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 affects HIV-1 replication in human lymphocytes. To understand the mechanism, we used Jurkat cells, human PBMCs, and human $CD4^+$ T cells. Our results showed that the inhibition of HIV-1 infection caused by disrupting CypA-capsid interactions is dependent on human TRIM5α (TRIM5α_{hu}), suggesting that TRIM5α_{hu} restricts HIV-1. Accordingly, we found that TRIM5α_{hu} binds to the HIV-1 core. Disruption of CypA-capsid interactions failed to affect HIV-1-A92E infection, correlating with the loss of TRIM5α_{hu} binding to HIV-1-A92E cores. Disruption of CypA-capsid interactions in PBMCs and $CD4^+$ T cells had a greater inhibitory effect on HIV-1 when compared to Jurkat cells. HIV-1-A92E infection of PBMCs and $CD4^+$ T cells was unaffected by disruption of CypA-capsid interactions. Consistent with TRIM5α restriction, disruption of CypA-capsid interactions in $CD4^+$ T cells inhibited reverse transcription. Overall, our results showed that CypA binding to the core protects HIV-1 from TRIM5α_{hu} restriction.

Keywords: HIV-1; CypA; TRIM5α_{hu}; core; capsid; restriction; $CD4^+$ T cells

52 **INTRODUCTION**

53 The early steps of human immunodeficiency virus (HIV-1) replication
54 involve delivery of the viral core into the host cytoplasm. The viral core is a
55 conical structure composed of ~1,500 monomers of the HIV-1 capsid protein,
56 which protects the viral RNA genome (Pornillos et al., 2009; Sundquist and Hill,
57 2007). The capsid protein plays a role in uncoating, reverse transcription, and
58 nuclear import. To perform these functions, the capsid protein has been shown to
59 functionally interact with several host dependency factors, including cyclophilin A
60 (CypA) (Luban et al., 1993), Nup153 (Buffone et al., 2018; Di Nunzio et al., 2013)
61 (Brass et al., 2008; Konig et al., 2008; Lee et al., 2010; Matreyek and Engelman,
62 2011; Matreyek et al., 2013; Zhou et al., 2008), RANBP2 (Di Nunzio et al., 2012;
63 Meehan et al., 2014; Ocwieja et al., 2011), and TNPO3 (Brass et al., 2008; Christ
64 et al., 2008; Konig et al., 2008; Krishnan et al., 2010; Levin et al., 2010; Ocwieja
65 et al., 2011; Thys et al., 2011; Valle-Casuso et al., 2012; Zhou et al., 2008).
66 Effective HIV-1 infection may occur only when these factors interact with the HIV-
67 1 core at the appropriate time and in the appropriate order, thus raising the
68 possibility that the initial binding of particular factors may modulate the
69 subsequent binding of other factors.
70

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

75 1998; Sokolskaja et al., 2004; Thali et al., 1994; Towers et al., 2003). Although
76 the ability of CypA to modulate HIV-1 infection is cell-type dependent, it is clear
77 that the disruption of CypA-capsid interactions negatively affects HIV-1 infection
78 of lymphocytes (Braaten and Luban, 2001; Li et al., 2009; Ptak et al., 2008;
79 Sherry et al., 1998; Sokolskaja et al., 2004; Towers et al., 2003; Wainberg et al.,
80 1988; Yin et al., 1998); however, the underlying mechanism remains elusive.
81 To investigate the molecular events that result in a decrease in HIV-1
82 infection upon disruption of CypA-capsid interactions in lymphocytes, we used
83 Jurkat cells, primary human peripheral blood mononuclear cells (PBMCs), and
84 primary human $CD4^+$ T cells as experimental models. Our experiments revealed
85 that inhibition of HIV-1 infection caused by disrupting CypA-capsid interactions is
86 dependent on the expression TRIM5α_{hu}. These results suggested that TRIM5α_{hu}
87 is restricting HIV-1 infection upon disruption of CypA-capsid interactions. In
88 agreement, we found that TRIM5α_{hu} binds to the HIV-1 core. Interestingly,
89 TRIM5α_{hu} does not bind to HIV-1 cores bearing the capsid mutant A92E (HIV-1-
90 A92E). Infection of HIV-1-A92E viruses was not affected by disruption of CypA-
91 capsid interactions, which correlates with the loss of TRIM5α_{hu} binding to HIV-1
92 cores bearing the A92E mutation. These experiments suggested that CypA
93 protects the HIV-1 core from TRIM5α_{hu}. Although the infectivity defect observed
94 in Jurkat cells is small, disruption of CypA-interactions in PBMCs and $CD4^+$ T
95 cells causes a major defect in infectivity. In agreement with our hypothesis that
96 CypA is protecting the core from TRIM5α_{hu}, infection of PBMCs and $CD4^+$ T cells
97 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}.

121 **RESULTS**

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

123 To understand the role of CypA in HIV-1 infection of $CD4^+$ T cells, we
124 used the human T-cell line Jurkat as an experimental model. Cyclosporin A
125 (CsA) is a molecule that binds to CypA, and thus blocks cellular CypA activity. To
126 measure the effect of CsA treatment on HIV-1 infectivity, we infected Jurkat cells
127 with HIV-1 tagged with green fluorescent protein (HIV-1-GFP) in the presence of
128 10 μM CsA. Consistent with results from previous studies (Braaten and Luban,
129 2001; Hatziioannou et al., 2005; Li et al., 2009; Ptak et al., 2008; Sokolskaja et
130 al., 2004; Towers et al., 2003; Wainberg et al., 1988; Yin et al., 1998), HIV-1-
131 GFP infection of human Jurkat T cells was sensitive to CsA treatment (Figure 1A,
132 upper panel). Experiments were repeated multiple times and the fraction of GFP-
133 positive cells normalized to the dimethyl sulfoxide (DMSO)-treated control is
134 shown (Figure 1A, lower panel). To further confirm the importance of CypA-
135 capsid interactions in HIV-1 infection, we challenged Jurkat T cells with p24-
136 normalized amounts of HIV-1-P90A and HIV-1-G89V, which are mutant versions
137 of HIV-1 that disrupt CypA-capsid interactions (Gamble et al., 1996; Thali et al.,
138 1994). Infection was determined by measuring the percentage of GFP-positive
139 cells at 48 h post-infection. We found that the mutant HIV-1 strains bearing
140 capsid changes that disrupted CypA-capsid interactions were less infectious than
141 wild-type HIV-1 (Figure 1B, upper panel). Experiments were repeated multiple
142 times and the fraction of GFP-positive cells normalized to control is shown
143 (Figure 1B, lower panel). Although Jurkat cells failed to show a dramatic

144 decrease in HIV-1 infectivity as a result of disrupted CypA-capsid interactions, we
145 still used this cell line as a model to mechanistically understand the contribution
146 of CypA to HIV-1 infection.
147
148 **Human TRIM5α (TRIM5α_{hu}) binds to the HIV-1 capsid.**
149 Previous studies have shown that CypA modulates the ability of rhesus
150 tripartite motif (TRIM) protein, TRIM5α (TRIM5α_{rh}), to restrict HIV-1 infection
151 (Berthoux et al., 2005; Burse et al., 2017; Keckesova et al., 2006; Lin and
152 Emerman, 2008; Stremlau et al., 2006b), which is not surprising because both
153 proteins interact with the HIV-1 core. It is well known that TRIM5α_{rh} cages the
154 HIV-1 core using cooperative binding during infection (Diaz-Griffero et al., 2009;
155 Ganser-Pornillos et al., 2011; Stremlau et al., 2006a). Because both CypA and
156 TRIM5α_{rh} bind to the HIV-1 core, it is possible that CypA modulates the binding
157 of TRIM5α_{rh} to the HIV-1 core and vice versa.
158 More recent studies have suggested that TRIM5α_{hu} blocks HIV-1 infection
159 (Jimenez-Guardeno et al., 2019; OhAinle et al., 2018), possibly by binding to the
160 HIV-1 core. Therefore, we tested the ability of TRIM5α_{hu} to bind to the HIV-1 core
161 using our capsid binding assay (Selyutina et al., 2018). We found that TRIM5α_{hu}
162 binds to the HIV-1 core (Figure 2). TRIM5α_{hu} showed a stronger interaction with
163 the HIV-1 core compared with TRIM5α_{rh} (Figure 2). Our results showed for the
164 first time that TRIM5α_{hu} interacted with the HIV-1 core. One possibility is that
165 CypA modulates the ability of TRIM5α_{hu} to interact with the HIV-1 core.

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

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

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

187 HIV-1-P90A is a mutant form of HIV-1 in which CypA-capsid interactions
188 are disrupted during infection. We tested whether HIV-1-P90A infection was

189 inhibited in TRIM5α_{hu} KD Jurkat cells. As shown in Figure 3C, we found that HIV-
190 1-P90A infection was not inhibited in TRIM5α_{hu} KD cells compared with control
191 cells. This result supports our hypothesis that expression of TRIM5α_{hu} is
192 important for the infection phenotype observed when CypA-capsid interactions
193 are disrupted. It is possible that CypA prevents the binding of TRIM5α_{hu} to the
194 HIV-1 core via steric hindrance, thus protecting the core from the restriction
195 factor during the early steps of infection.
196
197 **CypA prevents the interaction of TRIM5α_{hu} with the HIV-1 core.**
198 To test whether CypA modulates the binding of TRIM5α_{hu} to the HIV-1
199 core, we analyzed the binding of TRIM5α_{hu} to the HIV-1 core in the presence of
200 CsA, which binds to endogenously expressed CypA in cellular extracts and
201 prevents CypA binding to the HIV-1 core. As shown in Figure 4A, TRIM5α_{hu}
202 binding to the HIV-1 core increased 3- to 4-fold in the presence of CsA. We also
203 found that the binding of TRIM5α_{hu} to mutant P90A HIV-1 cores was 5- to 6-fold
204 higher than that of wild-type HIV-1 cores (Figure 4A) in the absence or presence
205 of CsA. These results suggested that CypA prevents the binding of TRIM5α_{hu} to
206 the HIV-1 core during infection. It is possible that CypA and TRIM5α_{hu} compete
207 with each other to interact with the incoming core. To test this hypothesis, we
208 investigated whether the addition of exogenous CypA decreased TRIM5α_{hu}
209 binding to the HIV-1 core. For this purpose, we assessed the ability of TRIM5α_{hu}
210 from cellular extracts to bind to the HIV-1 core in the presence of 10 μg of
211 recombinant CypA protein. We found that the addition of recombinant CypA

212 significantly decreased the binding of TRIM5α_{hu} to the HIV-1 core (Figure 4B). As
213 expected, recombinant CypA did not affect the binding of TRIM5α_{hu} to HIV-1
214 cores bearing the P90A mutation. Overall, these results suggested that CypA
215 protects the core from the restriction factor TRIM5α_{hu}.
216
217 **Mutant HIV-1 strains that are impaired for capsid-TRIM5α_{hu} interactions are**
218 **insensitive to CsA treatment.**
219 Previous studies have shown that infection of Jurkat cells by the HIV-1
220 strain bearing the capsid mutation A92E (HIV-1-A92E-GFP) was less sensitive to
221 CsA treatment when compared with wild-type HIV-1 (Hatziioannou et al., 2005;
222 Sokolskaja et al., 2004). Consistent with these findings, we observed that HIV-1-
223 A92E infection of Jurkat cells was insensitive to CsA treatment (Figure 5A). This
224 insensitivity may occur due to one or both of the following scenarios: 1) HIV-1-
225 A92E may have a higher affinity for CypA, which may better protect the virus
226 from TRIM5α_{hu}, and 2) HIV-1-A92E may have a lower affinity for TRIM5α_{hu},
227 which may prevent inhibition of infection. To explore these possibilities, we tested
228 the ability of CypA to interact with HIV-1 cores containing the A92E capsid
229 mutation. We found that CypA did not bind to the mutant A92E core, which
230 suggested no or poor interaction between HIV-1-A92E and CypA during infection
231 (Figure 5B). As a control, we showed that the binding of CypA to wild-type HIV-1
232 cores was CsA-sensitive (Figure 5B). Because CypA does not bind to HIV-1-
233 A92E, a stronger binding between HIV-1-A92E and TRIM5α_{hu} may result in
234 inhibition of infection. However, we found that the infection of Jurkat cells by HIV-

235 1-A92E was not affected when compared with that of wild-type HIV-1 (Figure
236 5A), suggesting that HIV-1-A92E may be unable to interact with TRIM5α_{hu}.
237 Consistent with our hypothesis, TRIM5α_{hu} did not interact with mutant HIV-1
238 cores bearing the capsid change A92E (Figure 5C). Overall, these results
239 indicated that mutant HIV-1 A92E cores bind neither CypA nor TRIM5$\alpha_{hu,}$ which
240 may explain the reason that CsA treatment does not affect HIV-1-A92E infection
241 of Jurkat cells.
242
243 **CypA-capsid interactions are required for HIV-1 infection of human PBMCs**
244 **and CD4$^+$ T cells.**
245 To understand the role of CypA in HIV-1 infection of human primary
246 PBMCs, we challenged PBMCs and CD4$^+$ T cells from different donors with HIV-
247 1 in the presence of increasing concentrations of CsA, which prevents the
248 binding of CypA to HIV-1 capsid (Diaz-Griffero et al., 2006; Luban et al., 1993).
249 As shown in Figure S1, CsA treatment inhibited HIV-1 infection in PBMCs and
250 CD4$^+$ T cells. This result suggested that CypA-capsid interactions are required
251 for HIV-1 infection of human PBMCs and CD4$^+$ T cells. To further corroborate
252 these findings, we challenged human PBMCs and CD4$^+$ T cells with increasing
253 amounts of HIV-1 particles bearing the capsid mutations P90A and G89V, both of
254 which prevent CypA-capsid interactions (Gamble et al., 1996; Thali et al., 1994).
255 Infection was determined by measuring the percentage of GFP-positive cells at
256 48 h post-infection. Wild-type and mutant HIV-1-GFP viruses were normalized by
257 quantifying the levels of p24. We found that HIV-1-P90A and HIV-1-G89V were

258 unable to infect human PBMCs and $CD4^+$ T cells (Figure 6A and B). Although
259 this experiment was performed in PBMCs and $CD4^+$ T cells obtained from six
260 different donors, all of which showed the same results, we have only shown
261 sample results obtained from three donors. Our results suggested that CypA-
262 capsid interactions are required for HIV-1 infection in human primary PBMCs and
263 $CD4^+$ T cells. Importantly, the lack of CypA appeared to have a stronger effect on
264 HIV-1 infection in primary human lymphocytes compared with that in Jurkat cells
265 (Figures 1B and 6A&B), thus indicating that CypA was crucial for HIV-1 infection
266 of human primary cells. Overall, these results suggested that the disruption of
267 CypA-capsid interaction is detrimental for HIV-1 infection of PBMCs and $CD4^+$ T
268 cells.
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270 **Disruption of HIV-1 binding to TRIM5α_{hu} does not affect viral infection in**
271 **human PBMCs and $CD4^+$ T cells.**

272 Mutant HIV-1 bearing the capsid change A92E lacks the ability to interact
273 with TRIM5α_{hu}. We examined the ability of HIV-1-A92E to infect PBMCs and
274 $CD4^+$ T cells to study the role of TRIM5α_{hu} during viral infection in these cells.
275 Consistent with the results observed in Jurkat cells, HIV-1-A92E infection levels
276 were comparable to that of wild-type HIV-1 in human PBMCs (Figure 7A). As a
277 control, we also challenged PBMCs with increasing concentrations of HIV-1-
278 P90A, an HIV-1 mutant that is completely inhibited in PBMCs (Figure 7A). We
279 observed similar results when we challenged $CD4^+$ T cells using HIV-1-A92E
280 (Figure 7B). Consistent with our previous binding experiments, we found that

281 HIV-1-A92E infection of PBMCs and $CD4^+$ T cells was not affected by TRIM5α_{hu},
282 which correlated with the inability of TRIM5α_{hu} to bind to mutant HIV-1-A92E
283 cores.
284
285 **Depletion of CypA expression in human $CD4^+$ T cells inhibits HIV-1**
286 **infection.**
287 We have previously shown that CypA-capsid interactions are important for
288 HIV-1 infection. To directly test the role of CypA in viral infection, we challenged
289 CypA knockout $CD4^+$ T cells with HIV-1_{NL4-3}-GFP. To create CypA knockouts,
290 $CD4^+$ T cells were separately electroporated with CRISPR/Cas9 complexes
291 containing five different guides against CypA (CypA-KO#1–5). Expression of
292 CypA was analyzed by western blotting using anti-CypA antibodies at 3 days
293 post-electroporation. As shown in Figure 8A, all five guides were able to
294 eliminate the expression of CypA. The CypA knockout cells were then challenged
295 with HIV-1_{NL4-3}-GFP. Consistent with our previous results, human $CD4^+$ T cells
296 depleted for CypA expression were resistant to HIV-1 infection (the results from
297 two donors are shown in Figure 8B). As a control, we used a CRISPR/Cas9
298 complex containing an RNA guide for CXCR4, which completely inhibits infection
299 of HIV-1_{NL4-3}-GFP. We also used a CRISPR/Cas9 complex containing a non-
300 targeting RNA guide. These results demonstrated that CypA expression is
301 essential for HIV-1 infection in human primary $CD4^+$ T cells.
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303 **CypA-capsid interactions are required for efficient HIV-1 reverse**
304 **transcription in human CD4$^+$ T cells.**
305 Our analysis showed that HIV-1 strains bearing capsid mutations that
306 disrupt CypA-capsid interactions are unable to infect human CD4$^+$ T cells. To
307 identify the step in the HIV-1 life cycle at which infection is disrupted, we
308 challenged human CD4$^+$ T cells with p24-normalized levels of GFP expressed by
309 wild-type and capsid mutant HIV-1 particles, and measured reverse transcription,
310 nuclear import, and integration using real-time polymerase chain reaction (PCR)
311 (Figure 9). Infection was measured by determining the percentage of GFP-
312 positive cells at 72 h post-infection (Figure 9A). Consistent with our previous
313 results, HIV-1-P90A and HIV-1-G89V strains showed weak infection in CD4$^+$ T
314 cells (Figure 9A). Reverse transcription was measured by determining the levels
315 of late reverse transcripts (LRT) at 10 h post-infection (Figure 9B). Interestingly,
316 reverse transcription was reduced in HIV-1-P90A and HIV-1-G89V, suggesting
317 that CypA-capsid interactions were important for HIV-1 reverse transcription in
318 CD4$^+$ T cells. As a control, we used Nevirapine (Nev), which inhibits reverse
319 transcription (Figure 9B). For nuclear import, we measured the levels of 2-long
320 terminal repeat (2-LTR) circles at 24 h post-infection (Figure 9C). Consistent with
321 a reverse transcription block, our results showed that both viruses were blocked
322 for nuclear import. Viral integration was determined using Alu-PCR at 48 h post-
323 infection (Figure 9D). As a control, the integration inhibitor Raltegravir (Ral) was
324 also used. As expected, the integration of HIV-1-P90A and HIV-1-G89V was
325 blocked. Overall, these results suggested that HIV-1 requires interaction with

326 CypA in order to undergo efficient reverse transcription in human $CD4^+$ T cells.
327 These results also suggested that in the absence of CypA, HIV-1 particles are
328 exposed to TRIM5α_{hu}, which inhibits reverse transcription.
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349 **DISCUSSION**

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

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

372 lymphocytes. Conversely, we also demonstrated that increasing the amounts of
373 CypA prevented the binding of TRIM5α_{hu} to the HIV-1 core. Based on these
374 results, we propose that the prevention of CypA binding allows TRIM5α_{hu} to bind
375 to the HIV-1 core, consequently inhibiting infection.

376 Our results indicated that the interaction of TRIM5α_{hu} with the HIV-1 core
377 leads to restriction of infection. However, several previous studies have shown
378 that overexpression of TRIM5α_{hu} has little or no effect on HIV-1 infection (Li et al.,
379 2006; Pham et al., 2010; Yap et al., 2005). One possibility is that restriction of
380 HIV-1 infection by TRIM5α_{hu} requires a specific cellular cofactor expressed only
381 in Jurkat cells and human primary lymphocytes. This cofactor may have been
382 absent or present only in insufficient amounts in other human cell lines that were
383 used for TRIM5α_{hu} overexpression experiments. The search for this type of
384 cofactors in the future is warranted. Alternatively, TRIM5α_{hu} might be recruited to
385 the HIV-1 core by other TRIM proteins through the formation of higher-order
386 complexes (Diaz-Griffero et al., 2009); TRIM5α dimers are known to associate
387 with other dimers and form higher-order self-association complexes. For
388 example, TRIM5α_{rh} forms higher-order complexes with human TRIM34 and
389 TRIM6 (Li et al., 2011). Alternatively, TRIM5α_{hu} might recruit other human TRIM
390 orthologs that are important for restriction. Future studies will explore the
391 existence of other human TRIM orthologs that may form higher-order complexes
392 with TRIM5α_{hu}.

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

395 wild-type HIV-1 infection of Jurkat cells; however, infection of HIV-1 viruses
396 bearing the capsid change A92E are insensitive to CsA treatment in Jurkat cells
397 (Hatziioannou et al., 2005; Sokolskaja et al., 2004). Here, we showed that this
398 insensitivity was due to the lack of TRIM5α_{hu} binding to HIV-1-A92E mutant
399 cores. Normal viral infectivity correlated with the inability of TRIM5α_{hu} to bind to
400 HIV-1-A92E cores. Additionally, the HIV-1-A92E mutant core also did not interact
401 with CypA, indicating that the A92E mutation abolishes interaction of the HIV-1
402 core with both TRIM5α_{hu} and CypA. Based on these results, it is possible that
403 TRIM5α_{hu} binds to the CypA-binding loop or in its proximity.

404 The disruption of CypA-capsid interactions had a stronger effect on viral
405 infectivity in human primary cells compared with Jurkat cells. Mutant HIV-1 (P90A
406 or G89V) infection of PBMCs or $CD4^+$ T cells was 10–20 fold weaker than that of
407 wild-type HIV-1. Similarly, KD of CypA expression in human primary $CD4^+$ T cells
408 potently blocked HIV-1 infection. In contrast, HIV-1-A92E infection of primary
409 lymphocytes was not affected when compared to that of wild-type HIV-1. Taken
410 together, these results suggested that TRIM5α_{hu} blocks HIV-1 infection of human
411 primary lymphocytes when CypA-capsid interactions are disrupted. Consistent
412 with this idea, we observed that the inhibition of infection caused by the
413 disruption of CypA-capsid interactions in $CD4^+$ T cells occurred prior to HIV-1
414 reverse transcription.

415 Our work has shown that CypA protects the core from TRIM5$\alpha_{hu.}$
416 Intriguingly, new- and old-world monkeys express a protein in which the TRIM
417 domain of TRIM5α and CypA form a fusion protein (Brennan et al., 2008;

418 Newman et al., 2008; Sayah et al., 2004). Based on our results, it is possible that
419 evolution may have taken advantage of a positive factor, in this case CypA, and
420 fused to the TRIM domain to develop a restriction factor.
421 Overall, this work showed the mechanism by which disruption of CypA-
422 capsid interactions affect HIV-1 infection of human primary lymphocytes. Future
423 experiments will explore whether a similar mechanism operates in macrophages
424 or dendritic cells.
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ACKNOWLEDGEMENTS

We thank the NIH AIDS repository for important reagents. We are very grateful to Kyusik Kim and Jeremy Luban for reagents and helpful discussions. This work was supported by an R01 grant from the NIH AI087390, to F.D.-G.

464 **MATERIALS AND METHODS**

465 **Cell lines and drugs**

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

473

474 **Infection using HIV-1-GFP reporter viruses**

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

481

482 **Capsid expression and purification**

483 pET-11a vectors were used to express the HIV-1 capsid protein containing
484 the A14C and E45C mutations. Point mutations P90A and A92E were introduced
485 using the QuikChange II site-directed mutagenesis kit (Stratagene) according to
486 the manufacturer's instructions. All proteins were expressed in *Escherichia coli*

487 one-shoot BL21star (DE3) cells (Invitrogen), as previously described (Selyutina et
488 al., 2018). Briefly, cells were inoculated in Luria-Bertani (LB) medium and
489 cultured at 30°C until mid-log phase (A_{600}, 0.6–0.8). Protein expression was
490 induced with 1 mM isopropyl-β-d-thiogalactopyranoside (IPTG) overnight at
491 18°C. Cells were harvested by centrifugation at 5,000 × g for 10 min at 4°C, and
492 pellets were stored at −80°C until purification. Purification of capsids was carried
493 out as follows: pellets from 2 L of Luria containing bacteria were lysed by
494 sonication (Qsonica microtip: 4420; A = 45; 2 min; 2 sec on; 2 sec off for 12
495 cycles), in 40 ml of lysis buffer (50 mM Tris pH = 8, 50 mM NaCl, 100 mM β-
496 mercaptoethanol, and Complete EDTA-free protease inhibitor tablets). Cell
497 debris was removed by centrifugation at 40,000 × g for 20 min at 4°C. Proteins
498 from the supernatant were precipitated by incubation with one-third the volume of
499 saturated ammonium sulfate containing 100 mM β-mercaptoethanol for 20 min at
500 4°C, and centrifugation at 8,000 × g for 20 min at 4°C. Precipitated proteins were
501 resuspended in 30 ml of buffer A (25 mM 2-(N-morpholino) ethanesulfonic acid
502 (MES) pH 6.5 and 100 mM β-mercaptoethanol) and sonicated 2–3 times
503 (Qsonica microtip: 4420; A = 45; 2 min; 1 sec on; 2 sec off). The protein sample
504 was dialyzed three times in buffer A (2 h, overnight, 2 h), sonicated, diluted in
505 500 ml of buffer A, and was chromatographed sequentially on a 5-ml HiTrap Q
506 HP column and on a 5-ml HiTrap SP FF column (GE Healthcare), which were
507 both pre-equilibrated with buffer A. The capsid protein was eluted from the
508 HiTrap SP FF column using a linear gradient of concentrations ranging from 0–2
509 M NaCl. The eluted fraction that had highest protein levels was selected based

510 on absorbance at 280 nm. Pooled fractions were dialyzed three times (2 h,
511 overnight, 2 h) in storage buffer (25 mM MES, 2 M NaCl, 20 mM β-
512 mercaptoethanol). The sample was reduced to a concentration of 20 mg/ml using
513 Centricon and stored at -80°C.
514
515 **Assembly of stabilized HIV-1 capsid tubes**
516 One milliliter of monomeric capsid (5 mg/ml) was dialyzed in SnakeSkin
517 dialysis tubing 10K MWCO (Thermo Scientific) using a buffer that is high in salt
518 and contains a reducing agent (buffer 1: 50 mM Tris, pH 8, 1 M NaCl, 100 mM β-
519 mercaptoethanol) at 4°C for 8 h. Subsequently the protein was dialyzed using
520 buffer 1 without the reducing agent β-mercaptoethanol (buffer 2: 50 mM Tris, pH
521 8, 1 M NaCl) at 4°C for 8 h. The absence of β-mercaptoethanol in the second
522 dialysis allows the formation of disulfide bonds between Cysteine 14 and 45
523 inter-capsid monomers in the hexamer. Finally, the protein was dialyzed using
524 buffer 3 (20 mM Tris, pH 8,0, 40 mM NaCl) at 4°C for 8 h. Assembled complexes
525 were kept at 4°C for up to 1 month.
526
527 **Capsid binding assay protocol.**
528 Human HEK293T cells were transfected for 24 h with a plasmid
529 expressing the protein of interest (TRIM5α_{hu},TRIM5α_{rh}, or CypA). The cell
530 medium was completely removed and cells were scraped off the plate and lysed
531 in 300 µl of capsid binding buffer (CBB: 10 mM Tris, pH 8,0, 1,5 mM $MgCl_2$, 10
532 mM KCl). Cells were rotated at 4°C for 15 min and then centrifuged to remove

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

556 and incubated at 4°C for 5 min. Thirty microliters of $CD4^+$ T-cell isolation buffer
557 and 20 µl of $CD4^+$ T-cell microbead cocktail were then added and further
558 incubated for 10 min at 4°C. Depending on the amount of PBMCs isolated, LS
559 Column (MACS Miltenyi Biotec, #130-042-401) or MS Column (MACS Miltenyi
560 Biotec, #130-042-201) attached to a MACS Separator was pre-washed with 3 ml
561 or 6 ml of ice-cold $CD4^+$ T-cell isolation buffer, respectively. PBMC suspension
562 was added to the column and the flow-through was collected in a 15-ml tube. The
563 LS or MS Columns were then washed with 3 ml or 6 ml of ice-cold $CD4^+$ T-cell
564 isolation buffer, respectively, and the flow-through was collected. The newly
565 isolated $CD4^+$ T cells were then centrifuged at 800 *x g* for 5 min and resuspended
566 in RPMI medium supplemented with IL-2 (100 U/ml).
567
568 **Knockdown of TRIM5α_{hu} expression in Jurkat cells**
569 Lentiviral particles were produced in human 293T cells by co-transfection
570 of 1 µg TAT, 1 µg REV, 1.5 µg VSVG, 1 µg HDM(codon optimized gag-pol), and
571 7.5 µg of the lentiviral vector pLKO1, or 7.5 µg the pLKO1 vector containing an
572 shRNA that targets TRIM5α_{hu} (pLKO1-shRNA-TRIM5α_{hu}). Viruses were
573 harvested at 48 h post-transfection and used to transduce Jurkat cells for 5 days
574 at a 1:1 virus to complete media ratio. Transduced cells were selected in 0.15
575 µg/ml of puromycin. Cells that stably contained pLKO1 or pLKO1-shRNA-
576 TRIM5α_{hu} were maintained in puromycin. Expression of TRIM5α_{hu} was evaluated
577 by challenging the cells with N-tropic murine leukemia virus (N-MLV) and B-tropic
578 murine leukemia virus (B-MLV).

579

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

581 Detailed protocols for RNP production and primary CD4+ T cell editing
582 have been previously published (Hultquist et al., 2016). Briefly, lyophilized crRNA
583 and tracrRNA (Dharmacon) were suspended at a concentration of 160 μM in 10
584 mM Tris-HCL (7.4 pH) with 150 mM KCl. 5μL of 160μM crRNA was mixed with
585 5μL of 160μM tracrRNA and incubated for 30 min at 37°C. The gRNA:tracrRNA
586 complexes were then mixed gently with 10μL of 40μM Cas9 (UC-Berkeley
587 Macrolab) to form Cas9 ribonucleoproteins (RNPs). Five 3.5μL aliquots were
588 frozen in Lo-Bind 96-well V-bottom plates (E&K Scientific) at -80°C until use. All
589 crRNA guide sequences used in this study were derived from the Dharmacon
590 pre-designed Edit-R library for gene knock-out, including the non-targeting (U-
591 007502, U-007501) and CYPA-targeting guides (CM-004979-01 through CM-
592 004979-05).

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

602 piperazineethanesulfonic acid (HEPES, Corning), 2mM Glutamine (UCSF Cell
603 Culture Facility), 50μg/mL penicillin/streptomycin (P/S, Corning), 5mM sodium
604 pyruvate (Corning), and 10% fetal bovine serum (FBS, Gibco). Media was
605 supplemented with 20 IU/mL IL-2 (Miltenyi) immediately before use. For
606 activation, bulk CD4+ T cells were immediately plated on anti-CD3 coated plates
607 [coated for 2 hours at 37°C with 20μg/mL anti-CD3 (UCHT1, Tonbo Biosciences)]
608 in the presence of 5μg/mL soluble anti-CD28 (CD28.2, Tonbo Biosciences). Cells
609 were stimulated for 72 hours at 37°C / 5% CO2 prior to electroporation.
610 Each electroporation reaction consisted of 4x10^5 T cells, 3.5 μL RNP,
611 and 20 μL electroporation buffer. After three days of stimulation, cells were
612 suspended and counted. RNPs were thawed and allowed to come to room-
613 temperature. Immediately prior to electroporation, cells were centrifuged at 400xg
614 for 5 minutes, supernatant was removed by aspiration, and the pellet was
615 resuspended in 20 μL of room-temperature P3 electroporation buffer (Lonza) per
616 reaction. 20 μL of cell suspension was then gently mixed with each RNP and
617 aliquoted into a 96-well electroporation cuvette for nucleofection with the 4D 96-
618 well shuttle unit (Lonza) using pulse code EH-115. Immediately after
619 electroporation, 80 μL of pre-warmed media without IL-2 was added to each well
620 and cells were allowed to rest for at least one hour in a 37°C cell culture
621 incubator. Cells were subsequently moved to 96-well flat-bottomed culture plates
622 pre-filled with 100 μL warm complete media with IL-2 at 40 IU/mL (for a final
623 concentration of 20 IU/mL) and anti-CD3/anti-CD2/anti-CD28 beads (T cell
624 Activation and Stimulation Kit, Miltenyi) at a 1:1 bead:cell ratio. Cells were

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

648 MH532: 5'-GAGTCCTGCGTCGAGAGAGC-3'; LRT-Probe: 5′-(FAM)-
649 CAGTGGCGCCCGAACAGGGA-(TAMRA)-3'. 2-LTR circles: MH535: 5'-
650 AACTAGGGAACCCACTGCTTAAG-3'; MH536: 5′-
651 TCCACAGATCAAGGATATCTTGTC-3′; 2-LTR probe : MH603: 5'-(FAM)-
652 ACACTACTTGAAGCACTCAAG-GCAAGCTTT-(TAMRA)-3'. Alu-PCR 1st round :
653 Alu 1: 5'-TCCCAGCTACTCGGGAGGCTGAGG-3'; Alu 2: 5'-
654 GCCTCCCAAAGTGCTGGGATTACAG-3'; Lambda U3: 5′-
655 ATGCCACGTAAGCGAAACTTTCCGCTGGGGACTTTCCAGGG-3′. Alu
656 quantitative PCR 2nd round: Lambda: (5'-ATGCCACGTAAGCGAAACT-3'); U5:
657 (5'-CTGACTAAAAGGGTCTGAGG-3'; Probe: 5'-(FAM)-
658 TTAAGCCTCAATAAAGCTTGCCTTGAGTGC-(TAMRA). β-actin: FX: 5'-
659 AACACCCCAGCCATGTACGT-3', RX: 5-CGGTGAGGATCTTCATGAGGTAGT,
660 Actin-Probe: (6FAM)-CCAGCCAGGTCCAGACGCAGGA-(BBQ).
661
662 **Western blot analysis**.
663 Cells were lysed in lysis buffer (50 mM Tris [pH 8.0], 280 mM NaCl, 0.5%
664 IGEPAL 40, 10% glycerol, 5 mM $MgCl_2$). Proteins were detected by western blot
665 using anti-FLAG (1:1,000 dilution; Sigma), anti-hemagglutinin (HA) (1:1,000
666 dilution; Sigma), anti-CypA (1:5,000 dilution; catalog number ab58144; Abcam),
667 or anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:5,000; Ambion),
668 or anti-p24 (1:1,000, catalog number 183-H12-5C; NIH) antibodies. Secondary
669 antibodies against rabbit and mouse IgG conjugated to IRDye 680LT or IRDye
670 800CW were obtained from Li-Cor (1:10,000 dilution). Bands were detected by

671 scanning blots using the Li-Cor Odyssey imaging system in the 700-nm or 800-
672 nm channels.
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694 **FIGURE LEGENDS**

695

696 **Figure 1. CypA is important for HIV-1 infection of Jurkat cells. (A)** Human
697 Jurkat cells were challenged with increasing amounts of HIV-1 expressing GFP
698 as a reporter of infection (HIV-1-GFP) in the presence of 10 μM cyclosporin A
699 (CsA) (upper panel). DMSO was used as control. Infection was determined at 48
700 h post-challenge by measuring the percentage of GFP-positive cells using a flow
701 cytometer. Similar results were obtained from three independent experiments
702 and a representative experiment is shown. The result of three independent
703 experiments normalized to wild-type HIV-1-GFP infection with standard deviation
704 is shown (lower panel). * indicates P-value < 0.005 as determined by the
705 unpaired t-test. **(B)** Jurkat cells were challenged with increasing amounts of p24-
706 normalized HIV-1, HIV-1-P90A, or HIV-1-G89V expressing GFP (upper panel).
707 Infection was determined at 48 h post-challenge by measuring the percentage of
708 GFP-positive cells using a flow cytometer. Similar results were obtained from
709 three independent experiments and a representative experiment is shown. The
710 results of three independent experiments normalized to wild-type HIV-1-GFP
711 infection with standard deviation is shown (lower panel). * indicates P-value <
712 0.005, ns indicates not significant as determined by the Two-way ANOVA
713 Tukey's multiple comparisons test.

714

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716

717 **Figure 2. TRIM5α$_{hu}$ binds to the HIV-1 core.** Human 293T cells were
718 transfected with plasmids expressing the wild-type human TRIM5α tagged with
719 an HA epitope (TRIM5α$_{hu}$-HA). At 24 h post-transfection, cells were lysed in
720 capsid binding buffer (CBB), as described in Materials and Methods. Inputs
721 containing different amounts of TRIM5α$_{hu}$ were prepared (INPUT). Cell extracts
722 containing different amounts of TRIM5α$_{hu}$-HA were mixed with 20 µl of stabilized
723 HIV-1 capsid tubes (5 mg/ml) and incubated for 1 h at room temperature.
724 Stabilized HIV-1 capsid tubes were collected by centrifugation and washed twice
725 using CBB. Pellets were resuspended in 1× Laemmli buffer (BOUND). INPUT
726 and BOUND fractions were analyzed by western blotting using anti-HA and anti-
727 p24 antibodies. As control, similar experiments were performed using the capsid
728 binder, rhesus TRIM5α tagged with an HA epitope (TRIM5α$_{rh}$-HA). Each
729 experiment was repeated at least six times and a representative experiment is
730 shown.
731
732 **Figure 3. Expression of TRIM5α$_{hu}$ is necessary for the inhibition of HIV-1**
733 **infection caused by the disruption of CypA-capsid interactions. (A)** Human
734 Jurkat cells stably expressing a specific shRNA against TRIM5α$_{hu}$ (Jurkat-
735 TRIM5α$_{hu}$-KD #1–4) or the empty vector (Jurkat-Control #4 and 5) were
736 challenged using increasing amounts of N-MLV-GFP, which are restricted by
737 TRIM5α$_{hu}$. Infection was determined at 48 h post-challenge by measuring the
738 percentage of GFP-positive cells using a flow cytometer. As a control, similar
739 infections were performed using B-MLV-GFP viruses, which are not restricted by

740 TRIM5α_{hu}. **(B)** Jurkat-TRIM5α_{hu}-knockdown (KD) and Jurkat-control cells were
741 challenged with increasing amounts of HIV-1-GFP in the presence of cyclosporin
742 A (CsA). Infection was determined at 48 h post-challenge by measuring the
743 percentage of GFP-positive cells using a flow cytometer. As a control, similar
744 experiments were performed using DMSO. **(C)** Jurkat-TRIM5α_{hu}-KD and Jurkat-
745 control cells were challenged with increasing amounts of p24-normalized HIV-1-
746 GFP or HIV-1-P90A-GFP. Infection was determined at 48 h post-challenge by
747 measuring the percentage of GFP-positive cells using a flow cytometer. Each
748 experiment was repeated at least three times and a representative experiment is
749 shown.
750
751 **Figure 4. CypA prevents the binding of TRIM5α_{hu} to the HIV-1 core. (A)**
752 Human 293T cells were transfected with plasmids expressing TRIM5α_{hu}-HA. At
753 24 h post-transfection, cells were lysed in capsid binding buffer (CBB) (INPUT).
754 Cell extracts containing TRIM5α_{hu}-HA were mixed with 20 µl of stabilized wild-
755 type or P90A HIV-1 capsid tubes (5 mg/ml) in the presence of 10 µM CsA.
756 Mixtures were incubated for 1 h at room temperature. Stabilized HIV-1 capsid
757 tubes were collected by centrifugation and washed twice using CBB. Pellets were
758 resuspended in 1X Laemmli buffer (BOUND). INPUT and BOUND fractions were
759 analyzed by western blotting using anti-HA, anti-CypA, and anti-p24 antibodies.
760 Experiments were repeated three times and a representative experiment is
761 shown. The bound fraction relative to input for three independent experiments
762 with standard deviation is shown. * indicates P-value < 0.005, ns indicates not

763 significant as determined by the Two-way ANOVA Tukey's multiple comparisons
764 test. **(B)** Cell extracts containing TRIM5α_{hu}-HA were mixed with 20 µl of stabilized
765 wild-type or P90A HIV-1 capsid tubes (5 mg/ml) in the presence of 10 µg
766 recombinant CypA. Mixtures were incubated for 1 h at room temperature.
767 Stabilized HIV-1 capsid tubes were collected by centrifugation and washed twice
768 using CBB. Pellets were resuspended in 1× Laemmli buffer (BOUND). INPUT
769 and BOUND fractions were analyzed by western blotting using anti-HA, anti-
770 CypA, and anti-p24 antibodies. Experiments were repeated three times and a
771 representative experiment is shown. The bound fraction relative to input for three
772 independent experiments with standard deviation is shown. * indicates P-value <
773 0.005, ns indicates not significant as determined by the Two-way ANOVA
774 Tukey's multiple comparisons test.
775
776 **Figure 5. HIV-1 viruses bearing a capsid mutant that does not interact with**
777 **TRIM5α_{hu} are insensitive to CsA. (A)** HIV-1-A92E infection of Jurkat cells.
778 Jurkat cells were challenged with increasing amounts of p24-normalized HIV-1-
779 GFP, HIV-1-P90A-GFP (left panel), or HIV-1-A92E-GFP (right panel) in the
780 presence of 10 µM Cyclosporin A (CsA). DMSO was used as a negative control.
781 Infection was determined at 48 h post-challenge by measuring the percentage of
782 GFP-positive cells using a flow cytometer. Experiments were repeated three
783 times and a representative experiment is shown. **(B)** Binding of CypA to HIV-1
784 cores bearing the capsid change A92E. Cell extracts from human 293T cells
785 containing CypA-V5 were mixed with 20 µl of stabilized wild-type or A92E HIV-1

786 capsid tubes (5 mg/ml) in the presence of 10 μM CsA. Mixtures were incubated
787 for 1 h at room temperature. Stabilized HIV-1 capsid tubes were collected by
788 centrifugation and washed twice using capsid binding buffer (CBB). Pellets were
789 resuspended in 1× Laemmli buffer (BOUND). INPUT and BOUND fractions were
790 analyzed by western blotting using anti-V5 and anti-p24 antibodies. Experiments
791 were repeated three times and a representative experiment is shown. The bound
792 fraction relative to input for three independent experiments with standard
793 deviation is shown. **(C)** Binding of TRIM5α_{hu} to HIV-1 cores bearing the capsid
794 change A92E. Similarly, cell extracts containing TRIM5α_{hu}-HA were mixed with
795 20 μl of stabilized wild-type or A92E HIV-1 capsid tubes (5 mg/ml) in the
796 presence of 10 μM CsA. Mixtures were incubated for 1 h at room temperature.
797 Stabilized HIV-1 capsid tubes were collected by centrifugation and washed twice
798 using CBB. Pellets were resuspended in 1× Laemmli buffer (BOUND). INPUT
799 and BOUND fractions were analyzed by western blot using anti-HA and anti-p24
800 antibodies. Experiments were repeated three times and a representative
801 experiment is shown. The bound fraction relative to input for three independent
802 experiments with standard deviation is shown.
803
804 **Figure 6. CypA-capsid Interactions are required for HIV-1 infection of**
805 **human peripheral blood mononuclear cells (PBMCs) and CD4$^+$ T cells. (A)**
806 PBMCs from two different donors were challenged with increasing amounts of
807 HIV-1-GFP, HIV-1-G89V-GFP, or HIV-1-P90A-GFP. Infection was determined at
808 72 h post-challenge by measuring the percentage of GFP-positive cells.

809 Experiments were repeated three times per donor and a representative
810 experiment is shown. **(B)** Purified $CD4^+$ T cells from two different donors were
811 challenged with increasing amounts of HIV-1-GFP, HIV-1-G89V-GFP, or HIV-1-
812 P90A-GFP. Infection was determined at 72 h post-challenge by measuring the
813 percentage of GFP-positive cells. Experiments were repeated three times per
814 donor and a representative experiment is shown.
815
816 **Figure 7. Infection of HIV-1 bearing the capsid change A92E, which does**
817 **not bind TRIM5α_{hu}, is not affected in human PBMCs and $CD4^+$ T cells. (A)**
818 PBMCs from three different donors were challenged with increasing amounts of
819 HIV-1-GFP, HIV-1-P90A-GFP, or HIV-1-P92E-GFP. Infection was determined at
820 72 h post-challenge by measuring the percentage of GFP-positive cells.
821 Experiments were repeated three times per donor and a representative
822 experiment is shown. **(B)** $CD4^+$ T cells from three donors were challenged with
823 increasing amounts of HIV-1-GFP, HIV-1-P90A-GFP, or HIV-1-P92E-GFP.
824 Infection was determined at 72 h post-challenge by measuring the percentage of
825 GFP-positive cells. Experiments were repeated three times per donor and a
826 representative experiment is shown.
827
828 **Figure 8. Depletion of CypA expression in human $CD4^+$ T cells inhibits HIV-**
829 **1 infection. (A)** Human primary $CD4^+$ T cells from two different donors were
830 knocked-out for CypA expression using the CRISPR-Cas9 system, as described
831 in methods. Briefly, $CD4^+$ T cells were electroporated using five different guide

832 RNAs (gRNAs) against CypA (gRNA#1-5) together with the Cas9 protein. At 72 h
833 post-electroporation, endogenous expression of CypA in $CD4^+$ T was analyzed
834 by western blotting using antibodies against CypA. As controls, we
835 electroporated a gRNA against CXCR4 and a non-targeting gRNA. Expression of
836 GAPDH was used as a loading control. Similar results were obtained using two
837 different donors and a representative blot is shown. **(B)** Human primary $CD4^+$ T
838 cells depleted for CypA expression were challenged with a replication-competent
839 HIV-1 expressing GFP as a reporter of infection (HIV-1_{NL4-3}-GFP). Infection was
840 determined at 72 h post-infection by measuring the percentage of GFP-positive
841 cells. Experiments were performed in triplicates, and results for two different
842 donors with standard deviation are shown.
843
844 **Figure 9. CypA-capsid interactions are required for HIV-1 reverse**
845 **transcription in human $CD4^+$ T cells.** Human primary $CD4^+$ T cells were
846 challenged with DNase-pretreated HIV-1-GFP, HIV-1-P90A-GFP, or HIV-1-
847 G89V-GFP at a multiplicity of infection (MOI) < 1.0. Infection was determined by
848 measuring the percentage of GFP-positive cells using flow cytometry at 72 h
849 post-challenge **(A)**. In parallel, total DNA was extracted from similar infections at
850 12, 36, and 72 h post-infection. The DNA samples collected at 12 h post-infection
851 were used to determine the levels of late reverse transcripts (LRTs) using real-
852 time PCR **(B)**, as described in methods. As a control, we inhibited reverse
853 transcription by using 10 μm of Nevirapine (Nev). HIV-1 2-LTR circles, which is
854 an indirect measure of nuclear import, were quantified using real-time PCR in

855 DNA samples collected 36 h post-infection **(C)**, as described in methods.
856 Integrated proviruses were measured by determining the amount of Alu-PCR
857 (Alu) products using real-time PCR in DNA samples collected at 72 h post-
858 infection **(D)**, as described in methods. As a control, we inhibited proviral
859 integration by using 10 μM Raltegravir (Ral). LRT, 2-LTR, and Alu products were
860 normalized to actin. Results were analyzed using the unpaired t-test. Differences
861 were considered statistically significant at $P < 0.01$ (**), $P < 0.001$ (***), $P <$
862 0.0001 (****).
863
864 **Figure S1. Inhibition of CypA-capsid interactions with CsA restricts HIV-1**
865 **infection in PBMCs and $CD4^+$ T cells. (A)** PBMCs from three different donors
866 were challenged with HIV-1-GFP in the presence of increasing concentrations of
867 CsA. DMSO was used as a control. Infection was determined at 72 h post-
868 challenge by measuring the percentage of GFP-positive cells. **(B)** $CD4^+$ T cells
869 from two different donors were challenged with HIV-1-GFP in the presence of 10
870 μM CsA. DMSO was used as a control. Infection was determined at 72 h post-
871 challenge by measuring the percentage of GFP-positive cells.
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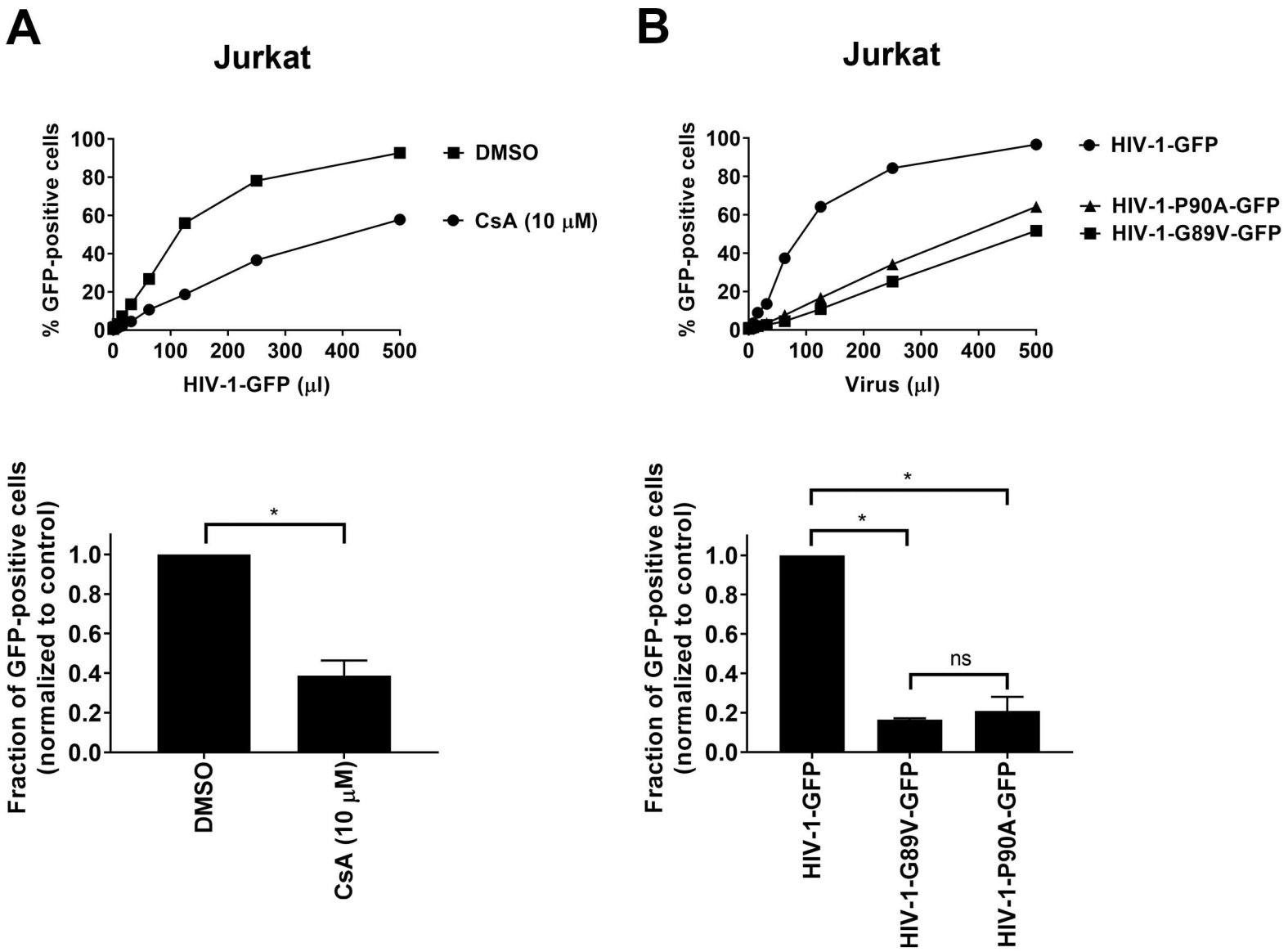

A
Jurkat
% GFP-positive cells
HIV-1-GFP (μl)
DMSO
CsA (10 μM)
Fraction of GFP-positive cells (normalized to control)
*
DMSO
CsA (10 μM)
B
Jurkat
% GFP-positive cells
Virus (μl)
HIV-1-GFP
HIV-1-P90A-GFP
HIV-1-G89V-GFP
Fraction of GFP-positive cells (normalized to control)
*
*
ns
HIV-1-GFP
HIV-1-G89V-GFP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

Figure 1

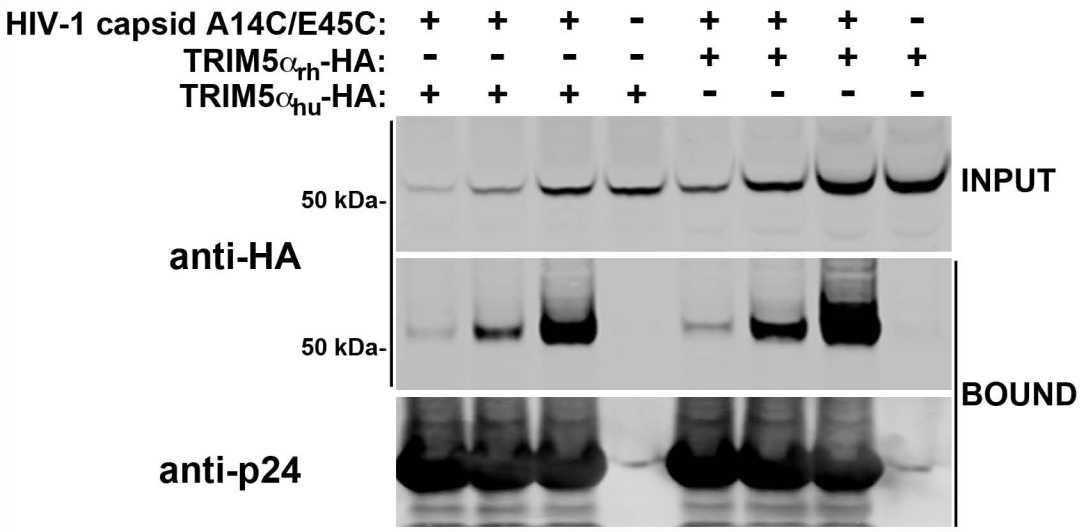

HIV-1 capsid A14C/E45C: + + + - + + + -
TRIM5αrh-HA: - - - - + + + +
TRIM5αhu-HA: + + + + - - - -
50 kDa-
anti-HA
50 kDa-
anti-p24
INPUT
BOUND

Figure 2

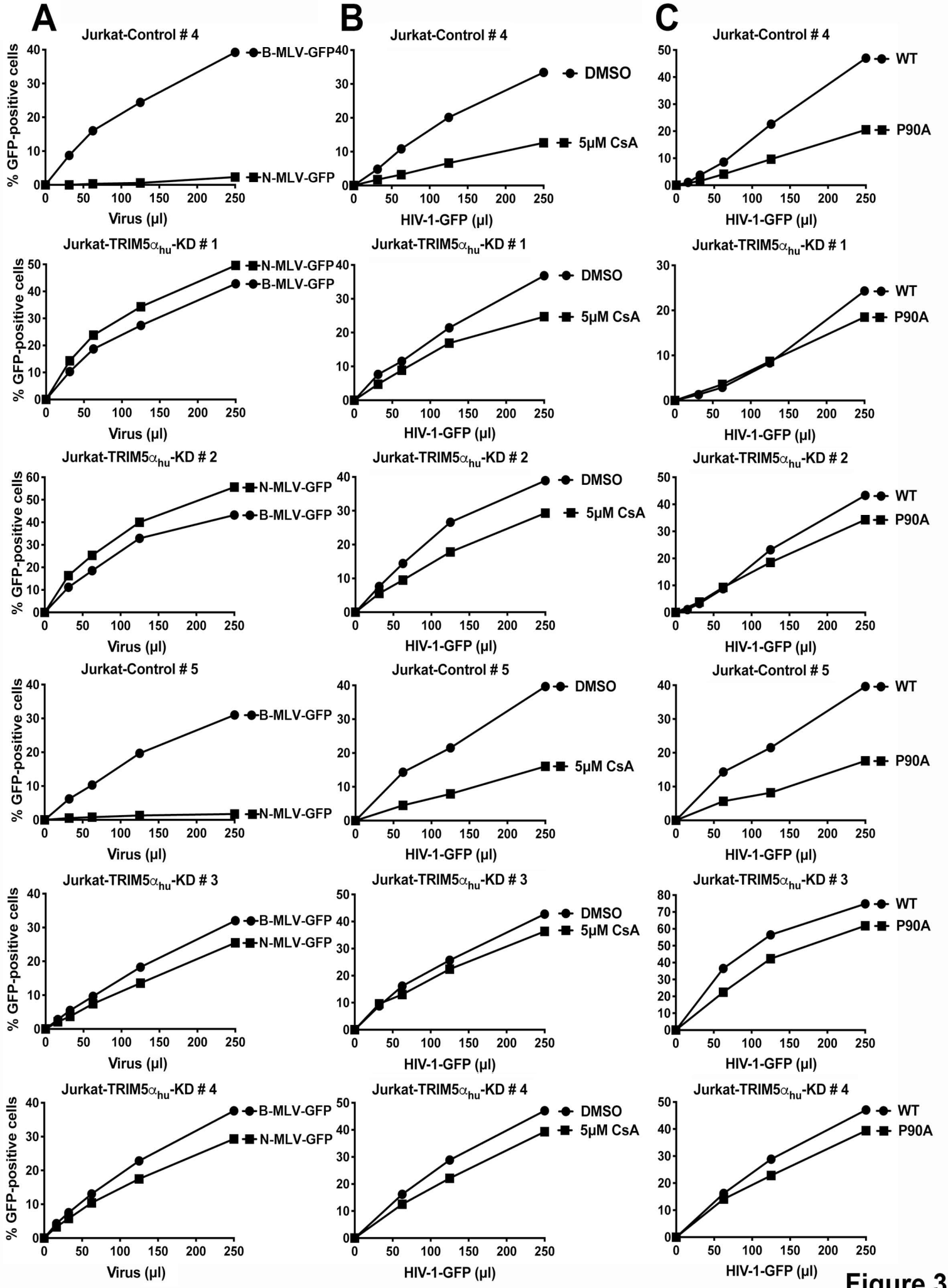

A
B
C
Jurkat-Control # 4
Jurkat-TRIM5αhu-KD # 1
Jurkat-TRIM5αhu-KD # 2
Jurkat-Control # 5
Jurkat-TRIM5αhu-KD # 3
Jurkat-TRIM5αhu-KD # 4
% GFP-positive cells
Virus (µl)
HIV-1-GFP (µl)
B-MLV-GFP
N-MLV-GFP
DMSO
5µM CsA
WT
P90A

Figure 3

A

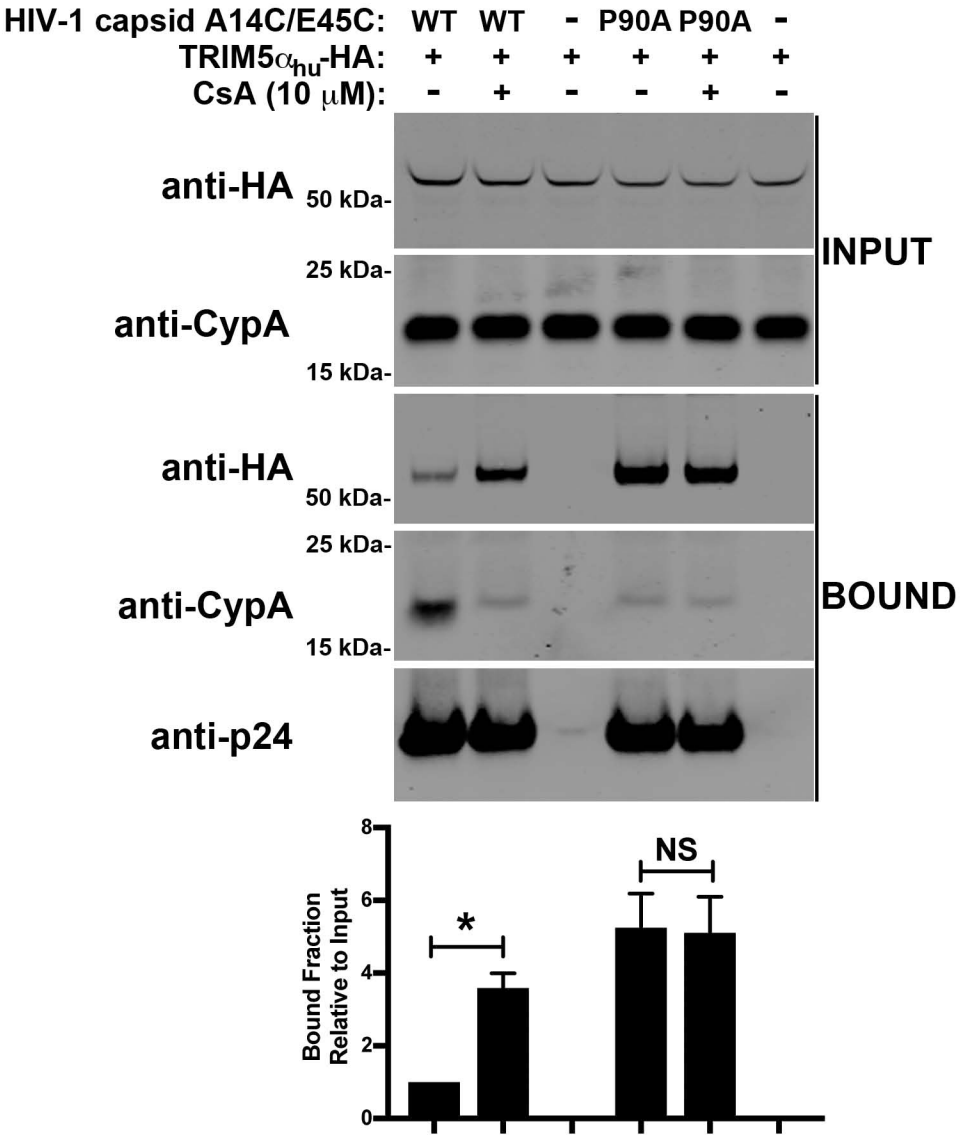

HIV-1 capsid A14C/E45C: WT WT - P90A P90A -
TRIM5αhu-HA: + + + + + +
CsA (10 μM): - + - - + -
anti-HA
50 kDa-
25 kDa-
anti-CypA
15 kDa-
INPUT
anti-HA
50 kDa-
25 kDa-
anti-CypA
15 kDa-
BOUND
anti-p24
Bound Fraction Relative to Input
0
2
4
6
8
*
NS

Figure 4

B

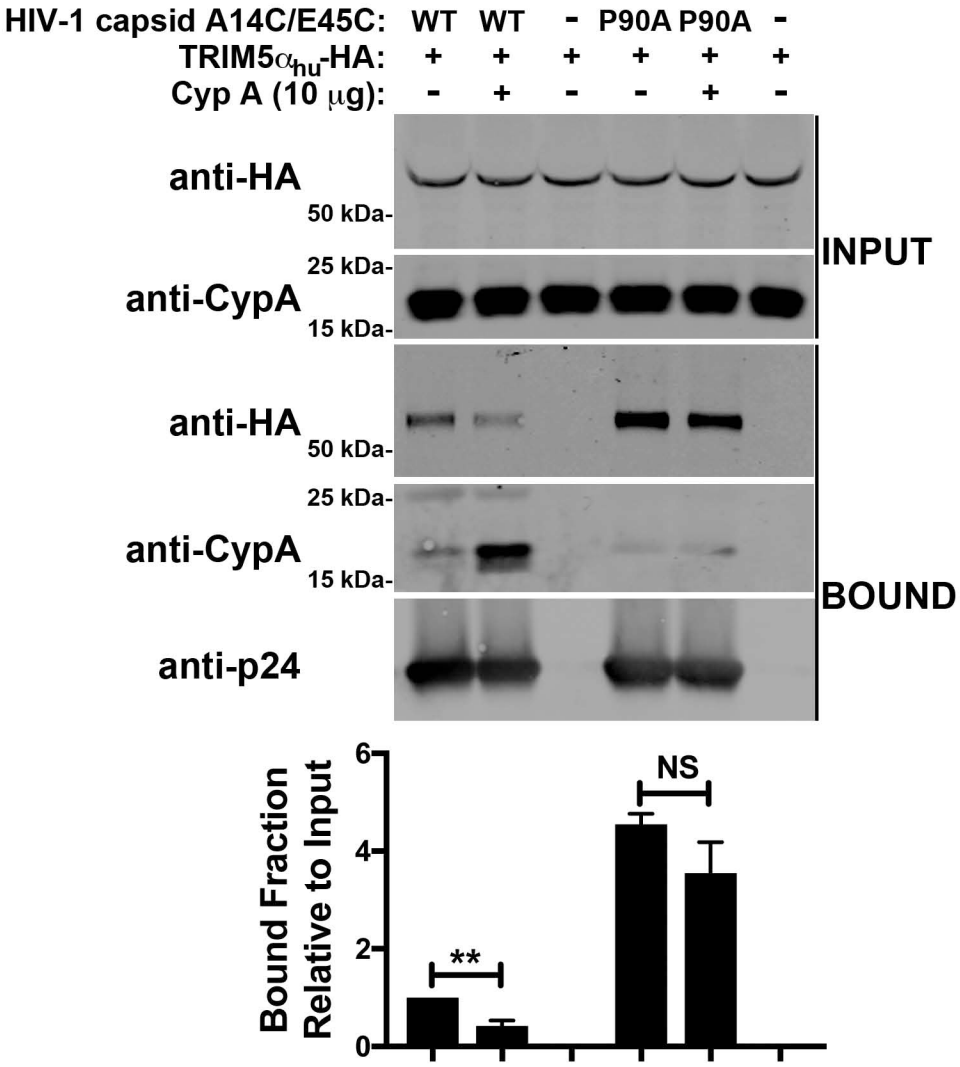

HIV-1 capsid A14C/E45C: WT WT - P90A P90A -
TRIM5αhu-HA: + + + + + +
Cyp A (10 μg): - + - - + -
anti-HA
50 kDa-
25 kDa-
anti-CypA
15 kDa-
INPUT
anti-HA
50 kDa-
25 kDa-
anti-CypA
15 kDa-
BOUND
anti-p24
Bound Fraction Relative to Input
6
4
2
0
**
NS

Figure 4

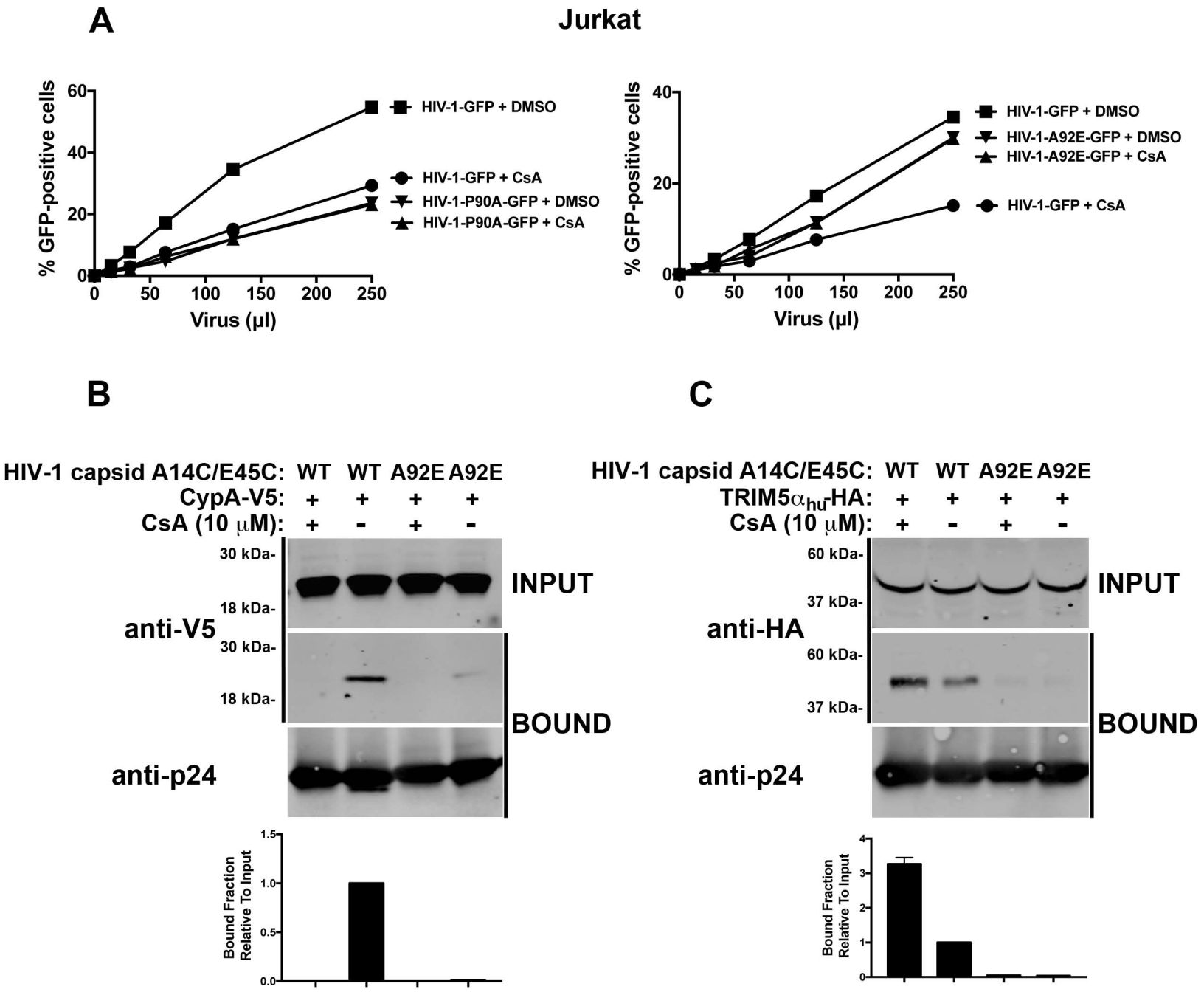

A
Jurkat
% GFP-positive cells
Virus (µl)
HIV-1-GFP + DMSO
HIV-1-GFP + CsA
HIV-1-P90A-GFP + DMSO
HIV-1-P90A-GFP + CsA
HIV-1-A92E-GFP + DMSO
HIV-1-A92E-GFP + CsA
B
C
HIV-1 capsid A14C/E45C: WT WT A92E A92E
CypA-V5: + + + +
CsA (10 µM): + - + -
TRIM5αhu-HA: + + + +
anti-V5
anti-HA
anti-p24
INPUT
BOUND
30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

Figure 5

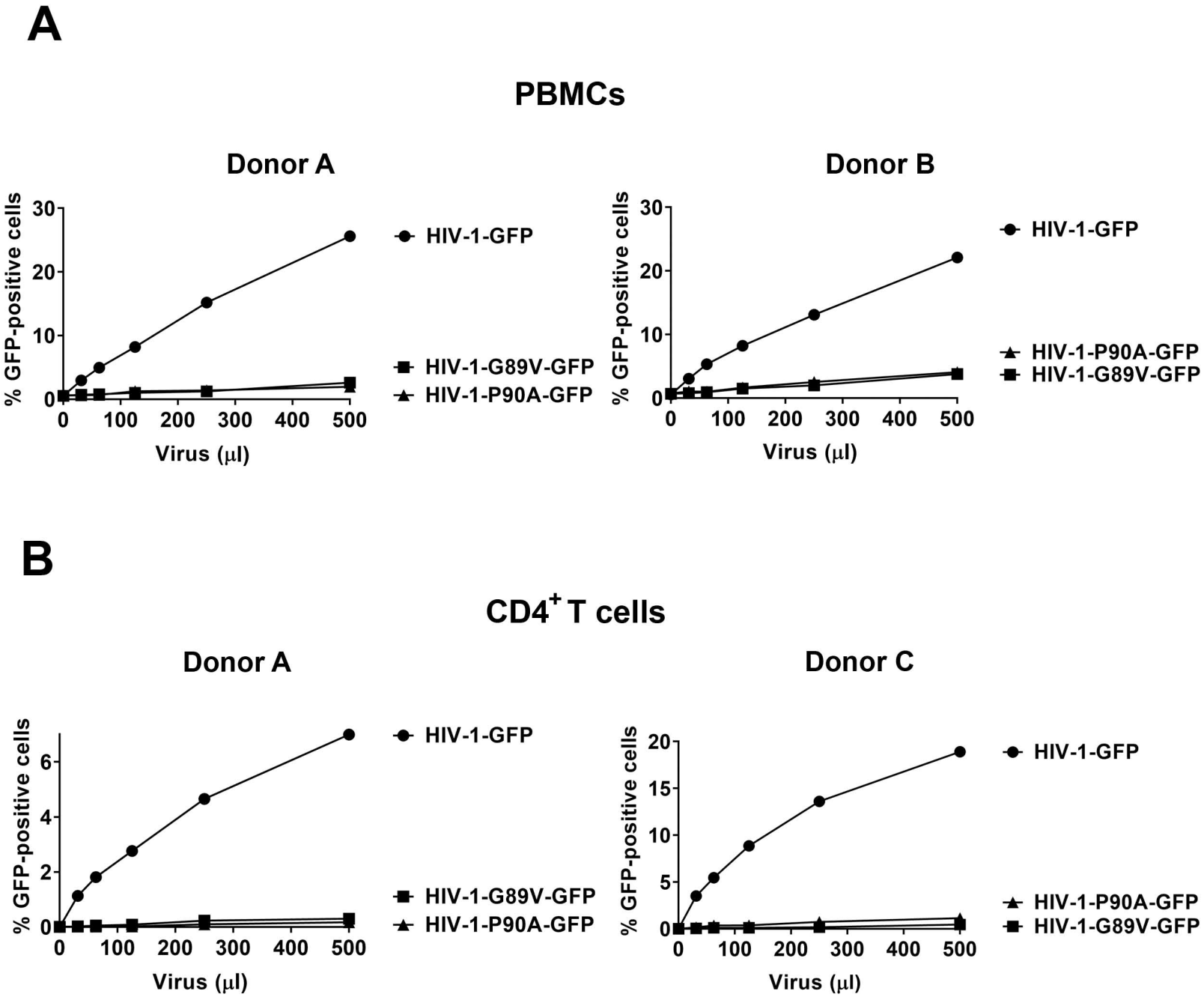

A
PBMCs
Donor A
Donor B
% GFP-positive cells
Virus (μl)
HIV-1-GFP
HIV-1-G89V-GFP
HIV-1-P90A-GFP
B
CD4+ T cells
Donor A
Donor C

Figure 6

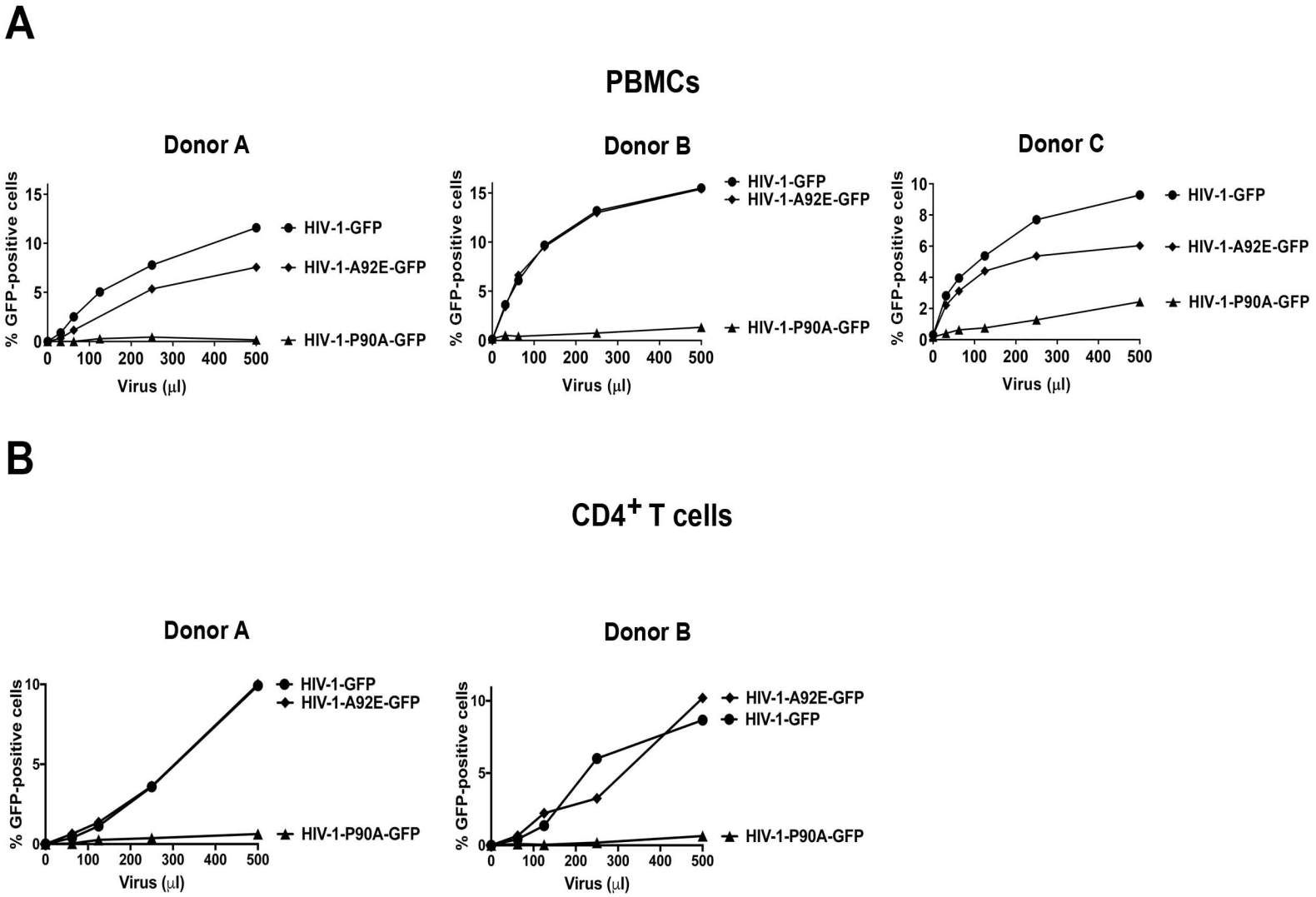
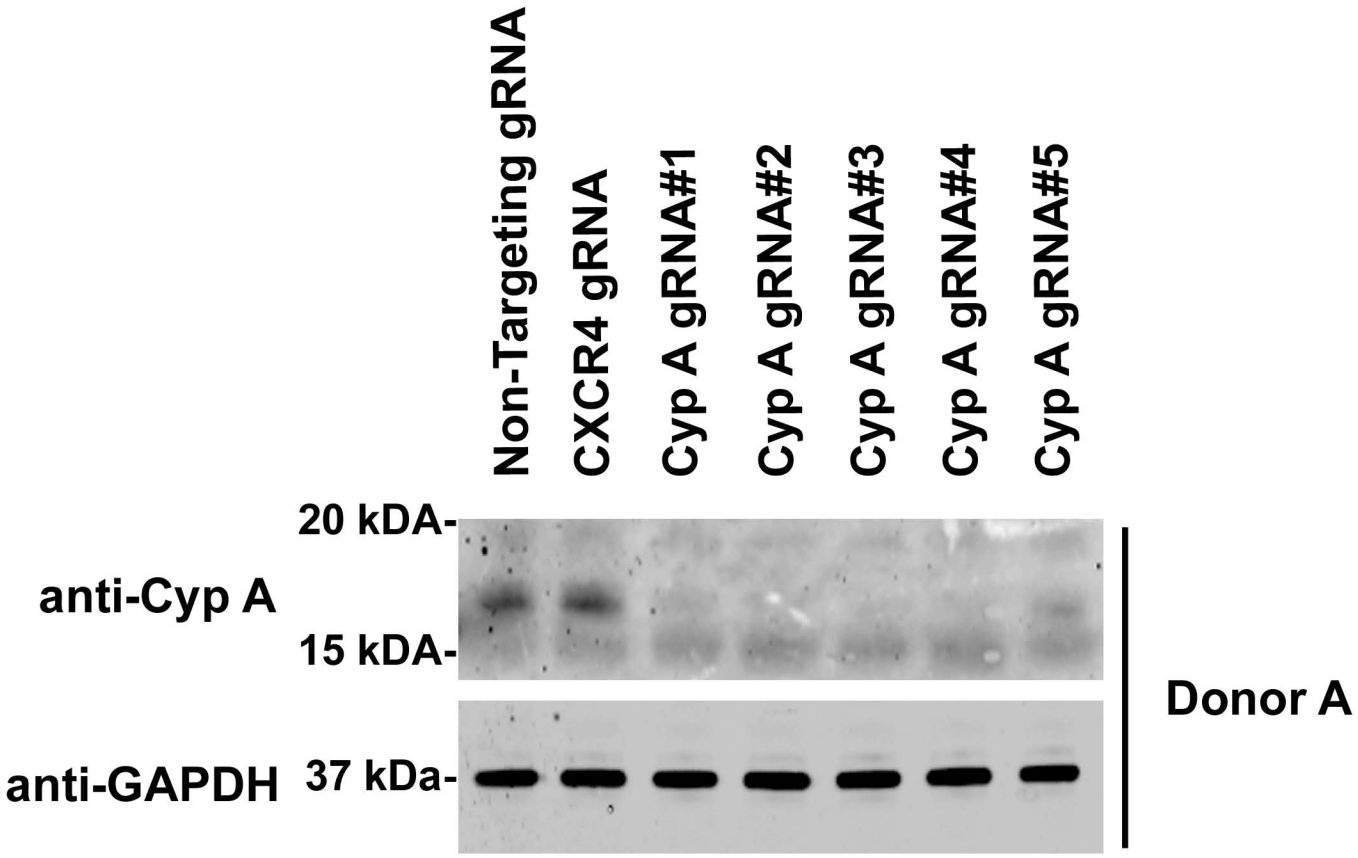

A
PBMCs
Donor A
Donor B
Donor C
% GFP-positive cells
Virus (μl)
HIV-1-GFP
HIV-1-A92E-GFP
HIV-1-P90A-GFP
B
CD4+ T cells
Donor A
Donor B

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

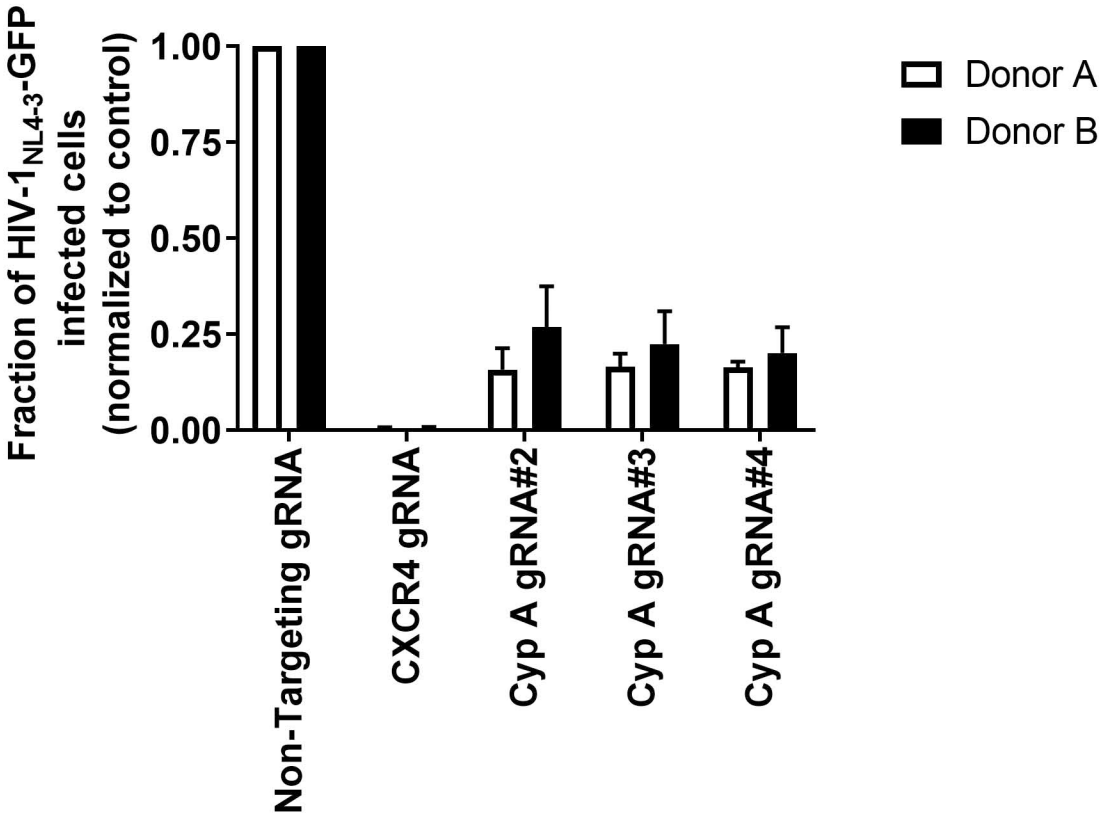

Fraction of HIV-1NL4-3-GFP infected cells (normalized to control)
1.00
0.75
0.50
0.25
0.00
Donor A
Donor B
Non-Targeting gRNA
CXCR4 gRNA
Cyp A gRNA#2
Cyp A gRNA#3
Cyp A gRNA#4

Figure 8

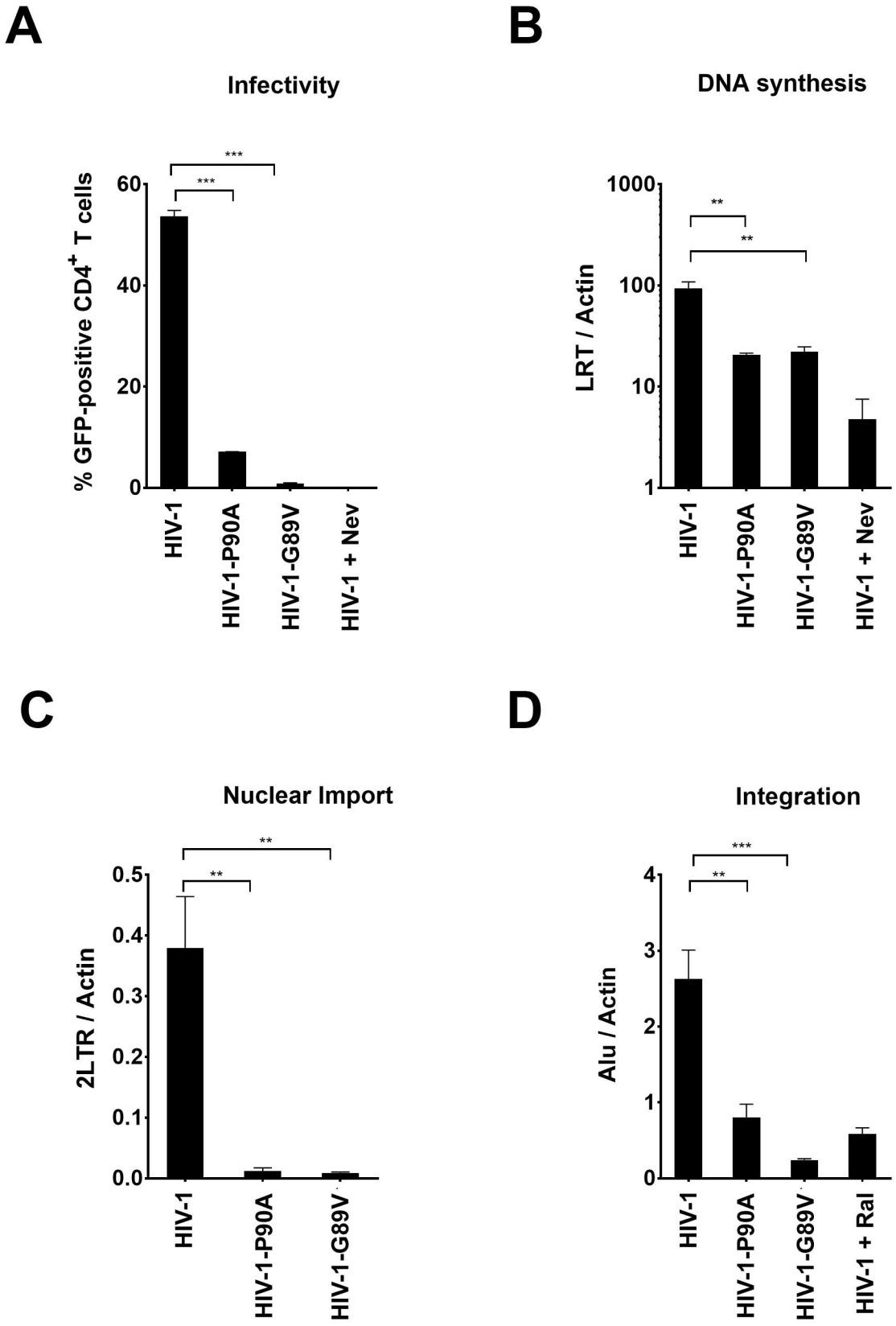

A
Infectivity
% GFP-positive CD4+ T cells
60
40
20
0
***
***
HIV-1
HIV-1-P90A
HIV-1-G89V
HIV-1 + Nev
B
DNA synthesis
LRT / Actin
1000
100
10
1
**
**
HIV-1
HIV-1-P90A
HIV-1-G89V
HIV-1 + Nev
C
Nuclear Import
2LTR / Actin
0.5
0.4
0.3
0.2
0.1
0.0
**
**
HIV-1
HIV-1-P90A
HIV-1-G89V
D
Integration
Alu / Actin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

Figure 9

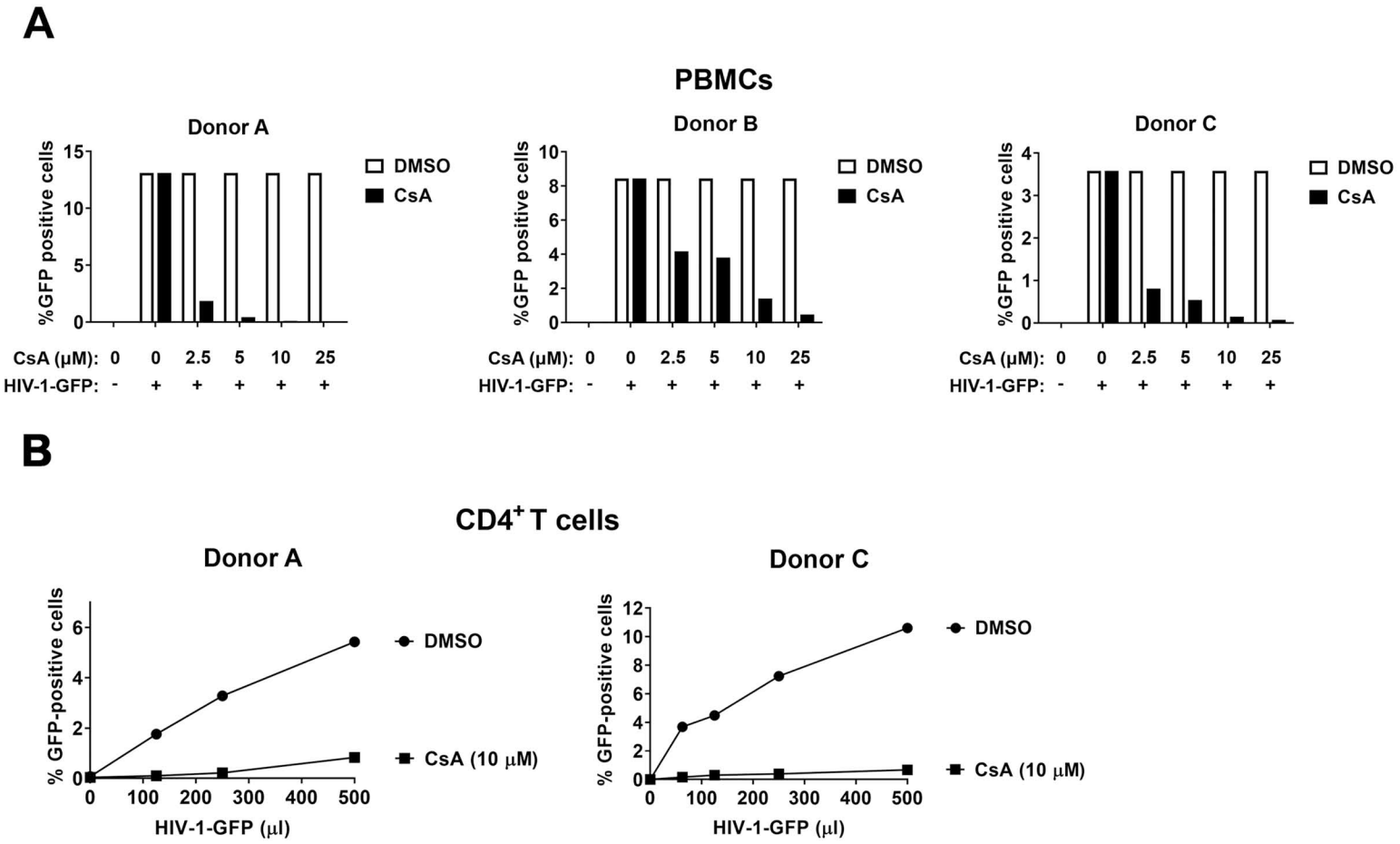

A
PBMCs
Donor A
Donor B
Donor C
%GFP positive cells
DMSO
CsA
CsA (μM): 0 0 2.5 5 10 25
HIV-1-GFP: - + + + + +
B
CD4+ T cells
Donor A
Donor C
% GFP-positive cells
DMSO
CsA (10 μM)
HIV-1-GFP (μl)

Figure S1