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Induction of APOBEC3G Ubiquitination and Degradation by an HIV-1 Vif-Cul5-SCF Complex

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HIV-1 Vif is essential for viral evasion of host antiviral factor CEM15/APOBEC3G. We now report that Vif interacts with cellular proteins Cul5, Elongins B and C, and Rbx1 to form an Skp1-Cullin-F-box (SCF)-like complex. The ability of Vif to suppress antiviral activity of APOBEC3G was specifically dependent on Cul5-SCF function, allowing Vif to interact with APOBEC3G and induce its ubiquitination and degradation. A Vif mutant that interacted with APOBEC3G but not with Cul5-SCF was functionally inactive. The Cul5-SCF was also required for Vif function in distantly related SIVmac/HIV-2. These results indicate that the conserved Cul5-SCF pathway used by Vif is a potential target for antiviral development.

The *vif* orf is present in all lentiviruses except EIAV and is required for viral replication and pathogenicity in vivo (*1*–*12*). In the absence of Vif, HIV-1 virions that are produced from non-permissive primary T lymphocytes and certain T-cell lines are defective and cannot initiate productive infection. Recently, CEM15/APOBEC3G (hereafter referred to as APOBEC3G), which is present only in non-permissive cells, has been identified as a mediator of anti-HIV-1 activity, and its activity has been shown to be suppressed by Vif (*13*). APOBEC3G belongs to a family of proteins that have cytidine deaminase activity and, when packaged into HIV-1 virions, induce modification of newly synthesized minus-strand viral DNA from cytosines to uracils (*13*–*18*). We sought to characterize cellular proteins that interact with Vif and regulate its function, as a means of understanding the mechanism of HIV-1 evasion of host antiviral defense.

To efficiently precipitate Vif from HIV-1-infected cells and subsequently identify the cellular protein(s) that might interact with Vif, we constructed an infectious HIV-1 clone (HXB2VifHA) in which the end of Vif carried an HA tag. This clone replicated as efficiently as the parental clone HXB2 (*8*) in H9 cells, which are not permissive for a Vif deletion mutant HXB2ΔVif (fig. S1, SOM Text). An anti-HA antibody immunoprecipitated HA-tagged Vif from lysates of HXB2VifHA-infected H9 cells, but not untagged Vif from control HXB2-infected H9 cells (Fig. 1). Three cellular proteins co-precipitated with Vif-HA but were absent from HXB2 control samples (Fig. 1A). These proteins, of ~85kDa, 18kDa, and 15 kDa (Fig. 1A), were identified by mass spectrometry as Cullin-5 (Cul5) and Elongins B and C, respectively. Their identities were confirmed by reactivity with Cul5-, Elongin B-, or Elongin C-specific antibodies (Fig. 1B). Although Rbx1 was not clearly visible by silver staining, it also co-precipitated with Vif-HA, as revealed by immunoblotting with anti-Rbx1 (Fig. 1B). In the converse experiment, HIV-1 Vif could also be immunoprecipitated with Cul5 (Fig. 1C). Moreover, when an SLQ → AAA mutation was introduced into the highly conserved lentiviral Vif motif SLQXLA, the interaction between HIV-1 Vif and Cul5-Elongin B-Elongin C was significantly decreased (Fig. 1D). Interaction of HIV-1 Vif with Cul5, Elongin B, and Elongin C was also detected in HXB2VifHA–infected Jurkat cells and transfected 293T cells (fig. S2).

These results indicate that HIV-1 Vif interacts with Cul5, Elongin B, Elongin C, and Rbx1 to form a complex reminiscent of an Skp1-Cullin-F box (SCF)-like, or more specifically a VCB-like, complex (*19*, *20*). These complexes represent one of the largest families of ubiquitin-protein ligases, ubiquitinating a broad range of proteins involved in cell cycle regulation, signal transduction, transcription, and other cellular processes (*21*). SCF and SCF-like complexes consist of cullin, Rbx1, adaptor proteins (Skp1 or Elongins B and C), and F-Box proteins that determine target protein specificity. The Vif-Cul5-containing SCF complex detected here bears strong similarity to the adenovirus E4orf6-Cul5-containing SCF complex (Cul5, Elongin B, Elongin C, and Rbx1), with the major difference being the involvement of HIV-1 Vif and E4orf6 (*22*, *23*).

To examine whether the Cul5-containing SCF complex is required for Vif function, we first established 293T cells expressing the host antiviral factor APOBEC3G (293T/APOBEC3G) and demonstrated that the production of infectious HIV-1 virions from 293T/APOBEC3G was Vif-dependent (fig. S3A, SOM Text). Rbx1 (Fig. 2A) is an integral component of the SCF complexes (*21*), and its overexpression has been shown to disrupt SCF function (*24, 25*). We therefore hypothesized that overexpression of Rbx1 should reduce HIV-1 infectivity in the presence of APOBEC3G. Overexpression of Rbx1 in 293T and 293T/APOBEC3G cells transfected with pRbx1-Myc was detected using an anti-Rbx1 antibody (fig. S3B). Consistent with a previous report (*25*), overexpression of Rbx1 increased the Nedd8 modification of Cul5 by about 50% (fig. S3C). When viral infection was evaluated in the MAGI-CCR5 system, we found that the HIV-1 infectivity was not affected by the overexpression of Rbx1 in 293T cells (Fig. 2B, lanes 1 and 2). However, in repeated experiments, HIV-1 infectivity was reduced by approximately 75% when Rbx1 was

overexpressed in the presence of APOBEC3G (Fig. 2B, lanes 3 and 4). Therefore, overexpression of Rbx1 compromised the ability of Vif to suppress the antiviral activity of APOBEC3G. When Vif function was not required (i.e., in the absence of APOBEC3G), disruption of SCF activity had little effect on HIV-1 infectivity.

Rbx1 has been shown to associate with various cullin SCF complexes (*21*). All cullin family members are known to be modified by the ubiquitin-like small molecule Nedd8 (*26*). To address more directly whether Cul5-containing SCF complexes are required for Vif function, we studied the effects of Cul5 mutants on HIV-1 infectivity by constructing Cul5 mutants defective for Nedd8 modification (Fig. 2C, SOM, Text) and Rbx1 binding (Fig. 2D, SOM, Text). We then evaluated the effect of these Cul5 mutants on HIV-1 infectivity using viruses from transfected 293T or 293T/APOBEC3G cells in MAGI-CCR5 cells. Virus infectivity was not affected by co-expression of these Cul5 mutants with HIV-1 in 293T cells (Fig. 2E, lanes 1–4). However, the infectivity was significantly reduced when the Nedd8 (pCul5ΔNedd8) or the Rbx1-binding mutant (pCul5ΔRbx1) of Cul5 was co-expressed with HIV-1 in 293T/APOBEC3G cells (Fig. 2E, lanes 7 and 8). We also observed that several N-terminal internal deletion mutants of Cul5 did not affect HIV-1 Vif function in 293T/APOBEC3G cells. The results obtained with one of these mutants (an internal deletion of amino acids 65 to 93, pCul5ΔN2) are shown here (Fig. 2E, lane 6). Expression of all these Cul5 mutants did not affect HIV-1 production from 293T/APOBEC3G cells (fig. S3D), nor were the released viral protein patterns altered (fig. S3E). Therefore, expression of only select Cul5 mutants specifically blocked the ability of HIV-1 Vif to suppress the antiviral activity of APOBEC3G.

HIV-2/SIVmac are highly related primate lentiviruses that are distantly related to HIV-1, but their replication in human cells is still Vif-dependent (*4*). We also observed that APOBEC3G blocked the production of infectious virions by the SIVmac ΔVif mutant and that SIVmac Vif could neutralize the antiviral activity of APOBEC3G (fig. S4). Furthermore, selected Cul5 mutants specifically blocked the ability of SIVmac Vif to suppress the antiviral activity of APOBEC3G (fig. S4). Therefore, both HIV-1 and SIVmac Vif proteins apparently mediate their function in human cells through the Cul5-containing SCF complex. This finding is particularly interesting because the Vif proteins of HXB2 and SIVmac251 share only ~30% amino acid identity.

It is possible that Vif recruits E3 ligase to ubiquitinate APOBEC3G and induce its degradation. Consistent with a previous report (*15*), interaction of APOBEC3G with Myc-tagged Vif (Fig. 3A, lane 3) but not untagged Vif (Fig. 3A, lane 4) was achieved by co-immunoprecipitation with the anti-Myc antibody. Interaction of Vif with APOBEC3G was further confirmed by the reverse immunoprecipitation experiment (Fig. 3B). Interestingly, the non-functional SLQ mutant Vif, which had lost the ability to interact with Cul5-Elongin B-Elongin C, was still able to interact with APOBEC3G (Fig. 3B, lane 6), suggesting that interaction between Vif and APOBEC3G alone is not sufficient to suppress the activity of APOBEC3G.

The effect of Vif on the stability of APOBEC3G was also evaluated. Pulse-chase experiments indicated that HIV-1 Vif induced rapid degradation of APOBEC3G (Fig. 3C). Therefore, the steady-state level of APOBEC3G in the presence of Vif (Fig. 3D, lane 2) was significantly lower than that in the absence of Vif (Fig. 3D, lane 1). Treatment with the proteasome inhibitor MG132 significantly increased the steady-state level of APOBEC3G, even in the presence of Vif (Fig. 3D, lane 4). More importantly, Cul5 mutants that blocked the function of Vif (Fig. 2) also inhibited Vif-mediated degradation of APOBEC3G, resulting in comparable steady-state levels of APOBEC3G in the presence (Fig. 3E, lanes 3 and 4) and absence of Vif (Fig. 3E, lane 1).

To assess whether Vif could promote the ubiquitination of APOBEC3G, we co-expressed HIV-1 Vif and APOBEC3G-HA in the presence or absence of Myc-tagged ubiquitin in 293T cells. HA-tagged APOBEC3G was immunoprecipitated by the anti-HA antibody, followed by the detection of ubiquitinated APOBEC3G using the anti-Myc antibody. Ubiquitinated APOBEC3G was not detected in cells lacking HA-tagged APOBEC3G (Fig. 3F, lanes 1 and 2) or in cells lacking Myc-tagged ubiquitin, either in the absence (Fig. 3F, lane 3) or presence of Vif (Fig. 3F, lane 4). A significantly higher level of ubiquitinated APOBEC3G was, however, detected in cells expressing the Myc-tagged ubiquitin in the presence of Vif (Fig. 3F, lane 6) than in its absence (Fig. 3F, lane 5). HIV-1 Vif-induced ubiquitination of APOBEC3G was inhibited by Cul5 mutants that blocked Vif function: Both Cul5ΔRbx1 (Fig. 3F, lane 8) and Cul5ΔNedd8 (Fig. 3F, lane 10) inhibited Vif-induced ubiquitination of APOBEC3G.

Vif deletion (HXB2ΔVif) resulted in efficient incorporation of APOBEC3G into released virions (Fig. 4A, lane 3). Consistent with a recent report (*15*), Vif blocked incorporation of APOBEC3G into wild-type virions (Fig. 4A, lane 2). Cul5 mutants, which inhibited Vif-induced ubiquitination and degradation of APOBEC3G, caused efficient incorporation of APOBEC3G even in the presence of Vif (Fig. 4B, lanes 3 and 4). Cul5ΔN2, which had little effect on Vif function (Fig. 2), did not influence incorporation of APOBEC3G (Fig. 4B, lane 2). These findings were in good agreement with the observed inhibition of HIV-1 infectivity by Cul5 mutants in the presence of APOBEC3G (Fig. 2). As expected, none of these Cul5 mutants influenced APOBEC3G incorporation into Vif mutant virions (Fig. 4B, lanes 5 to 7). The ability of Vif to exclude APOBEC3G from released virions was compromised in the presence of proteasome inhibitor (Fig. 4C, lane 5), which also interfered with the ability of HIV-1 Vif to allow production of infectious virus in cells expressing APOBEC3G (Fig. 4D, lane 6).

In this study, we have identified cellular proteins that interact with HIV-1 Vif and are critical to Vif's ability to suppress the antiviral activity of APOBEC3G. Cullins are a highly conserved family of proteins, capable of assembling into E3 ubiquitin ligases. These ligases are known to regulate the stability and activity of cell cycle regulators, transcription factors, signaling proteins, and DNA damage repair pathways. The cellular targets of the Cul5-containing E3 ubiquitin ligases remain to be identified. In the case of HIV-1, our data suggest that an SCF-like complex may be formed by the assembly of HIV-1 Vif with Cul5, Elongin B, Elongin C, and Rbx1. Since Vif can also interact with APOBEC3G, Vif may function as a link between Cul5-SCF and APOBEC3G or as an F-box-like protein that targets APOBEC3G for ubiquitination and degradation, activities reminiscent of Vpu-induced degradation of CD4 molecules (*27*).

The ability of Vif to induce ubiquitination and degradation of APOBEC3G also correlated with its ability to prevent virion incorporation of APOBEC3G, which is detrimental to HIV-1 (*13–18*). A recent report observed a less profound

degradation of APOBEC3G by Vif (*15*). It is conceivable that not all APOBEC3G and Vif molecules co-localize in the cell, and the APOBEC3G and Vif molecules that co-localize at the site of HIV-1 assembly could represent a small fraction of the total cellular APOBEC3G. This fraction would be targeted by the Vif-Cul5-SCF complex for ubiquitination and degradation. The extent of Vif-induced degradation of APOBEC3G would thus be dependent on the amount of co-localization of these proteins and their concentrations in the cell. Both Cul5 mutants and proteasome inhibitors blocked the ability of HIV-1 Vif to exclude APOBEC3G and to generate infectious virions, implicating Cul5 E3 ubiquitin ligases and proteasomes in the function of Vif. Since suppression of host antiviral activity by Vif is essential for HIV-1 infection, strategies that interfere with the interaction of Vif with the Cul5-SCF complex and/or degradation of APOBEC3G may lead to the design of novel anti-HIV therapies.

References and Notes

1. J. H. Simon, N. C. Gaddis, R. A. Fouchier, M. H. Malim, *Nature Med.* **4**, 1397 (1998).
2. N. Madani, D. Kabat, *J. Virol.* **72**, 10251 (1998).
3. A. G. Fisher *et al.*, *Science* **237**, 888 (1987).
4. J. S. Gibbs, D. A. Regier, R. C. Desrosiers, *AIDS Res. Hum. Retroviruses* **10**, 607 (1994).
5. D. H. Gabuzda *et al.*, *J. Virol.* **66**, 6489 (1992).
6. N. C. Kan *et al.*, *Science* **231**, 1553 (1986).
7. T. H. Lee *et al.*, *Science* **231**, 1546 (1986).
8. M. Dettenhofer, S. Cen, B. A. Carlson, L. Kleiman, X. F. Yu, *J. Virol.* **74**, 8938 (2000).
9. J. H. Simon, M. H. Malim, *J. Virol*, **70**, 5297 (1996).
10. J. Sodroski *et al.*, *Science* **231**, 1549 (1986).
11. K. Strebel *et al.*, *Nature* **328**, 728 (1987).
12. U. von Schwedler, J. Song, C. Aiken, D. Trono, *J. Virol.* **67**, 4945 (1993).
13. A. M. Sheehy, N. C. Gaddis, J. D. Choi, M. H. Malim, *Nature* **418**, 646 (2002).
14. H. Zhang *et al.*, *Nature* **424**, 94 (2003).
15. R. Mariani *et al.*, *Cell* **114**, 21 (2003).
16. B. Mangeat *et al.*, *Nature* **424**, 99 (2003).
17. D. Lecossier, F. Bouchonnet, F. Clavel, A. J. Hance, *Science* **300**, 1112 (2003).
18. R. S. Harris *et al.*, *Cell* **113**, 803 (2003).
19. K. Iwai *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 12436 (1999).
20. C. E. Stebbins, W. G. Kaelin, Jr., N. P. Pavletich, *Science* **284**, 455 (1999).
21. R. J. Deshaies, *Annu. Rev. Cell. Dev. Biol.* **15**, 435 (1999).
22. J. N. Harada, A. Shevchenko, D. C. Pallas, A. J. Berk, *J. Virol.* **76**, 9194 (2002).
23. E. Querido *et al.*, *Genes Dev.* **15**, 3104 (2001).
24. W. M. Gray, H. Hellmann, S. Dharmasiri, M. Estelle, *Plant Cell* **14**, 2137 (2002).
25. T. Kamura *et al.*, *Science* **284**, 657 (1999).
26. T. Hori *et al.*, *Oncogene* **18**, 6829 (1999).
27. F. Margottin *et al.*, *Mol.* Cel.l **1**, 565 (1998).

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S4
References and Notes

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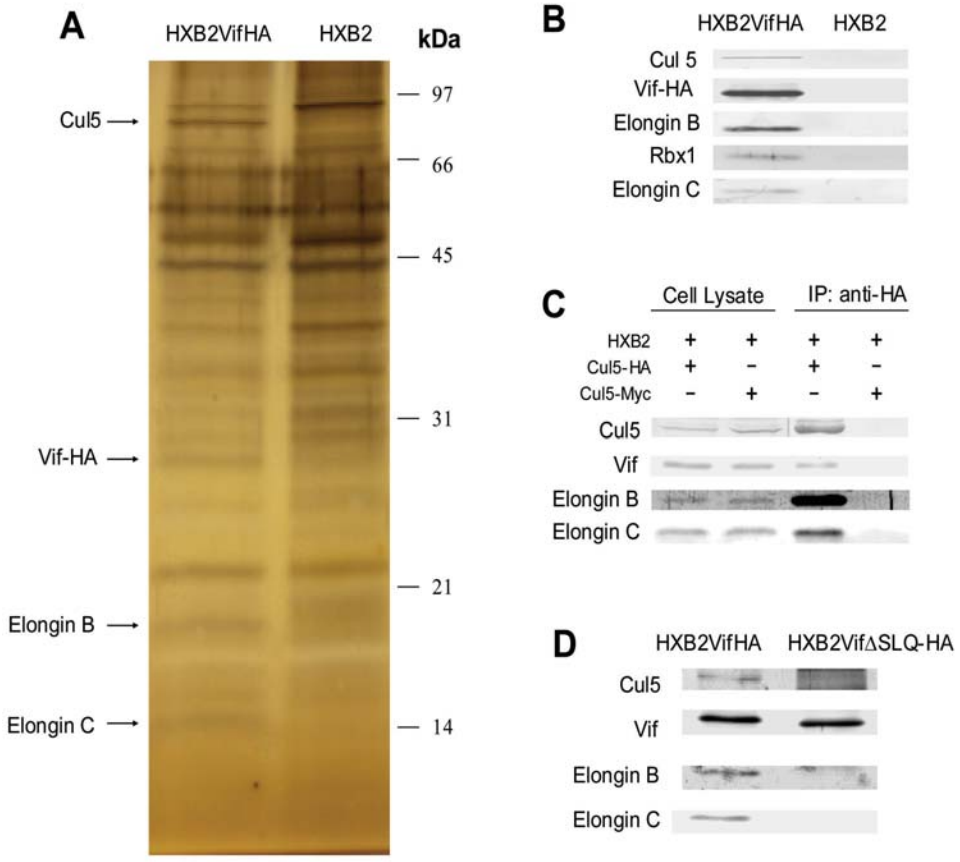
Fig. 1. Identification of cellular proteins that interact with HIV-1 Vif. (**A**) Co-immunoprecipitation of cellular proteins with Vif-HA. Cell lysates from HXB2- or HXB2VifHA-infected H9 cells were immunoprecipitated with anti-HA antibody, followed by SDS-PAGE and silver staining. The identification of Cul5, Elongin B, and Elongin C was achieved by mass spectroscopic analysis; these proteins were not detected in the control HXB2 samples. (**B**) Immunoblot of precipitated samples from HXB2 and HXB2VifHA-infected H9 cells. Precipitated samples as described above were separated by SDS-PAGE, transferred to nitrocellulose membranes and reacted with antibodies against Cul5, HIV-1 Vif, Elongin B, Rbx1, or Elongin C. (**C**) Co-immunoprecipitation of HIV-1 Vif with Cul5. 293T were co-transfected with HXB2 plus Cul5-HA or Cul5-Myc expression vectors. Cell lysates were prepared 48 h after transfection and immunoprecipitated with anti-HA antibody. Proteins in cell lysates and immunoprecipitated samples were detected by immunoblotting using antibodies against Cul5, Vif, Elongin B, or Elongin C. (**D**) Reduced interaction of VifΔSLQ with Cul5-SCF. Cell lysates from HXB2VifHA- or HXB2VifΔSLQ-HA-infected H9 cells were immunoprecipitated with anti-HA antibody. Precipitated samples were analyzed by immunoblotting with antibodies against Cul5, HIV-1 Vif, Elongin B, or Elongin C.

Fig. 2. Overexpression of Rbx1 and Cul5 mutants blocks Vif function in the presence of APOBEC3G. (**A**) Top panel: Diagram of the Cul5-SCF complex. Bottom panel: Partial amino acid sequences of Cul1, Cul5, and Cul5ΔNedd8. (**B**) Overexpression of Rbx1 blocks Vif function. HIV-1 (HXB2) viruses were produced from 293T cells or 293T/APOBEC3G cells co-transfected with the control vector VR1012 or pRbx1, and their infectivity was examined using MAGI-CCR5 cells. Virus input was normalized by the level of p24. The infectivity of HXB2 produced from 293T cells co-transfected with VR1012 was set as 100%. Results are the average of 5 independent experiments. (**C**) Immunoblotting of wild-type Cul5-Myc, Cul5ΔNedd8-Myc, and Cul5ΔRbx1-Myc. Both Neddylated and un-Neddylated Cul5-Myc were detected from pCul5-Myc-transfected 293T cells by the anti-Myc tag antibody (lane 1). Only the un-Neddylated Cul5-Myc was detected in pCul5ΔNedd8-Myc-transfected 293T cells (lane 2). (**D**) Interaction of Rbx1 was detected by co-immunoprecipitation with Cul5ΔNedd8-Myc (lane 1) and wild-type Cul5-Myc (lane 2), but not with Cul5ΔRbx1-Myc mutant (lane 3), using the anti-Myc-tag antibody. Immunoprecipitated samples were detected by

immunoblotting using antibodies against Cul5 (upper panel) or Rbx1 (lower panel). (**E**) Cul5 mutants blocked Vif function in the presence of APOBEC3G. HIV-1 (HXB2) viruses were produced from 293T cells or 293T/APOBEC3G cells co-transfected with control vector VR1012, pCul5ΔN2, pCul5Δnedd8 or pCul5ΔRbx1. Virus input was normalized by the level of p24. The infectivity of HXB2 produced from 293T cells co-transfected with VR1012 was set as 100%. Results are the average of 5 independent experiments.

Fig. 3. Vif induces degradation and ubiquitination of APOBEC3G. (**A**) and (**B**) Interaction between Vif and APOBEC3G. Data are representatives of at least three independent experiments. (A) 293T cells were transfected with APOBEC3G-HA and Vif-Myc or Vif expression vectors as indicated. Equal amounts of cell extract were immunoprecipitated with anti-Myc-tag antibody and analyzed by immunoblotting using antibodies against HA (upper panel) or Vif (lower panel). (B) 293T cells were transfected with Vif or the VifΔSLQ mutant and APOBEC3G-HA or APOBEC3G-Myc expression vectors as indicated. Equal amounts of cell extract were immunoprecipitated with anti-HA-tag antibody and analyzed by immunoblotting using antibodies against HA (upper panel) or Vif (lower panel). (**C**) Pulse-chase experiments of APOBEC3G-HA in the absence or presence of Vif. Cells were metabolically labeled for 15 min and chased for the indicated times. APOBEC3G-HA was immunoprecipitated, separated by SDS-PAGE, and analyzed by fluorography. The amount of APOBEC3G-HA at time 0 was normalized to 100%. (**D**) Inhibition of Vif-induced degradation of APOBEC3G by proteasome inhibitor MG132. 293T cells were transfected with APOBEC3G-HA plus Vif expression or the control vectors and treated with DMSO or MG132. Equal amounts of cell extract were analyzed by immunoblotting using antibodies against HA for the detection of APOBEC3G. Ribosomal P19 antigen was used as total protein loading control. (**E**) Inhibition of Vif-induced degradation of APOBEC3G by Cul5 mutants. 293T cells were transfected with APOBEC3G-HA and the control vectors or Vif plus Cul5 mutant expression vectors as indicated. Equal amounts of cell extract were analyzed by immunoblotting using antibodies against the HA tag for the detection of APOBEC3G-HA. (**F**) Ubiquitination of APOBEC3G induced by Vif and blocked by Cul5 mutants. 293T cells were transfected with expression vectors encoding APOBEC3G-HA, Vif, Myc-tagged ubiquitin, Cul5 mutants, or control vectors as indicated. Cells were lysed and immunoprecipitated with anti-HA tag antibody and analyzed by immunoblotting with anti-Myc tag antibody for the detection of ubiquitinated APOBEC3G (upper panel). To prevent the degradation of ubiquitinated APOBEC3G, all experiments were performed in the presence of proteasome inhibitor MG132. Unmodified APOBEC3G was detected using the anti-HA tag antibody (lower panel).

Fig. 4. Effects of Vif, proteasome inhibitor, and Cul5 mutants on virion packaging of APOBEC3G and infectivity of HIV-1. (**A**) HIV-1 Vif blocked virion packaging of APOBEC3G. (**B**) Cul5 mutants increased APOBEC3G virion packaging in the presence of Vif. 293T/APOBEC3G cells were transfected with HXB2 or HXB2ΔVif plus Cul5 mutant expression vectors as indicated. At 48 h after transfection, viruses were purified and analyzed by immunoblotting. Viral proteins were detected by anti-p24 antibody. APOBEC3G-HA was detected by anti-HA antibody. (**C**) Proteasome inhibitor increased APOBEC3G virion packaging in the presence of Vif. 293T/APOBEC3G cells were transfected with HXB2 or HXB2ΔVif. At 24 h after transfection, cells were treated with DMSO or 2.5 μM MG132 for 16 h, and viruses were purified and analyzed by immunoblotting. Viral proteins were detected by anti-p24 antibody. APOBEC3G-HA was detected by anti-HA antibody. (**D**) Proteasome inhibitor MG132 blocked Vif function in the presence of APOBEC3G. HXB2 and HXB2ΔVif viruses were produced from 293T cells or 293T/APOBEC3G cells in the absence or the presence of 2.5 μM MG132. Virus input was normalized by the level of p24. Infectivity of HXB2 produced from 293T cells in the absence of MG132 was set as 100%. Results are the average of 5 independent experiments.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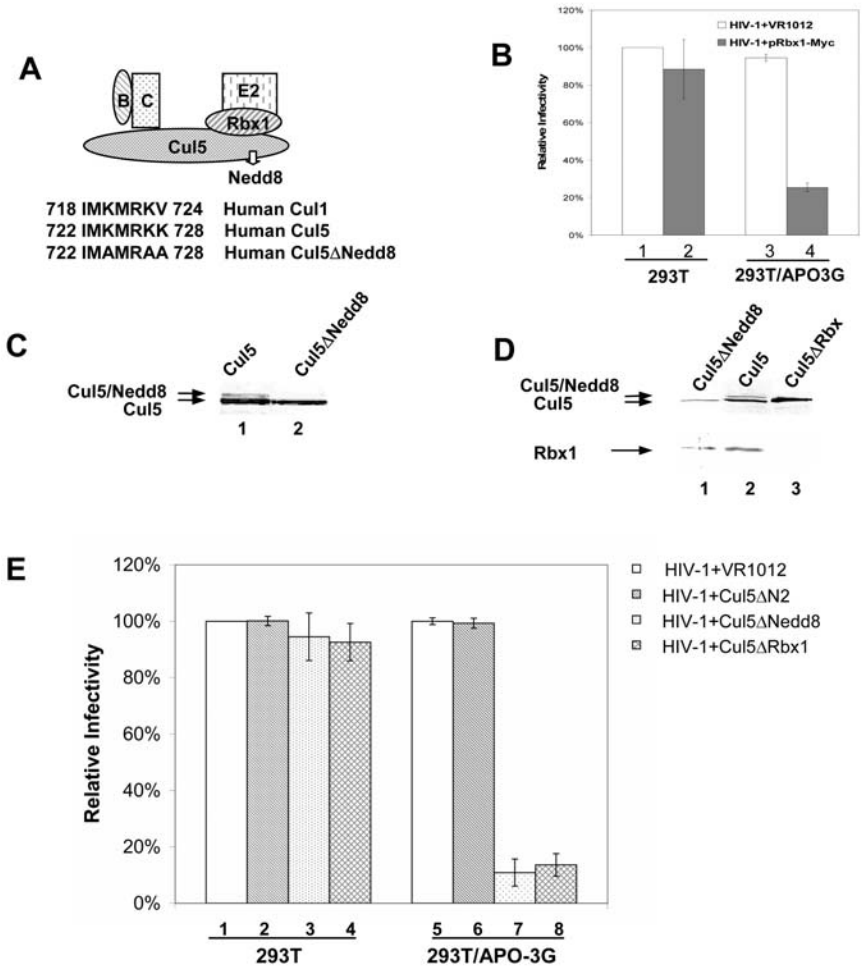
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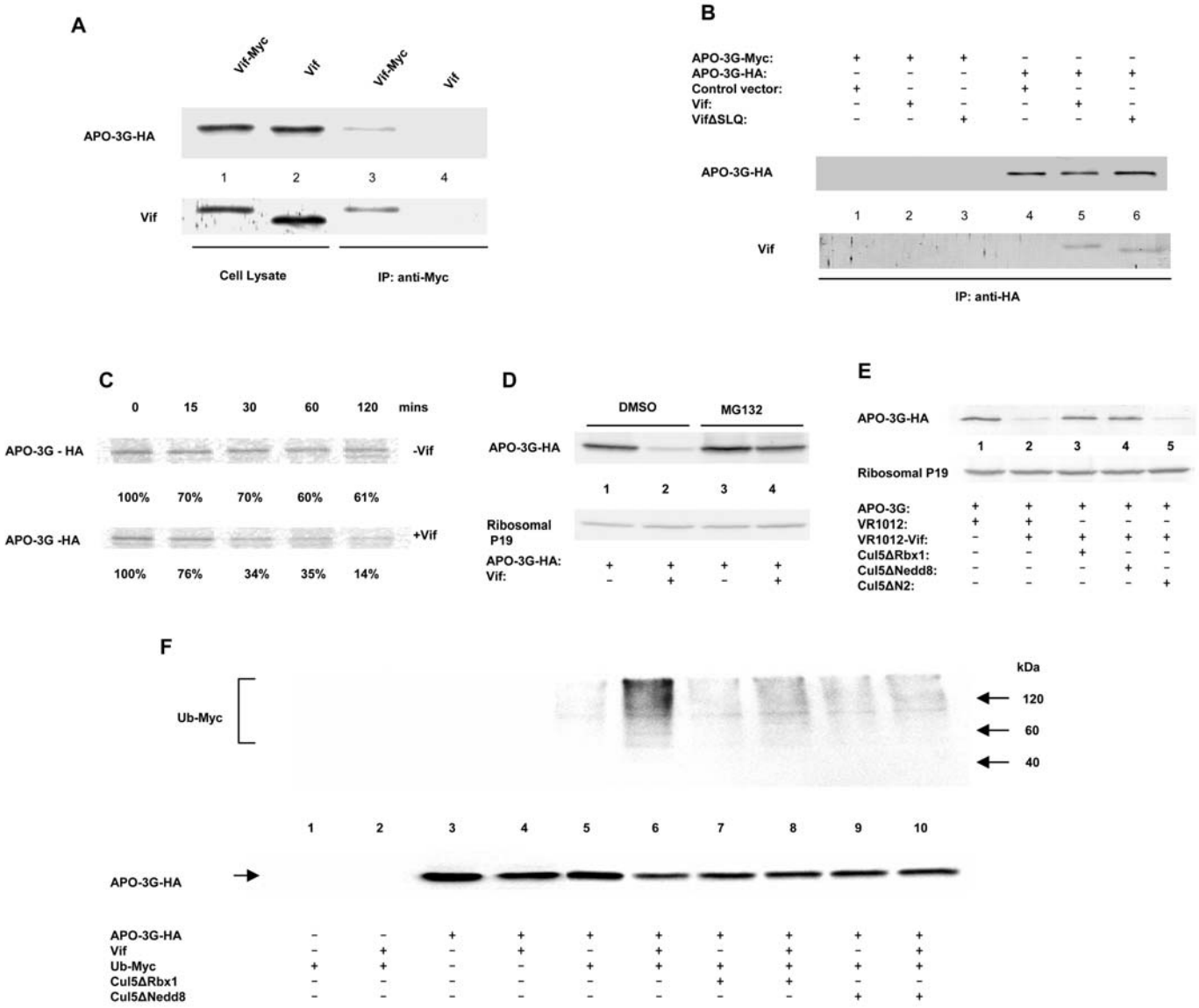
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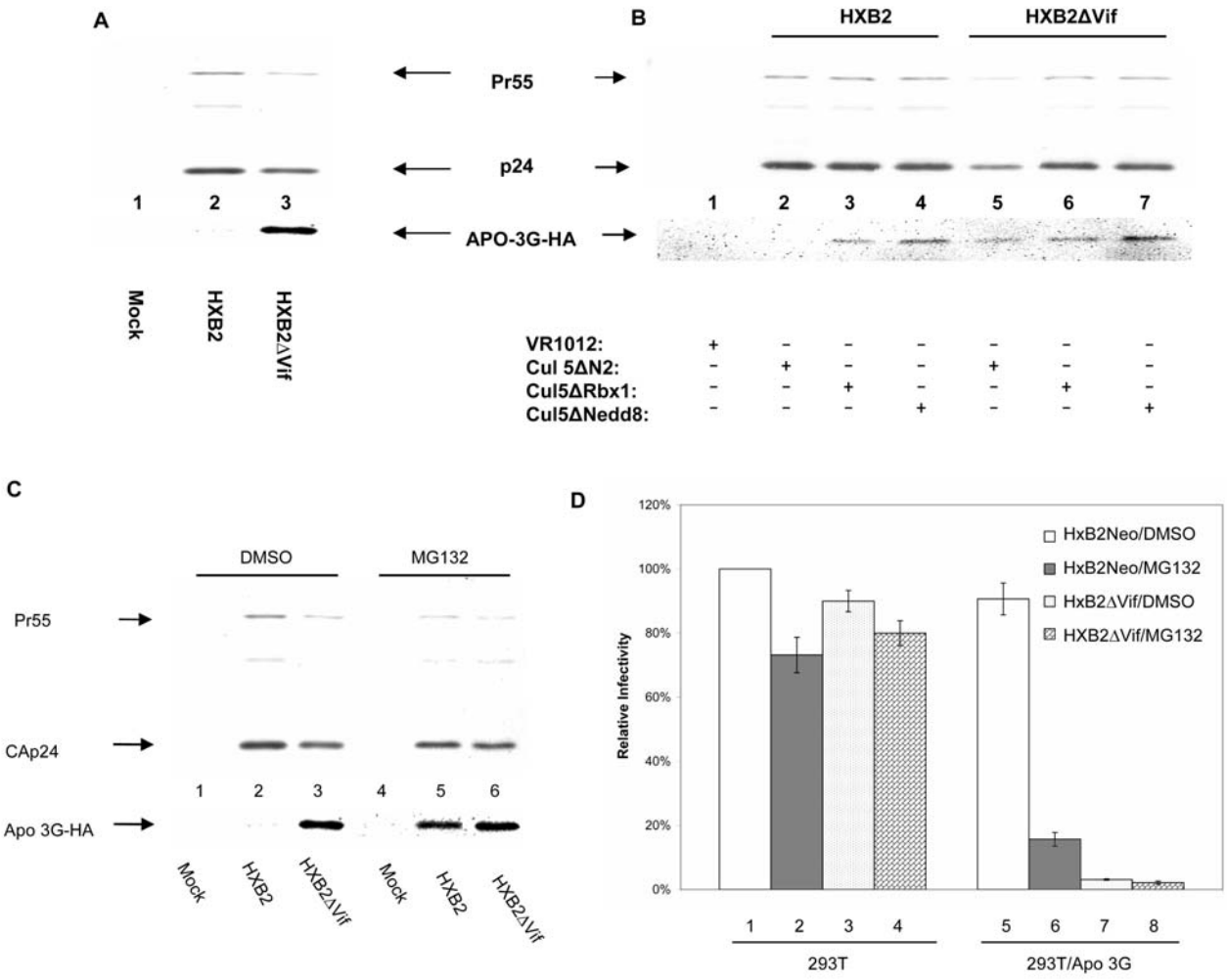
Cul5ΔRbx1 – – – – – – + + – –
Cul5ΔNedd8 – – – – – – – – + +


A
Pr55
p24
APO-3G-HA
1
2
3
Mock
HXB2
HXB2ΔVif
B
HXB2
HXB2ΔVif
1
2
3
4
5
6
7
VR1012:
Cul 5ΔN2:
Cul5ΔRbx1:
Cul5ΔNedd8:
C
DMSO
MG132
Pr55
CAp24
Apo 3G-HA
1
2
3
4
5
6
Mock
HXB2
HXB2ΔVif
Mock
HXB2
HXB2ΔVif
D
Relative Infectivity
120%
100%
80%
60%
40%
20%
0%
HxB2Neo/DMSO
HxB2Neo/MG132
HxB2ΔVif/DMSO
HXB2ΔVif/MG132
1
2
3
4
5
6
7
8
293T
293T/Apo 3G