Identification of the HIV-1 NC binding interface in Alix Bro1 reveals a role for RNA

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Abstract:

HIV-1 recruits members of ESCRT, the cell membrane fission machinery that promote virus exit. HIV-1 Gag protein gains access to ESCRT directly by binding Alix, an ESCRT-associated protein that promotes budding. The Alix Bro1 and V domains bind Gag NC and p6 regions, respectively. Whereas the V-p6 binding and function are well characterized, residues in Bro1 that interact with NC and their functional contribution to Alix-mediated HIV-1 budding are unknown. We mapped Bro1 residues that constitute NC binding interface and found they are critical for function. Intriguingly, residues involved in interactions on both sides of the Bro1-NC interface are positively charged, suggesting the involvement of a negatively charged cellular factor serving as a bridge. Nuclease treatment eliminated Bro1-NC interactions revealing involvement of RNA. These findings establish a direct role for NC in mediating interactions with ESCRT necessary for virus release, and report the first evidence of RNA involvement in such recruitments.

(151 words)
**Introduction:**

HIV-1 usurps members of the host cell fission machinery to promote virus release. Two conserved sequences located within the C-terminal p6 domain of Gag: PTAP and LYPXnL named Late (L) domains are utilized to fulfill such functions. They bind Tsg101 and Alix, respectively (14, 37, 40), two host cellular proteins that initiate a series of sequential interactions leading to the recruitment of members of the endosomal sorting complex required for transport (ESCRT) pathway (5, 9, 30). The latter is comprised of three multi-protein complexes named ESCRT-I, II and III that facilitate membrane-modeling events critical for multivesicular bodies (MVB) generation (2, 3), cytokinesis (7) and autophagy (32).

Tsg101 functions in HIV-1 release as part of ESCRT-I (26) and mediate access to members of ESCRT-III, the charged MVB proteins CHMP2 and CHMP4 isoforms as well as the VPS4 ATPase (29, 38, 41). Whereas interactions that link Tsg101 (and ESCRT-I) to ESCRT-III are still unknown, Alix binds CHMP4 isoforms directly thus linking Gag to ESCRT-III members (12, 19, 37, 39). Although the Tsg101/PTAP pathway is considered predominant in HIV-1 release, the Alix/LYPXnL pathway is also functional in 293T cells and appears to be more efficient in T lymphocytes (11-13, 37, 39). This pathway is also sufficient to drive the release of the Equine Infectious Anemia Virus (EIAV) (8, 37), a lentivirus that relies solely on cellular Alix for virus budding.

Alix structure revealed two well-ordered domains: the N-terminal boomerang-shaped Bro1 and the central V-shaped domains (12, 21); they interact with the NC and p6 domains of HIV-1 Gag, respectively (10-12, 31, 37). The binding interface between the LYPXnL motif and the V domain and its functional role has been well characterized (12). In contrast, residues in the Alix Bro1 domain that mediate interactions with NC (10, 11, 31) and their role in virus release are not known. We performed a mutational analysis and used a combination of binding and functional assays to map the Bro1-NC interface and examine its role in virus budding. Residues delineating the interface have been identified and their nature suggested a critical role for RNA in Bro1-NC interactions.
Material and Methods:

Proviral and expression vectors

We used the wild-type molecular clones of HIV-1 pNL4-3 (1) and EIAV UK (24). The L-domain HIV-1 mutant PTAP- and the PTAP-RKI and PTAP-RKII mutants were previously described (15). The HA-tagged version of Alix full length, Alix Bro1 domain and the Flag-tagged CHMP4B expression constructs were previously described (10, 36). The Alix full length was also cloned in pEXPR-IBA105 (IBA, BioTAGnology, Göttingen, Germany) between EcoR1 and Not1 sites to obtain the Strep-tagged version. Point mutations were introduced in Alix Bro1 domain using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, United States). Seventeen silent mutations that render HA-Alix resistant to siRNA (denoted Alix^RR in Figure 3B) were introduced into the wild-type Alix coding region. Residues in Alix Bro1 were selected following SAS analysis using the Alix Bro1 domain crystal structure (PDB 2OEW). SAS values were calculated using the AREAIMOL program (23, 33) that is part of the CCP4 suite (43). The N-terminal Flag-tagged Nedd4.1 was described in Sette et al 2010 (35) and The C-terminal HA-tagged APOBEC3G in (16). The Glutathione S-Transferase (GST) fusion plasmids encoding the EIAV UK NC-p9 region, the HIV-1 NC-p6 and its mutants NC_RKI-p6 and NC_RKII-p6 were previously described (4, 10, 36).

Virus release analysis

293T cells were maintained and transfected as previously described (36). Twenty-four hours after transfection, cells and culture media were harvested and their protein content was analyzed using the protocol previously described (36). HIV-1 proteins were detected using an anti-HIV-1 p24 monoclonal antibody (clone 183-H12-5C) or NEA-9306. EIAV proteins were detected using a horse anti-EIAV serum (28). EIAV release ratio (values in percentage) was calculated using the following: release ratio = virus-associated Gag/ cell-associated Gag, as determined by densitometry analysis of Western blot films using ImageJ software (W. S. Rasband, NIH, Bethesda, MD; http://rsb.info.nih.gov/ij). Alix, Bro1 domain and the respectively mutants were detected using an anti-HA monoclonal antibodies (Sigma, St. Louis, MO).
**Infectivity Assay**

Viral infectivity was quantified using TZM-bl cells assay (42). HeLa TZM-bl cells were obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. They were seeded (2×10⁴ cells) in 96-well plates and the following day infected in triplicate with HIV-1 PTAP- rescued virus stocks in presence of 20 μg/ml DEAE-dextran (Sigma, St. Louis, MO). After 48 hours, cells were assayed for luciferase activity using the Steady-Glo™ Reagent kit (Promega, Madison, WI) according to manufacturer’s instructions. Luminescence was quantified using a microplate reader (Turner BioSystems, Sunnyvale, CA).

**Immunoprecipitation Assays**

These assays were conducted as previously described (36). Immunoprecipitation complexes and cell lysates (input fractions) were analyzed by SDS-PAGE and western blot using anti-HA and anti-Flag M2 antibodies (Sigma, St. Louis, MO). To perform the immunoprecipitation assays using the Strep tagged proteins, cell lysates were incubated with Strep-Tactin Sepharose (IBA GmbH, Gottingen, Germany) for 2h at 4°C. The matrix was washed five times in lysis buffer and eluted using SDS-PAGE loading buffer.

**Pull down assays and Nuclease treatments**

The empty pGEX vector or that carrying the coding sequences of HIV-1 NC-p6 and EIAV NC-p9 were expressed in BL21(DE3) pLysS E. coli (Stratagene) and their interaction with HA-Bro1 and its mutants were examined in GST-pull down assays following the protocol previously described (36). Where indicated, protein complexes captured on beads were incubated for 30 min at 37°C in presence or absence of 50 μg/ml RNase A (EMD, Chemicals, Inc. San Diego, CA) or 75 U (0.75U/ul) benzonase/nuclease (Novagen) in benzonase buffer (1.2 mM MgCl₂, 50 mM Tris-HCl [pH 8.0]). Eluted complexes and cell lysates (input fractions) were analyzed by SDS-PAGE and western blot using the indicated antibodies.

**Alix knockdown and reconstitution**
293T cells (2.5 x 10⁶ cells/ml) were transfected with 250 pmol of a mixture of two RNAi oligonucleotides (Invitrogen life technologies, Grand Island, NY) against cellular Alix. After 36h, cells were cotransfected with the same amount of RNAi and 500 ng of EIAV UK proviral DNA and 150 ng of HA-Alix or HA-Alix mutants RNAi resistant (RR). Cells and virus were harvested and processed as described above.

Results:
Identification of the NC-Bro1 binding interface

The N-terminal Alix Bro1 domain binds NC while the central V domain binds the short conserved sequence LYPXₙL Late (L) domain in the p6 region, respectively (10-12, 31). Whereas binding determinants and role of the latter have been extensively studied and characterized (12), those of the former are not known. We sought to map residues in the Alix Bro1 domain that mediate binding to HIV-1 and EIAV NC and examined their role in virus release. Residues in Bro1 that are accessible to solvent and therefore likely to be exposed and engaged in protein-protein interactions have been selected using solvent accessible surface (SAS) prediction for mutational analysis. More than 20 residues displaying high SAS values (see material and methods) – indicating exposure -- were found in the first 202 residues of Alix Bro1 domain, a fragment sufficient to bind NC (10). Specifically, residues in this region were selected for mutational and further analysis based on several criteria including: i) high SAS values in comparison to residues known to be exposed in the Alix Bro1 domain such as those belonging to the Phe105 loop (36) and ii) the ability to bind CHMP4. Next mutants were assessed for capture of NC and the data obtained was summarized in Table 1. We found that substitutions of either Q8 [glutamine residue in position 8 in the Bro1 sequence] and K11 residues (Q8K mutant), or K48, R51 and R56 residues (K2R mutant) to alanines, caused a significant inhibition of the Bro1 domain interactions with NC-p6 domains in GST pull-down assays (Figure 1A, lanes 4 and 6). Binding became undetectable when
both sets of mutations were introduced in Bro1 (Q8K/K2R mutant) (Figure 1A, lane 8; see Table 1 for mutant nomenclature). Similar results were obtained with EIAV NC-p9 protein (Figure 1B). NC-Bro1 interactions had no effect on Alix V-p6 interactions since all defective Alix Bro1 mutants retained interactions with NC-p6 fragment (Figure 1C). Furthermore, NC-Bro1 interactions were inhibited specifically since all Alix mutants retained binding to their natural cellular partner CHMP4B, suggesting proper folding (Figure 2A and Table 1). Since Alix also binds the ubiquitin ligase Nedd4-1--its cellular partner important for function (35)--we tested the effect of mutations in Bro1 domain on Alix interactions with Nedd4-1. Q8K, K2R and Q8K/K2R mutants displayed a Nedd4-1 interaction pattern comparable to their wt Alix counterpart (Figure 2B). Together these results indicated that residues Q8, K11, K48, R51, R56 and K60 are part of the Bro1-NC binding interface.

**Disruption of Bro1-NC binding inhibits Alix function in virus release**

To examine the functional significance of the Bro1-NC interface, we disrupted its residues and assessed the effect on HIV-1 release using a virus rescue assay (12) (39). Whereas ectopic expression of wild type (WT) Alix rescued HIV-1 lacking access to ESCRT-I (HIV-1 PTAP- mutant) (Figure 3A, lanes 1-2), Alix mutants with a compromised NC binding interface displayed diminished virus stimulation abilities that were proportional to the number of mutated residues (Figure 3A, lanes 5-8). The effect of the Q8K/K2RK mutation on viral release was comparable to that of the I212D, which disrupts Alix binding to the essential CHMP4 factors (compare lanes 3 and 8). Similar results were obtained when Alix mutants were used to functionally replace cellular Alix and facilitate EIAV release (Figure 3B). Placing residues involved in NC-Bro1 interactions in the Bro1 crystal structure (12) revealed a defined interface that exposes a cluster of basic residues on one side of the boomerang (Figure 3C). Together these data draw a direct correlation between the Bro1 domain ability to bind NC and Alix function in virus release and suggest the first direct functional link between Bro1-NC interactions and virus release.

**Bro1-NC binding involves RNA**
Mapping of the Bro1-NC binding interface revealed that all residues involved are positively charged (Figure 3C). This finding was surprising because Bro1-NC interactions were previously reported to be RNA independent (31). Moreover we previously showed that residues in NC involved in association with the Alix Bro1 domain are also positively charged. Indeed, NC mutant mutants RKI and RKII carrying substitutions of lysine and arginine (Figure 4A) to alanine residues ceased to bind Bro1 and function in virus release [Figure 4B and C and (4, 10, 11, 36)] and these budding defects were alleviated by providing Gag with a parallel access via Nedd4.2 expression to the scission-inducing members of the ESCRT pathway (11). This indicated that mutations of basic residues in NC interfered with NC-Bro1 interactions and interrupted Alix function in scission events. Involvement of positively charged residues on both NC and Bro1 sides raised the possibility that their interaction might involve a negatively charged cellular factor. Since NC binds and incorporates viral (as well as cellular) RNA into virions (25), we elected to test its potential involvement in Bro1-NC interaction. GST-NC-p6 binding to Alix Bro1 domain was tested in presence or absence of nuclease. Whereas GST-NC-p6 captured the Bro1 domain as expected (Figure 5A left, lane 3), treatment of these complexes with nuclease (lane 4) or RNAse A (Figure 5B, lanes 5 and 6) abrogated binding. Similarly, GST-NC-p6 binding to APOBEC3G (34, 44), a host cell protein known to require RNA for interactions with NC (6, 20), was equally sensitive to nuclease treatment (Figure 5A right, lane 3) or RNAse treatment (data not shown). Similar results were obtained with the EIAV NC-p9 construct (Figure 5A center). Collectively, this data indicate that RNA is involved and important for NC-Bro1 interactions.

Discussion

HIV-1 interactions with the ESCRT-associated protein Alix is sufficient for virus production from T-lymphocytes (11, 13). Such recruitment provides the only direct access to the cell membrane fission machinery. Indeed, the Alix Bro1 domain binds
CHMP4 isoforms, which are members of ESCRT-III that play a key role in ringing and severing viral budding necks (29). Two interactions have been identified between Gag and Alix. The interaction between Gag p6 region and Alix V domain was identified and its role in virus release was clearly established (12, 37). The second interaction that links NC to the Alix Bro1 domain has been recently identified (10, 31). Here we identified residues in the Alix Bro1 domain that define the binding interface with NC and found that RNA is involved in such interactions.

Key determinants of NC-Bro1 interactions: Since the discovery of NC-Bro1 interactions, questions regarding their role in virus release arose. Several lines of evidence underscored the importance of NC-Bro1 interactions including the findings that a functional NC is required for Alix mediated virus release (4, 10, 11, 31) and mutant NC viruses that fail to bind the Bro1 domain were also defective in virus budding (4, 10, 11). The role of Bro1-NC interactions in virus release was further strengthened by the identification of residues that mediate binding. Their mutational analysis revealed that those critical for binding NC lie within the first beta sheet (β1) and the second alpha helix (α2) of Bro1. Residues interacting with NC are positively charged and cluster in a well-defined interface, whose disruption was sufficient to cause a dramatic loss of binding and Alix-mediated virus release (Figure 1 and 3). This provides direct evidence of a requirement for contact between Bro1 and NC to achieve efficient virus release.

Why Alix requires binding to both NC and p6 to promote virus exit is not clear [12, 39 and Figure 2]. However clues into the necessity for interactions between NC and Bro1 come from the latter’s ability to recruit CHMP4, which links Gag directly to membrane fission-inducing ESCRT-III members. Consistent with this notion, Bro1 can function as the smallest unit of Alix as its ectopic expression promoted virus release, provided it retained binding to both NC and CHMP4 (4, 10, 11). Moreover, an Alix mutant lacking binding to either NC or p6 failed to replace cellular Alix and promote EIAV budding (Figure 3), further reaffirming a key functional role for Bro1-NC interactions in virus release.

The contribution of Bro1 versus V binding to Gag in Alix function during budding appears to differ. Indeed, disruption of interactions with V was more detrimental to virus release than that of Bro1, since the V-binding devoid AlixF676D exhibited less
activity in virus release than the NC binding-defective AlixQ8/K2RK (Figure 3). Conversely, the latter’s defect mirrors interference with CHMP4 recruitment (I212D mutant) as Alix mutants lacking either determinants (Figure 3) displayed comparable virus release defects. Interestingly, AlixF676D lost the ability to locate to sites of assembly at the membrane (18) despite retaining a functional NC binding interface, suggesting NC-Bro1 interactions might take place only later in the viral egress process (i.e.: in the budding neck), a role in agreement with its functional importance (Figure 3). These findings suggest a model for Alix function in two steps (Figure 6). First, Alix is anchored to sites of assembly via p6-V interactions and Bro1 seems to be unavailable to capture CHMP4, possibly due to a structural masking (45). Next, Bro1 binds NC—possibly in the budding neck-- and becomes available to recruit CHMP4 during budding, a sequence of events that fits with the recent visualization of CHMP4 at the membrane once Gag assembly is complete (18).

RNA bridges interactions between NC and Bro1: Identification of NC-binding interface in Bro1 revealed that residues involved are positively charged and delineate a well-defined interface on one side of the Bro1 boomerang domain (Figure 3C). Moreover, mutation of basic residues in NC also eliminated binding to Bro1 (4, 10, 36), implying the involvement of a negatively charged factor. RNA however was excluded from Bro1-NC interactions in a previous report (31). This discrepancy is not clear but could be due to the method employed to capture Bro1 (see materials and methods for buffer composition in salt and detergent) and/or the fragment used. Indeed, Popov et al., used NC-p1 domains to capture Alix whereas we used NC-p1-p6 (Figure 5). RNA was nevertheless the obvious candidate to bridge interactions Bro1 and NC since the latter binds genomic RNA during assembly (17, 22). In agreement with this, RNA involvement was confirmed as nuclease and RNase treatments abrogated NC-Bro1 binding. Similarly, mutations of basic residues in NC—involvded in RNA recruitment—also eliminated NC-Bro1 interactions (Figure 5) and brought Alix function to a near halt (10, 35). Recent reports employing high-resolution imaging suggested that genomic RNA localizes to the plasma membrane in the initial steps of assembly, before a Gag nascent complex became “visible” (17, 22) whereas recruitment of Alix accompanies Gag accumulation during the early steps assembly at cell membrane (18). These observations and our findings suggest...
that Gag-RNA assembly complexes recruit Alix, which fits with the involvement of RNA in interactions with the ESCRT-binding Alix Bro1 domain and NC. The requirement for RNA in Alix Bro1-NC interactions is not surprising as NC binding of RNA early in viral nascent particle is critical for its three dimensional structure/folding (25), a crucial step for particle assembly as well as the subsequent steps of recruitment and utilization of ESCRT components necessary for virus budding and exit (this study).

In summary, we identified NC binding interface in Alix Bro1. The nature of residues involved revealed a critical role for RNA. Since interactions between Gag, genomic RNA and Alix precede CHMP4 recruitment to sites of assembly (17, 18), we propose a model (Figure 6 right) in which an “NC-RNA-Bro1” nucleoprotein complex recruits CHMP4 during budding (in the budding neck) in order for virus exit to proceed.

Acknowledgements:

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Figure legends:

Figure 1: Mapping of Alix Bro1 residues involved in the interaction with HIV-1 and EIAV NC domains. (A and B) GST, GST-NCp6 and GST-NCp9 fusion proteins were expressed in *E. coli*, captured on Glutathione conjugated beads and then subsequently incubated with lysates from 293T cells expressing WT HA-tagged Bro1 domain or the indicated Bro1 mutants (Q8K, K2R, Q8/K2R). Captured proteins and cell lysates were analyzed by SDS-PAGE and western blot. GST fusion proteins were visualized by Coomassie blue staining. (C) Mutations that compromise the NC-binding interface in Bro1 do not affect Alix V domain interaction with HIV-1 p6. Pull-down assays were performed as described above with the only difference that full-length HA-tagged Alix WT and the indicated mutants were used instead of the isolated Bro1 domain.

Figure 2: Alix Bro1 mutants retain binding to their natural cellular partners. (A) Alix Bro1 mutants bind CHMP4B. 293T cells were co-transfected with Flag-tagged CHMP4B alone (lane 1), or in combination with HA-tagged WT Bro1 (lane 2), or the indicated mutant (lanes 3–6). Cells were lysed in RIPA buffer and clear lysates were incubated with anti-HA antibody-conjugated beads. Both input and immunoprecipitated complexes were analyzed by SDS-PAGE and western blot using the indicated antibody. (B) Alix Bro1 mutants bind Nedd4-1. 293T cells were co-transfected with Flag-tagged Nedd4-1 alone (lane 1), or in combination with Strep-tagged WT Alix (lane 2), or the indicated mutant (lanes 3–5). Cells were lysed in RIPA buffer and clear lysates were incubated with Strep-Tactin Sepharose. The input and the purified complexes were probed with the anti-Flag and anti-Alix antibodies.

Figure 3: Alix Bro1 interface involved in NC binding plays a critical role in virus release. (A) The Alix Bro1 mutants with a compromised NC binding interface show a decreased ability to stimulate the release of the HIV-1 PTAP-mutant. 293T cells were transfected with HIV-1 PTAP-proviral DNA alone (lane 1), with WT HA-tagged Alix (lane 2) or the indicated Alix mutants (lanes 3–8). Cells and viruses were collected 24h post-transfection and their protein content was analyzed by SDS-PAGE and western
blot using the indicated antibodies. Release of infectious viral particles was quantified using HeLa TZM-bl assays from five independent experiments and expressed relative to WT Alix (lane 2). Error bars represent standard deviations (SD). (B) An intact NC binding interface is required for Alix function in EIAV release. 293T cells were transfected twice with Alix RNAi oligonucleotides at 36h intervals. At the second transfection, cells were co-transfected with EIAV provirus alone (lanes 1 and 2), with RNAi resistant (RR) version of Alix WT (lane 3) or the indicated mutants (lanes 4-6). Cells and viruses were collected 24h post-transfection and their protein content was analyzed by SDS-PAGE and western blot using the indicated antibodies. Release ratios were calculated as described in Materials and Methods from 3 independent experiments and expressed relative to WT Alix (lane 3), ± SD. (C) Alix Bro1 residues engaged in NC interaction define a new functional interface. Residues Q8, K11, K48, R51, R56, K60 placed in the Alix Bro1 domain crystal structure (2OEW) cluster within a positively-charged exposed surface (shown in red) on one side of the Bro1 domain. For reference, the Phe105 residue [required for Alix function in HIV-1 release, (36)] and the CHMP4B binding interface (27) are shown in green and yellow, respectively.

Figure 4: Basic residues in NC are required for the interaction with Alix Bro1 domain. (A) Schematic representation of the HIV-1 NC domain (amino acids 1 to 55). Lysine (K) and arginine (R) residues replaced with alanine in the RKI and RKII mutants are circled and underlined, respectively. (B) Mutation of basic residues at the N- or C-terminal of NC prevents NC-Bro1 interaction. GST, GST-NCp6, GST-NCp6RKI or GST-NCp6RKII fusion proteins were expressed in E. coli, captured on Glutathione conjugated beads and then subsequently incubated with lysates from 293T cells expressing WT HA-Bro1 domain. Captured proteins and cell lysates were analyzed by SDS-PAGE and western blot using an anti-HA antibody. GST fusion proteins were visualized by Coomassie blue staining. (C) Alix function in HIV-1 release requires an intact NC domain. 293T cells were transfected with HIV-1 PTAP- proviral DNA harboring a WT NC (lanes 1-2) or the indicated NC mutant (lanes 3-6) either alone (lanes 1, 3 and 5) or with WT HA-tagged Alix (lanes 2, 4 and 6). Cells and viruses were collected 24h post-transfection and their protein content was analyzed by SDS-PAGE and western blot using the indicated antibodies. Release of infectious viral particles was quantified using HeLa TZM-bl assays from five independent experiments and expressed relative to WT Alix (lane 2). Error bars represent standard deviations (SD).
western blot using the indicated antibodies. Release of viral particles was quantified from two independent experiments.

Figure 5: RNA is involved in the Alix Bro1-NC interaction. (A) GST, GST-NCp6 (left panel) and GST-NCp9 (center panel) fusion proteins expressed in E. coli and captured on glutathione beads were incubated with lysates from 293T cells expressing HA-tagged WT Bro1 domain or APOBEC3G (right panel), followed by incubation with or without benzonase/nuclease. (B) GST and GST-NCp6 fusion proteins purified by glutathione beads were incubated with lysates from 293T cells expressing HA-tagged WT Bro1, followed by treatment with or without benzonase (lanes 3 and 4) or RNAseA (lanes 5 and 6). In both experiments, captured proteins and cell lysates were analyzed by SDS-PAGE and western blot. GST fusion proteins were visualized by Coomassie blue staining.

Figure 6: Schematic representation of a two steps model for Alix function in virus assembly and budding. In a first step, Alix is recruited by Gag to the plasma membrane during assembly through interaction between its V-domain and the (L)YPXnL motif in p6 and Alix is thus anchored at the membrane (left panel), whether NC binds Bro1 during this step or not is not clear since it was insufficient to locate Alix to the membrane when the LYPXnL was disrupted (18). In a second step, we propose that the NC-Bro1 interaction occurs in the budding neck and is followed (or accompanied) with the recruitment of CHMP4B (right panel). Basic residues found in both NC and Bro1 proteins mediate binding and RNA (in red) plays the role of the negatively charged factor that bridges this interaction.
Table 1: List and characterization of Alix-Bro1 mutants tested in this study

Residues belonging to the Phe105 loop (36) are in grey box. ND: not determined because of low or no expression. Data for mutants in bold characters are shown in Figures 1, 2 and 3. (+) and (-) symbols indicate positive and negative (absence) binding, respectively.

SAS: Solvent Accessible Surface

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Fig 1
Fig 2
Fig 3
Fig 4
Fig 5
During Assembly

HIV-1 Gag NC PTAP LYPX

Bro1 Alix V Domain

PRD Zf1 Zf2

+++

HIV-1 Gag NC PTAP LYPX

 Bro1 Alix V Domain

PRD Zf1 Zf2

+++

VPS4 ESCRT-III CHMP4B

Fig 6

During Assembly

In the budding neck