIM5α_{hu} restriction.

Graphical Abstract
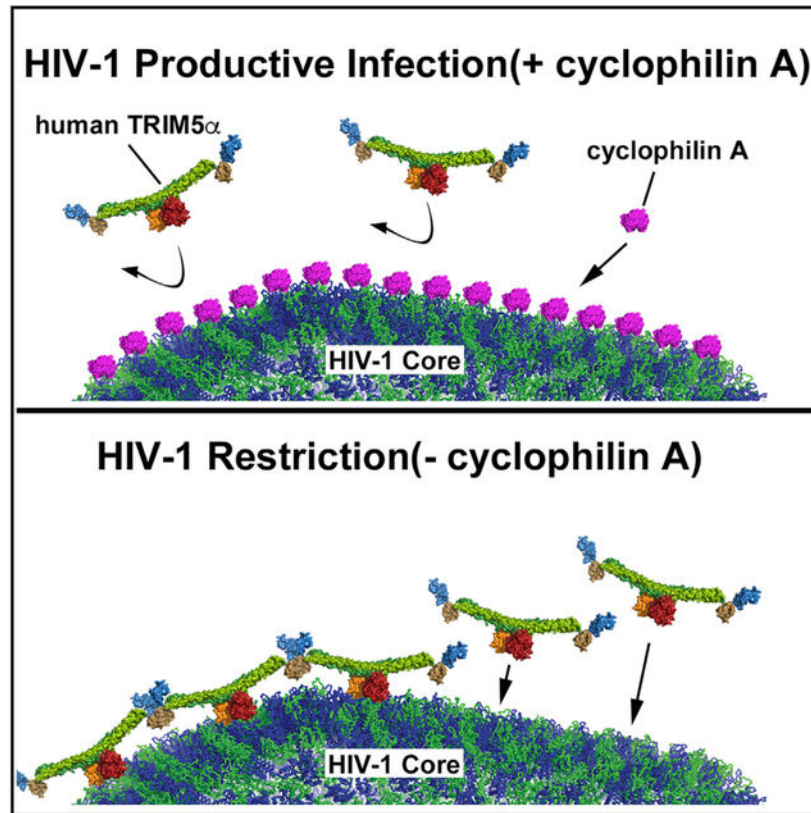
In Brief

Selyutina et al. show that, during HIV infection, the drug cyclosporine removes cyclophilin A bound to the HIV-1 core, facilitating the interaction between human TRIM5α and the core. This inhibits infection and explains the mechanism behind the long-standing observation that cyclosporine counteracts HIV.

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 (Brass et al., 2008; Buffone et al., 2018; Di Nunzio et al., 2013; König 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), TNPO3 (Brass et al., 2008; Christ et al., 2008; König 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), TNPO1 (Fernandez et al., 2019; Idica et al., 2017), and CPSF6 (Lee et al., 2010). 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; Sherry et al., 1998; 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; Sherry et al., 1998; Sokolskaja et al., 2004; Towers et al., 2003; Wainberg et al., 1988; Yin et al., 1998); however, the underlying mechanism remains elusive. This work will focus on the mechanism by which the disruption of CypA-capsid interactions negatively affects HIV-1 infection of lymphocytes.

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 of human tripartite motif 5a (TRIM5α_{hu}). Depletion of TRIM5α_{hu} expression in Jurkat and CD4$^+$ T cells rescued HIV-1 infection of viruses that cannot interact with CypA, such as HIV-1-P90A. These results suggest that TRIM5α_{hu} restricts 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) or G94D (HIV-1-G94D). Infection of HIV-1-A92E/G94D 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/G94D mutation. These experiments suggest that CypA protects the HIV-1 core from TRIM5α_{hu}. 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 protects the core from TRIM5α_{hu}, infection of PBMCs and CD4$^+$ T cells by HIV-1-A92E viruses was insensitive to the disruption of CypA-capsid interactions. Consistent 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 suggest 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 pseudotyped with the vesicular stomatitis virus G (VSV-G) envelope (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 S1A, top panel). Experiments were repeated multiple times, and the fraction of GFP-positive cells normalized to the dimethyl sulfoxide (DMSO)-treated control is shown in Figure S1A (bottom 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-GFP and HIV-1-G89V-GFP, 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 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 S1B, top panel). Experiments were repeated multiple times, and the fraction of GFP-positive cells normalized to control is shown in Figure S1B (bottom 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.

TRIM5α_{hu} Binds to the HIV-1 Capsid

Previous studies have shown that CypA modulates the ability of rhesus TRIM protein, 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. One model is 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 or vice versa.

More recent studies have suggested that TRIM5α_{hu} blocks HIV-1 infection (Jimenez-Guardeño 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 new capsid-binding assay that incorporates washing steps (Selyutina et al., 2018). This new capsid-binding assay has been extensively validated by using different capsid binding proteins (Selyutina et al., 2018). This capsid-binding assay utilizes capsid tubes that are stabilized by disulfide bonds, providing the necessary stability to sustain washing steps, which were not possible when using the capsid-binding assay that utilized *in vitro* assembled capsid-nucleocapsid (CA-NC) tubes. Using this new assay, we found that TRIM5α_{hu} binds

to the HIV-1 core (Figure 1A). TRIM5α$_{hu}$ showed a stronger interaction with the HIV-1 core when compared with TRIM5α$_{rh}$ (Figure 1A). To further test these results, we compared TRIM5α$_{hu}$ and TRIM5α$_{rh}$ binding to *in vitro* assembled HIV-1 CA-NC complexes (Figure 1B). Our results showed that TRIM5α$_{hu}$ interacts with the HIV-1 CA assembled tubes, suggesting that TRIM5α$_{hu}$ interacts with the HIV-1 core. One possibility is that CypA modulates the ability of TRIM5α$_{hu}$ to interact with the HIV-1 core in lymphocytes.

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 2A), as described in STAR Methods. To test for TRIM5α$_{hu}$ function, 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 S2), whereas, in shRNA control cells, N-MLV-GFP infection was blocked (Figure S2). As a control, we also showed that TRIM5α$_{hu}$ KD cells were susceptible to B-tropic murine leukemia virus GFP (BMLV-GFP), which is not restricted by TRIM5α$_{hu}$ (Figure S2). 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 (Figures 2B and S2B). TRIM5α$_{hu}$ KD cells treated with CsA did not show marked inhibition of HIV-1-GFP infection compared with control cells (Figures 2B and S2B), suggesting that TRIM5α$_{hu}$ is involved in the inhibition of viral infection observed in cells that CypA-capsid interactions are disrupted.

HIV-1-P90A-GFP is a mutant form of HIV-1 in which CypA-capsid interactions are disrupted during infection. We tested whether HIV-1-P90A-GFP infection was inhibited in TRIM5α$_{hu}$ KD Jurkat cells. As shown in Figures 2C and S2B, we found that HIV-1-P90A-GFP 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 in lymphocytes. 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 3A, 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 3A) in the absence

of CsA. These results suggest 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 mg 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 3B). As expected, recombinant CypA did not affect the binding of TRIM5α$_{hu}$ to HIV-1 cores bearing the P90A mutation. Overall, these results suggest 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 or G94D 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-A92EGFP infection of Jurkat cells was insensitive to CsA treatment (Figure S3). This insensitivity may occur due to HIV-1-A92EGFP inability to interact with TRIM5α$_{hu}$. To explore this possibility, we tested whether TRIM5α$_{hu}$ interacts with HIV-1 cores containing the A92E mutation. We found that TRIM5α$_{hu}$ did not interact with mutant HIV-1 cores bearing the capsid change A92E (Figure 3C). We observed similar results using the capsid mutant G94D (Figure 3C), which showed a similar pattern of infectivity when compared to HIV-1-A92E-GFP viruses. As a control, we showed that the binding of TRIM5α$_{hu}$ to wild-type HIV-1 cores increased when CsA was added (Figure 3C). Overall, these results indicate that TRIM5α$_{hu}$ does not bind to mutant HIV-1 capsids bearing the change A92E or G94D, which may explain the reason that CsA treatment does not affect the infection of Jurkat cells by HIV-1-A92E-GFP or HIV-1-G94DGFP viruses.

Endogenously Expressed TRIM5α$_{hu}$ Binds to the HIV-1 Capsid

Next, we tested whether the endogenously expressed TRIM5α$_{hu}$ binds to the HIV-1 capsid. As shown in Figure 3D, endogenously expressed TRIM5α$_{hu}$ from cellular extracts derived from HT1080 cells binds to stabilized HIV-1 capsid tubes. This showed the ability of endogenously expressed TRIM5α$_{hu}$ to bind to HIV-1 capsid. As a control, we utilized HT1080 cells that were knocked out for expression of TRIM5α$_{hu}$ (HT1080-TRIM5α$_{hu}$-KO). In agreement with our previous results, the use of CsA increased the binding of endogenously expressed TRIM5α$_{hu}$ to stabilized HIV-1 capsid tubes (Figure 3D). Furthermore, endogenously expressed TRIM5α$_{hu}$ also bound to stabilized HIV-1 capsid tubes bearing the P90A mutation (Figure 3E).

CypA-Capsid Interactions Are Required for HIV-1 Infection of Human PBMCs and CD4^{+} T Cells

To understand the role of CypA in HIV-1 infection of human primary cells, we challenged PBMCs and CD4^{+} T cells from different donors with HIV-1-GFP in the presence of increasing concentrations of CsA, which prevents the binding of CypA to HIV-1 capsid

(Diaz-Griffero et al., 2006; Luban et al., 1993). As shown in Figure S4A, CsA treatment inhibited HIV-1 infection in PBMCs. Similarly, CsA inhibited HIV-1-GFP infection of human primary CD4+ T cells (Figure S4B). This result suggests that CypA-capsid interactions are required for HIV-1-GFP infection of human PBMCs and CD4+ T cells. To further corroborate these findings, we challenged human PBMCs and CD4+ T cells with increasing amounts of HIV-1-GFP particles bearing the capsid mutations P90A and G89V, both of which prevent CypA-capsid interactions (Gamble et al., 1996; Thali et al., 1994). Infection was determined by measuring the percentage of GFP-positive cells at 72 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-GFP and HIV-1-G89V-GFP were unable to infect human PBMCs or CD4+ T cells (Figures 4A and S5). Although these experiments were performed in PBMCs and CD4+ T cells obtained from six different donors, all of which showed similar results, we have only shown sample results obtained from three donors. Our results suggest that CypA-capsid interactions are required for HIV-1 infection in human primary PBMCs and CD4+ T cells. Importantly, the lack of CypA binding appeared to have a stronger effect on HIV-1 infection in primary human lymphocytes when compared with that of Jurkat cells (Figure 2), thus indicating that CypA is crucial for HIV-1 infection of human primary cells. Overall, these results suggest 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 or G94D lacks the ability to interact with TRIM5α_hu (Figure 3C). In order to study the role of TRIM5α_hu during viral infection, we examined the ability of HIV-1-A92E-GFP and HIV-1-G94D-GFP to infect CD4+ T cells (Figures 4B and 4C). Consistent with results observed in Jurkat cells, infection levels by the mutant viruses were slightly affected when compared to that of wild-type HIV-1-GFP in primary human CD4+ T cells (Figures 4B and 4C). As a control, we also challenged CD4+ T cells with increasing concentrations of HIV-1-P90A-GFP, an HIV-1 mutant that is completely inhibited in CD4+ T cells (Figures 4B and 4C). Consistent with our previous binding experiments, these experiments suggest that infection of CD4+ T cells by HIV-1-A92E-GFP or HIV-1-G94D-GFP was not affected by TRIM5α_hu, which correlated with the inability of TRIM5α_hu to bind to HIV-1 cores bearing the mutation A92E or G94D. We also demonstrate that CsA does not affect the ability of HIV-1-A92E-GFP viruses to infect human primary CD4+ T cells (Figure 4D), suggesting that HIV-1-A92E-GFP viruses are insensitive to TRIM5α_hu.

Depletion of CypA Expression in Human CD4+ T Cells Inhibits HIV-1 Infection

We have previously shown indirectly 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-GFP (VSV-G pseudotyped virus) or HIV-1NL4–3-GFP (replication competent virus). 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 5A, all five guides were able

to eliminate the expression of CypA. The CypA knockout cells were then challenged with HIV-1-GFP (Figure 5B) or replication competent HIV-1NL4–3-GFP (Figure 5C). Consistent with our previous results, human CD4^{+} T cells depleted for CypA expression were resistant to HIV-1 infection. As a control, we used a CRISPR/Cas9 complex containing a non-targeting RNA guide (Figures 5B and 5C). As an additional control in experiments using HIV-1NL4–3-GFP virus, we used a CRISPR/Cas9 complex containing an RNA guide for CXCR4, which completely inhibits infection of the replication-competent virus HIV-1NL4–3-GFP (Figure 5C). These results demonstrate that CypA expression is essential for HIV-1 infection of human primary CD4^{+} T cells.

Depletion of TRIM5α$_{hu}$ Expression in Human CD4^{+} T Cells Rescues Infection by HIV-1-P90A Viruses

Our results show that TRIM5α$_{hu}$ does not interact with A92E cores. In addition, we show that HIV-1-A92E viruses do not exhibit a replication defect in primary CD4^{+} T cells in the absence or presence of CsA. Altogether, these results suggest that TRIM5α$_{hu}$ blocks HIV-1 replication in primary CD4^{+} T cells where CypA-capsid interactions have been disrupted. To test whether TRIM5α$_{hu}$ blocks the infection of viruses that do not interact with CypA, we challenged TRIM5α$_{hu}$ knockout CD4^{+} T cells with increasing amounts of HIV-1-P90A viruses. As shown in Figure 6A, gRNA#6 and gRNA#7 efficiently depleted TRIM5α$_{hu}$ expression in CD4^{+} T cells. Consistent with our hypothesis, depletion of TRIM5α$_{hu}$ expression completely rescued infection of HIV-1-P90A viruses (Figure 6B). Considering that HIV-1-P90A-GFP does not bind CypA protein but does bind TRIM5α$_{hu}$, these results demonstrate that TRIM5α$_{hu}$ is the restriction factor blocking infection of primary CD4^{+} T cells by HIV-1-P90A viruses. Furthermore, these experiments suggest that CypA protects the HIV-1 core from TRIM5α$_{hu}$ restriction.

CypA-Capsid Interactions Are Required for Efficient HIV-1 Reverse Transcription in Human CD4^{+} T Cells

Our analysis shows 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 wild-type and mutant HIV-1-GFP viruses and measured reverse transcription, nuclear import, and integration using real-time quantitative polymerase chain reaction (qPCR) (Figure S6). Infection was measured by determining the percentage of GFP-positive cells at 72 h post-infection (Figure S6A). Consistent with our previous results, HIV-1-P90AGFP and HIV-1-G89V-GFP strains showed weak infection of CD4^{+} T cells (Figure S6A). Reverse transcription was measured by determining the levels of early reverse transcripts (ERTs) and late reverse transcripts (LRTs) at 12 h post-infection (Figure S6B). Interestingly, reverse transcription was reduced for HIV-1-P90A-GFP and HIV-1-G89V-GFP, 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 S6B). For nuclear import, we measured the levels of 2-long terminal repeat (2-LTR) circles at 36 h post-infection (Figure S6C). Consistent with a reverse transcription block, our results show that both viruses were blocked for nuclear import. Viral integration was determined using Alu-PCR at 66 h post-infection (Figure

S6D). 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 suggest that HIV-1 requires interaction with CypA to undergo efficient reverse transcription in human CD4+ T cells. These results also suggest that, in the absence of CypA, HIV-1 particles are exposed to TRIM5α_{hu}, 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 explore 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-Guardeño 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. We also show that endogenously expressed TRIM5α_{hu} binds to the HIV-1 core. 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. We show that depletion of TRIM5α_{hu} expression in Jurkat and primary CD4+ T cells rescue the HIV-1 infectivity phenotype 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 suggest 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 demonstrate that increasing the amounts of CypA prevents the binding of TRIM5α_{hu} to the HIV-1 core. Based on these results, we propose that prevention of CypA binding allows TRIM5α_{hu} to bind to the HIV-1 core, consequently inhibiting infection. While this manuscript was under revision, Kim et al. (2019) showed similar results using immunofluorescence microscopy.

Our results indicate 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 (Li et al., 2006; Pham et al., 2010; Yap et al., 2005). One possibility is that restriction of HIV-1 infection by TRIM5α_{hu} requires specific cellular cofactors expressed only in human 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. The search for these cofactors in the future is warranted. 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). A third possibility is that TRIM5α_{hu} might be recruiting other human TRIM orthologs to the HIV-1 core, which are causing 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 wild-type HIV-1 infection of Jurkat and CD4$^+$ T cells; however, infection of HIV-1 viruses bearing the capsid change A92E or G94D results in an infection that is insensitive to CsA treatment in Jurkat cells (Hatziioannou et al., 2005; Sokolskaja et al., 2004). Here, we show that this insensitivity is due to the lack of TRIM5α_{hu} binding to HIV-1-A92E or HIV-1-G94D mutant cores. Normal viral infectivity correlated with the inability of TRIM5α_{hu} to bind to HIV-1-A92E and HIV-1-G94D cores. Based on these results, it is possible that TRIM5α_{hu} binds to the CypA-binding loop or in its proximity.

The disruption of CypA-capsid interactions had a stronger effect on viral infectivity in human primary cells compared with cell lines. Mutant HIV-1 (P90A or G89V) infection of PBMCs or CD4$^+$ T cells was 10–20-fold weaker than that of wild-type HIV-1. Similarly, KD of CypA expression in human primary CD4$^+$ T cells potently blocked HIV-1 infection. In contrast, infection of primary lymphocytes by HIV-1-A92E or HIV-1-G94D was not affected when compared to that of wild-type HIV-1. In addition, depletion of TRIM5α_{hu} expression in CD4$^+$ T cells rescued infectivity of HIV-1-P90A viruses. Taken together, these results show that TRIM5α_{hu} blocks HIV-1 infection of human primary lymphocytes when CypA-capsid interactions are disrupted. Consistent with this idea, we observe that the inhibition of infection caused by the disruption of CypA-capsid interactions in CD4$^+$ T cells occurred prior to HIV-1 reverse transcription.

Our work has shown that CypA protects the core from TRIM5α_{hu}. Intriguingly, new- and old-world monkeys express a protein in which the TRIM domain of TRIM5α and CypA form a fusion protein (Brennan et al., 2008; Newman et al., 2008; Sayah et al., 2004). 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 (i.e., TRIMCyp) in response to CypA protecting the virus against TRIM5α.

Although this work explains the infection phenotype observed when disrupting CypA-capsid interactions during infection, it does not explain the role of CypA in HIV-1 infection. It has been suggested that CypA is involved in nuclear import and integration (Schaller et al., 2011). CypA has also been observed to enter the nucleus with capsid (Burdick et al., 2017). Future experiments will provide mechanistic insights on the works of CypA in HIV-1 infection.

Overall, this work shows 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.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE Antibodies	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal anti-FLAG M2	Sigma-Aldrich	Cat# F1804; RRID:AB_262044
Mouse monoclonal anti-hemagglutinin (HA)	Sigma-Aldrich	Cat# H3663; RRID:AB_262051
Mouse monoclonal anti-Cyclophilin A (human)	Abcam	Cat# ab58144; RRID:AB_941220
Rabbit monoclonal anti-TRIM5a (human, monkey)	Cell Signaling Technology	Cat# 14326S; RRID:AB_2798451
Mouse monoclonal anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Invitrogen (Thermo Fisher Scientific)	Cat# AM4300; RRID:AB_437392
Mouse monoclonal anti-HIV-1 p24 (183-H12-5C)	NIH AIDS Reagent Program	Cat# 3637; RRID:CVCL_9N48
Goat anti-Mouse IRDye 680LT	LI-COR	Cat# 925–68020; RRID:AB_2687826
Goat anti-Rabbit IRDye 680LT	LI-COR	Cat# 926–68021; RRID:AB_10706309
Goat anti-Mouse IRDye 800CW	LI-COR	Cat# 926–32210; RRID:AB_621842
Goat anti-Rabbit IRDye 800CW	LI-COR	Cat# 925–32211; RRID:AB_2651127
Rabbit monoclonal anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Cell Signaling Technology	Cat #2118S; RRID:AB_437392
Bacterial and Virus Strains		
E. Coli one-shoot BL21star (DE3)	Invitrogen	Cat#C601003
E. Coli DH5α competent cells	Zymo Research	Cat# T3007
N-MLV	Diaz-Griffero et al., 2008	N/A
B-MLV	Diaz-Griffero et al., 2008	N/A
HIV-1 $_{NL4-3}$	This paper	N/A
HIV-1-G89V	This paper	N/A
HIV-1-P90A	This paper	N/A
HIV-1-A92E	This paper	N/A
HIV-1-G94D	This paper	N/A
HIV-1 NL4-3 Nef:IRES:GFP	Hultquist et al., 2016	N/A
Biological Samples		
Healthy adult Peripheral Blood Mononuclear Cells (PBMCs)	LifeSource/Vitalant	N/A
Chemicals, Peptides, and Recombinant Proteins		
Cyclosporin A	Millipore Sigma	Cat#: PHR1092; CAS: 59865-13-3
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	Cat#: D2438; CAS: 67-68-5
Difco LB Broth, Miller (Luria-Bertani)	Fisher Scientific	Cat#: BD244610
Isopropyl β-D-1-thiogalactopyranoside	Sigma-Aldrich	Cat#: I5502; CAS: 367-93-1
cOmplete EDTA-free protease inhibitor cocktail	Millipore Sigma	Cat#: 11873580001,

REAGENT or RESOURCE Antibodies	SOURCE	IDENTIFIER
Tris (TRIS(HYDROXYMETHYL)AMINOMETHANE)	Crystalgen	Cat#300-844-5000; CAS: 77-86-1
Sodium Chloride (NaCl)	Crystalgen	Cat# 300-747-5000; CAS: 767-14-5
β-Mercaptoethanol (BME)	Acros organics	Cat# 125470010; CAS: 60-24-2
2-(N-morpholino) ethanesulfonic acid (MES)	Calbiochem	Cat# 475893; CAS: 4432-31-9
Magnesium Chloride (MgCl$_2$)	Sigma-Aldrich	Cat# M2670; CAS: 7791-18-6
Potassium Chloride (KCl)	Fisher Scientific	Cat# BP366-1; CAS: 7447-40-7
Dithiothreitol (DTT)	VWR	Cat# 97061-340; CAS: 3483-12-3
D-(+)-Sucrose	VWR	Cat# 97061-432; CAS: 57-50-1
Human IL-2	Cell Signaling Technology	Cat# 8907SF
Phytohemagglutinin-L (PHA-L)	Millipore Sigma	Cat# 11249738001
Human IL-2	MACS Miltenyi Biotec	Cat# 130-097-742
Anti-Human CD3 (UCHT1)	Tonbo Biosciences	Cat# 70-0038-U100; RRID:AB_2621475
Anti-Human CD28 (CD28.2)	Tonbo Biosciences	Cat# 70-0289-U100; RRID:AB_2621493
Cas9-NLS Recombinant Protein, purified, 40uM	MacroLab Berkeley	N/A
Dulbecco's Phosphate-Buffered Salt (PBS) Solution 1X	Corning	21031CV
EDTA, pH 8.0, 0.5M	Corning	46034CI
Critical Commercial Assays		
QuikChange II site-directed mutagenesis kit	Agilent	Cat#: 200523
CD4+ T Cell Isolation Kit, human	MACS Miltenyi Biotec	Cat# 130-096-533
EasySep Human CD4+ T Cell Isolation Kit	STEMCELL	Cat# 17952
P3 Primary Cell 96-well Nucleofector Kit	Lonza	Cat# V4SP-3096
T Cell Activation/Expansion Kit, human	MACS Miltenyi Biotec	Cat# 130-091-441
QIAamp DNA Micro Kit	QIAGEN	Cat# 56304
Experimental Models: Cell Lines		
Human: Jurkat E6.1	ATCC	TIB-152
Human: 293T/17	ATCC	CRL-11268
Human: HT1080	ATCC	CCL-121
Oligonucleotides		
All standard cloning primers for site-directed mutagenesis	Integrated DNA Technologies	N/A
Early reverse transcripts (ERT) hRU5-F, 5′-GCCTCAATAAAGCTTGCCTTGA-3′	Mbisa et al., 2009	N/A
Early reverse transcripts (ERT) hRU5-R, 5′-TGACTAAAAGGGTCTGAGGGATCT-3′	Mbisa et al., 2009	N/A
ERT probe, 5′-(FAM)-AGAGTCACACAAC AGACGGGCACACACTA-(TAMRA)-3′	Mbisa et al., 2009	N/A

REAGENT or RESOURCE Antibodies	SOURCE	IDENTIFIER
Late Reverse Transcripts (LTR) MH531, 5′-TGTGTGCCCGTCTGTTGTGT-3′,	Butler et al., 2001	N/A
Late Reverse Transcripts (LTR) MH532, 5′-GAGTCCTGCGTCGAGAGAGC-3′	Butler et al., 2001	N/A
LRT-Probe, 5′-(FAM)-CAGTGGCGCCCGAACAGGGA-(TAMRA)-3′	Butler et al., 2001	N/A
2-LTR circles MH535, 5′-AACTAGGGAACCCACTGCTTAAG-3′	Butler et al., 2001	N/A
2-LTR circles MH536, 5′-TCCACAGATCAAGGATATCTTGTC-3'	Butler et al., 2001	N/A
2-LTR probe MH603, 5′-(FAM)-ACACTAC TTGAAGCACTCAAG-GCAAGCTTT-(TAMRA)-3′	Butler et al., 2001	N/A
Table S1 Primers and RNA guides	N/A	N/A
Recombinant DNA		
pET-11a	EMD Mollipore	Cat # 69436
Plasmid: CA A14C/E45C in pET-11a	Selyutina et al., 2018	N/A
Plasmid: CA A14C/E45C/P90A in pET-11a	Selyutina et al., 2018	N/A
Plasmid: CA A14C/E45C/A92E in pET-11a	This paper	N/A
Plasmid: CA A14C/E45C/G94D in pET-11a	This paper	N/A
pLKO1	Sigma	Cat# 10878
pLKO1-shRNA-TRIM5a$_{hu}$	Sigma	Cat# 13478
Plasmid: human TRIM5a-HA in pLPCX	Perron et al., 2007	N/A
Plasmid: rhesus TRIM5a-HA in pLPCX	Stremlau et al., 2006a	N/A
Plasmid: Tat	Stremlau et al., 2006a	N/A
Plasmid: Rev	Stremlau et al., 2006a	N/A
Plasmid: VSVg	Stremlau et al., 2006	N/A
Other		
HiTrap Q HP 5mL	GE Healthcare	Cat# 17115401
HiTrap SP HP 5mL	GE Healthcare	Cat# 17115201
Centriprep Centrifugal Filter Unit 30 kDa	Millipore Sigma	Cat# 4306
SnakeSkin Dialysis Tubing, 10KMWCO	Fisher Scientific	Cat# 68100
GE Healthcare Ficoll-Paque PLUS Media	Fisher Scientific	Cat# 45001-749
Hanks' Balanced Salt solution	Sigma-Aldrich	Cat# H6648
LS Columns	MACS Miltenyi Biotec	Cat# 130-042-401
MS Columns	MACS Miltenyi Biotec	Cat# 130-042-201
SepMate PBMC Isolation Tubes	STEMCELL	Cat# 85450
Maxima Probe/ROX qPCR Master Mix (2X)	ThermoScientific	Cat#K0231
TURBO DNase (2 U/μL)	Invitrogen	Cat#AM2238
Platinum SuperFi DNA Polymerase	Invitrogen	Cat#12351050
iTaq Universal SYBR® Green Supermix	Biorad	Cat#1725124
0.2 ml PCR 8-tube with attached clear flat caps, natural	USA Scientific	1402–3900
Fetal Bovine Serum (FBS, heat-inactivated)	GIBCO	16140–071
RPMI-1640 (high glucose)	Corning	MT10-017-CV

REAGENT or RESOURCE Antibodies	SOURCE	IDENTIFIER
Penicillin-streptomycin (5 mg/mL)	Corning	MT10-040-CV
Sodium pyruvate (100×)	Corning	MT25-000-CI
HEPES (100×)	Fisher / HyClone	SH3023701
DMEM (high glucose)	Corning	MT10-017-CV

LEAD CONTACT AND MATERIALS AVAILABILITY

This study generated the following new plasmids: pET11A-A14C/E45C/A92E, pET11A-A14C/E45C/G94D, HIV-1-P90A, HIV-1-G89V, HIV-1-A92E and HIV-1-G94D. Plasmids generated and used in this study are available upon request. Further information and requests for plasmids, resources, and reagents should be directed to and will be fulfilled by the lead contact Felipe Diaz-Griffero (Felipe.Diaz-Griffero@einsteinmed.org)

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Bacterial strains—All molecular cloning was carried out in *E. coli* DH5α competent cells. All recombinant proteins were expressed and purified from *E. coli* one-shot BL21star (DE3) strain. Both bacterial stains were routinely grown in Luria-Bertani (LB) medium at 37°C (or at the indicated temperatures) while shaking at 200 RPM.

Human cell lines—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/17 and HT1080 (ATCC) cells were grown at 37°C in Dulbecco's modified eagle medium (DMEM) supplemented with 10% FBS and 1% penicillin-streptomycin.

Human PBMCs—Adult Peripheral Blood Mononuclear Cells (PBMCs) from anonymous, healthy donors were isolated from donated buffy coat products, a post-centrifugation product from a single whole blood donation that contains white blood cells and platelets (Lifesource/ Vitalant, Chicago, and New York Blood Center, New York).

METHOD DETAILS

Infection using HIV-1-GFP reporter viruses—Recombinant human immunodeficiency virus-1 (HIV-1) strains such as HIV-1-G89V, HIV-1-P90A, HIV-1-A92E and HIV-1-G94D expressing GFP and pseudotyped with VSV-G were prepared as previously described (Diaz-Griffero et al., 2008). To produce replication competent HIV-1$_{NL4-3}$-GFP virus, we transfected human 293T/17 cells with a full molecular clone containing the sequence of HIV-1$_{NL4-3}$ that expresses Nef-IRES-GFP as a reporter of infection (Hultquist et al., 2016). All viruses were titered by p24 levels and infectivity. 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 (BD FACSCelesta).

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, A92E and G94D 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-shot 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 $5,000 \times g$ 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 cultures were lysed by sonication (Qsonica microtip: 4420; A = 45; 2 min; 2 s on; 2 s 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 $40,000 \times g$ 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 $8,000 \times g$ 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 s on; 2 s 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 Centriprep Centrifugal Filter Unit and stored at −80°C.

Assembly of stabilized HIV-1 capsid tubes—One milliliter of monomeric capsid (5 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 (TRIM5α_{hu} or TRIM5α_{rh}). 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 centrifuged to remove cellular debris (21,000 3 g, 15 min, 4°C). Cell lysates were incubated with stabilized HIV-1 capsid tubes for 1 h at 25°C.

Subsequently, the stabilized HIV-1 capsid tubes were centrifuged at $21,000 \times g$ for 2 min. Pellets were washed 2–3 times by resuspension and centrifugation in CBB or phosphate-buffered saline (PBS). Pellets were resuspended in Laemmli buffer $1 \times$ and analyzed by western blot using anti-p24 and the indicated antibodies.

Capsid binding assay using *in vitro* assembled HIV-1 CA-NC complexes— Capsid binding assays were performed as described earlier (Lienlaf et al., 2011). Briefly, HIV-1 CA-NC particles were assembled *in vitro* by incubating in buffer A (50 mM Tris (pH 8,0), 0.5 mM NaCl, 2 mg/ml DNA oligo-(TG)50) at 4°C overnight. Human HEK293T cells were transfected for 24 h with a plasmid expressing the protein of interest (TRIM5α$_{hu}$ or TRIM5α$_{rh}$,). The cell medium was completely removed and cells were scraped off the plate and lysed in 300 μL of capsid binding buffer with DTT (CBB+: 10 mM Tris, pH 8,0, 1,5 mM MgCl$_2$, 10 mM KCl, 0,5 mM DTT). Cells were rotated at 4°C for 15 min and then centrifuged to remove cellular debris ($21,000 \times g$, 15 min, 4°C). Cell lysates were incubated with HIV-1 CA-NC complexes capsid tubes for 1 h at 25°C. The mixture was spun through a 70% sucrose cushion (70% sucrose, $1 \times$ PBS) at $100,000 \times g$ in an SW55 rotor (Beckman), 1 h, 4°C. After centrifugation pellets were resuspended in Laemmli buffer $1 \times$ and analyzed by western blot using anti-p24 and anti-HA antibodies.

Preparation of PBMCs and CD4$^+$ T cells—PBMCs from healthy donor whole blood were isolated by density gradient centrifugation using Ficoll-Paque Plus (GE Health Care). 40 mL of whole blood was centrifuged at $300 \times g$ for 10 min, and the plasma layer was removed and replaced with Hank's Balanced Salt solution (HBSS; Sigma Aldrich). The whole blood was then diluted to a ratio of 1:2 with HBSS with 20 mL of the diluted blood layered on top of 20 mL Ficoll-Paque Plus and centrifuged at $300 \times g$ for 30 min. The resulting buffy coat layer was collected, washed twice with HBSS, and resuspended in RPMI medium containing 10% (vol/vol) FBS and 1% (vol/vol) penicillin-streptomycin, and activated with IL-2 (100 U/ml) (Human IL-2; Cell Signaling Technology, #8907SF) and phytohemagglutinin (1 μg/ml) for 3 days. CD4$^+$ T cells were obtained via negative selection from PBMCs using a human CD4$^+$ T cell isolation kit (MACS Miltenyi Biotec, #130-096-533). Per 1×10^7 total cells, PBMCs were resuspended in 40 μl CD4$^+$ T cell isolation buffer (PBS, pH 7.2, 0.5% bovine serum albumin, and 2 mM EDTA). Ten microliters of CD4$^+$ T cell biotin-antibody cocktail was then added and incubated at 4°C for 5 min. Thirty microliters of CD4$^+$ T cell isolation buffer and 20 μl of CD4$^+$ T cell microbead cocktail were then added and further incubated for 10 min at 4°C. Depending on the amount of PBMCs isolated, LS or MS Column attached to a MACS Separator was pre-washed with 3 mL or 6 mL of ice-cold CD4$^+$ T cell isolation buffer, respectively. PBMC suspension was added to the column and the flow-through was collected in a 15 mL tube. The LS or MS Columns were then washed with 3 mL or 6 mL of ice-cold CD4$^+$ T cell isolation buffer, respectively, and the flow-through was collected. The newly isolated CD4$^+$ T cells were then centrifuged at $800 \times g$ for 5 min and resuspended in RPMI medium supplemented with IL-2 (100 U/ml).

Knockdown of TRIM5α expression in Jurkat cells—Lentiviral particles were produced in human 293T/17 cells by co-transfection of 1 μg TAT, 1 μg REV, 1.5 μg VSVG,

1 mg HDM (codon optimized gag-pol), and 7.5 μg of the lentiviral vector pLKO1 or 7.5 μg the pLKO1 vector containing an shRNA that targets TRIM5α (pLKO1-shRNA-TRIM5α). Viruses were harvested at 48 h post-transfection and used to transduce Jurkat cells for 5 days at a 1:1 virus to complete media ratio. Transduced cells were selected in 0.15 μg/ml of puromycin. Cells that stably contained pLKO1 or pLKO1-shRNA-TRIM5α were maintained in puromycin. Expression of TRIM5α was evaluated by western blotting and by challenging the cells with N-tropic murine leukemia virus (N-MLV) and B-tropic murine leukemia virus (B-MLV).

Generation of TRIM5α knockout HT1080 cell line—Human TRIM5α knockout (KO) HT1080 cell line was generated by using the clustered regulatory interspaced short palindromic repeat (CRISPR)-Cas9 gene system (Edit-R CRISPR-Cas9 gene engineering with SMARTCas9 nucleases; GE Healthcare). The CRISPR genomic guide RNA sequence for human TRIM5α was designed to target exon 1 near the start codon. The guide RNA construct, human cytomegalovirus (hCMV)-Cas9, and CMV-GFP were co-transfected into HT-1080 cells. After 48 h of transfection, GFP-positive cells were single-cell sorted by using the FACSAria system (BD Biosciences, Franklin Lakes, NJ, USA). Clonal HT-1080 colonies were expanded and characterized for the loss of TRIM5α protein expression by western blot analysis using anti-TRIM5α antibodies and by challenging the cells with N-tropic murine leukemia virus (N-MLV) and B-tropic murine leukemia virus (B-MLV).

CRISPR-Cas9 Knock-outs in Primary CD4+ T Cells—Detailed protocols for the production of CRISPR-Cas9 ribonucleoprotein complexes (crRNPs) and primary CD4+ T cell editing have been previously published (Hultquist et al., 2016; Hultquist et al., 2019). Briefly, lyophilized crRNA and tracrRNA (Dharmacon) were suspended at a concentration of 160 μM in 10 mM Tris-HCl (7.4 pH), 150 mM KCl. 5μL of 160μM crRNA was mixed with 5μL of 16μM tracrRNA and incubated for 30 min at 37°C. The gRNA:tracrRNA complexes were then mixed gently with 10μL of 40μM purified Cas9-NLS protein (UC-Berkeley Macrolab) to form CRISPR-Cas9 ribonucleoprotein complexes (crRNPs). Five 3.5μL aliquots were frozen in 0.2 mL PCR tubes (USA Scientific) at −80°C until use. crRNA guide sequences used in this study were a combination of sequences derived from the Dharmacon pre-designed Edit-R library for gene knock-out and custom ordered sequences as indicated.

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Ficoll-Paque Plus (GE Health Care, #17-1440-02). PBMCs were washed with PBS 1× three times to remove platelets and suspended at a final concentration of 5*10^8 cells/mL in PBS 1×, 0.5% BSA, 2mM EDTA. 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), 50 μg/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 12 hours at 4°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. After stimulation, cell purity and activation was verified by CD4/CD25 immunostaining and flow cytometry as previously described (Hultquist et al., 2016; Hultquist et al., 2019).

After three days of stimulation, cells were suspended and counted. Each electroporation reaction consisted of between 5×10^5 and 1×10^6 T cells, 3.5 μL RNP, and 20 μL electroporation buffer. crRNPs were thawed and allowed to come to room-temperature. Immediately prior to electroporation, cells were centrifuged at 400×g 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, 100 μL of pre-warmed media without IL-2 was added to each well and cells were allowed to rest for 30 minutes 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 U/mL (for a final concentration of 20 U/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 cultured at 37°C / 5% CO$_2$ in a dark, humidified cell culture incubator for 4 days to allow for gene knock-out and protein clearance, with additional media added on day 2. To check knock-out efficiency, 50 μL of mixed culture was removed to a centrifuge tube. Cells were pelleted, supernatant was removed, and pellets were resuspended in 100 μL 2.5x Laemmli Sample Buffer. Protein lysates were heated to 98°C for 20 min before storage at −20°C until western blotting.

Detection of Reverse Transcripts, 2-LTR circles, and integration in CD4$^+$ T cells—Total DNA was extracted from 1×10^6 infected cells. Samples were processed at 12, 36, and 66 h post-infection using QIAmp DNA micro kit (QIAGEN). Viral DNA forms of wild-type and mutant HIV-1 were amplified using real-time PCR (Butler et al., 2001; Valle-Casuso et al., 2012). β-actin amplification was used for normalization. ERT, which refers to the early reverse transcripts (prior the minus strand transfer), and LRT, which refers to the whole synthesis of DNA by HIV-1, were measured at 12 h post-infection. 2-LTR circles were measured at 36 h post-infection to assess nuclear import. Provirus integration in cellular genome was measured at 66 h post-infection using Alu-PCR. Reactions were performed in 1X FastStart Universal Probe Master Mix (Rox) 2 × (Roche) in 20 μl volume. The PCR reaction consisted of the following steps: initial annealing (50°C 2 min), denaturation step (95°C 15 min), 40 cycles of amplification (95°C 15 s, 58°C 30 s, 72°C 30 s). Primer or probe sequences are as follows: ERT hRU5-F2: 5′-GCCTCAA TAAAGCTTGCCTTGA-3′; hRU5-R: 5′-TGACTAAAAGGGTCTGAGGGATCT-3′; ERT-Probe: 5′-(FAM)-AGAGTCACACAACAGACGGGCACACACTA-(TAMRA)-3′. LRTMH531: 5′-TGTGTGCCCGTCTGTTGTGT-3′; MH532: 5′-GAGTCCTGCGTCGAGAGAGC-3′; LRT-Probe: 5′-(FAM)-CAGTGGCGCCCGAACAGGGA-(TAMRA)-3′. 2-LTR circles: MH535: 5′-

AACTAGGGAACCCACTGCTTAAG-3′; MH536: 5′-TCCACAGATCAAGGATATCTTGTC-3′; 2-LTR probe: MH603: 5′-(FAM)-ACACTACTTGAAGCACTCAAG-GCAAGCTTT-(TAMRA)-3′. Alu-PCR 1st round: Alu 1: 5′-TCCCAGCTACTCGGGAGGCTGAGG-3′; Alu 2: 5′-GCCTCCCAAAGTGCTGGGATTACAG-3′; Lambda U3: 5′-ATGCCACGTAAGCGAAACTTTCCGCTGGGGACTTTCCAGGG-3′. Alu quantitative PCR 2nd round: Lambda: (5′-ATGCCACGTAAGCGAAACT-3′); U5: (5′-CTGACTAAAAGGGTCTGAGG-3′; Probe: 5′-(FAM)-TTAAGCCTCAATAAAGCTTGCCTT GAGTGC-(TAMRA)-3′. β-actin: FX: 5′-AACACCCCAGCCATGTACGT-3′, RX: 5-CGGTGAGGATCTTCATGAGGTAGT-3′, Actin-Probe: (6FAM)-CCAGCCAGGTCCAGACGCAGGA-(BBQ)-3′.

Western blot analysis—Cells were lysed in lysis buffer (50 mM Tris [pH 8.0], 280 mM NaCl, 0.5% IGEPAL 40, 10% glycerol, 5 mM MgCl$_2$). Proteins were detected by western blot using anti-FLAG (1:1,000 dilution; Sigma), anti-hemagglutinin (HA) (1:1,000 dilution; Sigma), anti-CypA (1:5,000 dilution; catalog number ab58144; Abcam), anti-TRIM5α$_{hu}$ (1:1,000 dilution; catalog number 14326S; Cell Signaling Technology), anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:5,000 dilution; Invitrogen), or anti-p24 (1:1,000 dilution, catalog number 3637; NIH) antibodies. Secondary antibodies against rabbit and mouse IgG conjugated to IRDye 680LT or IRDye 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.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis was performed using unpaired t test. Information on sample number, amount of repeats and p value can be found in the corresponding figure legend. Quantification of the intensity of western blot bands was performed using ImageJ. For all experiments the mean and standard deviation values were calculated using GraphPad Prism 7.0c.

DATA AND CODE AVAILABILITY

This study did not generate any datasets or code.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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Highlights

- Depletion of cyclophilin A (CypA) expression in CD4$^+$ T cells blocks HIV-1 infection

- CypA binding to the core prevents HIV-1 restriction by TRIM5a during T cell infection

- Endogenously expressed TRIM5α binds to the HIV-1 core and blocks reverse transcription

- Human TRIM5α binds to HIV-1 cores but not to cores bearing capsid changes A92E/G94D
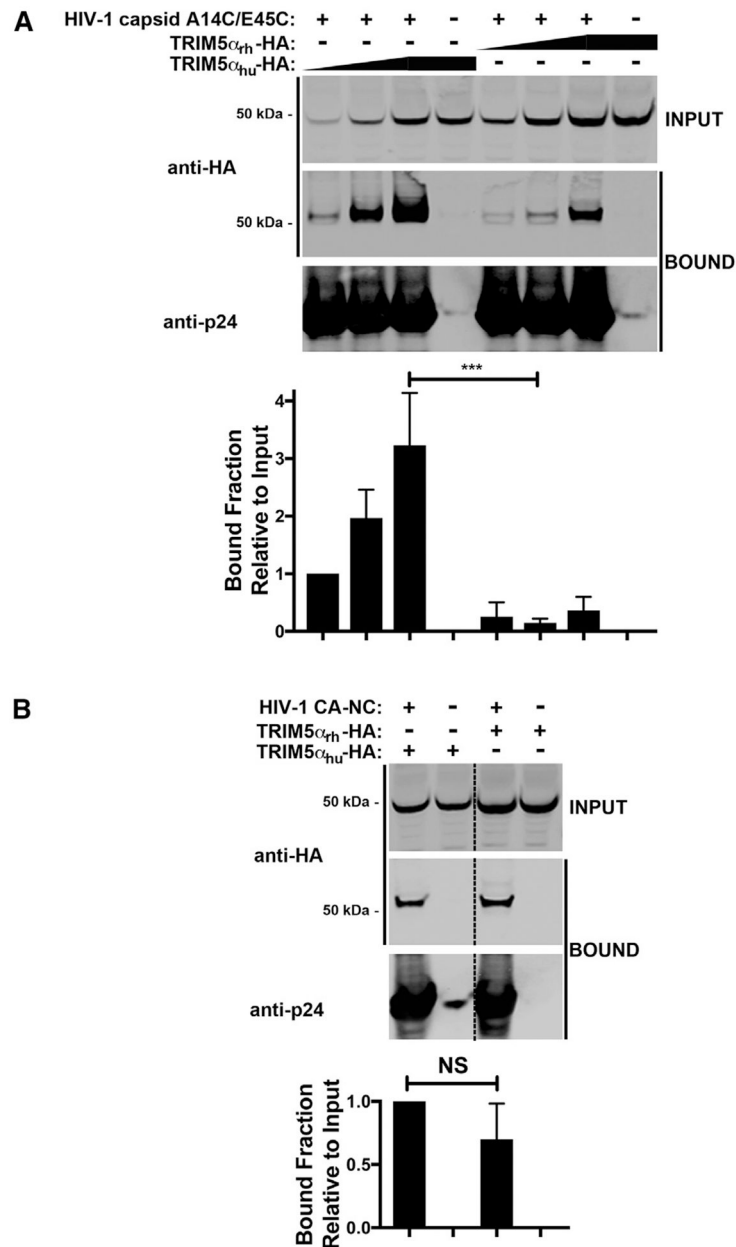
Figure 1. TRIM5α_hu Binds to the HIV-1 Core

Human 293T cells were transfected with plasmids expressing the wild-type human TRIM5α tagged with an HA epitope (TRIM5α_hu-HA).

(A) 24 h post-transfection, cells were lysed in capsid binding buffer (CBB), as described in STAR 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.

(B) Alternatively, cells were lysed in CBB with DTT, and a fraction of the cell lysate was stored (INPUT). Cell extracts were mixed with 5 μL of capsid-nucleocapsid (CA-NC)

particles assembled *in vitro* and incubated for 1 h at room temperature. Subsequently, the mixture was spun through a 70% sucrose cushion. Pellets were resuspended in 1 3 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).
Experiments were repeated six times and a representative experiment is shown. The bound fraction relative to input for six independent experiments with standard deviations is shown. ***p < 0.0005 as determined by the unpaired t test.
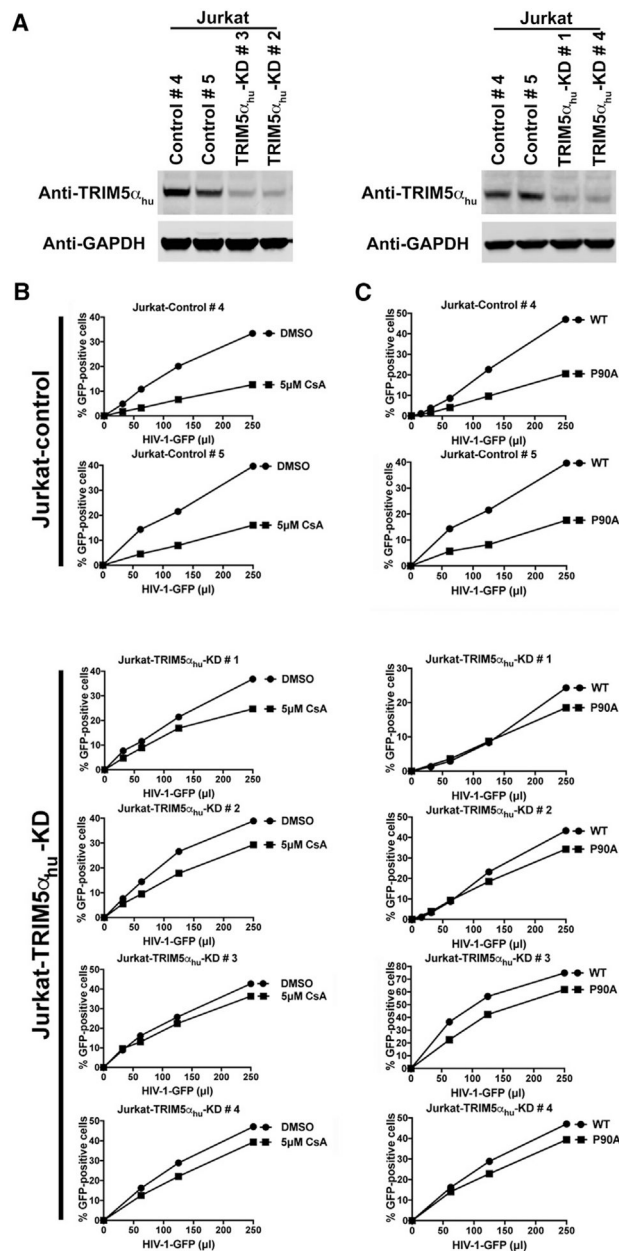
Figure 2. Expression of TRIM5α_hu Is Necessary for the Inhibition of HIV-1 Infection Caused by the Disruption of CypA-Capsid Interactions

(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 analyzed for endogenous expression of TRIM5α_hu by western blotting using antibodies against TRIM5α_hu. Expression of GAPDH was used as a loading control.

(B) Jurkat-TRIM5α_hu-KD and Jurkat-control cells were challenged with increasing amounts of HIV-1 expressing GFP as a reporter of infection (HIV-1-GFP) in the presence of 5 μM cyclosporin A (CsA). 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. Statistical analysis of these experiments is shown in Figure S2B.
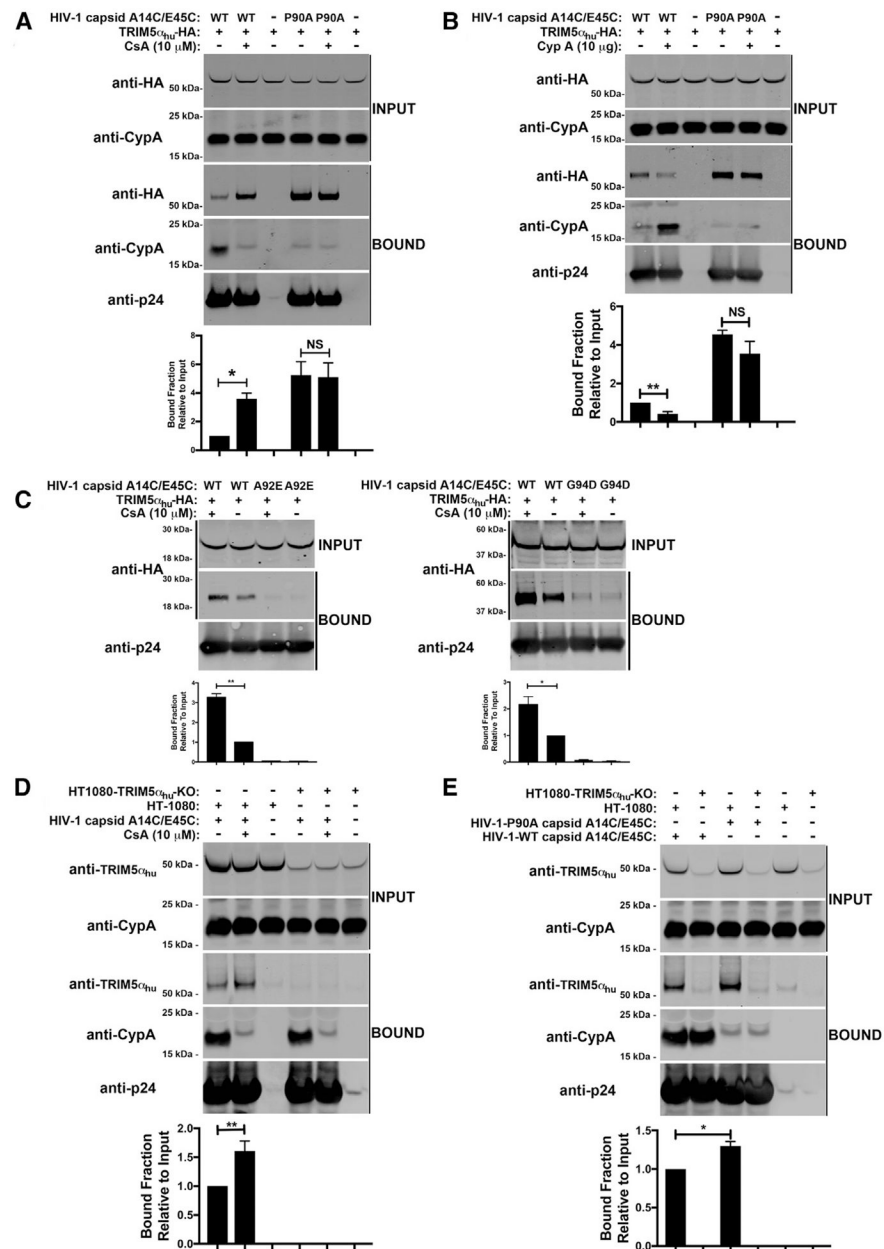
Figure 3. CypA Prevents the Binding of TRIM5α_hu to the HIV-1 Core
(A–C) Human 293T cells were transfected with plasmids expressing TRIM5α_hu-HA. 24 h post-transfection, cells were lysed in capsid binding buffer (CBB) (INPUT). Cell extracts containing TRIM5α_hu-HA were mixed with 20 μL of stabilized wild-type or P90A HIV-1 capsid tubes (5 mg/mL) in the presence of 10 mM CsA (A) or 10 μg recombinant CypA (B).
(C) Cell extracts containing TRIM5α_hu-HA were mixed with 20 μL of stabilized wild-type, A92E, or G94D HIV-1 capsid tubes (5 mg/mL) in the presence of 10 mM CsA.
(D and E) HT1080 cells (control or TRIM5α_hu CRISPR/Cas9 knockout) were lysed in capsid binding buffer (CBB) (INPUT). Cell extracts were mixed with either 20 μL of stabilized wild-typeor P90A HIV-1 capsid tubes (5 mg/mL) in the presence of 10 μM CsA (D) or 20 μL of stabilized wild-type or P90A HIV-1 capsid tubes (5 mg/mL) (E). 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 1× Laemmli buffer (BOUND). INPUT and BOUND fractions were analyzed by western blotting using anti-HA, anti-TRIM5α_{hu}, anti-CypA, and anti-p24 antibodies. Experiments were repeated three times and a representative experiment is shown. The bound fraction relative to input for three independent experiments with standard deviation is shown. *p < 0.005, **p < 0.001, ns indicates not significant as determined by the unpaired t test.
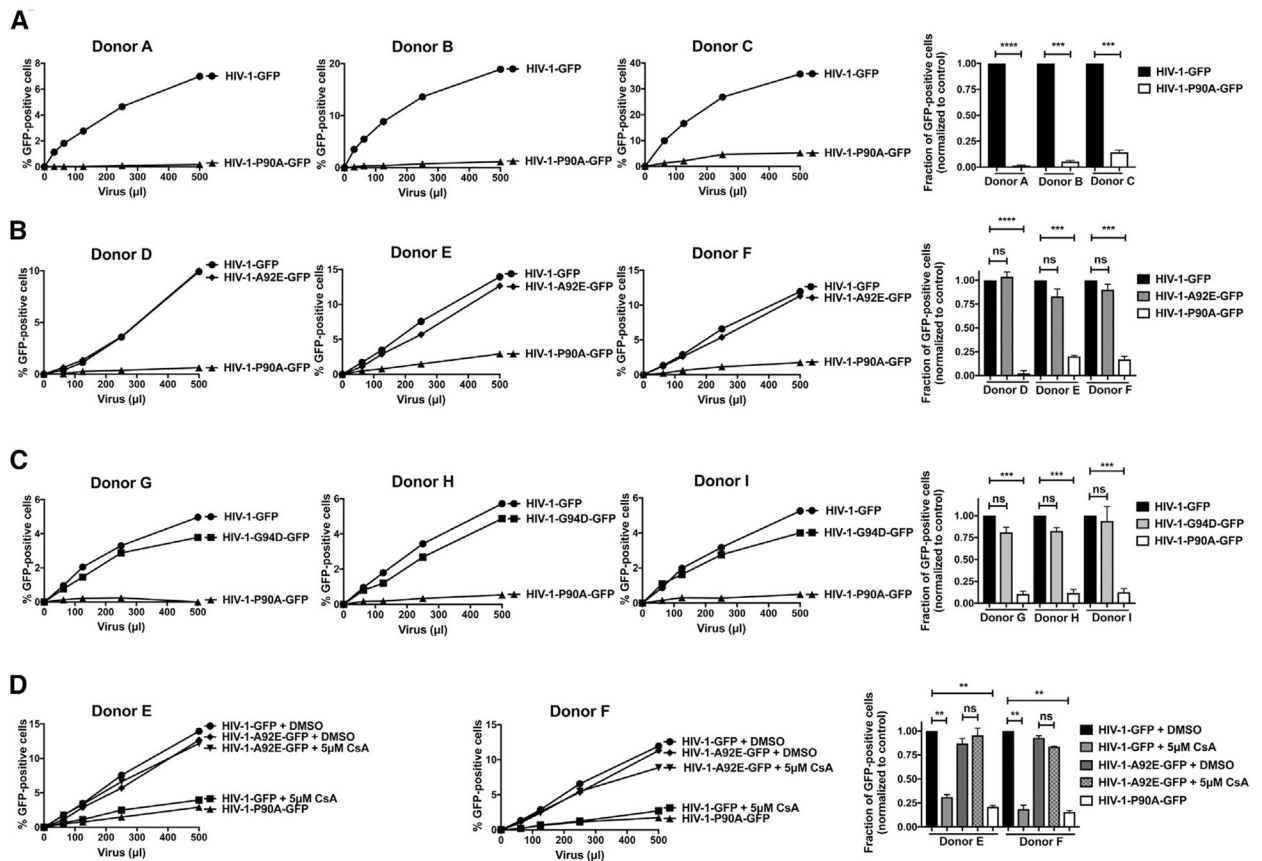
Figure 4. CypA-Capsid Interactions Are Required for HIV-1 Infection of Human CD4$^+$ T Cells

(A–C) Purified CD4$^+$ T cells were challenged with increasing amounts of p24-normalized HIV-1-GFP or HIV-1-P90A-GFP (A) and either HIV-1-A92E-GFP (B) or HIV-1-G94D-GFP (C). Alternatively, purified CD4$^+$ T cells were challenged with increasing amounts of p24-normalized HIV-1-GFP or HIV-1-A92E-GFP in the presence of 5 μM cyclosporin A (CsA). (D) DMSO was used as control. Infection was determined at 72 h post-challenge by measuring the percentage of GFP-positive cells.

Experiments were repeated three times per donor and a representative experiment is shown. The results of three independent experiments with standard deviation are shown; statistical analysis was performed using an intermediate value taken from the infection curves. **p < 0.001, ***p < 0.0005, ****p < 0.0001, ns indicates not significant as determined by the unpaired t test.

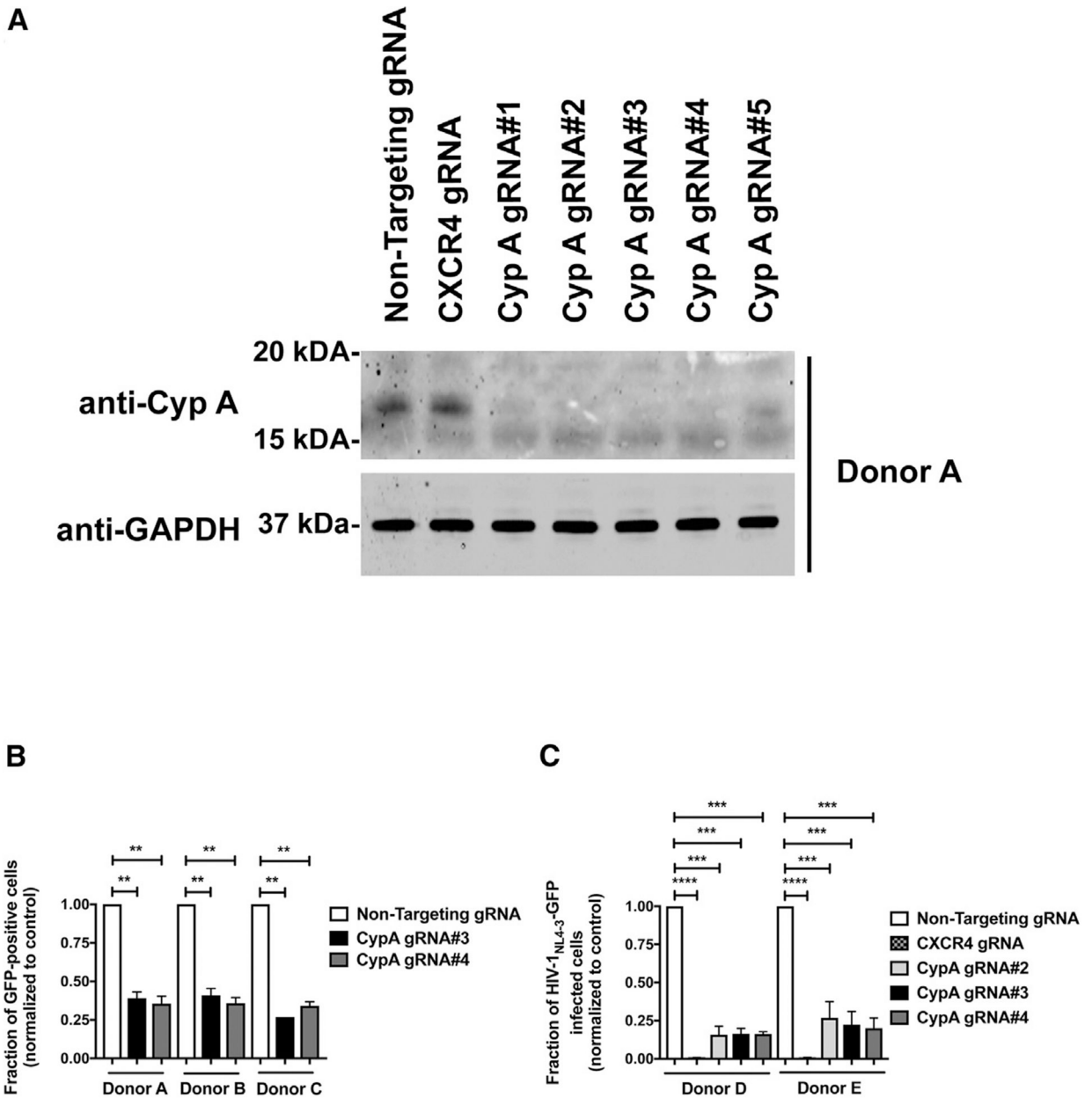**A**

B

C

Figure 5. Depletion of CypA Expression in Human CD4+ T Cells Inhibits HIV-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 STAR 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. For 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 three different donors and a representative blot is shown.

(B and C) Human primary CD4+ T cells depleted for CypA expression were challenged with HIV-1-GFP pseudotyped with the VSV-G envelope (B) or with a replication-competent

HIV-1 expressing GFP as a reporter of infection (HIV-1$_{NL4-3}$-GFP) (C). Infection was determined at 72 h post-infection by measuring the percentage of GFP-positive cells. Experiments were performed in triplicates and results for three (B) or two (C) different donors with standard deviation are shown. **p < 0.001, ***p < 0.0005, ****p < 0.0001 determined by the unpaired t test.
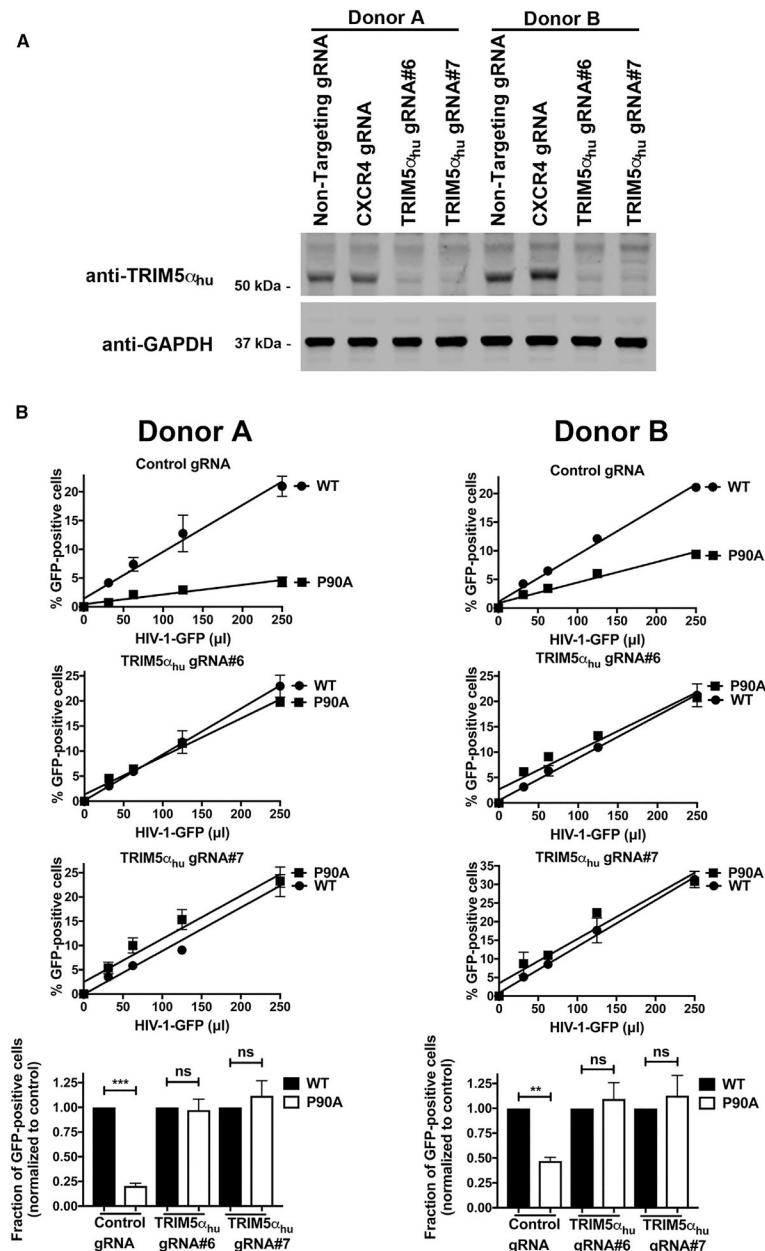
Figure 6. Depletion of TRIM5α_hu Expression in Human CD4+ T Cells Rescues Infection of HIV-1-P90A Viruses

(A) Human primary CD4+ T cells from two different donors were knocked out for TRIM5α_hu expression using the CRISPR/Cas9 system, as described in STAR Methods. Briefly, CD4+ T cells were electroporated using two different guide RNAs (gRNAs) against TRIM5α_hu (gRNA#6 and #7) together with the Cas9 protein. 72 h post-electroporation, endogenous expression of TRIM5α_hu in CD4+ T was analyzed by western blotting using antibodies against TRIM5α_hu. For controls, we electroporated a gRNA against CXCR4 and a non-targeting gRNA. Expression of GAPDH was used as a loading control.

(B) Human primary CD4$^+$ T cells depleted for TRIM5α$_{hu}$ expression were challenged with increasing amounts of HIV-1-GFP or HIV-1-P90A-GFP viruses. Infection was measured at 72 h post-infection by determining the percentage of GFP-positive cells.

Experiments were performed in duplicates and a representative experiment is shown. The results of two independent experiments normalized to wild-type HIV-1-GFP infection with standard deviation are shown. **p < 0.001, ***p < 0.0005, ns indicates not significant as determined by the unpaired t test.