Virus-producing cells determine the host protein profiles of HIV-1 virion cores

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Abstract

Background: Upon HIV entry into target cells, viral cores are released and rearranged into reverse transcription complexes (RTCs), which support reverse transcription and also protect and transport viral cDNA to the site of integration. RTCs are composed of viral and cellular proteins that originate from both target and producer cells, the latter entering the target cell within the viral core. However, the proteome of HIV-1 viral cores in the context of the type of producer cells has not yet been characterized.

Results: We examined the proteomic profiles of the cores purified from HIV-1 NL4-3 virions assembled in Sup-T1 cells (T lymphocytes), PMA and vitamin D3 activated THP1 (model of macrophages, mMΦ), and non-activated THP1 cells (model of monocytes, mMN) and assessed potential involvement of identified proteins in the early stages of infection using gene ontology information and data from genome-wide screens on proteins important for HIV-1 replication. We identified 202 cellular proteins incorporated in the viral cores (T cells: 125, mMΦ: 110, mMN: 90) with the overlap between these sets limited to 42 proteins. The groups of RNA binding (29), DNA binding (17), cytoskeleton (15), cytoskeleton regulation (21), chaperone (18), vesicular trafficking-associated (12) and ubiquitin-proteasome pathway-associated proteins (9) were most numerous. Cores of the virions from SupT1 cells contained twice as many RNA binding proteins as cores of THP1-derived virus, whereas cores of virions from mMΦ and mMN were enriched in components of cytoskeleton and vesicular transport machinery, most probably due to differences in virion assembly pathways between these cells. Spectra of chaperones, cytoskeletal proteins and ubiquitin-proteasome pathway components were similar between viral cores from different cell types, whereas DNA-binding and especially RNA-binding proteins were highly diverse. Western blot analysis showed that within the group of overlapping proteins, the level of incorporation of some RNA binding (RHA and HELIC2) and DNA binding proteins (MCM5 and Ku80) in the viral cores from T cells was higher than in the cores from both mMΦ and mMN and did not correlate with the abundance of these proteins in virus producing cells.

Conclusions: Profiles of host proteins packaged in the cores of HIV-1 virions depend on the type of virus producing cell. The pool of proteins present in the cores of all virions is likely to contain factors important for viral functions. Incorporation ratio of certain RNA- and DNA-binding proteins suggests their more efficient, non-random packaging into virions in T cells than in mMΦ and mMN.

Background

HIV-1 viral particles released from infected cells have been shown to incorporate many cellular proteins during the assembly and budding steps of morphogenesis. Findings from earlier studies, summarized in a web-based database (http://web.ncifcrf.gov/research/avp/protein_db), identified more than three hundred cellular proteins in HIV-1 particles. HIV-1, as well as other lentiviruses, incorporates components of the cellular endosomal sorting machinery and cytoskeleton proteins involved in the process of particle assembly [1-5], surface proteins captured with the plasma membrane during budding [6,7], RNA-binding proteins associated with incorporated viral RNA and RNA-Gag complexes [4,8-10], chaperones [11], and multiple concomitant proteins (reviewed in [12]) whose functions in viral morphogenesis and infectivity are still unknown.
Upon fusion of an HIV-1 particle with a target cell, viral cores are released into the cytoplasm and rearranged into sub-viral particles called reverse transcription complexes (RTCs), which subsequently mature into pre-integration complexes (PICs). These nucleoprotein structures support reverse transcription and also protect and transport viral cDNA to the site of integration. RTCs are composed of both viral and cellular proteins. Since RTCs are formed from the viral cores, their initial composition is identical to that of viral cores. Other than the key enzymatic components, reverse transcriptase (RT) and integrase (IN), at least five other viral proteins involved in structural organization, cytoplasmic trafficking and nuclear import (matrix [MA], nucleocapsid [NC], capsid [CA], Nef and viral protein R [Vpr]), have been identified as components of HIV-1 RTCs [13-19], reviewed in [20-22].

Although the early steps of HIV replication, reverse transcription and integration, are relatively autonomous, the participation of cellular proteins in early infection events has been demonstrated in previous studies [23-25]. After release from the viral particles, RTCs are still encapsulated in the shells formed by p24CA molecules which are stable in the cytoplasm for at least several hours [26,27]. The shell is believed to protect the reverse transcription machinery and all encapsulated proteins from the cytoplasmic environment to provide optimal conditions for their functional activity [28] and may contribute to the nuclear import of PICs [19,29-31]. The shell likely limits the access of host cell proteins to the RTC interior. Thus, most cellular factors which may contribute to the functional competence of early RTCs should be expected to get into the complexes from the cores of infecting virions.

The cellular proteins, which are known to be hijacked by assembling virus particles from virus-producing cells and are involved in the early post-entry stages of HIV-1 infection, can be grouped into the following categories. (1) Factors involved in the spatial organization and correct folding of viral proteins in the virion and probably RTC: clathrin [5,32] and heat shock proteins (Hsp70, Hsc70, Hsp60) [11,33] are probably critical for structural organization of Gag and Pol proteins and regulation of proteolytic processing and folding of the Pol products – RT and integrase; thioltransferase is found in HIV-1 virions and may be important for dimerization and activation of the viral protease [34], (reviewed in [12]); staufen1, an RNA-binding protein is packaged into virions and is involved in incorporation of HIV-1 RNA [8]. Interaction of staufen1 with Pr55Gag zing finger motifs may also be important for Gag multimerization and formation of the viral capsids [35]. (2) Proteins which have an effect on cDNA synthesis/accumulation: lysyl-tRNA synthetase is incorporated through the interaction with Gag and is critical for the priming of reverse transcription [36]; uracil DNA glycosylase 2 (UNG2), a cellular DNA repair enzyme that binds HIV-1 integrase and Vpr [37,38]. The role of this enzyme in early post-entry steps of infection remains controversial. The hypothesis that the catalytic activity of Vpr-associated UNG could modulate virus mutation rate and APOBEC3G-mediated G-to-A hypermutations [39-41] was not supported by subsequent studies [42]. However, recently published work of Guenzel and co-authors showed that the virion-incorporated nuclear form of UNG2 facilitated reverse transcription through a non-enzymatic mechanism involving direct interaction with the p32 subunit of the replication protein A (RPA) complex [43]. RNA helicase A (RHA or DHX9) is packaged into HIV-1 virions probably through the interaction with an RNA or Gag polypeptide and facilitates reverse transcription [10]. The protein INI1/hSNF5, a member of SWI/SNF chromatin remodeling complex, has been shown to be packaged into virions through the direct binding to integrase [44], and is involved in the synthesis of reverse transcription products [45]. Later studies demonstrated that INI1/hSNF5 selectively recruits into HIV-1 virions the components of Sin3a-HDAC1 cellular complex, whose presence is critical for the early reverse transcription stage [46]. Furthermore, interaction of HIV-1 integrase with INI1 has been shown to be essential for the nucleosome remodeling of host chromatin and hence overcoming the structural nucleosome barrier for viral integration [47]. (3) Proteins involved in RTC formation, protection and transport: cyclophilin A, which is incorporated into virions via binding to the CA domain of Pr55Gag [23]. The role of CA-bound cyclophilin A in the viral life cycle is still unclear [48] Recently published data showed this protein to be critical for protection and stabilization of HIV-1 cores [49]. It may also be involved in PIC nuclear transport [31]. (4) Restriction factors of the early stages of HIV-1 infection: members of the APOBEC3 family of DNA/RNA editing cytidine deaminases, APOBEC3G (A3G) and APOBEC3F (A3F), are incorporated in Vif-negative virus particles (a small amount of these factors may be present also in Vif-positive virions) and then restrict reverse transcription by carrying out hypermutation of newly synthesized HIV-1 DNA [41,50-52]. Numerous studies have shown that A3G molecules from the target cell have no effect on cDNA deamination, and only virion-incorporated A3G affects viral DNA (reviewed in [53]), suggesting that the cDNA synthesis and accumulation machineries are effectively isolated from the environment of the target cell cytoplasm, but can be affected by factors which are encapsulated in cores and found within RTCs. Initiation of uncoating or disintegration of the capsid shell is believed to be dependent on the completion of reverse transcription
acetate (PMA) and vitamin D3. This treatment activates and THP1 cells treated with phorbol 12-myristate 13-acetate (PMA) and vitamin D3. Thus, the activated THP1 cells may be considered as a model of macrophages [61,62].

To isolate core structures from HIV-1 virions produced by infected cells, we engaged a technique of “spin-thru” equilibrium density gradient sedimentation described earlier [63-66]. This method of ultra-speed centrifugation of previously concentrated HIV-1 virions through a sucrose density gradient overlaid with a detergent layer (1% Triton X-100) allows for the purification of mature lentiviral cores whose density varies from 1.23 to 1.27 g/ml [67,68] (Figure 1A, lower panel), whereas intact viral particles display buoyant density 1.18-1.20 mg/ml (Figure 1A, upper panel). To establish the purity of our viral core preparations from cellular vesicles, which have density similar to that of virions (1.14-1.20 g/ml) and may contaminate virus preparations [69], and to compare maturation of capsid cores in the viruses produced by T lymphocytes and MDM model cells, we engaged electron microscopy and Western blot analysis. Examination of the negatively stained concentrated virion samples in a transmission electron microscope revealed the presence of both extracellular vesicles and viral particles with diameter from 120 to 130 nm (Figure 1B1-B3). Analysis of ultrathin sections of the viral particles used for core isolation showed that the population represented a mix of immature (Figure 1B4, black double arrows) and mature virions (Figure 1B4, single arrow). The lipid membrane-covered structures were not found in purified viral core preparations. Only the conical-shape and oval structures with length about 80–110 nm were found in the samples of purified viral cores, indicating that the preparations after “spin-thru” purification contained only mature capsid cores (Figure 1B5-B8). Previous studies of cores from HIV-1 [64] and HIV-2 virions [63] isolated by “spin-thru” centrifugation method showed the presence of mature products of Gag and GagPol proteolytic processing (CA, MA, Vpx [for HIV-2] and RT with high enzymatic activity). Immunoblotting of our core preparations obtained after “spin-thru” purification of the same amounts of Sup-T1- and THP1-derived viral particles, carried out with human IgG prepared from pooled plasma of HIV antibody positive donors, revealed similar amounts of major products of Gag and GagPol processing, such as MA, CA, IN and RT proteins (Figure 1C). The Pr55Gag and Pr41Gag (MA + CA) have also been identified in the core samples from both viruses, suggesting that mature cores may contain some amounts of unprocessed Gag polypeptide. Taken together, our data indicate that the selected method of purification allows for the isolation of mostly mature cores from the pools of virions produced by both T lymphocytes and MDM model cells.

We also performed Western blotting of our samples using the anti-CD45 antibody (Figure 2A). CD45 is
known to be abundant in microvesicles, but is apparently excluded from HIV-1 virions [70]; the lack of this protein in our core preparations would confirm their purity from the vesicular fraction. Indeed, we did not detect CD45 in the samples of cores from HIV-1 virions produced by both Sup-T1 and THP1 cells (Figure 2A, two right lanes), whereas the specimens of culture media from these cells concentrated only through a 30% sucrose cushion contained detectable amounts of CD45. Western blotting of the samples of culture media from untransfected and NL4-3 proviral clone-transfected 293 T/17 cells using anti-RNA helicase A (RHA or DHX9) antibody showed presence of this DEAD box RNA helicase in the preparations of media from both transfected and untransfected cells after purification through 30% sucrose. However, in the “spin-thru”
purified samples, RHA was detected only in the preparations of media containing HIV-1 (Figure 2B). Since RHA is known to be present in both vesicles [71] and HIV-1 virions [10,72,73], our analysis confirmed that the method of “spin-thru” centrifugation removed extracellular membranous structures from the 30% sucrose-concentrated cell culture supernatants, but retained intravirion core structures.

To further prove that “spin-thru” centrifugation purifies cores from intact virions, we tested the presence of VSV-G envelope protein in the samples of VSV-G-pseudotyped HIV-1 produced by co-transfected 293 T/17 cells after concentration through 30% sucrose cushion and “spin-thru” centrifugation (Figure 2C). The VSV-G was clearly detected in the samples of concentrated pseudotyped virus, but was not found in the core samples after “spin-thru” purification, confirming purity of the core preparations from the envelope glycoproteins.

The SDS-PAGE separation of our core preparations (Figure 2D) revealed major bands corresponding to proteins with molecular weights of 24 and 31 kDa (corresponding to HIV-1 CA and IN, respectively), indicating the presence of mature viral Gag and GagPol products in the analyzed core structures. On the other hand, multiple bands corresponding to the polypeptides of different molecular weights, which do not represent known HIV-1 proteins, suggest incorporation of many cellular proteins in the core structures of the viral particles produced by different cell types. The data of proteomic analysis (shown below) confirmed this suggestion. The staining of SDS-PAGE with Coomassie also revealed
multiple protein bands in the control preparations, suggesting that the culture media from uninfected THP1 and especially Sup-T1 cells contained protein-rich, non-viral, non-membranous particles with buoyant density ≥1.23 mg/ml, probably the products of disintegrated dead cells (Figure 2D). Thus, to obtain proteomic profiles of the host proteins associated with HIV-1 viral cores, both viral cores and uninfected control preparations from each cell type were subjected to SDS-PAGE protein separation, trypsin digestion and subsequent LC-MS/MS analysis. The protein profile of each viral core sample was then compared with the corresponding control sample. Overlapping proteins were eliminated from the protein spectra of the viral cores, except the proteins whose scores were >5-fold higher in the preparations of viral cores than in control samples (proteins such as chaperones Hsp70 and Hsp90, and cytoskeletal proteins β actin, α and β tubulin, whose presence in core samples was confirmed by Western blot [Figure 4A]). As a result, a total of 202 cellular proteins were found to be associated with the cores of HIV-1 virions.

Proteomic profiling of HIV-1 viral cores

The proteins obtained from LC-MS/MS analysis of peptide preparations and filtered as described above and in Materials and Methods were categorized according to their functions and subcellular localization using NCBI protein database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=Protein), NCBI RefSeq database (www.ncbi.nlm.nih.gov/RefSeq/) and DAVID Bioinformatics Resources 6.7 (NIAID NIH) (http://david.abcc.ncifcrf.gov). We compared our data with published results of the genome-wide analyses of cellular proteins involved in HIV-1 infection [57–59,74] and with global network of HIV-human protein–protein interactions [75] to assess the potential role of identified proteins in HIV-1 infection and putative mechanisms of their incorporation into the virion. The HIV-1 proteins identified in the viral cores are summarized in Table 1, the cellular proteins – in Tables 2 and S1 in Additional file 1.

Viral proteins

The major proteins constituting the HIV-1 nucleocapsid core, CA, NC, IN and RT (both p51 and p66 subunits) were identified in the preparations from virions generated by all cell types (Table 1). All core samples also contained MA protein, which is located in retroviral virions mostly outside the capsid and forms a matrix between the viral capsid and envelope [76,77]. However, presence of MA in the RTCs and PICs [13,14,17,78,79] suggests that this protein is physically associated with the cores of HIV-1 virions. Comparison of our list of identified viral proteins with the MS/MS analysis data of whole MDM-produced HIV-1 particles [4] shows characteristic differences between the protein profiles of viral cores and whole virions. The gp120Env glycoprotein detected in whole virions is absent in our samples; however, the gp41 transmembrane (TM) Env product was identified in the cores from virions assembled in T lymphocytes and MDM-like activated THP1 cells. Since gp41 has been found to be associated with MA during virion assembly and probably in mature viral particles [80,81] (reviewed in [82]), a low amount of this glycoprotein in detergent-purified core preparations was expected. The fact that we could not identify gp41 in the cores of virions from non-activated THP1 cells by LC-MS/MS confirms our suggestion that the concentration of this glycoprotein in our preparations is negligible. The absence of gp120Env in our samples confirms purity of isolated core structures from the viral envelope and intact virions, shown also in Figure 2C.

The Tat, Nef and Vif proteins were identified earlier in the viral particles produced by infected MDM using proteomic methods [4]. We did not detect Tat in our core preparations, but MS/MS analysis revealed Nef in the viral cores from all analyzed cell types. Earlier studies indicated that Nef is incorporated into viral particles, stably associates with virion cores and facilitates reverse transcription and early steps of replication [16,64,83,84]. The Vif and Rev proteins were identified with low scores only in the cores from T lymphocyte-derived virus, suggesting their very low concentrations in the cores. HIV-1 Vif has been shown to interact with Pr55Gag and viral protease during the assembly of virus particles [85]. Rev protein can be incorporated into cores in the complex with viral RNA. Surprisingly, we did not identify Vpr in the viral core samples. Numerous studies revealed Vpr in HIV-1 virions [86–88] and RTCs [15,89–91]. However, while traditional methods of identification using specific antibodies recognize Vpr in HIV-1 particles, LC-MS/MS analysis performed by Chertova and co-authors [4] also did not reveal this protein in the preparations of whole highly-purified virions. The visibility of protein for LC-MS is defined by a few factors, including affinity to C18 column (hydrophobicity) and ionization efficiency of the peptides. Typically, about 10% of tryptic peptides of a long protein are visible. Thus, some proteins could be invisible for LC-MS/MS in spite of overall high sensitivity of a method. Moreover, although trypsin cleaves Vpr to 14 peptides, most of them are not charged or have a negative total charge in solution with neutral pH (analyzed using Innovagen Peptide property calculator tool [http://www.innovagen.se/]), which may cause additional problem with their detection by mass spectrometry.

HIV-1 protease (PR) was observed in all our core preparations. At the same time, detection of Gag and Gag-Pol polyprotein-precursors in respective gel fractions suggests that not all precursor molecules are subjected
Cores of virions assembled in:
- Sup-T1 (125)
- Thp1 (90)
- PMA-activated Thp1 (110)

Cores of virions assembled in:
- Sup-T1 infected-NL4-3 Env(MLV)
- Sup-T1 infected-NL4-3 VSV-G

Protein categories (for C1-C4)
- DNA binding: chromatin organization, damage repair
- RNA binding: organization, modif., splicing, transport
- Transcription, transcription regulation
- Chaperones
- Ubiquitination, proteasome
- Cytoskeleton
- Regulation of cytoskeleton
- Vesicular transport
- Nuclear import
- Nuclear export
- Regulation of cell cycle and differentiation
- Signal transduction (incl. apoptosis pathways)
- Metabolism, metabolism regulation
- Ribosome
- Intracellular transport
- Nuclear structure, lamina, nucleoli
- Extracellular signaling
- Translation and translation regulation
- Protein post-translation modification
- Cell adhesion

Figure 3 (See legend on next page.)
to the proteolytic cleavage during the virion assembly and a subset of unprocessed polyproteins is present in the mature viral cores.

**Cellular proteins**

Within 202 unique cellular proteins revealed in our preparations of purified cores from HIV-1 virions, the samples from Sup-T1-derived virus included 125 proteins, while the virion cores from activated and non-activated THP1 cells contained 110 and 90 proteins, respectively (Table 2). A similar number of common proteins was found between cores from viruses produced by activated and non-activated THP1 cells (51), non-activated THP1 and Sup-T1 cells (63), and activated THP1 and Sup-T1 (52) (Figure 3A). Forty two proteins were common to all viral cores, which equates to 34%, 38% and 47% of the proteins found in the cores of viruses derived from Sup-T1, activated and non-activated THP1 cells, respectively.

We identified 125 host cell proteins in the cores of virions assembled in Sup-T1 cells; cores of the viruses from activated THP1 cells contained 12% less host proteins, whereas the number of host proteins in the cores of non-activated THP1-derived virus was 28% less than in the cores of virus from Sup-T1. Interestingly, only 20% of all cellular proteins identified in the cores of virus from non-activated monocytic cells were unique, other proteins were also found in the cores of the virions derived from Sup-T1 or activated THP1 cells. The cores of activated THP1 and Sup-T1-derived viruses, in contrast, contained 45% and 42% of unique host proteins, respectively (Figure 3A). Since the T lymphocytes and MDM (activated THP1 is a model of MDM) naturally

| Protein | Contained in samples from: |
|---------|-----------------------------|
| Pr 160GagPol | 
| Pol TF | 
| Pol TF (PR, RT domains) | 
| RT p66 subunit | 
| Pr55Gag | 
| RT p51 subunit | 
| gp41TM Env | 
| Gag 41 kDa | 
| IN p31Pol | 
| CA p24Gag | 
| Nef | 
| Vif | 
| MA p17Gag | 
| RNase H 15 kDa Pol polypeptide | 
| 15 kDa partially-processed Gag precursor (NC, sp1, p6) | 
| Rev | 
| PR p10Pol | 
| NC p7Gag | 

*Proteins are indicated as being within the core from a specific producer cell type by a check symbol (✓).*
### Table 2 Cellular Proteins in HIV-1 cores

| Location | Gene Name | Accession Number | Protein Name | Contained in cores produced by: |
|----------|-----------|-----------------|--------------|---------------------------------|
|          |           |                 |              | SupT1 | Act. | N-Act. |
| N,C      | RUVBL1    | 4506753         | TATA binding protein interacting protein 49 kDa | ✓ ✓ ✓ |
| M,C,N    | MCM5      | 1232079         | Minichromosome maintenance complex component 5 | ✓ ✓ ✓ |
| N        | RCC2      | 11360295        | Regulator of chromosome condensation protein 2 | ✓ ✓ |
| N,C      | RUVBL2    | 5730023         | TBP-interacting protein, 48-KD | ✓ ✓ |
| N        | TOP2B     | 288565          | DNA topoisomerase II | ✓ |
| N,C      | SSRP1     | 4507241         | Structure specific recognition protein 1 | ✓ |
| N        | H2AFY     | 32492946        | H2A histone family, member Y | ✓ |
| N,C      | ERVK-6    | 3600071         | Reverse transcriptase encoded by human endogenous HERV-K retrovirus | ✓ |
| N        | MCM7      | 2134885         | Replication licensing factor MCM7 | ✓ |
| N,M      | TEP1      | 1848277         | telomerase-associated protein 1; TP-1 | ✓ |
| N        | H2AFY     | 32492946        | H2A histone family, member Y | ✓ |
| N,C      | XRCC5     | 35038           | Nuclear factor IV - KU80, ATP-dependant DNA helicase II | ✓ ✓ ✓ |
| N        | PRKDC     | 1362789         | DNA-activated protein kinase | ✓ ✓ |
| N        | DDB1      | 12643730        | DNA damage binding protein 1 | ✓ |
|          |           |                 |              | 6 3 4 |

### DNA Damage Repair

| N        | XRCC5     | 35038           | Nuclear factor IV - KU80, ATP-dependant DNA helicase II | ✓ ✓ ✓ |
| N        | PRKDC     | 1362789         | DNA-activated protein kinase | ✓ ✓ |
| N        | DDB1      | 12643730        | DNA damage binding protein 1 | ✓ |
|          |           |                 |              | 1 3 2 |

### DNA Binding: Transcription Regulation

| N        | UPF1      | 1575536         | Regulator of nonsense transcript stability | ✓ ✓ ✓ |
| N        | DHX9      | 3915658         | ATP-dependent RNA helicase A - DEAD box protein 9 | ✓ ✓ ✓ |
| N        | EFTUD2    | 24474791        | Small nuclear ribonucleoprotein component | ✓ ✓ ✓ |
| N        | SNRNP20014043179 | Helicase hBrr2 200 kDa | ✓ ✓ ✓ |
| N        | HNRNPM    | 14141154        | Heterogeneous nuclear ribonucleoprotein M isoform b | ✓ ✓ |
| N,C      | EIF3A     | 32449796        | Eukaryotic translation initiation factor 3, subunit A | ✓ ✓ |
| N        | PABPC1    | 29743688        | Poly(A) binding protein, cytoplasmic 1 | ✓ ✓ |
| N        | PRPF8     | 17999537        | U5 snRNP-specific protein | ✓ ✓ |
| N,C      | HNRNPH1   | 5031753         | Heterogeneous nuclear ribonucleoprotein H1 | ✓ ✓ |
| N        | PDCD7     | 4416183         | ES18 - U12-type spliceosome component | ✓ ✓ |
| N,C      | FLII      | 2135121         | Flightless-I homolog | ✓ ✓ |
| C        | DDX3X     | 13514813        | Helicase like protein 2 - DEAD/H box polypeptide 3 | ✓ |
| N,C,Mit  | DDX17     | 5453840         | RNA-dependent helicase p72 - DEAD box polypeptide 17 isoform 1 | ✓ |
| N        | RENT1     | 1575536         | Regulator of nonsense transcript stability | ✓ |
| N,C      | SYNCRIP   | 26454828        | Synaptotagmin-binding, cytoplasmic RNA-interacting protein | ✓ |
| N,C      | PABP2     | 12229876        | Polyadenylate-binding protein 2 | ✓ |
| N        | HNRNPR    | 13629286        | Heterogeneous nuclear ribonucleoprotein R | ✓ |
| N        | SNRNP200  | 14043179        | Small nuclear ribonucleoprotein 200 kDa (US) | ✓ |
Table 2 Cellular Proteins in HIV-1 cores* (Continued)

| Cell Type | Gene | Protein Name | Description | Presence |
|-----------|------|--------------|-------------|----------|
| C         | ACO1 | Iron regulatory protein 1 | ✓           |
| CM        | FHL1 | Four and a half LIM domains 1 protein isoform C | ✓           |
| N         | PCBP1| Poly(C) binding protein 1 | ✓           |
| NC        | HNRNPF| Heterogeneous nuclear ribonucleoprotein F | ✓           |
| NC        | SRSF3| Splicing factor, arginine/serine-rich 3 | ✓           |
| N         | RALY | Autoantigenic RNA binding protein | ✓           |
| N         | DDX21| RNA helicase II/Gu protein - DEAD box polypeptide 21 | ✓           |
| NCMit     | RTCD1| RNA 3'-terminal phosphate cyclase | ✓           |
| NC        | HNRNPA1| Heterogeneous nuclear ribonucleoprotein A1 | ✓           |
| VN,C      | EIF5A2| Eukaryotic translation initiation factor 5A2; eIF-5A2 protein | ✓           |
| NCMit     | SNUPN| D3b subcomplex of human core snRNP domain | ✓           |

Number of Proteins per Cell Type:

| Cytoskeleton       | 24 | 11 | 9 |
|--------------------|----|----|---|
| MYH10              | ✓  | ✓  | ✓ |
| MYH9               | ✓  | ✓  | ✓ |
| TUBA1A             | ✓  | ✓  | ✓ |
| TUBA1C             | ✓  | ✓  | ✓ |
| TUBB               | ✓  | ✓  | ✓ |
| TUBB3              | ✓  | ✓  | ✓ |
| ACTBL2             | ✓  | ✓  | ✓ |
| TUBB4              | ✓  | ✓  | ✓ |
| TUBB1              | ✓  | ✓  | ✓ |
| TUBG1              | ✓  | ✓  | ✓ |
| MYO1F              | ✓  | ✓  | ✓ |
| MSN                | ✓  | ✓  | ✓ |
| CORO1A             | ✓  | ✓  | ✓ |
| CNP                | ✓  | ✓  | ✓ |
| FLII               | ✓  | ✓  | ✓ |
| TUBG1              | ✓  | ✓  | ✓ |
| TPM2               | ✓  | ✓  | ✓ |

Number of Proteins per Cell Type:

| Cytoskeleton Regulation | 10 | 11 | 12 |
|-------------------------|----|----|----|
| HSPB1                   | ✓  | ✓  | ✓  |
| MSN                     | ✓  | ✓  | ✓  |
| CORO1A                  | ✓  | ✓  | ✓  |
| CNP                     | ✓  | ✓  | ✓  |
| FLII                    | ✓  | ✓  | ✓  |
| TUBG1                   | ✓  | ✓  | ✓  |
| MYO1G                   | ✓  | ✓  | ✓  |
| TTLL10                  | ✓  | ✓  | ✓  |
| ACTR2                   | ✓  | ✓  | ✓  |
| B-SEP                   | ✓  | ✓  | ✓  |
| PFP1                    | ✓  | ✓  | ✓  |
| ACTR3                   | ✓  | ✓  | ✓  |
| DNAJA1                  | ✓  | ✓  | ✓  |
| RHOA                    | ✓  | ✓  | ✓  |
| ARPC4-2                 | ✓  | ✓  | ✓  |

*Table continued from previous page.*

Number of Proteins per Cell Type:

| Cytoskeleton       | 24 | 11 | 9 |
|--------------------|----|----|---|
| MYH10              | ✓  | ✓  | ✓ |
| MYH9               | ✓  | ✓  | ✓ |
| TUBA1A             | ✓  | ✓  | ✓ |
| TUBA1C             | ✓  | ✓  | ✓ |
| TUBB               | ✓  | ✓  | ✓ |
| TUBB3              | ✓  | ✓  | ✓ |
| ACTBL2             | ✓  | ✓  | ✓ |
| TUBB4              | ✓  | ✓  | ✓ |
| TUBB1              | ✓  | ✓  | ✓ |
| TUBG1              | ✓  | ✓  | ✓ |
| MYO1F              | ✓  | ✓  | ✓ |
| MSN                | ✓  | ✓  | ✓ |
| CORO1A             | ✓  | ✓  | ✓ |
| CNP                | ✓  | ✓  | ✓ |
| FLII               | ✓  | ✓  | ✓ |
| TUBG1              | ✓  | ✓  | ✓ |
| TPM2               | ✓  | ✓  | ✓ |

Number of Proteins per Cell Type:

| Cytoskeleton Regulation | 10 | 11 | 12 |
|-------------------------|----|----|----|
| HSPB1                   | ✓  | ✓  | ✓ |
| MSN                     | ✓  | ✓  | ✓ |
| CORO1A                  | ✓  | ✓  | ✓ |
| CNP                     | ✓  | ✓  | ✓ |
| FLII                    | ✓  | ✓  | ✓ |
| TUBG1                   | ✓  | ✓  | ✓ |
| MYO1G                   | ✓  | ✓  | ✓ |
| TTLL10                  | ✓  | ✓  | ✓ |
| ACTR2                   | ✓  | ✓  | ✓ |
| B-SEP                   | ✓  | ✓  | ✓ |
| PFP1                    | ✓  | ✓  | ✓ |
| ACTR3                   | ✓  | ✓  | ✓ |
| DNAJA1                  | ✓  | ✓  | ✓ |
| RHOA                    | ✓  | ✓  | ✓ |
| ARPC4-2                 | ✓  | ✓  | ✓ |

*Table continued from previous page.*
Table 2 Cellular Proteins in HIV-1 cores* (Continued)

| Cell Type | Protein | Description | Number of Proteins per Cell Type |
|-----------|---------|-------------|----------------------------------|
| C         | RAC2    | RAS-related C3 botulinum toxin substrate 2 | ✓ |
| M,V,C     | TLN1    | Talin       | ✓ |
| M,C       | FLNA    | Filamin     | ✓ |
| M         | SPG8    | Strumpellin  | ✓ |
| C         | NCKAP1L  | HEM1 protein | ✓ |
| C         | TBCD    | Tubulin folding cofactor D | ✓ |
| M,C       | CAP1    | Human adenylyl cyclase-associated CAP protein homolog 1 (S. cerevisiae, S. pombe) | ✓ |
|           |         |             | **9** 10 10** |
| Cell Signaling | | | |
| N,C       | GNB2L1  | Guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1 | ✓ ✓ ✓ |
| N,C       | MYADM   | Myeloid-associated differentiation marker | ✓ ✓ |
| C,V       | PTPRC   | Protein tyrosine phosphatase | ✓ ✓ |
| M         | SEMA7A  | Semaphorin L | ✓ |
| M,C       | PKN1    | Serine/threonine protein kinase | ✓ |
| C,M       | MMP14   | Matrix metalloproteinase 14 | ✓ |
| M         | TIMP3   | Tissue inhibitor of metalloproteinases-3 | ✓ |
|           |         |             | **3 5 3** |
| Nuclear Import | | | |
| N,C       | TNPO1   | Karyopherin β2; importin β 2; transportin; transportin 1 | ✓ ✓ |
| V,N       | TNPO3   | Transportin-5R; importin 12; transportin-5R2 | ✓ |
| N,C       | KPNB1   | Karyopherin β1; importin 90; importin β-1 | ✓ |
| N         | KPNA2   | Karyopherin a2; RAG cohort 1, importin a1 | ✓ |
|           |         |             | **1 3 1** |
| Nuclear Export | | | |
| N         | XPOS    | RANBP21/exportin 5 | ✓ ✓ |
| N,C       | XPO5    | Exportin T (tRNA exportin) | ✓ ✓ |
|           |         |             | **2 0 2** |
| Apoptosis | C       | HP95        | Programmed cell death 6 interacting protein | ✓ ✓ ✓ |
|           |         |             | **1 1 1** |
| Extracellular Signaling | | | |
| V,C,M     | PTPRC   | Protein tyrosine phosphatase, receptor type, C | ✓ |
| C         | AIMPI   | Aminoacyl tRNA synthetase complex-interacting multifunctional protein 1 | ✓ |
| M         | CLEC1A  | C-type lectin domain family 1, member A | ✓ |
|           |         |             | **2 1 0** |
| Intracellular Transport | | | |
| C,M       | CLTC    | Clathrin, heavy chain (Hc) | ✓ ✓ ✓ |
| M,C       | RAB8A   | RAB8A, member RAS oncogene family | ✓ ✓ ✓ |
| M         | CPNE1   | Copine I | ✓ ✓ |
| C,N       | TNIP1   | TNFAIP3 interacting protein 1; HIV-1 Nef interacting protein | ✓ |
| M,N,C     | GC      | Vitamin D binding protein | ✓ |
| M,C       | AP2B1   | Adaptor-related protein complex 2, β1 subunit | ✓ |
|           |         |             | **3 4 4** |
Table 2 Cellular Proteins in HIV-1 cores* (Continued)

| Cellular Proteins | Gene | Description | Number of Proteins per Cell Type |
|-------------------|------|-------------|---------------------------------|
| RNA Stability Regulation | | | |
| CN | RNH1 | 15029922 | Ribonuclease/angiogenin inhibitor 1 | ✓ |
| | | | Number of Proteins per Cell Type: | 1 0 0 |
| Nuclear Lamina, intranuclear components | | | |
| N | LMNB1 | 15126742 | Lamin B1 | ✓ |
| N | NCL | 21750187 | Nucleolin | ✓ |
| N | DKC1 | 14602859 | Dyskerin; dyskeratosis congenita 1 | ✓ |
| N | RPL3 | 18606060 | Ribosomal protein L3 | ✓ |
| | | | Number of Proteins per Cell Type: | 4 0 0 |
| Cell adhesion | | | |
| M,C | PCDHGA7 | 14196477 | Protocadherin gamma subfamily A, 7 | ✓ |
| M | HABP2 | 4758502 | Hyaluronan binding protein 2 | ✓ |
| | | | Number of Proteins per Cell Type: | 2 0 0 |
| Lipid Biosynthesis | | | |
| C,N | FDFT1 | 11514495 | Farnesyl-diphosphate farnesyltransferase 1 | ✓ |
| M,C | FASN | 15779138 | Fatty acid synthase | ✓ |
| | | | Number of Proteins per Cell Type: | 1 1 0 |
| Transmembrane Ion Transport | | | |
| N,M | SLC2A6 | 113463 | Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6 | ✓ |
| C,M | SLC4A10 | 7513341 | Sodium bicarbonate cotransport protein 2 | ✓ |
| C,M | ATP6V0E2 | 542837 | ATPase, H+ transporting V0 subunit e2 | ✓ |
| | | | Number of Proteins per Cell Type: | 1 1 0 |
| Cell-Cell Transport | | | |
| M,C | ESYT1 | 7512911 | Extended synaptotagmin-like protein 1 | ✓ |
| | | | Number of Proteins per Cell Type: | 0 1 0 |
| Ribosomal | | | |
| R | RPL7A | 4506661 | 60 S-L7A | ✓ ✓ ✓ |
| R | RPL18A | 11415026 | 60 S-L18A | ✓ ✓ ✓ |
| R | RPL7A | 17456110 | 60 S-L7 | ✓ ✓ |
| R | 27483402 | 40 S-S2 | ✓ ✓ |
| R | RPL7L1 | 27498574 | 60 S-L7Like1 | ✓ ✓ |
| R | RPL13 | 15431295 | 60 S-L13 | ✓ ✓ |
| R | RPL24 | 4506619 | 60 S-L24 | ✓ ✓ |
| R | RPS8 | 4506743 | 40 S-S8 | ✓ |
| | | | Number of Proteins per Cell Type: | 7 5 5 |
| Ubiquitin/Proteasome | | | |
| C,N | UBA1 | 24485 | Ubiquitin-like modifier activating enzyme 1 | ✓ ✓ ✓ |
| C | BFAR | 27675450 | Bifunctional apoptosis regulator | ✓ ✓ ✓ |
| N,C | PSMC3 | 107855 | Proteasome (prosome, macropain) 26 S subunit, ATPase, 3 | ✓ ✓ ✓ |
| C | PSMD7 | 2134660 | Proteasome (prosome, macropain) 26 S subunit, non-ATPase, 7 | ✓ |
| M,C | PSMB1 | 12653473 | Proteasome (prosome, macropain) subunit, β type, 1 | ✓ |
| N,C | PSMD11 | 2150046 | Proteasome (prosome, macropain) 26 S subunit, non-ATPase, 11 | ✓ |
| C,N | PAAF1 | 33150632 | Proteasomal ATPase-associated factor 1 | ✓ |
Table 2 Cellular Proteins in HIV-1 cores* (Continued)

| Number of Proteins per Cell Type: | 6 | 6 | 3 |

**Metabolism, Metabolism Regulation**

| Number of Proteins per Cell Type: | 4 | 4 | 3 |

**Cell Cycle Regulation/Cell Differentiation**

| Number of Proteins per Cell Type: | 1 | 0 | 0 |

**Nucleotide Biosynthesis**

| Number of Proteins per Cell Type: | 1 | 1 | 1 |

**Amino Acid Biosynthesis**

| Number of Proteins per Cell Type: | 5 | 10 | 7 |
### Table 2 Cellular Proteins in HIV-1 cores (Continued)

#### Aminoacyl tRNA synthetases

| Code | Uniprot ID | Protein                          | CN     | C,N   | C,Mit | M,C   | N,C   |
|------|------------|----------------------------------|--------|-------|-------|-------|-------|
| AARS | 31873336   | Isoleucyl-tRNA synthetase       | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| MARS | 15929104   | Methionyl-tRNA synthetase       | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| RARS | 2118344    | Arginyl-tRNA synthetase         | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| GARS | 3845409    | Glycyl-tRNA synthetase          | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| WARS | 8439415    | Tryptophanyl-tRNA synthetase    | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| DARS | 4557513    | Aspartyl-tRNA synthetase        | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| QARS | 11493441   | Glutaminyl-tRNA synthetase      | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |

**Number of Proteins per Cell Type:**

| CN | 5 | C,N | 3 | C,Mit | 2 |

#### Translation and Translation Regulation

| Code | Uniprot ID | Protein                                      | CN     | C,N   | C,Mit | M,C   | N,C   |
|------|------------|----------------------------------------------|--------|-------|-------|-------|-------|
| EIF3A| 32449796   | Eukaryotic translation initiation factor 3, subunit A | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| EEF2 | 4503483    | Eukaryotic translation elongation factor 2; polypeptidyl-tRNA translocase | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| CC2D1B| 27715655  | Coiled-coil and C2 domain containing 1B      | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| CYFIP1| 24307969  | Cytoplasmic FMR1 interacting protein 1 (Sra1) | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| AIMP2| 27662300   | Aminoacyl tRNA synthetase complex-interacting multifunctional protein 2 | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |

**Number of Proteins per Cell Type:**

| N,C | 3 | C | 4 | M | 0 |

#### Protein Post-Translation Modification

| Code | Uniprot ID | Protein                                      | CN     | C,N   | C,Mit | M,C   | N,C   |
|------|------------|----------------------------------------------|--------|-------|-------|-------|-------|
| PAFAH1B| 4505587  | Platelet-activating factor acetylhydrolase 1b, catalytic subunit 3 | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| PARP1 | 130781     | Poly [ADP-ribose] polymerase-1               | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| USP14 | 4827050    | Ubiquitin specific protease 14               | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| SERPINC1| 4502261  | Serpin peptidase inhibitor, clade C (antithrombin), member 1 | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| LPL   | 15030193   | Lipoprotein lipase                          | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| NMT1  | 345862     | N-myristoyltransferase 1                     | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| CPD   | 21903712   | Carboxypeptidase D                          | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |

**Number of Proteins per Cell Type:**

| M | 4 | C | 3 | N | 0 |

#### Protein Degradation (non-proteasomal)

| Code | Uniprot ID | Protein                                      | CN     | C,N   | C,Mit | M,C   | N,C   |
|------|------------|----------------------------------------------|--------|-------|-------|-------|-------|
| ANPEP| 28678      | Alanyl (membrane) aminopeptidase              | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |

**Number of Proteins per Cell Type:**

| M | 0 | C | 1 | N | 1 |

#### Chaperones/Molecular Folding

| Code | Uniprot ID | Protein                                      | CN     | C,N   | C,Mit | M,C   | N,C   |
|------|------------|----------------------------------------------|--------|-------|-------|-------|-------|
| DNAJC13| 7513063  | DnaJ (Hsp40) homolog, subfamily C, member 13 | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| HSP90A120149594| Heat shock protein 90 kDa α (cytosolic), class B member 1 | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| HSP8A8| 24234686   | Heat shock 70 kDa protein 8                   | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| CCT3 | 2136253    | Chaperonin containing t-complex polypeptide 1 (TCP1), subunit 3 (γ); TCP1 ring complex protein TRiC5 | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| CCT6A| 4502643    | Chaperonin containing t-complex polypeptide 1 (TCP1), subunit 6A (C1) | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| CCT6B| 22654493   | Chaperonin containing t-complex polypeptide 1 (TCP1), subunit 2 | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| HSPB1| 662841     | Heat shock 27 kDa protein 1                   | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| PP1A | 2624881    | Human Cyclophilin A                           | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| NAP1| 5174613    | Nucleosome assembly protein 1-like 4; nucleosome assembly protein 2 | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| NAP1L4| 13540473  | t-complex polypeptide 1 (TCP1)                | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| CCT5 | 12804225   | Chaperonin containing t-complex polypeptide 1 (TCP1), subunit 5 (ε) | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| P4HB | 20070125   | Prolyl 4-hydroxylase, β polypeptide           | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| CCT7 | 5453607    | Chaperonin containing t-complex polypeptide 1 (TCP1), subunit η | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |
| SERPINH1| 123576  | Serpin peptidase inhibitor, clade H (heat shock protein 47), member 1 | ✓✓✓    | ✓✓✓   | ✓✓✓   | ✓✓✓   | ✓✓✓   |

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spectra and number of proteins within each group of overlapping proteins could also be observed. Nevertheless, the proteins identified in all viral cores included the spectra of molecular chaperones (7), cytoskeleton components (7), and vesicular transport-associated proteins (5) were the most numerous (Figure 3C4). These functional groups of proteins have been shown to be involved in the folding of viral proteins and HIV-1 virion assembly (reviewed in [12,93]). Some of these proteins (clathrin, transferrin receptor 1, RAB7, RAB5C, EHD4, Hsp70, Hsp90, cyclophilin A, β actin, tubulin α1) are very typical for HIV-1 virions and were registered earlier in the samples of purified viral particles (summarized in the database of Host Proteins in HIV-1 [http://web.ncifcrf.gov/research/avp/protein_db.asp]). Probably some of these proteins, such as β actin, α and β tubulin, moesin and major vault protein 1 are incorporated into HIV-1 virions non-specifically, due to their close proximity to a budding site, as suggested earlier [4,94], whereas other cellular proteins may be incorporated due to specific interactions with viral proteins, such as Gag and GagPol [11,23,33], viral genomic RNA [8], or tRNA^Lys^3 primer [73]. These host proteins, called “Captives” in a recent review [12], may be involved in the virion assembly and budding process or be important for post-assembly steps of HIV-1 life cycle. Below we attempt to assess the packaging of selected cellular proteins in the cores of HIV-1 virions depending on the type of producer cells.

### Semi-quantitative analysis of selected cellular proteins packaging into the cores of HIV-1 virions assembled in different types of cells

Although the spectra of the cellular proteins incorporated in the viral cores depended on the type of virus-producing cells, the quantitative differences within the group of overlapping proteins could also be observed. We compared the abundance of certain cellular proteins, selected from the group of 42 common proteins, in the viral cores and lysates of the producer cells. Since the group of 42 cellular proteins identified in all viral cores included the members which are likely involved in viral replication (Table 3), we selected 7 proteins from...
Figure 4 (See legend on next page.)
different functional categories for analysis by Western blot. Within the group of RNA-binding proteins, we analyzed incorporation of RNA helicase A, because the function of this protein in HIV-1 replication has been shown earlier [10,95], and small nuclear ribonucleoprotein 200 kDa (U5) (SNRNP200 or HELIC2), which currently has no known role in HIV replication but has been detected with a high score in our MS/MS preparations. Within DNA-binding group of proteins, we analyzed the Minichromosome maintenance complex component 5 (MCM5) involved in initiation of DNA replication, 80-kilodalton subunit of the Ku heterodimer protein or ATP-dependant DNA helicase II (Ku80 or XRCC5), which is involved in the repair of DNA double-strand breaks and telomerase function [96,97], Pontin52 (RUVBL1) and Reptin52 (RUVBL2) DNA helicases, both being the components of several high molecular weight protein complexes involved in chromatin remodeling, transcription regulation, DNA damage sensing and repair [98]. All these DNA-binding proteins were detected with high scores by LC-MS/MS in the cores of virions assembled in Sup-T1 cells, whereas in the cores of THP1-derived virions (both activated and non-activated) these proteins were less abundant. Incorporation of these proteins in the viral cores did not correlate with their abundance in the producer cells: XRCC5, MCM5 and SNRNP200 were presented in all types of cells at a similar level, whereas the RNA helicase A (DHX9) was abundant in dividing Sup-T1 and non-activated THP1 cells and decreased in non-dividing activated THP1 (Figure 4B,C). Interestingly, the incorporation of RNA helicases DHX9 and SNRNP200 into the cores did not correlate with the packaging of viral genomic RNA, as quantitative RT-PCR of the RNA isolated from CA p24Gag-normalized purified core samples, subjected to reverse transcription with oligo-dT primer and then to quantitative real-time PCR with the primer set specific for positive-strand HIV-1 DNA. The data represents analysis of three independent preparations. Each point shows mean RNA copy number ± SD per 1 ng of p24Gag in the viral core sample.

Discussion

The proteomic analysis of the cores isolated from HIV-1 virions assembled in T lymphocytes and activated (model of MDM) and non-activated monocytic cells revealed more than thirty cellular proteins which have been previously shown to be involved in different stages of HIV-1 replication and/or incorporated in HIV-1 virions (Table 3). Since HIV-1 is a retrovirus with a RNA genome of less than 10 kb and encodes only nine polypeptides, it engages numerous cellular factors and pathways at all stages of its life cycle. Some of the factors, especially proteins involved in RNA splicing and nuclear export, multi-vesicular bodies (MVB) and late endosomal pathway, as well as the proteins directly involved in the HIV-1 budding process, such as AIP1/Alix, may be incorporated into virions by being associated with the viral RNA and proteins, but do not play a visible role in subsequent stages of viral replication [4,125,130]. For
Table 3 Previously discovered viral proteins with a known role in HIV-1 Replication

| Protein                                    | Gene name            | Score in virion cores from | Known role in HIV-1 replication                                                                 |
|--------------------------------------------|----------------------|---------------------------|-------------------------------------------------------------------------------------------------|
| DNA Binding: Chromatin Organization, Replication, Topoisomerases |                      |                           |                                                                                                 |
| Regulator of chromosome condensation 2     | RCC2                 | 3.32 1.78                | RCC proteins interact with Rac1 and Arf6 subnetworks and limit signaling required for membrane protrusion and delivery [99]; RCC2 acts as a Rac1 guanine nucleotide exchange factor (GEF); RCC1 is involved in HIV-1 RNA nuclear export through activation of RanGAP [100] and hence facilitates dissociation of RNA nuclear export complex [101]. |
| Helicase like protein 2 - DEAD/H box polypeptide 3 | DDX3X                | 2.05 -                    | RNA helicases that catalyze ATP-dependent unwinding of double-stranded RNA and DNA-RNA complexes; localize in both nucleus and cytoplasm and function as transcriptional regulators; may also be involved in expression and nuclear export of retroviral RNAs, particularly in post-transcriptional regulation of HIV-1 [104]. DDX9 is member of DEAD box RNA helicases, important for nuclear export of non-spliced HIV-1 RNA [103]. |
| RNA-dependent helicase p72 - DEAD box polypeptide 17 isoform 1 | DDX17                | 6.36 -                    | Members of ATP-dependent DEAD box RNA helicases, potentially involved in interaction with HIV-1 RNA. |
| RNA helicase II/Gu protein - DEADDDX21 box polypeptide 21 | DDX17                | - 2.07 -                  |                                                                                                 |
| ATP-dependent RNA helicase A - DEAD box protein 9 | DHX9                 | 6.36 6.09 27.18           | RNA helicases that catalyze ATP-dependent unwinding of double-stranded RNA and DNA-RNA complexes; localize in both nucleus and cytoplasm and function as transcriptional regulators; may also be involved in expression and nuclear export of retroviral RNAs, particularly in post-transcriptional regulation of HIV-1 [104]. DDX9 is member of DEAD box RNA helicases, important for nuclear export of non-spliced HIV-1 RNA [103]. |
| Heterogeneous ribonucleoprotein A1         | nuclearHNRNA1-       | 1.92 -                    | RNA binding proteins that complex with heterogeneous nuclear RNA (hnRNA) and are involved in pre-mRNA processing in the nucleus: alternative splicing regulation, polyadenylation, nuclear-cytoplasmic transport and other aspects of mRNA metabolism and transport; hnRNPA1 is involved in HIV-1 mRNA splicing [105,106]; hnRNPA2 is found to be important for trafficking of HIV-1 mRNA out of the nucleus and through the cytoplasm [105]; hnRNPH and hnRNPK interact directly with HIV-1 RNA and are involved in alternative splicing [107]. |
| Heterogeneous ribonucleoprotein F           | nuclearHNRNPF        | 3.47 -                    |                                                                                                 |
| Heterogeneous ribonucleoprotein H1          | nuclearHNRNPH13.04   | 8.26 -                    |                                                                                                 |
| Heterogeneous ribonucleoprotein M isoform b | nuclearHNRNPM1.9     | - -                      |                                                                                                 |
| Heterogeneous ribonucleoprotein R           | nuclearHNRNPRA       | 6.31 -                    |                                                                                                 |
| Nonsense-mediated decay (NMD)/UPF1 factor   |                      | 5.65 6.48 6.32            |                                                                                                 |
| Cytoskeleton                                |                      |                           |                                                                                                 |
| β Actin                                    | ACTBL2               | 60.1235.05 99.59          | Actin microfilaments are important for RTC formation and RTC transport in cytoplasm [1]; interaction with NC domain of Gag is required for HIV-1 assembly [109]. |
| Tubulin α 1                                | TUBA1A               | 69.3958.56 66.49          | Microtubules are shown to be important for RTC cytoplasmic trafficking [18,110] assembly of Gag polyprotein molecules [111] and viral genomic RNA trafficking [112]. |
| Tubulin α 6                                | TUBA1C               | 57.1141.39 61.72          |                                                                                                 |
| Tubulin β 5                                | TUBB                 | 29.8227.85 49.68          |                                                                                                 |
| Tubulin β 3                                | TUBB3                | 13.849.07 30.56           |                                                                                                 |
| Tubulin γ 1                                | TUBG1                | - - 4.96                 |                                                                                                 |
| Dynein                                     | DYNC1H1              | 11.68 10.11               |                                                                                                 |
| Cytoskeleton Regulation                     |                      |                           |                                                                                                 |
| ARP3 actin-related homolog (yeast)          | 3ACTR3               | 9.52 -                    | Major constituent of the ARP2/3, a 7 subunit complex, responsible for actin polymerization [113]. The complex is required for early phase of HIV-1 replication [114]. |
Table 3 Previously discovered viral proteins with a known role in HIV-1 Replication* (Continued)

| Protein Function | Gene Symbol | Xcorr          | M+H | M+Na+ |
|------------------|-------------|----------------|-----|-------|
| Nuclear Import   |             |                |     |       |
| TNPO3            | Unidentified| 5.75           | -   | -     |
| Rab5C GTP protease| Rab5C       | 3.68           | -   | -     |
| Rab7A GTP protease| Rab7A       | 3.68           | -   | -     |
| Rab8A GTP protease| Rab8A       | 3.68           | -   | -     |
| Rab11A GTP protease| Rab11A     | 3.68           | -   | -     |
| XPOS             | XPOS        | 5.10           | -   | -     |
| CLTC protein - clathrin | CLTC | 37.6 7.77 | 7   |       |
| Rab5C GTP protease | Rab5C       | 3.68           | -   | -     |
| Rab7A GTP protease | Rab7A       | 3.68           | -   | -     |
| Rab8A GTP protease | Rab8A       | 3.68           | -   | -     |
| Rab11A GTP protease | Rab11A     | 3.68           | -   | -     |
| PDCD6IP           | PDCD6IP     | 3.68           | -   | -     |
| TNFAIP3 interacting protein 1; HIV-1 Nef interacting protein; NAF1 | TNIP1 | 1.0 - | - |
| Hsp70 protein 8    | HSPA8       | 7.85           | 64.2| 31.35 |
| Hsp70 protein 9 (mortalin) | HSPA9      | 5.75           | -   | -     |
| Cyclophilin A, CyPA | PPIA        | 2.35           | 7.85| 3.35  |

* Proteins are listed by function category.

** Full Protein name as listed in NCBI Protein database (http://www.ncbi.nlm.nih.gov/protein).

*** Official Gene Symbol as listed by HGNIC.

### Nuclear Import

| Protein Function | Gene Symbol | Xcorr          | M+H | M+Na+ |
|------------------|-------------|----------------|-----|-------|
| Impontin-β family member, binds catalytic core domain close to the N terminus of IN and promotes nuclear entry of PICs [115,116]; might serve as a chaperone that associates with PIC post-entry to guide it through nuclear pore [117]. | HSPA8 | 7.85 26.4 31.35 |
| Directly interacts with central core domain of HIV-1 integrase, facilitates PIC nuclear import [118-120]. | KPN2A | - - 7.91 |
| Importin α/β heterodimer interacts with HIV-1 integrase and probably MA protein and Vpr to translocate PIC into the nucleus [121] | TNPO1 | - 3.63 1.95 |
| Rab GTP proteases are important for vesicular trafficking. They are activated by guanine nucleotide exchange factor (GFE), RCC2 protein revealed in HIV-1 cores can act as a GEF. Rab11 is important for HIV-1 production [123]; Rab1 potentially associates with HIV-1 Rev and is involved in nuclear export of viral RNA [124]; Rab9 is required for Gag trafficking to the site of assembly [123]; Rab7-interacting lysosomal protein promotes vRNA clustering at the MTOC [112]; Rab6 is probably involved in viral entry [58]. | RAB5C | - 6.78 6.58 |
| Association of RanBP1 and 2 with Rev-CRM1-RanGTP complex has been shown [122], thus RanBP is required for dissociation of nuclear export complex during HIV-1 RNA nuclear export [101]. | RAB7A | - 9.08 21.97 |
| Clathrin is incorporated in HIV-1 particles probably through interaction with Pol, especially IN domain [32]; it facilitates the accurate morphogenesis of infectious particles probably by contribution to spatial organization of Gag and Pol proteins and proteolytic processing of virion components during particle assembly [5]. | CLTC | 37.6 7.7 7 |
| Rab5C GTP protease | Rab5C       | 3.68           | -   | -     |
| Rab7A GTP protease | Rab7A       | 3.68           | -   | -     |
| Rab8A GTP protease | Rab8A       | 3.68           | -   | -     |
| Rab11A GTP protease | Rab11A     | 3.68           | -   | -     |
| Programmed cell death 6 interacting protein; HP95; AIP1/ALIX | PDCD6IP     | 6.43 13.62 29.94 |
| An ERK binding protein, Naf1, attenuates EGF/ERK2 nuclear signaling, binds HIV-1 Nef and increases cell surface CD4 expression [126]. ERK2 interacts with HIV-1 matrix, packaged into virions and responsible for MA phosphorylation [24]. | TNFAIP3 | 1.0 - |
| Incorporates into virions via binding to the CA domain of Pr55Gag [23]. The role of CA-bound CyPA is still unclear [48]. It is critical for protection and stabilization of HIV-1 cores as a chaperone [49] and is probably involved in PIC nuclear transport [31] | Hsp70 protein 8 | HSPA8 |
| Heat shock protein 70 family members are shown to be incorporated in HIV-1 particles. This is important for subsequent viral cDNA synthesis [11,122]; they can also interfere with Vpr in HIV-1 nuclear import in macrophages [128,129]. | Hsp70 protein 9 (mortalin) | HSPA9 |
| Incorporates into virions via binding to the CA domain of Pr55Gag [23]. The role of CA-bound CyPA is still unclear [48]. It is critical for protection and stabilization of HIV-1 cores as a chaperone [49] and is probably involved in PIC nuclear transport [31] | Cyclophilin A, CyPA | PPIA | 22.3520.00 7.68 |

the other factors, such as Hsp70, CLTC protein/clathrin and Rab GTP proteases, their important role in the molecular organization of mature virions and probably viral entry into the target cells has been proposed earlier [5,11,32,33,58]. Here, we focus on the proteins identified in the core structures of HIV-1 virions assembled in different cell types. These proteins can be potentially involved in post-entry stages of the viral replication.

The HIV-1 morphogenesis is known to be different in T lymphocytes and myeloid cells. In T cells, the viral particle budding and assembly have been shown to take place directly at the plasma membrane (reviewed in [131]), whereas in macrophages, earlier studies detected assembling HIV-1 particles in the late endosomes [132] (reviewed in [131]) or in internally sequestered plasma membrane domains that contain late endosomal markers but are connected to the cell surface [133]. Recent reports revealed an extensive tubular network and large sheet-like structures which extended to the cell surface from vesicular compartments and contained HIV-1
virions, released into the extracellular media [134,135]. Indeed, the cores of model MDM-derived virions contained twelve vesicular trafficking-associated proteins, whereas only six were detected in the T cell-derived viral cores. The larger proportion of cytoskeleton and cytoskeleton regulatory proteins in the cores of virions assembled in THP1 cells than in Sup-T1-derived cores may be dependent on the abundance of these proteins in producer cells. Uninfected THP1 cells contain larger amounts of actin and β tubulin than Sup-T1 (Figure 3A), suggesting that other cytoskeleton and associated cytoskeleton regulatory proteins may also be more abundant in these myeloid cells; hence, increasing the probability that the virus will hijack this subset of proteins. Our data suggest that the unique core-incorporated proteins, which are different in the viruses assembled in different cell types, are mostly indiscriminately hijacked during virion assembly and likely not important for subsequent infection.

The group of forty-two cellular proteins identified in the cores of virions produced by all types of cells contains at least thirteen proteins whose involvement in different stages of HIV-1 infection has been shown previously (Table 2 and 3). On the other hand, many proteins within this group have never been found to be implicated in any infection event. Meanwhile, incorporation of these proteins into the viral cores from different types of producer cells suggests that at least some of them may be important for successful infection. For instance, within the functional category of vesicular trafficking-associated proteins, the member of ESCRT pathway AIP1/ALIX detected in all our core preparations has been shown earlier to be interacting with HIV-1 Gag late domain and to be important for the release of viral particles [125,136]. Clathrin has also been found to be abundant in HIV-1 viral particles and important for the correct assembly and maturation of viral particles through the regulation of proteolytic processing of virus components [5,32]. Members of the RAB family of proteins were found to be important for different steps of HIV-1 particle assembly and probably RNA incorporation [112,123,124] (Table 3). Available data suggest that the proteins of this group are important factors of assembly and maturation of the viral particle and get into the viral cores in association with Gag and GagPol proteins or viral RNA. Some of the factors of vesicular trafficking may be potentially involved in the early stage of HIV-1 infection: the functional genomic screening of factors involved in HIV-1 infection showed that the vesicular RAB6A protein is important for the late phase of reverse transcription in infected cells [58].

The cytoskeletal proteins were also abundant in all core preparations, however, the role of these proteins in HIV-1 virions is still questionable. The actin microfilaments form the plasma membrane cortex, and both actin and microtubular networks are involved in HIV-1 particle assembly [111,112,137], so that the proportion of these proteins in viral particles may be up to 15% of the molar level of Gag [94]. Actin is packaged in the virions probably in association with the NC domain of Gag [111,137,138]. Thus, actin and actin-associated proteins coronin, moesin, filamin, and FLII can get into the viral cores due to the actin interaction with Gag or GagPol. However, involvement of these virion-packaged molecules in post-assembly events of the virus life cycle has not been shown [12]. Our data indicate that the ratio of β tubulin between the viral cores reproduced the concentration ratio of this protein between the producer cells, suggesting the capturing, but not specific incorporation, of this cytoskeletal protein into assembling virions. On the contrary, the cytoskeleton regulation proteins were found to be mostly different in the viral cores from different cell types, which may reflect variability of the profiles of these proteins in virus-producing cells. Some of them, particularly Hsp27, a protein containing a nuclear localization signal [139] and found in the cores of virions from all cell types, can be potentially involved in post-entry steps of infection, although the role of this protein in viral replication remains unknown.

The other category of proteins abundant in HIV-1 viral cores is the molecular chaperones. The profiles of these proteins are very similar in all analyzed samples. Indeed, Hsp27, Hsp40 (DnaJ) co-chaperone, Hsp70, Hsp90, numerous members of TCP1 (Hsp60) tetradecameric complex, as well as peptidylprolyl isomerase cyclophilin A were found in all core preparations (Table 2). Previously, these proteins were identified in purified samples of whole HIV-1 virions and Gag preparations [4,10,23]. Hsp70 was also found in HIV-2, SIVmac and SIVagm [11,56]. Since the major function of these proteins is to regulate folding of newly synthesized polypeptides, facilitate intracellular protein transport and assemble multisubunit protein structures [140,141], they likely play an important role in HIV-1 particle assembly, processing and folding of the viral proteins during virion core maturation and maintain structural integrity of the viral core and RTCs [11,33,49]. The early RTC functions, especially organization of reverse transcription, may also depend on the proper activity of incorporated chaperones.

The RNA-binding proteins represent the most diverse group of cellular factors in viral cores. Although we identified 29 RNA-binding proteins in the core preparations, only four of them were found in all core samples (Table 2). These are (1) regulator of nonsense transcript stability (UPF1), (2) ATP-dependent RNA helicase A (RHA or DHX9), (3) small nuclear ribonucleoprotein component (Snpr116 or EFTUD2), and RNA helicase hBrr2 200 kDa (SNRNP200 or HELIC2). The role of the first two factors in HIV-1 infection was thoroughly characterized before. The UPF1 protein, RNA helicase from the
SFI superfamily, involved in translation of Gag polypeptide, was found in virus-producing cells in association with HIV-1 ribonucleoprotein (RNP) along with Pr55Gag, viral RNA and cellular protein Staufen 1 [108]. Thus, UPF1 can be packaged in the virions in association with both Gag and the viral genomic RNA. RNA helicase A (RHA), a member of the DEAD family of proteins which are capable of unwinding the double-stranded RNA structure, was earlier found to be associated with HIV-1 Gag and incorporated into HIV-1 virions in an RNA-dependent manner. Packaging of this protein into HIV-1 virions was important for endogenous reverse transcription [10]. Jeang and Yedavalli suggested that RHA incorporated into HIV-1 viral cores might be important for the reverse transcription in RTCs [95]. A recent study that revealed an important role of this enzyme in the annealing of tRNA\textsuperscript{Lys}3 primer [73], confirmed this suggestion. Two other proteins, SNRNP200 (member of the family of U5 DEXH-box RNA helicases) and Snpr116 (U5 snRNP specific protein, 116 kD), are both members of the U5 group of small nuclear RNA proteins, the spliceosome components, and have not been detected in HIV-1 virions before. Since these proteins are known as important components of splicing machinery required for a spliceosome catalytic activity [142,143], they can be associated with HIV-1 pre-mRNA and remain associated with a mature viral RNA molecule.

Interestingly, our analysis showed higher level of DHX9 (RHA) and SNRNP200 (HELIC2) in the cores of virions assembled in T cells, as compared with the viral cores from the monocyte and MDM models, which did not correlate with the abundance of these proteins in producer cells. Since we did not find significant differences in the RNA and CA protein count between the virions from analyzed cells, observed differences suggest that the mechanism of incorporation of these proteins into the virions (binding to viral RNA or interaction with Gag or/and GagPol) is more effective and likely selective in T lymphocytes, than in monocyte and MDM model cells. Because of importance of RNA helicase A for the reverse transcription in HIV-1 virions and RTCs, we expect that SNRNP200 protein may also be involved in cDNA synthesis or accumulation.

Analysis of earlier published genome-wide screens performed by Warrilow and co-authors [22] to select the host factors potentially implicated in HIV reverse transcription showed that the proteins involved in DNA replication, transcription and repair, as well as proteins of the ubiquitin-proteasome pathway may also be important. Within the 17 DNA-binding proteins detected in our core preparations, only two, ATP-dependent DNA helicase II (XRCC5 or Ku80) and TATA binding protein interacting protein 49 kDa (RUVBL1 or Pontin52), were found in the core of all virions. Two other proteins, minichromosome maintenance complex component 5 (MCM5 or CDC46) and regulator of chromosome condensation protein 2 (RCC2), were identified only in Sup-T1 and activated THP1 cells (Table 2), although Western blot showed presence of MCM5 also in non-activated THP1. The protein RUVBL2 (Reptin52) was not identified by MS/MS in activated THP1, but the fact that in cells this DNA helicase is complexed with the closely related RUVBL1 protein in hetero-dodecamers [144] suggests incorporation of this protein in the virions from all studied cells. Our analysis showed that among DNA binding proteins present in the viral cores from different cell types only XRCC5 and especially MCM5 displayed an increased level of incorporation from the T cells, similar to RNA-binding DHX9 and SNRNP200, whereas core incorporation of RUVBL1 and RUVBL2 reproduced their level in virion-producing Sup-T1 and THP1 cells. The DNA helicase MCM5, a member of the MCM family of chromatin-binding proteins is involved in the initiation of DNA replication and was found to be upregulated during the transition from the G0 to G1/S phase of the cell cycle (RefSeq database). Interaction of this protein with HIV has not been shown before. Another DNA helicase, XRCC5 or Ku80, which is involved in repairing DNA double-strand breaks, was earlier found to be important for viral cDNA circularization, nuclear import and integration [145,146]. However, this function was shown for the protein expressed in the infected target cells, but not for virion-incorporated Ku80. Packaging of both MCM5 and XRCC5 (Ku80) DNA helicases in all viral cores and their high levels in the cores of T lymphocyte-derived virions suggest that the core-incorporated molecules of these proteins can also be involved in processes associated with cDNA processing and/or integration during post-entry steps of infection, especially in T cells.

Within the group of ubiquitin-proteasome pathway associated proteins a total of 9 proteins were detected; three of them were identified in viral cores from all producer cells (Table 2). Earlier, numerous 26 S proteasome-associated proteins were found in HIV-1 and SIV particles [4,56]. Involvement of the ubiquitin-proteasome system in the budding of lentiviral particles was shown earlier (reviewed in [147]). Since all major domains of the membrane-associated HIV-1 Gag molecules have been shown to be ubiquitinated during virion budding [148], the ubiquitination factors could package into virions in association with Gag and then get into the viral cores. However, the role of virion-associated factors of the ubiquitin-proteasome system in the early steps of HIV-1 infection is unknown.

Conclusions
Taken together, results of our study indicate that the profile of host cell proteins packaged in the cores of HIV-1 virions depends on the type of producer cell. High
abundance of certain proteins in the cell increases the probability of their capturing by the virions and hence their presence in the viral cores. However, certain members of functional groups of DNA- and RNA-binding proteins, molecular chaperones, cytoskeletal, vesicular trafficking-associated and ubiquitin-proteasome pathway-associated proteins were found in the cores of virions from all analyzed cells, suggesting that their incorporation is non-random and that they can be directly or indirectly involved in either the virus assembly/budding or early infection events. Our findings that the abundance of cellular proteins DHX9, (RHA) SNRNP200, MCM5, and XRCC5 (Ku80) within virus-producing cells did not correlate with the abundance seen in cores of produced virions, specifically their unexpected higher packaging in T cells, suggests that the incorporation of these factors in T lymphocytes is more efficient than in myeloid cells. These differences may be associated with variability of localization of these host proteins relative to the sites of virion assembly in different cell types and/or with different localization of virion assembly complexes. The host factors abundant in the viral cores may play a role in subsequent steps of HIV-1 infection, specifically in T cells. Further analysis of the role of these proteins in viral replication might reveal new mechanisms of the modulation of HIV infection by the host proteins and identify new targets for antiretroviral therapeutic interventions.

Methods

Cells and viruses
The acute monocytic leukemia cell line THP1 (from S. Tsuchiya) and T lymphoblastoma Sup-T1 cells (from James Hoxie) were provided by the NIH AIDS Research & Reference Reagent Program. The human kidney fibroblasts 293 T/17 was purchased from ATCC (Manassas, VA). All cells were maintained at 37°C and 5% CO2 in RPMI-1640, the media was changed again and the cells were cultured in fresh media for an additional 48 h at 37°C and 5% CO2, the cells were washed from the virus-containing media, re-suspended in RPMI-1640 (pre-warmed to 37°C) and seeded in a regular (Sup-T1 and non-activated THP1) or polylsine-treated (THP1 for activation) 75 cm² tissue culture flasks at a concentration of 4 x 10⁶ cells per ml (Sup-T1, non-activated THP1) or of 1 x 10⁶ cells per ml (THP1 for activation). To get activated THP1 cells, the PMA and vitamin D₃ solutions were added to cells to a final concentration of 100 nM. Then, the cells were incubated at 37°C and 5% CO₂ for 72 h.

Virus-containing culture media from infected Sup-T1, activated and non-activated THP1 cells, as well as the media from the same types of non-infected cells (control) were harvested after incubation with virus (or equivalent volume of the virus-negative culture media) and purified from cell debris by being centrifuged at 2,500 rpm and 4°C for 5 minutes and filtered through 0.45 µm syringe filters. Then, filtered samples were centrifuged at 100,000 x g and 4°C for 3 h through 2 ml cushions of 30% sucrose in STE buffer (10 mM Tris–HCl [pH 7.4], 100 mM NaCl, and 1 mM EDTA) in a Beckman SW-41 rotor. The pellets were re-suspended in 300 µl of STE buffer and the viral cores were then isolated by “spin-thru” purification as described earlier [63-66]. Briefly, 3.8 ml of a 30-50% linear density gradient of sucrose in STE buffer was overlaid with 1 ml of 15% sucrose containing 1% Triton X-100 and then covered with a 0.4-ml cushion of 7.5% sucrose in STE. The HIV-1 positive and negative samples, concentrated through 30% sucrose and resuspended in STE (0.3 ml) were carefully layered on top of the 7.5% sucrose layer and centrifuged in a Type 100 Ti rotor (Beckman Coulter) at 100,000 x g and 4°C for 16–18 h. The pellets were re-suspended in 26 µl of STE buffer and replaced to polypropylene, non-siliconized Eppendorf microtubes; 4 µl aliquots were set aside for the p24CA ELISA assay. The CA p24₄₅₀-GA₈-normalized suspensions of HIV-1 cores and control suspensions were subjected to SDS-PAGE protein separation for subsequent LC-MS/MS analysis.
Western blotting, or to In-solution protein digestion with trypsin for the LC-MS/MS analysis of unseparated protein samples.

In order to test purity of the “spin-thru” isolated cores from undestroyed viral particles, 400 μl aliquots of the suspensions of viral cores and concentrated whole virions were separately subjected to centrifugation in a 30-70% sucrose gradient for 5 h at 125,000×g and 4°C in a SW-60Ti rotor (Beckman Coulter). Ten fractions of the gradient (each 400 μl) were then collected from the bottom of the tubes and densities were determined. All fractions were dialyzed versus 1 L of ice-cold PBS using Tube-O-DIALYZER 1 kDa MEDI Kit (G Biosciences, St. Louis, MO) according to the manufacturer’s protocol and then applied for p24 enzyme-linked immunosorbent assay using Alliance HIV-1 p24 ELISA Kit (PerkinElmer, Waltham, MA).

Additionally, electron microscopy (EM) was applied to test purity of the viral and core preparations. For EM, the virions concentrated through 30% sucrose and “spin-thru”-purified core preparations were resuspended in 20 μl of STE buffer, incubated 20 minutes on formware carbon film-coated 100 square mesh nickel grids (Electron Microscopic Sciences) at room temperature, and then incubated with 4% glutaraldehyde fixing solution for 10 minutes. After five-time wash in molecular grade water (Mediatech, Manassas, VA), samples were stained with 2% uranyl acetate. For analysis of virion structure, fractions of the gradient (each 400 μl) were then collected from the bottom of the tubes and densities were determined. All fractions were dialyzed versus 1 L of ice-cold PBS using Tube-O-DIALYZER 1 kDa MEDI Kit (G Biosciences, St. Louis, MO) according to the manufacturer’s protocol and then applied for p24 enzyme-linked immunosorbent assay using Alliance HIV-1 p24 ELISA Kit (PerkinElmer, Waltham, MA).

For “in-solution” protein digestion, the suspensions of HIV-1 cores after “spin-thru” centrifugation were treated with 10 mM DTT (60°C for 1 h) and 150 mM iodoacetamide (1 h at room temperature in the dark) in 20 μl of 0.1% trifluoroacetic acid (TFA) in 80% ACN and subjected to HPLC separation. The resulted peptides were extracted three times: (1) with 25 mM of NH4HCO3; ACN (1:1); (2) 5% formic acid (FA); (3) 5% FA:ACN (1:1). After pooling all the extracts together, samples were purified through ZipTip pipette tips C18 (Millipore), eluted with 30 μl of 0.1% trifluoroacetic acid (TFA) in 80% ACN and subjected to HPLC separation and MS/MS analysis.

For Western blot analysis, the aliquots of the lysates of HIV-1 infected Sup-T1 and THP1 cells, the virus samples and culture media from non-infected cells taken before and after the isolation were subjected to SDS-PAGE, subsequently transferred to a PVDF membrane and then detected using anti-HIV-1 p24 (24–3) mouse monoclonal antibody and human HIV immunoglobulin (HIV-IgG) from NIH AIDS Research & Reference Reagent Program; anti-RNA Helicase A (ab70777) rabbit polyclonal antibody from Abcam; anti-CD45, clone F10-89-4 monoclonal antibody from Millipore (Temecula, CA); monoclonal anti-Actin clone AC-40 from Sigma; anti-β tubulin (D-10), anti-Reptin 52 (D-6), anti-Ku80 (B-1) and anti-MCM5 (G-1) mouse monoclonal antibodies from SantaCruz Biotechnology (Santa Cruz, CA); anti-HELIC2 (N-20) and anti-Pontin 52 (N-15) goat polyclonal antibodies also from Santa Cruz. Specific bands were visualized by ECL (Thermo Scientific, Rockford, IL). Quantification of the Western blotting results was performed using ImageJ software.
HPLC-MS/MS of tryptic digests and database search

The peptides in each sample were separated by microcapillary reversed-phase liquid chromatography (HPLC), coupled online to an ion trap mass spectrometer Thermo LTQ Orbitrap XL. The mass spectrometer was operated in a data-dependent MS/MS mode using a normalized collision-induced dissociation (CID) energy of 35%. The CID spectra were compared against those of the EMBL non-redundant protein database. Only peptides having cross-correlation ($X_{corr}$) cutoffs of 2.6 for [M + 2 H]$^{2+}$, 3.0 for [M + 3 H]$^{3+}$ and higher charge state were considered. These SEQUEST criteria thresholds resulted in a 1-2% of False Discovery Rate. The proteome analysis of the spectra was made by Proteome Discoverer 1.2 software (Thermo Fisher Scientific). The protein profiles of the samples of viral cores were compared with identically prepared samples from non-infected cells. The sub-cellular localization and function of each filtered protein was determined using gene ontology (GO) information obtained from cross-referencing each protein’s Swiss-Prot accession number to the GO localization information available on the NCBI protein database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=Protein) and The Human Protein Atlas database (www.proteomAtlas.org). The involvement of the proteins in known cellular pathways associated with major biological processes such as cell cycle, intracytoplasmic transport, cytoplasm organization, nuclear transport, chromatin structure maintenance/regulation, RNA splicing and reorganization, transcription, apoptosis, proteasomal degradation, etc. were assessed using NCBI RefSeq database (www.ncbi.nlm.nih.gov/RefSeq/) and DAVID Bioinformatics Resources 6.7 (NIAID NIH) (http://david.abcc.ncifcrf.gov).

RNA purification and RT reaction

RNA was purified from suspensions of “spin-thru” purified viral cores containing 250 ng of p24CA using TRI Reagent-LS (MRC, Cincinnati, OH) according to the manufacturer’s protocol. A total of 0.5 μg of RNA from the RNA fraction was treated with 0.25 mg/ml DNase I RNase-free (Roche, Mannheim, Germany) for 60 minutes in the presence of 5 mM MgCl$_2$, followed by the heat inactivation at 65°C for 15 minutes. A 250 ng aliquot of total RNA was used to generate cDNA with the GoScript Reverse Transcription System (Promega, Madison, WI) using oligo-dT reverse primers.

DNA isolation and quantitative real-time PCR

Lysates of HIV-1 infected (72 h p.i.) Sup-T1, activated and non-activated THP1 cells were normalized to the total protein count using DC Protein Assay (BioRad) following manufacturer’s protocol. The total DNA was isolated using an IsoQuick Nucleic Acid Extraction Kit (ISC BioExpress, Kaysville, UT) following manufacturer’s recommendations. After isolation, the cellular DNA samples were analyzed by quantitative TaqMan real-time PCR to quantify chromosomal DNA. Set of primers specific for the β-globin gene has been used: forward primer BGF1 (5’-CAACCTCAAACAGACACATGG-3’), reverse primer BGR1 (5’-TCCACGTTACCTTGGCC-3’), and probe BGX1 (5’-FAM-CTCTGAGGAAGATTCTGCCGTATCTGC-3’). The 0.2 μl aliquots of RT reaction mixtures of the RNA samples from isolated viral cores (see above) were diluted to 10-fold and 100-fold and subjected to quantitative real-time PCR analysis with the set of primers specific for late HIV-1 reverse transcription product as described earlier [152]. The primers FOR-LATE (5’-TGTGTGCCCCTTCTTGTTGT-3’), REV-LATE (5’-GAGTCCTCGTCTCGAGAGATC-3’), and probe Lt-LTR-Prb (5’-FAM-CAGTGGCGCCCGAACAGGG-TAMRA-3’) recognized the positive-strand DNA, specific for the U5-Ψ LTR region. PCR reactions were performed with PerfeCta qPCR FastMix, UNG (Quanta Biosciences, Gaithersburg, MD) using 300 nM of each primer and 200 nM of probe according to the manufacturer protocol. Serial dilutions of DNA from 8E5 cells (CEM cell line containing a single copy of HIV-1 LAV provirus per cell) were used as the quantitative standards. Real-time PCR reactions were carried out at least in triplicate using the PTC-200 Peltier Thermal Cycler with Chromo4 Continuous Fluorescence Detector (both from MJ Research) and Opticon Monitor 2.03 software.

Additional file

**Additional file 1: Table S1:** Overlapping and unique high scored cellular proteins within viral cores isolated from the virus produced by Sup-T1 cells infected with HIV-1 NL4-3 strain pseudotyped with MLV Env (blue symbols) or VSV-G (green symbols) envelope glycoproteins.

Competing interests

The authors declare that they have no competing interests.

Author’s contributions

SS participated in the design of experiments, carried out most of the experiments, prepared samples for mass spectrometry and electron microscopy, analyzed data and contributed to manuscript preparation. YO performed LC-MS/MS data collection and analyzed raw mass spectrometry data. SN participated in the design of the study, supervised LC-MS/MS experiments and contributed to drafting of the manuscript. MB participated in the study design and coordination and contributed to manuscript preparation. SI conceived of the study, designed and coordinated experiments, participated in data analysis and prepared the manuscript. All authors read and approved the final manuscript.

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