Supplementary materials

HIV genome–wide protein associations:

A review of 30 years of research

Guangdi Li[1,2], Erik De Clercq[2]

1. Institute of Metabolism and Endocrinology, Metabolic Syndrome Research Center, the Second Xiangya Hospital, Central South University, Hunan 410011, China.
2. Department of Microbiology and Immunology, Rega Institute for Medical Research, School of Medicine, KU Leuven – University of Leuven, Leuven, 3000, Belgium

Correspondence to Dr. Guangdi Li (email: liguangdi.research@gmail.com) and

Dr. Erik De Clercq (email: erik.declercq@kuleuven.be)

Supplementary tables

Table S 1: Summary of HIV-1 PDB codes used for our structural visualization

Gene	*gag*						*pol*			*vif*	*vpr*	*tat*	*rev*	*Vpu*	*env*		*nef*
Protein	Matrix	Capsid	SP1	Nucleocapsid	SP2	p6	Protease	RT	Integrase	Vif	Vpr	Tat	Rev	Vpu	GP120	GP41	Nef
Number of units	3	5,6	1	1	1	1	2	2	4	2,3,4	1	2	2,6	1	3	3	2
Multimeric protein	1HIW #	3H4E 4XFZ	-	-	-	-	1A30 4LL3	3V4I 3V81	1K6Y	-	-	-	3LPH		4NCO 4TVP	2XRA 4TVP	-
Monomeric protein	-	-	1U57	1A1T	-	2C55	-	-	-	4N9F	1M8L	1K5K	-	1VPU 1PJE	-	-	4EMZ 1AVV

#: HIV PDB codes were extracted from the RCSB Protein Data Bank (http://www.rcsb.org/) using the protein sequence search.

-: a PDB code does not exist or is not available.

For GP41, we used 4TVP to visualize the pre-fusion state, and 2XRA to visualize the post-fusion state. Most protein structures are crystalized from HIV-1 strains, except for SIV Vpx (PDB: 4CC9) and prototype foamy virus integrase (PDB: 3L2V).

In **Figure 5A**, the following HIV-1 PDB codes are used to show the structure of Env in complex with CD4 and CXCR4.

(A) GP120 positions: 31–505 (PDB codes: 4JM2, 4TVP, 4NCO);

(B) GP41 positions: 518–664 (PDB codes: 4JM2, 4TVP, 4NCO);

(C) CXCR4 positions: 27–328 (PDB code: 3ODU);

(D) CD4 positions: 26–388, 397–458 (PDB codes: 1WIO, 2KLU).

In **Figure 10B**, the following HIV-1 PDB codes are used to visualize protease structures crystalized with 6 substrate peptides derived from Gag and GagPol cleavage sites.

(A) The MA–CA cleavage site (PDB: 1KJ4);

(B) The SP1–NC cleavage site (PDB: 1KJ7);

(C) The NC–SP2 cleavage site (PDB: 1TSQ);

(D) The SP2–p6 cleavage site (PDB: 1KJF);

(E) The p51–p15 cleavage site (PDB: 1KJG);

(F) The p15–IN cleavage site (PDB: 1KJH).

Table S2: Summary of publication citations implying the citation level of HIV pairwise protein interactions.

Protein interaction	References	Citation numbers extracted from Google Scholar	Level
GP120 – GP41	(1-11)	228+130+19+3+71+93+24+274+235+6+63=1146	High
$GP41^{Env}$ – Matrix	(12-19)	190+45+290+18+15+2+13+323=896	High
GP120 – Tat	(20-23)	41+4+28+4=77	Median
RT – Integrase	(24-31)	72+54+20+222+6+174+2+115=665	High
RT – Nucleocapsid	(32-35)	86+99+190+97=472	High
RT – Vif	(36)	20	Low
RT – Tat	(37)	28	Low
RT – Nef	(38, 39)	16+5=21	Median
Integrase – Rev	(40-42)	34+56+21=111	High
Integrase – Matrix	(43)	421	High
Matrix – Vpr	(44)	32	Low
Integrase – Nef	(39)	5	Low
Tat – Vpr	(45)	111	Median
Tat – Rev	(46)	1	Low
Tat – Nef	(47)	34	Low
Tat – Nucleocapsid	(48)	1	Low
Vif – Vpr	(49)	18	Low
NC^{Gag} – Vif	(50-53)	155+68+6+109=338	High
NC^{Gag} – Vpr	(54-56)	33+83+119=235	High
$p6^{Gag}$ – Vpr	(56-67)	178+87+77+104+409+112+18+119+62+14+29+136=1345	High
$p6^{Gag}$ – Vpx	(56, 57, 68, 69)	87+119+15+26=247	High
$GP41^{Env}$ – Nef	(70)	53	Low
Gag – RT	(71, 72)	12+20=32	Median
Protease – Gag/GagPol	(73-75)	225+8+1=234	High
Protease – Vif	(76-78)	17+28+13=58	Median

Protease – RT	(79)	36	Low
Protease – Tat	(80)	42	Low
Protease – Nef	(81-85)	28+45+55+94+20=242	High
Protease – GP41CT	(86, 87)	33+14=47	Median

(i) Protein interaction, HIV pairwise protein interactions listed in our main text; **(ii)** References, publications that have characterized HIV protein interactions; **(iii)** Citation numbers extracted from Google Scholar, the total number of citations of references based on Google Scholar search on March 1, 2016. **(iv)** Level, the citation level measured by the number of publications and their citation numbers. (a) High: well-known interactions that have been cited more than 300 times, or have been recorded by at least 3 publications with more than 100 citations in total. (b) Low, little-known interactions that have been reported by a single paper with less than 100 citations. (c) Median, lesser-known interactions include the remaining interactions.

Supplementary text S1

In this supplementary, we will summarize the basic functions of 16 HIV proteins. Additional information about their reference sequences, natural polymorphisms, protein structures and genomic localization is provided in our online platform (http://www.virusface.com/).

Matrix: HIV matrix, encoded by the *gag* gene, is a structural protein that builds the basic infrastructure of HIV particles. After the protease–mediate cleavage of Gag and Gagpol precursors, HIV matrix trimmers organize into ordered hexamers to create a structural layer beneath the viral membrane, which protects the integrity of HIV particles (88). The myristoylated N–terminal domain of HIV matrix is critical for targeting plasma membrane and for promoting viral assembly (89). To prevent nonspecific binding, matrix^{Gag} in the Gag polyprotein binds to nucleic acids in a PIP2-dependent manner (PIP2: phosphatidylinositol 4,5-bisphosphate) (88).

Capsid: HIV capsid, encoded by the *gag* gene, is a structural protein that builds the basic infrastructure of viral particles (88). The hexamer and pentamer forms of HIV capsid constitute the conical fullerene core of mature viral particles (90). The interactions between HIV capsid and host proteins allow for the packaging of host proteins (e.g. cyclophilin A) (91). HIV capsid also interacts with the host restriction factor TRIM5α to prevent the viral uncoating at the early stage (92). Multifaceted functions of HIV capsid have been summarized in a recent review (93).

Nucleocapsid: Nucleocapsid is a structural protein encoded by the *gag* gene (88, 94-96). To prevent viral RNA from nucleases, HIV nucleocapsid coats the genomic RNA within the viral core (97). Nucleocapsid also interacts with many host proteins (e.g. the ESCRT–associated protein ALIX) to promote viral budding (98). As a RNA chaperone, nucleocapsid enhances nucleic acid–dependent steps in the HIV life cycle. For instance, it not only promotes the DNA strand–transfer reaction during reverse transcription, but also stimulates viral integration (99).

p6: p6 is a structural protein located at the C terminus of the *gag* gene (88). During viral budding, HIV $p6^{Gag}$ recruits the host machinery to release the virus outwards from the cell surface (100). Moreover, HIV-1 $p6^{Gag}$ binds to Vpr and host proteins (e.g. AIP1/ALIX) for their viral packaging (88).

Protease: HIV protease is the first viral enzyme encoded by the *pol* gene. During viral maturation, protease cleaves Gag polyproteins at the cleavage sites to produce structural proteins (matrix, capsid, nucleocapsid, p6). In a similar fashion, protease cleaves the GagPol precursors to produce viral enzymes (protease, reverse transcriptase, integrase). Moreover, the protease activity depends on the concentration of GagPol precursors, whilst the rate of protease–mediated autoprocessing is modulated by the adjacent p6* sequence (101).

Reverse transcriptase (RT): RT is the second enzyme encoded by the *pol* gene. To produce dsDNA from viral single-stranded RNA genome, RT in the reverse transcriptase complex (RTC) undertakes both the RNA-dependent and the DNA-dependent polymerization reactions. During reverse transcription, RT jumps from one template to another on two copies of single–stranded genomic RNAs. The frequent template switch promotes the generation of new recombinant genomes derived from two parental RNA sequences (99). Numerous mutations occur because of the error-prone reverse transcription.

Integrase: Integrase is the third enzyme encoded by the *pol* gene. After the nuclear import of the pre-integration complex (PIC), viral integrase performs two major reactions (3'-processing and strand–transfer reactions) to insert the double-stranded viral DNA into human chromosomes. Inside mature viral particles, HIV integrase is cleaved from GagPol polyproteins by viral protease. Moreover, reverse transcriptase interacts with integrase to prevent the catalytic activity of integrase before viral integration (29). As part of the reverse transcriptase complex (RTC), integrase also plays a crucial role during reverse transcription (99).

Vif: Viral infectivity factor is an accessory protein encoded by all lentiviruses except the equine infectious anemia virus (102). Vif is notorious for hijacking the human ubiquitin ligase complex CBF-β to counteract the antiviral activity of host proteins such as APOBEC-3G and APOBEC-3F (103, 104). APOBEC3 proteins from the human APOBEC3 family of DNA cytosine deaminases are known as anti–HIV proteins that potently inhibit HIV-1 by introducing G–to–A hypermutaiton of the viral genome to impair DNA synthesis and integration (102, 105-107). Vif also interacts with Gag polyproteins to modulate the protease–mediated proteolytic processing (102). Notably, Vif is incorporated into HIV particles during viral budding (102).

Vpr: Viral protein R is an accessory protein which enhances HIV-1 replications in the non-dividing cells (e.g. macrophages). During the HIV-1 life cycle, Vpr plays multiple functions such as the modulation of viral reverse transcription, the nuclear import of the HIV-1 pre-integration complex, the transactivation of HIV-1 long terminal repeat (LTR) promoter, and the induction of apoptosis and G2/M cell cycle arrest (see review (108)). Notably, Vpr is incorporated into HIV-1 particles during viral budding (109).

Vpu: HIV-1 viral protein U (Vpu) is a membrane-associated accessory protein with two major functions: CD4 downregulation and tetherin antagonism (110). First, Vpu hijacks the human ubiquitin machinery to target CD4, and induces the downregulation of CD4 receptors in the endoplasmic reticulum (ER). Second, Vpu antagonizes tetherin, an interferon–regulated human restriction factor, to enhance the release of viral particles. Notably, Vpu is not incorporated into HIV particles during viral budding (111).

Vpx: Vpx is an accessory protein in HIV-2 and SIV, which marks a distinct difference compared to HIV-1. Major functions of Vpx include: (i) Vpx induces the ubiquitin-proteasome-dependent degradation of SAMHD1, which is a host protein that restricts HIV-2 replication in myeloid cells (112-114). (ii) Vpx is required for HIV-2 reverse transcription (115). (iii) Vpx assists nuclear import of the viral pre-integration complex (PIC) (112-114). Notably, Vpx is incorporated into viral particles during viral budding.

Rev: Rev is an accessory protein that controls the nuclear export of unspliced and partially spliced viral RNAs from the nucleus to the cytoplasm (116). Rev multimers bind to the stem-loop structure of Rev response element (RRE) in the *env* coding region of viral RNA, forming a large oligomeric ribonucleoprotein (RNP) (99). The RNP complex interacts with human export factor CRM1 (exportin 1 or Xpo1) to shuttle from the nucleus to the cytoplasm through the nuclear pore complex (NPC). Overall, Rev activity exerts a strong influence on viral RNA transport, translation and packaging (117). Notably, Rev is not incorporated into viral particles (111).

Tat: HIV trans-activator of transcription (Tat) is a regulatory protein that plays essential roles in viral replication. Tat exists in all lentiviruses and is the first eukaryotic transcription factor known to interact with TAR (transactivating response element) in RNA instead of DNA (99). Tat interacts with various human proteins to execute multiple functions (99, 118, 119). (i) Tat activates the transcription initiation and elongation of HIV-1 LTR promoter, preventing the premature termination of transcription and polyadenylation. (ii) Tat acts as a nucleic acid chaperone to regulate the capping of HIV-1 mRNA. (iii) Tat induces the T cell apoptosis, neurodegeneration and oxidative stress. (iv) Tat regulates the expression of major histocompatibility complex (MHC) and downregulates many cell surface receptors. (v) Tat suppresses the activity of reverse transcriptase to prevent the premature synthesis of viral DNA. (vi) Extracellular Tat upregulates the CXCR4 expression on CD4+ T cells, stimulates the expression of cytokines and interacts with cell-surface receptors to activate cellular signal transduction pathways. Notably, Tat is not incorporated into viral particles.

GP120: Encoded by the *env* gene, the surface glycoprotein GP120 is exposed on the surface of HIV particles (120). On the virion surface, there are approximately 14 envelope spikes consisting of three molecules of GP120 and GP41 each, connected by non-covalent interactions (121). During viral entry, GP120 interacts with specific receptors (e.g. CD4) on the cell surface (122). Specifically, the binding of CD4 to GP120 induces the conformational changes of GP120, therefore exposing the V3 loop

of GP120 to interact with cellular coreceptors (e.g. CCR5). Many human neutralizing antibodies have been found to target GP120, whereas a few antibodies (e.g. PG9, PG16) have a broad neutralization activity against different HIV-1 strains (123-125).

GP41: The transmembrane glycoprotein GP41 is the second envelope protein encoded by the *env* gene. GP41 contains a glycine-rich region that is essential for membrane fusion activity (126). HIV GP41 plays multiple activities during the viral life cycle (126). (i) Env intracellular trafficking is regulated by the cytoplasmic tail of GP41 (GP41CT) which interacts with various cellular proteins. (ii) GP41CT interacts with viral Matrix to regulate Env incorporation. (iii) GP41CT regulates internalization exerted by the clathrin-mediated endocytosis. (iv) GP41CT regulates the cellular activation of host transcription factors (e.g. NF-kB). (v) GP41 interacts with host proteins to regulate the activity of the actin cytoskeleton. (vi) HIV-1 GP41 membrane-proximal external region is targeted by human antibodies (e.g. 10E8) (127).

Nef: HIV negative regulatory factor (Nef) is an accessory protein which enhances viral pathogenesis (128). During the viral life cycle, Nef can play multiple roles (128). (i) Nef downregulates CD4 receptors and MHC molecules. (ii) Nef promotes the viral release and the cell-to-cell transmission. (iii) Nef activates the apoptosis and takes part in the clathrin-dependent endocytic pathways. Notably, Nef is incorporated into HIV particles (128).

References

1. **Pancera M, Majeed S, Ban YE, Chen L, Huang CC, Kong L, Kwon YD, Stuckey J, Zhou T, Robinson JE, Schief WR, Sodroski J, Wyatt R, Kwong PD.** 2010. Structure of HIV-1 gp120 with gp41-interactive region reveals layered envelope architecture and basis of conformational mobility. Proc Natl Acad Sci U S A **107:**1166-1171.
2. **Pancera M, Zhou T, Druz A, Georgiev IS, Soto C, Gorman J, Huang J, Acharya P, Chuang GY, Ofek G, Stewart-Jones GB, Stuckey J, Bailer RT, Joyce MG, Louder MK, Tumba N, Yang Y, Zhang B, Cohen MS, Haynes BF, Mascola JR, Morris L, Munro JB, Blanchard SC, Mothes W, Connors M, Kwong PD.** 2014. Structure and immune recognition of trimeric pre-fusion HIV-1 Env. Nature **514:**455-461.
3. **Do Kwon Y, Pancera M, Acharya P, Georgiev IS, Crooks ET, Gorman J, Joyce MG, Guttman M, Ma X, Narpala S, Soto C, Terry DS, Yang Y, Zhou T, Ahlsen G, Bailer RT, Chambers M, Chuang GY, Doria-Rose NA, Druz A, Hallen MA, Harned A, Kirys T, Louder MK, O'Dell S, Ofek G, Osawa K, Prabhakaran M, Sastry M, Stewart-Jones GB, Stuckey J, Thomas PV, Tittley T, Williams C, Zhang B, Zhao H, Zhou Z, Donald BR, Lee LK, Zolla-Pazner S, Baxa U, Schon A, Freire E, Shapiro L, Lee KK, Arthos J, Munro JB, Blanchard SC, Mothes W, Binley JM, et al.** 2015. Crystal structure, conformational fixation and entry-related interactions of mature ligand-free HIV-1 Env. Nat Struct Mol Biol **22:**522-531.
4. **Alsahafi N, Debbeche O, Sodroski J, Finzi A.** 2015. Effects of the I559P gp41 change on the conformation and function of the human immunodeficiency virus (HIV-1) membrane envelope glycoprotein trimer. PLoS One **10:**e0122111.
5. **Bartesaghi A, Merk A, Borgnia MJ, Milne JL, Subramaniam S.** 2013. Prefusion structure of trimeric HIV-1 envelope glycoprotein determined by cryo-electron microscopy. Nat Struct Mol Biol **20:**1352-1357.
6. **Finzi A, Xiang SH, Pacheco B, Wang L, Haight J, Kassa A, Danek B, Pancera M, Kwong PD, Sodroski J.** 2010. Topological layers in the HIV-1 gp120 inner domain regulate gp41 interaction and CD4-triggered conformational transitions. Mol Cell **37:**656-667.
7. **Guttman M, Lee KK.** 2013. A functional interaction between gp41 and gp120 is observed for monomeric but not oligomeric, uncleaved HIV-1 Env gp140. J Virol **87:**11462-11475.
8. **Julien JP, Cupo A, Sok D, Stanfield RL, Lyumkis D, Deller MC, Klasse PJ, Burton DR, Sanders RW, Moore JP, Ward AB, Wilson IA.** 2013. Crystal structure of a soluble cleaved HIV-1 envelope trimer. Science **342:**1477-1483.
9. **Lyumkis D, Julien JP, de Val N, Cupo A, Potter CS, Klasse PJ, Burton DR, Sanders RW, Moore JP, Carragher B, Wilson IA, Ward AB.** 2013. Cryo-EM structure of a fully glycosylated soluble cleaved HIV-1 envelope trimer. Science **342:**1484-1490.

10. **Drummer HE, Hill MK, Maerz AL, Wood S, Ramsland PA, Mak J, Poumbourios P.** 2013. Allosteric modulation of the HIV-1 gp120-gp41 association site by adjacent gp120 variable region 1 (V1) N-glycans linked to neutralization sensitivity. PLoS Pathog **9:**e1003218.
11. **Mao Y, Wang L, Gu C, Herschhorn A, Desormeaux A, Finzi A, Xiang SH, Sodroski JG.** 2013. Molecular architecture of the uncleaved HIV-1 envelope glycoprotein trimer. Proc Natl Acad Sci U S A **110:**12438-12443.
12. **Murakami T, Freed EO.** 2000. Genetic evidence for an interaction between human immunodeficiency virus type 1 matrix and alpha-helix 2 of the gp41 cytoplasmic tail. J Virol **74:**3548-3554.
13. **West JT, Weldon SK, Wyss S, Lin X, Yu Q, Thali M, Hunter E.** 2002. Mutation of the dominant endocytosis motif in human immunodeficiency virus type 1 gp41 can complement matrix mutations without increasing Env incorporation. J Virol **76:**3338-3349.
14. **Freed EO, Martin MA.** 1995. Virion incorporation of envelope glycoproteins with long but not short cytoplasmic tails is blocked by specific, single amino acid substitutions in the human immunodeficiency virus type 1 matrix. J Virol **69:**1984-1989.
15. **Tedbury PR, Ablan SD, Freed EO.** 2013. Global Rescue of Defects in HIV-1 Envelope Glycoprotein Incorporation: Implications for Matrix Structure. PLoS Pathog **9:**e1003739.
16. **Chan WE, Wang YL, Lin HH, Chen SS.** 2004. Effect of extension of the cytoplasmic domain of human immunodeficiency type 1 virus transmembrane protein gp41 on virus replication. J Virol **78:**5157-5169.
17. **Tedbury PR, Mercredi PY, Gaines CR, Summers MF, Freed EO.** 2015. Elucidating the Mechanism by which Compensatory Mutations Rescue an HIV-1 Matrix Mutant Defective for Gag Membrane Targeting and Envelope Glycoprotein Incorporation. J Mol Biol **427:**1413-1427.
18. **Brandano L, Stevenson M.** 2012. A highly conserved residue in the C-terminal helix of HIV-1 matrix is required for envelope incorporation into virus particles. J Virol **86:**2347-2359.
19. **Freed EO, Martin MA.** 1996. Domains of the human immunodeficiency virus type 1 matrix and gp41 cytoplasmic tail required for envelope incorporation into virions. J Virol **70:**341-351.
20. **Marchio S, Alfano M, Primo L, Gramaglia D, Butini L, Gennero L, De Vivo E, Arap W, Giacca M, Pasqualini R, Bussolino F.** 2005. Cell surface-associated Tat modulates HIV-1 infection and spreading through a specific interaction with gp120 viral envelope protein. Blood **105:**2802-2811.
21. **Poon S, Moscoso CG, Xing L, Kan E, Sun Y, Kolatkar PR, Vahlne AG, Srivastava IK, Barnett SW, Cheng RH.** 2013. Putative role of Tat-Env interaction in HIV infection. AIDS **27:**2345-2354.
22. **Monini P, Cafaro A, Srivastava IK, Moretti S, Sharma VA, Andreini C, Chiozzini C, Ferrantelli F, Cossut MR, Tripiciano A, Nappi F, Longo O, Bellino S, Picconi O, Fanales-Belasio E, Borsetti A, Toschi E, Schiavoni I,**

Bacigalupo I, Kan E, Sernicola L, Maggiorella MT, Montin K, Porcu M, Leone P, Leone P, Collacchi B, Palladino C, Ridolfi B, Falchi M, Macchia I, Ulmer JB, Butto S, Sgadari C, Magnani M, Federico MP, Titti F, Banci L, Dallocchio F, Rappuoli R, Ensoli F, Barnett SW, Garaci E, Ensoli B. 2012. HIV-1 tat promotes integrin-mediated HIV transmission to dendritic cells by binding Env spikes and competes neutralization by anti-HIV antibodies. PLoS One **7:**e48781.

23. **Cardaci S, Soster M, Bussolino F, Marchio S.** 2013. The V1/V2 loop of HIV-1 gp120 is necessary for Tat binding and consequent modulation of virus entry. FEBS Lett **587:**2943-2951.
24. **Tasara T, Maga G, Hottiger MO, Hubscher U.** 2001. HIV-1 reverse transcriptase and integrase enzymes physically interact and inhibit each other. FEBS Lett **507:**39-44.
25. **Wilkinson TA, Januszyk K, Phillips ML, Tekeste SS, Zhang M, Miller JT, Le Grice SF, Clubb RT, Chow SA.** 2009. Identifying and characterizing a functional HIV-1 reverse transcriptase-binding site on integrase. J Biol Chem **284:**7931-7939.
26. **Herschhorn A, Oz-Gleenberg I, Hizi A.** 2008. Quantitative analysis of the interactions between HIV-1 integrase and retroviral reverse transcriptases. Biochem J **412:**163-170.
27. **Wu X, Liu H, Xiao H, Conway JA, Hehl E, Kalpana GV, Prasad V, Kappes JC.** 1999. Human immunodeficiency virus type 1 integrase protein promotes reverse transcription through specific interactions with the nucleoprotein reverse transcription complex. J Virol **73:**2126-2135.
28. **Chakraborty A, Sun GQ, Mustavich L, Huang SH, Li BL.** 2013. Biochemical interactions between HIV-1 integrase and reverse transcriptase. FEBS Lett **587:**425-429.
29. **Zhu K, Dobard C, Chow SA.** 2004. Requirement for integrase during reverse transcription of human immunodeficiency virus type 1 and the effect of cysteine mutations of integrase on its interactions with reverse transcriptase. J Virol **78:**5045-5055.
30. **Tekeste SS, Wilkinson TA, Weiner EM, Xu X, Miller JT, Le Grice SF, Clubb RT, Chow SA.** 2015. Interaction between Reverse Transcriptase and Integrase Is Required for Reverse Transcription during HIV-1 Replication. J Virol **89:**12058-12069.
31. **Hehl EA, Joshi P, Kalpana GV, Prasad VR.** 2004. Interaction between human immunodeficiency virus type 1 reverse transcriptase and integrase proteins. J Virol **78:**5056-5067.
32. **Druillennec S, Caneparo A, de Rocquigny H, Roques BP.** 1999. Evidence of interactions between the nucleocapsid protein NCp7 and the reverse transcriptase of HIV-1. J Biol Chem **274:**11283-11288.
33. **Lener D, Tanchou V, Roques BP, Le Grice SF, Darlix JL.** 1998. Involvement of HIV-I nucleocapsid protein in the recruitment of reverse transcriptase into nucleoprotein complexes formed in vitro. J Biol Chem **273:**33781-33786.

34. **Peliska JA, Balasubramanian S, Giedroc DP, Benkovic SJ.** 1994. Recombinant HIV-1 nucleocapsid protein accelerates HIV-1 reverse transcriptase catalyzed DNA strand transfer reactions and modulates RNase H activity. Biochemistry **33:**13817-13823.
35. **Cameron CE, Ghosh M, Le Grice SF, Benkovic SJ.** 1997. Mutations in HIV reverse transcriptase which alter RNase H activity and decrease strand transfer efficiency are suppressed by HIV nucleocapsid protein. Proc Natl Acad Sci U S A **94:**6700-6705.
36. **Kataropoulou A, Bovolenta C, Belfiore A, Trabatti S, Garbelli A, Porcellini S, Lupo R, Maga G.** 2009. Mutational analysis of the HIV-1 auxiliary protein Vif identifies independent domains important for the physical and functional interaction with HIV-1 reverse transcriptase. Nucleic Acids Res **37:**3660-3669.
37. **Apolloni A, Meredith LW, Suhrbier A, Kiernan R, Harrich D.** 2007. The HIV-1 Tat protein stimulates reverse transcription in vitro. Curr HIV Res **5:**473-483.
38. **Fournier C, Cortay JC, Carbonnelle C, Ehresmann C, Marquet R, Boulanger P.** 2002. The HIV-1 Nef protein enhances the affinity of reverse transcriptase for RNA in vitro. Virus Genes **25:**255-269.
39. **Ciuffi A, Munoz M, Bleiber G, Favre M, Stutz F, Telenti A, Meylan PR.** 2004. Interactions of processed Nef (58-206) with virion proteins of HIV type 1. AIDS Res Hum Retroviruses **20:**399-407.
40. **Levin A, Rosenbluh J, Hayouka Z, Friedler A, Loyter A.** 2010. Integration of HIV-1 DNA is regulated by interplay between viral rev and cellular LEDGF/p75 proteins. Mol Med **16:**34-44.
41. **Rosenbluh J, Hayouka Z, Loya S, Levin A, Armon-Omer A, Britan E, Hizi A, Kotler M, Friedler A, Loyter A.** 2007. Interaction between HIV-1 Rev and integrase proteins: a basis for the development of anti-HIV peptides. J Biol Chem **282:**15743-15753.
42. **Levin A, Hayouka Z, Brack-Werner R, Volsky DJ, Friedler A, Loyter A.** 2009. Novel regulation of HIV-1 replication and pathogenicity: Rev inhibition of integration. Protein Eng Des Sel **22:**753-763.
43. **Gallay P, Swingler S, Song J, Bushman F, Trono D.** 1995. HIV nuclear import is governed by the phosphotyrosine-mediated binding of matrix to the core domain of integrase. Cell **83:**569-576.
44. **Sato A, Yoshimoto J, Isaka Y, Miki S, Suyama A, Adachi A, Hayami M, Fujiwara T, Yoshie O.** 1996. Evidence for direct association of Vpr and matrix protein p17 within the HIV-1 virion. Virology **220:**208-212.
45. **Sawaya BE, Khalili K, Gordon J, Taube R, Amini S.** 2000. Cooperative interaction between HIV-1 regulatory proteins Tat and Vpr modulates transcription of the viral genome. J Biol Chem **275:**35209-35214.
46. **Lata S, Ali A, Sood V, Raja R, Banerjea AC.** 2015. HIV-1 Rev downregulates Tat expression and viral replication via modulation of NAD(P)H:quinine oxidoreductase 1 (NQO1). Nat Commun **6:**7244.

47. **Joseph AM, Ladha JS, Mojamdar M, Mitra D.** 2003. Human immunodeficiency virus-1 Nef protein interacts with Tat and enhances HIV-1 gene expression. FEBS Lett **548:**37-42.
48. **Hong HW, Lee SW, Myung H.** 2013. Induced degradation of Tat by nucleocapsid (NC) via the proteasome pathway and its effect on HIV transcription. Viruses **5:**1143-1152.
49. **Wang J, Shackelford JM, Selliah N, Shivers DK, O'Neill E, Garcia JV, Muthumani K, Weiner D, Yu XF, Gabuzda D, Finkel TH.** 2008. The HIV-1 Vif protein mediates degradation of Vpr and reduces Vpr-induced cell cycle arrest. DNA Cell Biol **27:**267-277.
50. **Khan MA, Aberham C, Kao S, Akari H, Gorelick R, Bour S, Strebel K.** 2001. Human immunodeficiency virus type 1 Vif protein is packaged into the nucleoprotein complex through an interaction with viral genomic RNA. J Virol **75:**7252-7265.
51. **Huvent I, Hong SS, Fournier C, Gay B, Tournier J, Carriere C, Courcoul M, Vigne R, Spire B, Boulanger P.** 1998. Interaction and co-encapsidation of human immunodeficiency virus type 1 Gag and Vif recombinant proteins. J Gen Virol **79 (Pt 5):**1069-1081.
52. **Syed F, McCrae MA.** 2009. Interactions in vivo between the Vif protein of HIV-1 and the precursor (Pr55(GAG)) of the virion nucleocapsid proteins. Arch Virol **154:**1797-1805.
53. **Bouyac M, Courcoul M, Bertoia G, Baudat Y, Gabuzda D, Blanc D, Chazal N, Boulanger P, Sire J, Vigne R, Spire B.** 1997. Human immunodeficiency virus type 1 Vif protein binds to the Pr55Gag precursor. J Virol **71:**9358-9365.
54. **Li MS, Garcia-Asua G, Bhattacharyya U, Mascagni P, Austen BM, Roberts MM.** 1996. The Vpr protein of human immunodeficiency virus type 1 binds to nucleocapsid protein p7 in vitro. Biochem Biophys Res Commun **218:**352-355.
55. **de Rocquigny H, Petitjean P, Tanchou V, Decimo D, Drouot L, Delaunay T, Darlix JL, Roques BP.** 1997. The zinc fingers of HIV nucleocapsid protein NCp7 direct interactions with the viral regulatory protein Vpr. J Biol Chem **272:**30753-30759.
56. **Selig L, Pages JC, Tanchou V, Preveral S, Berlioz-Torrent C, Liu LX, Erdtmann L, Darlix J, Benarous R, Benichou S.** 1999. Interaction with the p6 domain of the gag precursor mediates incorporation into virions of Vpr and Vpx proteins from primate lentiviruses. J Virol **73:**592-600.
57. **Accola MA, Bukovsky AA, Jones MS, Gottlinger HG.** 1999. A conserved dileucine-containing motif in p6(gag) governs the particle association of Vpx and Vpr of simian immunodeficiency viruses SIV(mac) and SIV(agm). J Virol **73:**9992-9999.
58. **Accola MA, Ohagen A, Gottlinger HG.** 2000. Isolation of human immunodeficiency virus type 1 cores: retention of Vpr in the absence of p6(gag). J Virol **74:**6198-6202.
59. **Bachand F, Yao XJ, Hrimech M, Rougeau N, Cohen EA.** 1999. Incorporation of Vpr into human immunodeficiency virus type 1 requires a

direct interaction with the p6 domain of the p55 gag precursor. J Biol Chem **274:**9083-9091.

60. **Paxton W, Connor RI, Landau NR.** 1993. Incorporation of Vpr into human immunodeficiency virus type 1 virions: requirement for the p6 region of gag and mutational analysis. J Virol **67:**7229-7237.
61. **Kondo E, Mammano F, Cohen EA, Gottlinger HG.** 1995. The p6gag domain of human immunodeficiency virus type 1 is sufficient for the incorporation of Vpr into heterologous viral particles. J Virol **69:**2759-2764.
62. **Kondo E, Gottlinger HG.** 1996. A conserved LXXLF sequence is the major determinant in p6gag required for the incorporation of human immunodeficiency virus type 1 Vpr. J Virol **70:**159-164.
63. **Salgado GF, Marquant R, Vogel A, Alves ID, Feller SE, Morellet N, Bouaziz S.** 2009. Structural studies of HIV-1 Gag p6ct and its interaction with Vpr determined by solution nuclear magnetic resonance. Biochemistry **48:**2355-2367.
64. **Jenkins Y, Pornillos O, Rich RL, Myszka DG, Sundquist WI, Malim MH.** 2001. Biochemical analyses of the interactions between human immunodeficiency virus type 1 Vpr and p6(Gag). J Virol **75:**10537-10542.
65. **Salgado GF, Vogel A, Marquant R, Feller SE, Bouaziz S, Alves ID.** 2009. The role of membranes in the organization of HIV-1 Gag p6 and Vpr: p6 shows high affinity for membrane bilayers which substantially increases the interaction between p6 and Vpr. J Med Chem **52:**7157-7162.
66. **Zhu H, Jian H, Zhao LJ.** 2004. Identification of the 15FRFG domain in HIV-1 Gag p6 essential for Vpr packaging into the virion. Retrovirology **1:**26.
67. **Lu YL, Bennett RP, Wills JW, Gorelick R, Ratner L.** 1995. A leucine triplet repeat sequence (LXX)4 in p6gag is important for Vpr incorporation into human immunodeficiency virus type 1 particles. J Virol **69:**6873-6879.
68. **Jin L, Zhou Y, Ratner L.** 2001. HIV type 2 Vpx interaction with Gag and incorporation into virus-like particles. AIDS Res Hum Retroviruses **17:**105-111.
69. **Pancio HA, Ratner L.** 1998. Human immunodeficiency virus type 2 Vpx-Gag interaction. J Virol **72:**5271-5275.
70. **Schiavoni I, Trapp S, Santarcangelo AC, Piacentini V, Pugliese K, Baur A, Federico M.** 2004. HIV-1 Nef enhances both membrane expression and virion incorporation of Env products. A model for the Nef-dependent increase of HIV-1 infectivity. J Biol Chem **279:**22996-23006.
71. **Liao WH, Huang KJ, Chang YF, Wang SM, Tseng YT, Chiang CC, Wang JJ, Wang CT.** 2007. Incorporation of human immunodeficiency virus type 1 reverse transcriptase into virus-like particles. J Virol **81:**5155-5165.
72. **Saadatmand J, Guo F, Cen S, Niu M, Kleiman L.** 2008. Interactions of reverse transcriptase sequences in Pol with Gag and LysRS in the HIV-1 tRNALys3 packaging/annealing complex. Virology **380:**109-117.
73. **Prabu-Jeyabalan M, Nalivaika E, Schiffer CA.** 2002. Substrate shape determines specificity of recognition for HIV-1 protease: analysis of crystal structures of six substrate complexes. Structure **10:**369-381.

74. **Alvizo O, Mittal S, Mayo SL, Schiffer CA.** 2012. Structural, kinetic, and thermodynamic studies of specificity designed HIV-1 protease. Protein Sci **21:**1029-1041.
75. **Ozen A, Lin KH, Kurt Yilmaz N, Schiffer CA.** 2014. Structural basis and distal effects of Gag substrate coevolution in drug resistance to HIV-1 protease. Proc Natl Acad Sci U S A **111:**15993-15998.
76. **Baraz L, Hutoran M, Blumenzweig I, Katzenellenbogen M, Friedler A, Gilon C, Steinitz M, Kotler M.** 2002. Human immunodeficiency virus type 1 Vif binds the viral protease by interaction with its N-terminal region. J Gen Virol **83:**2225-2230.
77. **Khan MA, Akari H, Kao S, Aberham C, Davis D, Buckler-White A, Strebel K.** 2002. Intravirion processing of the human immunodeficiency virus type 1 Vif protein by the viral protease may be correlated with Vif function. J Virol **76:**9112-9123.
78. **Hutoran M, Britan E, Baraz L, Blumenzweig I, Steinitz M, Kotler M.** 2004. Abrogation of Vif function by peptide derived from the N-terminal region of the human immunodeficiency virus type 1 (HIV-1) protease. Virology **330:**261-270.
79. **Bottcher M, Grosse F.** 1997. HIV-1 protease inhibits its homologous reverse transcriptase by protein-protein interaction. Nucleic Acids Res **25:**1709-1714.
80. **Apolloni A, Hooker CW, Mak J, Harrich D.** 2003. Human immunodeficiency virus type 1 protease regulation of tat activity is essential for efficient reverse transcription and replication. J Virol **77:**9912-9921.
81. **Gaedigk-Nitschko K, Schon A, Wachinger G, Erfle V, Kohleisen B.** 1995. Cleavage of recombinant and cell derived human immunodeficiency virus 1 (HIV-1) Nef protein by HIV-1 protease. FEBS Lett **357:**275-278.
82. **Miller MD, Warmerdam MT, Ferrell SS, Benitez R, Greene WC.** 1997. Intravirion generation of the C-terminal core domain of HIV-1 Nef by the HIV-1 protease is insufficient to enhance viral infectivity. Virology **234:**215-225.
83. **Chen YL, Trono D, Camaur D.** 1998. The proteolytic cleavage of human immunodeficiency virus type 1 Nef does not correlate with its ability to stimulate virion infectivity. J Virol **72:**3178-3184.
84. **Bukovsky AA, Dorfman T, Weimann A, Gottlinger HG.** 1997. Nef association with human immunodeficiency virus type 1 virions and cleavage by the viral protease. J Virol **71:**1013-1018.
85. **Schorr J, Kellner R, Fackler O, Freund J, Konvalinka J, Kienzle N, Krausslich HG, Mueller-Lantzsch N, Kalbitzer HR.** 1996. Specific cleavage sites of Nef proteins from human immunodeficiency virus types 1 and 2 for the viral proteases. J Virol **70:**9051-9054.
86. **Waheed AA, Ablan SD, Roser JD, Sowder RC, Schaffner CP, Chertova E, Freed EO.** 2007. HIV-1 escape from the entry-inhibiting effects of a cholesterol-binding compound via cleavage of gp41 by the viral protease. Proc Natl Acad Sci U S A **104:**8467-8471.

87. **Waheed AA, Ablan SD, Sowder RC, Roser JD, Schaffner CP, Chertova E, Freed EO.** 2010. Effect of mutations in the human immunodeficiency virus type 1 protease on cleavage of the gp41 cytoplasmic tail. J Virol **84:**3121-3126.
88. **Bell NM, Lever AM.** 2013. HIV Gag polyprotein: processing and early viral particle assembly. Trends Microbiol **21:**136-144.
89. **Waheed AA, Freed EO.** 2012. HIV type 1 Gag as a target for antiviral therapy. AIDS Res Hum Retroviruses **28:**54-75.
90. **Zhao G, Perilla JR, Yufenyuy EL, Meng X, Chen B, Ning J, Ahn J, Gronenborn AM, Schulten K, Aiken C, Zhang P.** 2013. Mature HIV-1 capsid structure by cryo-electron microscopy and all-atom molecular dynamics. Nature **497:**643-646.
91. **Schaller T, Ocwieja KE, Rasaiyaah J, Price AJ, Brady TL, Roth SL, Hue S, Fletcher AJ, Lee K, KewalRamani VN, Noursadeghi M, Jenner RG, James LC, Bushman FD, Towers GJ.** 2011. HIV-1 capsid-cyclophilin interactions determine nuclear import pathway, integration targeting and replication efficiency. PLoS Pathog **7:**e1002439.
92. **Luban J.** 2012. TRIM5 and the Regulation of HIV-1 Infectivity. Mol Biol Int **2012:**426840.
93. **Campbell EM, Hope TJ.** 2015. HIV-1 capsid: the multifaceted key player in HIV-1 infection. Nat Rev Microbiol **13:**471-483.
94. **Mougel M, Houzet L, Darlix JL.** 2009. When is it time for reverse transcription to start and go? Retrovirology **6:**24.
95. **Darlix JL, Godet J, Ivanyi-Nagy R, Fosse P, Mauffret O, Mely Y.** 2011. Flexible nature and specific functions of the HIV-1 nucleocapsid protein. J Mol Biol **410:**565-581.
96. **Thomas JA, Gorelick RJ.** 2008. Nucleocapsid protein function in early infection processes. Virus Res **134:**39-63.
97. **Didierlaurent L, Racine PJ, Houzet L, Chamontin C, Berkhout B, Mougel M.** 2011. Role of HIV-1 RNA and protein determinants for the selective packaging of spliced and unspliced viral RNA and host U6 and 7SL RNA in virus particles. Nucleic Acids Res **39:**8915-8927.
98. **Sette P, Dussupt V, Bouamr F.** 2012. Identification of the HIV-1 NC binding interface in Alix Bro1 reveals a role for RNA. J Virol **86:**11608-11615.
99. **Xue B, Mizianty MJ, Kurgan L, Uversky VN.** 2012. Protein intrinsic disorder as a flexible armor and a weapon of HIV-1. Cell Mol Life Sci **69:**1211-1259.
100. **Morita E, Sundquist WI.** 2004. Retrovirus budding. Annu Rev Cell Dev Biol **20:**395-425.
101. **Zybarth G, Carter C.** 1995. Domains upstream of the protease (PR) in human immunodeficiency virus type 1 Gag-Pol influence PR autoprocessing. J Virol **69:**3878-3884.
102. **Henriet S, Mercenne G, Bernacchi S, Paillart JC, Marquet R.** 2009. Tumultuous relationship between the human immunodeficiency virus type 1 viral infectivity factor (Vif) and the human APOBEC-3G and APOBEC-3F restriction factors. Microbiol Mol Biol Rev **73:**211-232.

103. **Jager S, Kim DY, Hultquist JF, Shindo K, LaRue RS, Kwon E, Li M, Anderson BD, Yen L, Stanley D, Mahon C, Kane J, Franks-Skiba K, Cimermancic P, Burlingame A, Sali A, Craik CS, Harris RS, Gross JD, Krogan NJ.** 2012. Vif hijacks CBF-beta to degrade APOBEC3G and promote HIV-1 infection. Nature **481:**371-375.
104. **Harris RS, Liddament MT.** 2004. Retroviral restriction by APOBEC proteins. Nat Rev Immunol **4:**868-877.
105. **Henderson S, Fenton T.** 2015. APOBEC3 genes: retroviral restriction factors to cancer drivers. Trends Mol Med **21:**274-284.
106. **Simon V, Bloch N, Landau NR.** 2015. Intrinsic host restrictions to HIV-1 and mechanisms of viral escape. Nat Immunol **16:**546-553.
107. **Holmes RK, Malim MH, Bishop KN.** 2007. APOBEC-mediated viral restriction: not simply editing? Trends Biochem Sci **32:**118-128.
108. **Zhao RY, Li G, Bukrinsky MI.** 2011. Vpr-host interactions during HIV-1 viral life cycle. J Neuroimmune Pharmacol **6:**216-229.
109. **Cohen EA, Dehni G, Sodroski JG, Haseltine WA.** 1990. Human immunodeficiency virus vpr product is a virion-associated regulatory protein. J Virol **64:**3097-3099.
110. **Dube M, Bego MG, Paquay C, Cohen EA.** 2010. Modulation of HIV-1-host interaction: role of the Vpu accessory protein. Retrovirology **7:**114.
111. **Giroud C, Chazal N, Briant L.** 2011. Cellular kinases incorporated into HIV-1 particles: passive or active passengers? Retrovirology **8:**71.
112. **Zhu C, Gao W, Zhao K, Qin X, Zhang Y, Peng X, Zhang L, Dong Y, Zhang W, Li P, Wei W, Gong Y, Yu XF.** 2013. Structural insight into dGTP-dependent activation of tetrameric SAMHD1 deoxynucleoside triphosphate triphosphohydrolase. Nat Commun **4:**2722.
113. **Fujita M, Otsuka M, Nomaguchi M, Adachi A.** 2010. Multifaceted activity of HIV Vpr/Vpx proteins: the current view of their virological functions. Rev Med Virol **20:**68-76.
114. **Ayinde D, Maudet C, Transy C, Margottin-Goguet F.** 2010. Limelight on two HIV/SIV accessory proteins in macrophage infection: is Vpx overshadowing Vpr? Retrovirology **7:**35.
115. **Fujita M, Otsuka M, Miyoshi M, Khamsri B, Nomaguchi M, Adachi A.** 2008. Vpx is critical for reverse transcription of the human immunodeficiency virus type 2 genome in macrophages. J Virol **82:**7752-7756.
116. **Fernandes J, Jayaraman B, Frankel A.** 2012. The HIV-1 Rev response element: an RNA scaffold that directs the cooperative assembly of a homo-oligomeric ribonucleoprotein complex. RNA Biol **9:**6-11.
117. **Jeang K-T.** 2012. Multi-Faceted Post-Transcriptional Functions of HIV-1 Rev. Biology **1:**165-174.
118. **Strebel K.** 2003. Virus-host interactions: role of HIV proteins Vif, Tat, and Rev. AIDS **17 Suppl 4:**S25-34.
119. **Brady J, Kashanchi F.** 2005. Tat gets the "green" light on transcription initiation. Retrovirology **2:**69.

120. **Engelman A, Cherepanov P.** 2012. The structural biology of HIV-1: mechanistic and therapeutic insights. Nat Rev Microbiol **10:**279-290.
121. **Zhu P, Liu J, Bess J, Jr., Chertova E, Lifson JD, Grise H, Ofek GA, Taylor KA, Roux KH.** 2006. Distribution and three-dimensional structure of AIDS virus envelope spikes. Nature **441:**847-852.
122. **Caffrey M.** 2011. HIV envelope: challenges and opportunities for development of entry inhibitors. Trends Microbiol **19:**191-197.
123. **Walker LM, Huber M, Doores KJ, Falkowska E, Pejchal R, Julien JP, Wang SK, Ramos A, Chan-Hui PY, Moyle M, Mitcham JL, Hammond PW, Olsen OA, Phung P, Fling S, Wong CH, Phogat S, Wrin T, Simek MD, Protocol GPI, Koff WC, Wilson IA, Burton DR, Poignard P.** 2011. Broad neutralization coverage of HIV by multiple highly potent antibodies. Nature **477:**466-470.
124. **Wu X, Yang ZY, Li Y, Hogerkorp CM, Schief WR, Seaman MS, Zhou T, Schmidt SD, Wu L, Xu L, Longo NS, McKee K, O'Dell S, Louder MK, Wycuff DL, Feng Y, Nason M, Doria-Rose N, Connors M, Kwong PD, Roederer M, Wyatt RT, Nabel GJ, Mascola JR.** 2010. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science **329:**856-861.
125. **Walker LM, Phogat SK, Chan-Hui PY, Wagner D, Phung P, Goss JL, Wrin T, Simek MD, Fling S, Mitcham JL, Lehrman JK, Priddy FH, Olsen OA, Frey SM, Hammond PW, Protocol GPI, Kaminsky S, Zamb T, Moyle M, Koff WC, Poignard P, Burton DR.** 2009. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science **326:**285-289.
126. **Postler TS, Desrosiers RC.** 2013. The tale of the long tail: the cytoplasmic domain of HIV-1 gp41. J Virol **87:**2-15.
127. **Huang J, Ofek G, Laub L, Louder MK, Doria-Rose NA, Longo NS, Imamichi H, Bailer RT, Chakrabarti B, Sharma SK, Alam SM, Wang T, Yang Y, Zhang B, Migueles SA, Wyatt R, Haynes BF, Kwong PD, Mascola JR, Connors M.** 2012. Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. Nature **491:**406-412.
128. **Laguette N, Bregnard C, Benichou S, Basmaciogullari S.** 2010. Human immunodeficiency virus (HIV) type-1, HIV-2 and simian immunodeficiency virus Nef proteins. Mol Aspects Med **31:**418-433.