Moloney leukemia virus 10 (MOV10) inhibits the degradation of APOBEC3G through interference with the Vif-mediated ubiquitin–proteasome pathway

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Abstract

Background: MOV10 protein has ATP-dependent 5′–3′ RNA helicase activity and belongs to the UPF1p superfamily. It can inhibit human immunodeficiency virus type 1 (HIV-1) replication at multiple stages and interact with apolipoprotein-B-mRNA-editing enzyme catalytic polypeptide-like 3G (APOBEC3G or A3G), a member of the cytidine deaminase family that exerts potent inhibitory effects against HIV-1 infection. However, HIV-1-encoded virion infectivity factor (Vif) protein specifically mediates the degradation of A3G via the ubiquitin–proteasome system (UPS).

Results: We demonstrate that MOV10 counteracts Vif-mediated degradation of A3G by inhibiting the assembly of the Vif-CBF-β-Cullin 5-ElonginB-ElonginC complex. Through interference with UPS, MOV10 enhances the level of A3G in HIV-1-infected cells and virions, and synergistically inhibits the replication and infectivity of HIV-1. In addition, the DEAG-box of MOV10 is required for inhibition of Vif-mediated A3G degradation as the DEAG-box mutant significantly loses this ability.

Conclusions: Our results demonstrate a novel mechanism involved in the anti-HIV-1 function of MOV10. Given that both MOV10 and A3G belong to the interferon antiviral system, their synergistic inhibition of HIV-1 suggests that these proteins may play complicated roles in antiviral functions.

Keywords: MOV10, A3G, Vif, HIV-1, Ubiquitin–proteasome system (UPS)
MOV10, which is a member of the UPF1p family and has ATP-dependent 5′–3′ RNA helicase activity [17, 18]. MOV10 has complicated functions and features. For example, MOV10 is found to interact with Argonaute proteins and plays a role in microRNA (miRNA)-mediated regulation [19, 20]. MOV10 is also involved in polycomb-mediated repression of the tumor-suppressor INK4a [21]. Moreover, it has been reported that MOV10 is a type I interferon stimulated gene and several reports have indicated that this protein has broad antiretroviral activity against various viruses, such as HIV-1, murine leukemia virus (MLV), and equine infectious anemia virus (EIAV) [22–25]. Its inhibitory effect on LINE-1 retrotransposition has also been investigated [26, 27]. MOV10 can also be packaged into HIV-1 particles and affects HIV-1 replication at multiple stages [22, 28–30]. MOV10 expresses in varieties of human cells. And according to the data from GEO profile, we found that the expression profile of MOV10 is in moderate or high level in CD4 + T cells and monocytes (https://www.ncbi.nlm.nih.gov/geo/profiles, GEO Profiles ID: G9710126, 106167926, 52933168, 51070326).

Recently, several studies have reported that MOV10 interacts with A3G and both of these proteins are located in P-bodies and can be induced by interferon-α [23, 29, 31]. Based on the similar features of MOV10 and A3G, a research group has studied the possible relationship between these two restriction factors in HIV-1 infection [29]. They co-expressed MOV10 with A3G, but failed to find any functional synergistic effects on viral replication. Conversely, after knocking down endogenous MOV10 by siRNA in the presence of A3G, they did not find any significant impact on HIV-1 infectivity. Nevertheless, they detected the possible synergy of these two inhibitors in the absence of HIV-1 Vif protein. Given that MOV10 can bind with A3G, we hypothesize that MOV10 may affect the process of Vif-mediated degradation of A3G.

Thus, in this study, we aim to elucidate the correlations between MOV10 and A3G in the presence of Vif, which could occur during natural infection by HIV-1. Our findings provide important insights into the role of MOV10 in Vif-mediated A3G degradation and the mechanism through which MOV10 mediates the functional assembly of the Vif-CBF-β-Cullin 5-ElonginB-ElonginC complex to affect the Vif-mediated ubiquitin–proteasome pathway.

### Results

**MOV10 counteracts Vif-mediated degradation of A3G by interfering with the ubiquitin–proteasome pathway**

In order to examine the relationships among MOV10, A3G, and Vif, we co-transfected MOV10-FLAG-, A3G-HA-, and Vif-HA-expressing plasmids into 293T cells, and then evaluated the expression levels of A3G and Vif. Interestingly, significant increases in A3G and Vif protein expression were observed in cells overexpressing MOV10 (Fig. 1a). To confirm this phenomenon, we analyzed changes in expression of A3G and Vif in the presence of different levels of MOV10. We found that the enhancement of A3G and Vif expression was correlated with the level of MOV10 (Fig. 1b). We also observed the same phenotype by depleting endogenous MOV10 with MOV10-specific siRNA (Fig. 1c) [32]. To exclude the possibility of off-target effects of siRNA, a restoration experiment was conducted. Co-transfection of rMOV10-FLAG-expressing plasmid, a MOV10 construct that is resistant to siRNA-targeting, with the MOV10-specific siRNA restored the expression of A3G (Fig. 1d), indicating that MOV10-specific siRNA does not have off-target effects.

To further validate this phenotype, a similar experiment was performed in H9 cells infected with wild-type HIV-1. MOV10-knockdown H9 cells were constructed by the infection of MOV10-specific shRNA-expressing lentivirus. The cells were then infected with wild-type HIV-1 viruses. The culture supernatants were collected at different days after infection, and HIV-1 p24 was detected by ELISA kit. After culture for 12 days, HIV-1 p24-positive cells were sorted by flow cytometry (Fig. 2). In these HIV-1 p24-positive cells, the expression of endogenous A3G was significantly down-regulated by endogenous MOV10 depletion. HIV-1 replication and the expression of Gag protein were also enhanced by the depletion of MOV10 (Fig. 2d). The results demonstrate that MOV10 and A3G synergistically inhibit the replication of HIV-1.

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**Fig. 1** MOV10 counteracts Vif-mediated degradation of A3G. a, b MOV10 overexpression inhibits Vif-mediated A3G degradation. Human 293T cells were transfected with pcDNA3.1-A3G-HA (0.8 μg), pcDNA3.1-Vif-HA (0.5 μg), and pcDNA3.1-MOV10-FLAG (1.5 μg) (a) or different amounts of pcDNA3.1-MOV10-FLAG (from 0.5 to 2 μg) (b). Then, cells were collected and lysed at 48 h, and analyzed by western blotting with anti-HA, anti-FLAG, and anti-GAPDH antibodies. c, d The effect of MOV10 depletion on Vif-mediated degradation of A3G. Cells were transfected with pcDNA3.1-A3G-HA (0.8 μg), pcDNA3.1-Vif-HA (0.5 μg), MOV10-specific siRNA (50 nM) (c) and/or siRNA-resistant MOV10 construct (MOV10-FLAG) (0.4 μg) (d). After 48 h, cells were collected and analyzed by western blotting assay with anti-HA, anti-FLAG, anti-MOV10 and anti-GAPDH antibodies. Empty vector pcDNA3.1 was used in each transfection to normalize DNA amounts. Values in a–d represent percentages of A3G or MOV10 normalized against GAPDH and compared with control. The bar graphs represent the average expression of A3G with different treatment and relative to the A3G-only reaction control (set to 100%). All the data represent mean ± S.D from three independent experiments. Statistical significance was determined using t-test: *p < 0.05, **p < 0.01, ***p < 0.001.
MOV10 protects A3G from Vif-mediated degradation in wild-type HIV-1. a H9 cells were infected with pLKO.1-MOV10-shRNA or pLKO.1-Scr-shRNA lentivirus for 8 h and then selected with puromycin for 2 weeks. MOV10-knockdown H9 cells and control cells were infected with wild-type HIV-1 for 3 h and cultured with fresh medium for 12 days. The culture supernatants were collected at the indicated time points. Then HIV-1 p24 was detected using HIV-1 p24 ELISA kit at different time points (b). And at 12th day, these cells were analyzed by flow cytometer (c). HIV-1 p24 positive H9 cells were sorted and detected by western blotting with anti-MOV10, anti-A3G, anti-Vif, anti-HIV-1 p24, and anti-GAPDH antibodies (d). Values in d represent percentages of A3G or MOV10 normalized against GAPDH and compared with control. The bar graphs in d represent the average expression of A3G with different treatment and relative to the A3G-only reaction control (set to 100%). Data in a, b, and d represent mean ± SD from three independent experiments. *, statistically significant, p ≤ 0.05 (t test). All the results are representative of at least three independent experiments.
Considering the relationships between Vif/A3G and the ubiquitin–proteasome pathway [6–8, 10, 33], we evaluated the effects of MOV10 on the expression levels of A3G and Vif in the presence of the proteasome inhibitor MG132. After treatment with MG132 for 16 h, the expression levels of A3G and Vif were not affected by MOV10 overexpression (Fig. 3a). Further study showed that MOV10 could decrease the ubiquitination of A3G directly (Fig. 3b). Taken together, these data indicate that MOV10 can protect A3G from Vif-mediated degradation by interfering with the ubiquitin–proteasome pathway.

**MOV10 affects the assembly of the Vif-CBF-β-Cullin 5-ElonginB-ElonginC complex**

Previous studies have reported that A3G can bind with Vif (Fig. 4a) [10]. And, A3G protein contains two domains: the N-terminal domain is responsible for encapsidation and the C-terminal domain is responsible for deamination activity [34, 35]. Only the N-terminal domain of A3G can bind with Vif and the binding initiates the degradation process of A3G [36]. Because A3G can also interact with MOV10 (Fig. 4b) [20], the interaction of Vif with A3G may be affected by MOV10. To this end, we investigated the effects of MOV10 on the interaction between Vif and A3G in the presence of MG132. Human 293T cells were transfected with MOV10-FLAG-, A3G-HA-, and Vif-FLAG-expressing plasmids and then treated with MG132 for 16 h. However, we did not detect any changes in the levels of Vif-FLAG in the A3G-HA-immunoprecipitated samples with or without MOV10 (Fig. 4c). These results suggest that MOV10 does not affect the binding of Vif with A3G and therefore it may interfere with other steps in the A3G degradation process.

The interaction of Vif with CBF-β, Cullin 5, ElonginB, and ElonginC can facilitate the formation of a ubiquitin ligase complex, which is required for Vif to induce the degradation of A3G [11–13, 37, 38]. Therefore, we next examined
the effects of MOV10 on the interaction between Vif and different components in the complex. We transfected Vif-HA- and MOV10-FLAG-expressing plasmids with pcDNA3.1-ElonginB-FLAG, pcDNA3.1-ElonginC-FLAG, pcDNA3.1-Cullin 5-FLAG, or pcDNA3.1-CBF-β-FLAG into 293T cells. After immunoprecipitation, we found that the interactions of Vif with ElonginB, ElonginC, Cullin 5, and CBF-β significantly decreased when MOV10 was overexpressed (Fig. 5a–d), indicating that MOV10 affects Vif-ElonginB, Vif-ElonginC, Vif-Cullin 5, and Vif-CBF-β interactions during the assembly of the Vif-CBF-β-Cullin 5-ElonginB-ElonginC complex.

According to the above results, we suspected that MOV10 could interact with ElonginB, ElonginC, Cullin 5, or CBF-β. To test this hypothesis, we co-transfected 293T cells with pcDNA3.1-MOV10-FLAG plus pcDNA3.1-ElonginB-FLAG, pcDNA3.1-ElonginC-FLAG, pcDNA3.1-ElonginC-FLAG, pcDNA3.1-Cullin 5-FLAG or pcDNA3.1-CBF-β-FLAG. Previous study has demonstrated that ElonginC, ElonginB, and Cullin 5 can interact with each other [39]. To eliminate the influence of these endogenous proteins, siRNAs specific to ElonginB, ElonginC, and Cullin 5 mRNA were also co-transfected into cells at the same time (Fig. 6a). After immunoprecipitation and western blotting, significant binding was found between MOV10 and ElonginC or Cullin 5 (Fig. 6c, d). To further confirm the binding, we detected the interaction between MOV10-FLAG and endogenous ElonginC or Cullin 5. As shown in the Fig. 6f, g, the same phenomenon was observed. After treatment with an RNase mixture,
we found that the binding of MOV10 with Cullin 5 was partially dependent on RNA, whereas the interaction between MOV10 and ElonginC was not (Fig. 6h, i). However, the interaction between MOV10 and ElonginB or CBF-β was not detected (Fig. 6b, e).

The helicase activity center of MOV10 is required for its inhibitory effects on Vif-mediated A3G degradation

MOV10 contains a DEAG-box (D-E-A-G = Asp-Glu-Ala-Gly) motif and the DEAG-box mutant impairs the helicase activity of MOV10 [18, 28]. To examine whether the DEAG-box motif was required for the effects of MOV10 on Vif-mediated degradation of A3G, we used a MOV10-DEAG mutant (a point mutation in the DEAG-box motif, from DEAG to DQAG) to repeat the experiment shown as Fig. 1a [18, 22, 23, 32, 40]. Compared with wild-type MOV10, the MOV10-DEAG mutant almost lost the ability to prevent the degradation of A3G mediated by Vif (Fig. 7a), suggesting that the DEAG-box motif is involved in regulating this inhibitory effects of MOV10. To confirm this conclusion, we further detected the binding of the MOV10-DEAG mutant with ElonginC or Cullin 5. Compared with wild-type MOV10, the bindings of MOV10-DEAG mutant with ElonginC or Cullin 5 decreased significantly (Fig. 7b, c), suggesting that the DEAG-box motif of MOV10 plays an important role in the interaction between MOV10 with ElonginC or Cullin 5.
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MOV10 counteracts Vif-mediated A3G degradation in the context of HIV-1 replication

All of the above experiments were performed in the context of lack of other HIV-1 proteins. To verify whether the effect of MOV10 on Vif-mediated A3G degradation could be observed in the context of HIV-1 replication, we used two types of HIV-1 pNL4-3ΔEnv-GFP clones. Human 293T cells were transfected with pNL4-3ΔEnv-GFP plus pcDNA3.1-MOV10-HA or pcDNA3.1-FLAG, and pcDNA3.1-A3G-HA, and different amounts of MOV10-FLAG-expressing plasmid. Consistent with Fig. 1b, the expression levels of A3G and Vif were correlated with the expression levels of MOV10 in the presence of other HIV-1 proteins (Fig. 8a). Moreover, the same results were observed when we co-transfected 293T cells with pNL4-3ΔEnv-GFP, pcDNA3.1-A3G-HA, and different amounts of pcDNA3.1-MOV10-FLAG (Fig. 8b). To further confirm this, we examined the effect of MOV10 depletion on Vif-mediated A3G degradation in the context of HIV-1 replication. The same phenotypes as shown in Fig. 1c were recapitulated by MOV10-specific siRNAs (Fig. 8c, d). These data indicate that MOV10 can inhibit Vif-mediated degradation of A3G in the context of HIV-1 replication.

MOV10 increases the quantity of A3G in HIV-1 virions by protecting A3G from Vif-mediated degradation

As noted above, we demonstrated that MOV10 could increase the quantity of A3G by interfering with the proteasome pathway in virus-producing cells. Given that A3G can be packaged into HIV-1 virions and exert anti-HIV-1 activity [2, 35, 41–44]. We next evaluated the effects of MOV10 on the quantity of A3G in HIV-1 virions. We co-transfected 293T cells with pNL4-3ΔEnv-GFP, pcDNA3.1-A3G-HA, and pcDNA3.1-MOV10-FLAG, subsequently collected the supernatants and cells of each sample. Interestingly, although MOV10 enhanced A3G levels in cell lysates, the quantity of A3G was also increased in the supernatant viral particles (Fig. 9a). To further confirm this, the A3G levels were analyzed in virus-producing cells and viral particles following depletion of endogenous MOV10 with MOV10-specific siRNAs. MOV10 knockdown could reduce the quantity of A3G in both virus producing cells and viral particles (Fig. 9b). Previous studies showed that MOV10 can be packaged into virions and affects HIV-1 replication at multiple stages. Here, we overexpressed MOV10 in a dose-dependent manner, and we found that there was a synergy between the packaging levels of MOV10 and A3G (Fig. 9c). Taken together, these results demonstrate that MOV10 increases the quantity of A3G in HIV-1 virions by interfering with the Vif-mediated ubiquitin–proteasome pathway.

Furthermore, to explore the potential synergistic effect of MOV10 and A3G on the infectivity of the newly-produced virions, NL4-3ΔEnv-GFP and NL4-3ΔEnv-GFP-ΔVif particles were produced with increasing amounts of MOV10 in either the presence or absence of A3G. After normalization for HIV-1 p24, TZM-bl cells were infected with these viral particles and then the infectivity of viruses was determined (Fig. 10a, b). For NL4-3ΔEnv-GFP-ΔVif particles, compared with the group of single MOV10 or A3G treatment, the inhibitory effect of MOV10 or MOV10-DEAG mutant plus A3G group was equal to the effect of single A3G treatment group. And, the depletion of endogenous MOV10 with siRNA in the presence of A3G also has no impact on HIV-1 infectivity. It is consistent with previous study that co-expression of MOV10 did not enhance the inhibitory effect of A3G on the infectivity of ΔVif HIV-1 (Fig. 10a) [29]. However, for NL4-3ΔEnv-GFP particles, the fold reductions in infectivity at various amounts of MOV10 were different in the presence or absence of A3G and endogenous MOV10 was helpful for A3G to decrease the infectivity of HIV-1. But, MOV10-DEAG mutant lost its ability to help A3G decrease the infectivity of HIV-1 (Fig. 10b). This data further confirms that DEAG-box motif of MOV10 plays an important role in protecting A3G from Vif-mediated degradation. These results indicate that the inhibitory effect of A3G on the infectivity of HIV-1 can be synergistically enhanced by MOV10.
Discussion

In this report, we studied the relationship between MOV10 and A3G in the presence of HIV-1 Vif. Interestingly, we observed that MOV10 increased the levels of A3G and Vif in a concentration-dependent manner. The phenomenon is consistent with the previous study that the expression levels of A3G and Vif can be increased simultaneously in the presence of the proteasome inhibitor MG132 [45, 46]. Vif is an E3 ubiquitin ligase substrate receptor that interacts with host factors ElonginB, ElonginC, Cullin 5, and CBF-β to form an E3 ubiquitin ligase complex, which results in the polyubiquitylation of both Vif and A3G. And then, the Vif-A3G complex can be degraded together via the ubiquitin–proteasome system.
**a**

Western Blotting Results:

| Treatment            | Lanes |
|----------------------|-------|
| MOV10-FLAG           | 1-5   |
| pNL4-3ΔEnv-GFP-ΔVif  | +     |
| A3G-HA               | +     |
| Vif-HA               | +     |

Relative A3G expression:

- Lane 1: 10%
- Lane 2: 19%
- Lane 3: 46%
- Lane 4: 72%
- Lane 5: 100%

**b**

Western Blotting Results:

| Treatment            | Lanes |
|----------------------|-------|
| MOV10-FLAG           | 1-5   |
| pNL4-3ΔEnv-GFP       | +     |
| pNL4-3ΔEnv-GFP-ΔVif  | -     |
| A3G-HA               | +     |

Relative A3G expression:

- Lane 1: 9%
- Lane 2: 13%
- Lane 3: 28%
- Lane 4: 41%
- Lane 5: 100%

**c**

Western Blotting Results:

| Treatment            | Lanes |
|----------------------|-------|
| MOV10-siRNA          | 1-4   |
| NC-siRNA             | -     |
| pNL4-3ΔEnv-GFP-ΔVif  | +     |
| A3G-HA               | +     |
| Vif-HA               | +     |

Relative A3G expression:

- Lane 1: 100%
- Lane 2: 39%
- Lane 3: 45%
- Lane 4: 16%

**d**

Western Blotting Results:

| Treatment            | Lanes |
|----------------------|-------|
| MOV10-siRNA          | 1-4   |
| NC-siRNA             | -     |
| pNL4-3ΔEnv-GFP-ΔVif  | +     |
| pNL4-3ΔEnv-GFP       | -     |
| A3G-HA               | +     |

Relative A3G expression:

- Lane 1: 100%
- Lane 2: 38%
- Lane 3: 31%
- Lane 4: 14%
HIV-1 Vif has at least 4 conserved motifs, which are required for interactions with host proteins. The HCCH motif can bind to Cullin 5, the BC-box motif (144-SLQYLA-149) binds to ElonginC, 101-DVMK-104 binds to ElonginB, and 88-EW-89 is crucial for binding with CBF-β [38, 49–51]. Through a series of co-immunoprecipitation analyses, we found that MOV10 can disrupt the interaction of Vif with ElonginB, ElonginC, Cullin 5, or CBF-β and then decrease the ubiquitination of A3G. Finally, the degradations of A3G and Vif are blocked and their expression levels in cells are increased subsequently. These results suggest that MOV10 functions to mediate assembly of the Vif-CBF-β-ElonginB-ElonginC-Cullin 5 complex.

Previous studies have shown that Cullin 5 functions as a scaffold protein for the E3 ubiquitin ligase [52, 53] ubiquitin–proteasome. ElonginB, ElonginC, and CBF-β are adaptor proteins that function to maintain this complex. Moreover, Vif acts as a substrate acceptor to modulate the degradation of A3G [52, 54]. Therefore, reduced binding of Vif with Cullin 5 could affect the complex assembly efficiency. Moreover, researchers have verified the interactions between the different components of the complex. The binding of Cullin 5 to Vif enhances the stability of the Vif-CBF-β interaction [55]. Conversely, CBF-β is also crucial for the binding of Vif with Cullin 5, ElonginB, and ElonginC [37, 56, 57]. ElonginB and ElonginC play important roles in the interaction between Vif and CBF-β [16]. To clarify the mechanisms through which MOV10 disrupts the assembly of the Vif-CBF-β-ElonginB-ElonginC-Cullin 5 complex, we examined whether there were direct interactions between MOV10 and different components of the CBF-β-Cullin 5-ElonginB-ElonginC complex. The results demonstrate that MOV10 can bind with ElonginC or Cullin 5 and that binding between MOV10 and Cullin 5 is partially dependent on RNA. Our own study and previous studies have shown that MOV10 usually interacts with numerous RNA-associated proteins, such as AGO1/2, A3G, and HIV-1 Rev [20, 32]. Thus, it is not surprising that MOV10 interacts with Cullin 5 in an RNA-dependent manner. Accordingly, significant decreases in the binding of Vif with ElonginB, ElonginC, Cullin 5, and CBF-β were observed when MOV10 was overexpressed. For the inhibitory effects of MOV10 on the binding of Vif with ElonginB or CBF-β, the interactions of MOV10 with ElonginC, Cullin 5, and Vif may induce structural changes in the Vif-CBF-β-ElonginB-ElonginC-Cullin 5 complex, subsequently disrupting the interactions between Vif and ElonginB and between Vif and CBF-β. Several studies have shown that DEAG-box motif of MOV10 is crucial for its helicase activity [18, 28]. In our report, we also explored the
MOV10 knockdown in the context of HIV-1 replication. by MOV10 overexpression and significantly reduced by that the A3G level was significantly enhanced in virions of endogenous MOV10 in permissive human T cell line (CEM-SS). Previous study also showed that MOV10 and A3G. In 2010, Wang et al. [22] have shown that the replication of HIV-1 was enhanced by the deple-
tion of MOV10 and A3G. In 2010, Wang et al. [22] have shown the synergistic anti-HIV-1 activities. These results will help us to get more comprehensive and profound understanding of MOV10 and A3G.

Conclusions

Therefore, our results reveal a novel anti-HIV-1 mechanism of MOV10: it prevents A3G from Vif-induced proteasomal degradation and then increases the levels of A3G both in cells and in newly-synthesized virions. In addition, because both MOV10 and A3G have anti-HIV-1 activity and belong to the interferon antiviral system, our findings suggested that these proteins are of synergistic anti-HIV-1 activities. These results will help us to get more comprehensive and profound understanding of MOV10 and A3G.

Methods

Plasmid construction and siRNAs synthesis
pcDNA3.1-A3G-HA, pcDNA3.1-GFP-HA, pcDNA3.1-MOV10-FLAG, pcDNA3.1-MOV10-HA, pcDNA3.1-rMOV10-FLAG, pcDNA3.1-MOV10-DEAG-mutant-HA, pcDNA3.1-MOV10-DEAG-mutant-FLAG and pcDNA3.1-Ub-FLAG were constructed as described previously [20, 32, 59]. pcDNA3.1-Vif-HA, pcDNA3.1-Vif-FLAG, pcDNA3.1-ElonginB-FLAG, pcDNA3.1-Cullin 5-FLAG, pcDNA3.1-CBF-β-FLAG, and were constructed by our lab [60]. HA or FLAG epitope tag sequenced as described previously [20, 32, 59]. pcDNA3.1-Vif-HA, pcDNA3.1-Vif-FLAG, pcDNA3.1-ElonginB-FLAG, pcDNA3.1-Cullin 5-FLAG, pcDNA3.1-CBF-β-FLAG, and were constructed by our lab [60]. HA or FLAG epitope tag sequenced as described previously [20, 32, 59]. pcDNA3.1-Vif-HA, pcDNA3.1-Vif-FLAG, pcDNA3.1-ElonginB-FLAG, pcDNA3.1-Cullin 5-FLAG, pcDNA3.1-CBF-β-FLAG, and were constructed by our lab [60]. HA or FLAG epitope tag sequenced as described previously [20, 32, 59]. pcDNA3.1-Vif-HA, pcDNA3.1-Vif-FLAG, pcDNA3.1-ElonginB-FLAG, pcDNA3.1-Cullin 5-FLAG, pcDNA3.1-CBF-β-FLAG, and were constructed by our lab [60]. HA or FLAG epitope tag sequenced as described previously [20, 32, 59].
terminus of ElonginB, Cullin 5, or CBF-β was amplified through reverse transcription-polymerase chain reaction (RT-PCR) with the mRNA of 293T cells as the template. ElonginC with FLAG tag sequence at its 3′ terminus was amplified via PCR from the pElonginC-HA plasmid, which was generously provided by Dr. Xianghui Yu in Jilin University [62]. Then, the tagged ElonginB, ElonginC, Cullin 5, or CBF-β was inserted into pcDNA3.1 vector. HIV-1 proviral construct pNL4-3-ΔEnv-GFP has been described in our previous reports [63, 64]. pNL4-3-ΔEnv-GFP-ΔVif, a vif defective construct, was generated from pNL4-3-ΔEnv-GFP. The vif gene in pNL4-3-ΔEnv-GFP-ΔVif was disrupted by PCR-mediated site-directed mutagenesis and introduced nonsense mutations at codon positions 26, 27 (AAA, CAC → TAA, TAG) and/or 33, 34 (ACT, AAA → TAA, TAG) [65]. The vector pLKO.1-TRC, which contains a U6 promoter → selection gene and was obtained from Addgene (plasmid # 10878), was used for expression of MOV10-shRNA or scrambled control (Scr)-shRNA. Forward oligo of MOV10-shRNA (TRCN0000425452): 5′-CCGGGCGCCAGTTTTCGAGAGTTTCTCGAGGAAACTCTCGAAGACACTCTCGAAACACTGCGCCTTTTTG-3′; reverse oligo of MOV10-shRNA: 5′-AATTCAGAAAGGGCTGTTTCGAGGAATCTCTCGAAACTGCGCCTTTTTT-3′. Scr-shRNA forward oligo: 5′-CCCGAAGCCTACGCGGAATCTTTTGGAGTTCGCCGTACGTCTTTTTG-3′; Scr-shRNA reverse oligo: 5′-AATTCAGAAAGGGCTGTTTCGAGGAATCTCTCGAAACTGCGCCTTTTTT-3′ [40]. All oligos were synthesized from Ribobio (Guangzhou, China).

The siGENOME SMART pool against MOV10 and siRNA for negative control were designed by Dharmacon and the target sequences for MOV10-specific siRNAs were chosen as described previously [20, 32], ElonginB-specific siRNA, ElonginC-specific siRNA, and Cullin 5-specific siRNA were designed and synthesized by Ribobio (Guangzhou, China).

**Cell culture and transfection**

Human 293T cells were obtained from American Type Culture Collection (ATCC) and grown at 37 °C with 5% CO2 in Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen) supplemented with 10% fetal bovine serum (FBS) (Invitrogen) and 1% penicillin–streptomycin (Invitrogen). The cells were transfected with the indicated plasmids or siRNAs by lipofectamine 2000 (Invitrogen). The procedures described by the manufacturer were followed.

**Co-immunoprecipitation and western blotting**

Co-immunoprecipitation and western blotting assays were performed as previously described [20, 32]. In brief, human 293T cells were lysed with the lysis buffer (150 mM NaCl, 50 mM Tris–HCl [pH 7.5], 1 mM EDTA, 1% Triton X-100, 0.5% NP-40, plus PMSF and protease inhibitor cocktail [Sigma]) for 30 min at 4 °C. The cell lysates were clarified by centrifugation at 18,000g for 30 min at 4 °C, then mixed with anti-HA agarose beads (Sigma) and incubated at 4 °C for 4 h, followed by washing four times with cold lysis buffer and eluting in gel loading buffer. As indicated, the beads were treated with RNase mixture (DNase-free, Roche) (20 μg/ml) and incubated at 37 °C for 30 min. The immunoprecipitated samples were analyzed by SDS-PAGE and detected by western blotting. Anti-HA antibody (mouse monoclonal, Covance), anti-FLAG antibody (rabbit polyclonal, MBL), anti-GAPDH antibody (rabbit polyclonal, MBL), anti-MOV10 antibody (rabbit polyclonal, Abcam), anti-ElonginC antibody (rabbit polyclonal, Abcam), anti-Cullin 5 antibody (rabbit polyclonal, Abcam), anti-A3G antibody (rabbit polyclonal, Abcam), anti-Vif antibody (mouse monoclonal, Abcam), and anti-HIV-1 p24 antibody (rabbit polyclonal antibodies made by our lab) were used as primary antibodies [64]. Quantity One program (Bio-Rad) was used to quantify the western blotting results.

**HIV-1 virus-like particle (VLP) purification**

Human 293T cells were transfected with pNL4-3-ΔEnv-GFP or pNL4-3-ΔEnv-GFP-ΔVif and other indicated plasmids. After 48 h of transfection, cell supernatants were collected, centrifuged at 4 °C for 10 min at 8000 rpm (≈ 7000 g) and filtered through a 0.45 μm filter to remove cellular debris. And the cell-free supernatants were concentrated by ultracentrifugation through a 20% sucrose cushion at 4 °C for 2 h at 45,000 rpm (≈ 40,000 g) (HITACHI Preparative Ultracentrifuge, CP80WX). Then, the pellets were re-suspended in RIPA buffer containing protease inhibitor cocktail and subjected to immunoblotting.

**Construction of MOV10-knockdown H9 cells**

The pLKO.1-MOV10-shRNA or pLKO.1-Scr-shRNA was co-transfected with pSAX2 and pCMV-VSV-G into 293T cells. After 48 h, the supernatants were harvested and filtered with 0.45 μm filters (Millipore). Then, H9 cells were infected with MOV10-specific-shRNA-expressing or Scr-shRNA-expressing lentivirus respectively for 8 h and cultured with fresh medium. After 48 h, virus-infected H9 cells were selected by puromycin (1 μg/ml) for 2 weeks and subjected to the following experiments.
**Wild-type HIV-1 infection**

MOV10-knockdown H9 cells and negative control cells were infected with HIV-1NL4-3 (p24 titer of 5 ng ml⁻¹) for 3 h and then cultured with fresh medium and detected p24 in culture supernatant at different days. After 12 days, cells were collected and treated with the transcription factor buffer set including fixation/permeabilization and fixation/wash buffers (BD Biosciences) according to the manufacturer supernatant at different days. After 12 days, cellFITC-conjugated anti-HIV-1 p24 antibody (Santa Cruz Biotechnology) for intracellular HIV-1 Gag (p24) expression. Then, p24 positive cells were sorted with BD Aria sorter for further analysis. Data was analyzed with FlowJo software (Tree Star, Ashland, OR).

**Virus infectivity assay**

Human 293T cells were co-transfected with pCMV-VSV-G, pNL4-3ΔEnv-GFP,ΔVif (or pNL4-3ΔEnv-GFP), and increasing amounts of pcDNA3.1-MOV10-FLAG or pcDNA31-MOV10-mutant-FLAG in the presence or absence of A3G-HA expressing plasmid. The virus-containing supernatant were collected at 48 h after transfection and filtered by a 0.45 μm filter. After normalization for HIV-1 p24 by enzyme-linked immunosorbent assay (ELISA, Clonetech), TZM-bl cells (2.5 × 10⁵ cells per well in 24-well plates) were infected with virus which containing 5 ng of p24 capsid. And then, luciferase enzyme activity determinations at 72 h post infection were carried out.

**Abbreviations**

MOV10: Moloney leukemia virus 10, A3G: Apolipoprotein-B-mRNA-editing enzyme catalytic polypeptide-like 3G, USP: Ubiquitin–proteasome system.

**Authors’ contributions**

CL and HZ designed this project. CC, XM, QH, and CL performed most of the experiments. CC, CL, and HZ analyzed the results and wrote the paper. XL, FH, JZ, and XJ participated in some experiments. TP constructed ElonginB- and Cullin 5-expressing plasmids. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

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