Generation and validation of a highly sensitive bioluminescent HIV-1 reporter vector that simplifies measurement of virus release

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**SUBJECT AREAS**

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**KEYWORDS**

Reporter virus, nano-luciferase, virus release
Abstract
Background The continued persistence of HIV-1 as a public health concern due to the lack of a cure calls for the development of new tools for studying replication of the virus. Here, we used NanoLuc, a small and extremely bright luciferase protein, to develop an HIV-1 bioluminescent reporter virus that simplifies functional measurement of virus particle production.

Results The reporter virus encodes a Gag protein containing NanoLuc inserted between the matrix (MA) and capsid (CA) domains of Gag, thereby generating virus particles that package high levels of the NanoLuc reporter. We observe that inserting the NanoLuc protein within HIV-1 Gag has minimal impact on Gag expression and virus particle release. We show that the reporter virus recapitulates inhibition of HIV-1 particle release by Gag mutations, the restriction factor tetherin, and the small-molecule inhibitor amphotericin-B methyl ester.

Conclusion These results demonstrate that this vector will provide a simple and rapid tool for functional studies of virus particle assembly and release and high-throughput screening for cellular factors and small-molecules that promote or inhibit HIV-1 particle production.

Background
HIV-1 remains a major global public health challenge despite the advances made in our understanding of its replication and in the development of antiviral drugs. The HIV field continues to require innovative research tools and techniques to provide new insights on virus-host interactions that can guide the development of novel therapies.

Reporter viruses are an example of innovative tools that have been used to study HIV-1 replication. These viruses are engineered to carry reporter genes that enable the detection and quantification of virus infection and replication. An early HIV-1 reporter virus encoded a firefly luciferase gene in place of the nef gene (1). Several more HIV-1 reporter viruses have been developed using this approach (2, 3). With this strategy, the reporter virus infects target cells and expresses the reporter protein, allowing for detection and quantification of virus infection. However, because the reporter protein is not packaged into progeny virions, the reporter cannot be used to detect virus released from the cell. Another strategy that has been used to generate HIV-1 reporter virus involves inserting the reporter
gene into the gene encoding the structural protein Gag, often between the matrix (MA) and capsid (CA) domains but in other regions of Gag as well (4-6). Fluorescent reporter viruses made using the latter strategy have been used to detect viral transfer to target cells. A dual reporter virus encoding both a fluorescent protein-tagged Gag and a second fluorescent protein in place of Nef was used to study dynamics of HIV-1 replication in single cell infections (7).

The HIV-1 reporter viruses available today are highly useful tools for studying the early stages of the HIV-1 replication cycle. The early phase of the virus replication cycle begins with virus attachment and entry and ends with integration of the newly synthesized viral DNA into the host cell genome; the late phase begins with viral gene expression and culminates in virus release and maturation. Viruses that express a reporter gene (e.g., luciferase or GFP) in place of nef are suitable for studying the early steps of HIV-1 infection leading up to viral gene expression but not later steps. Conversely, viruses with the reporter protein embedded in the Gag protein can be used to study the late stages of HIV-1 replication. Gag-GFP viruses, for example, can be used to track Gag trafficking but are not suitable for quantification of virus release from the cells. Additional strategies have been devised to engineer reporter viruses that carry the reporter protein, allowing quantification of released virus. These include co-packaging a Vpr-GFP fusion protein with HIV-1 Gag during virus assembly, leading to the production of viruses containing Vpr-GFP (8). Another is the expression of a membrane-anchored Gaussia luciferase upstream of the HIV-1 nef gene resulting in the labeling of the virus envelope with the reporter protein (9).

To enable simple and highly sensitive measurement of virus release from transfected cells, we generated HIV-1 reporter viruses in which Nano-luciferase (NLuc) was inserted between the MA and CA domains of Gag (Gag-iNLuc). NLuc is smaller (~19kDa) and brighter (~150-fold) than firefly (~61kDa) or Renilla (~36kDa) luciferases (10). We demonstrate that the HIV-1 Gag-iNLuc vector releases virus particles at similar levels to the WT HIV-1 molecular clone, enabling facile and highly sensitive detection of virus particle production from transfected cells. We further demonstrate the utility of the HIV-1 Gag-iNLuc vector as a functional tool to study HIV-1 release by using it to recapitulate disruption mediated by Gag mutations, a small-molecule inhibitor, and expression of the
restriction factor tetherin. We show that although the infectivity of the Gag-iNLuc reporter virus is impaired, infectivity can be rescued by complementing with the WT HIV-1 molecular clone. These results highlight the potential of Gag-iNLuc as a tool for high throughput screening of host factors and compounds that specifically target the late stages of the HIV-1 replication cycle.

Results

**Generation of bioluminescent HIV-1 reporter vectors.**

To generate an HIV-1 reporter virus that enables quantification of the reporter protein directly from virus supernatant, we employed the previously reported strategy (4, 5) of introducing the reporter gene between the MA and CA domains of Gag, a location shown to tolerate genetic insertions with minimal effects on Gag protein expression and processing (4). The NLuc gene was introduced between MA and CA domains of HIV-1 Gag flanked by PR cleavage sites at the N-terminus or C-terminus, or both termini of NLuc or not flanked by any PR cleavage site (Fig. 1A). Upon PR-mediated Gag cleavage, the resulting NLuc products generated from these vectors are the NLuc protein and MA-NLuc, NLuc-CA and MA-NLuc-CA fusion proteins, respectively (Fig. 1A).

To assess the effect of the NLuc insertion into HIV-1 Gag on Gag protein expression and PR-mediated Gag cleavage, we transfected the HIV-1 Gag-NLuc vectors into HEK293T cells and after 48hrs, lysed the cells and pelleted virus from the supernatant. We performed western blot analysis to evaluate the expression of HIV-1 Gag-NLuc fusion proteins in the cells and the pelleted virus. We observed no adverse effect on Gag expression and processing and, importantly, the expected Gag PR cleavage products were generated, including some cleavage intermediates (Fig. 1B). We also measured NLuc activity from the cell lysate and virus supernatant and observed that all the vectors yielded robust NLuc activity; i.e., up to 2.0x10^7 relative light units (RLUs) (Fig. 1C). Finally, we calculated virus release efficiency (VRE) from the same samples and found release efficiency was similar to that of WT Gag and the Gag-iGFP construct (Fig. 1D).

**HIV-1 Gag-iNLuc enables highly sensitive measurement of virus release.**
To assess the sensitivity in the detection of virus release using the HIV-1 Gag-iNLuc vector, we transfected HEK293T cells with either an NLuc expression vector, the WT HIV-1 molecular clone pNL4-3, or decreasing amounts of the HIV-1 Gag-iNLuc vector (i.e. 1.0, 0.5, 0.25, 0.125 and 0.0625mg). At 48hrs post-transfection, we lysed the cells and purified virions from the supernatant, analyzed Gag levels by western blot (Fig. 2A), and measured NLuc activity from the cell lysates and supernatants (Fig. 2B). We were able to detect NLuc signal under all conditions tested, including at the lowest DNA input at which virion-associated Gag was undetectable by western blot. pNL4-3 Gag-iNLuc vector-transfected cells produced significantly higher levels of NLuc activity in both the cell lysate (>10-fold) and supernatant (>1000-fold) relative to the NLuc expression vector control. This implies that the NLuc activity in the supernatant is derived from the NLuc protein released with the HIV-1 Gag during virus release. We also measured RT activity (Fig. 2C) and p24 protein levels (Fig. 2D) in the virus supernatant and correlated both with supernatant NLuc activity (Fig. 2E & F). We observed that the supernatant NLuc activity was positively correlated with RT activity and p24 abundance, further reinforcing the specificity of the assay.

**The defect in HIV-1 Gag-iNLuc particle infectivity can be rescued by co-expression with WT.**

We generated virus using either WT pNL4-3, the HIV-1 Gag-iGFP or the HIV-1 Gag-iNLuc vectors by transfecting them into HEK293T cells and collecting the supernatants containing the progeny virions at 48hrs post-transfection. We quantified the relative amounts of virus in the supernatant by RT activity (Fig. 3A). We observed that RT activity of supernatants from cells transfected with the HIV-1 Gag-iGFP and HIV-1 Gag-iNLuc vectors was about 2-fold less than that of supernatants from cells transfected with WT pNL4-3. To test the infectivity of the virions produced from the HIV-1 Gag-iNLuc vectors, we infected TZM-bl cells with the RT-normalized virus supernatants and measured the infectivity by quantifying the firefly luciferase activity (Fig. 3B). We observed that the HIV-1 Gag-iNLuc viruses were approximately 10-fold less infectious than the WT virus. We also transfected the SupT1 T-cell line with the HIV-1 Gag-iNLuc vectors and monitored virus replication
kinetics over several days and observed that replication was significantly impaired compared to the WT HIV-1 (data not shown). We generated viruses using the pNL4-3 Gag-iNLuc vector complemented with different ratios of the WT HIV-1 molecular clone pNL4-3 and tested their infectivity. We observed that infectivity of the viruses generated with the pNL4-3 Gag-iNLuc vector was rescued when complemented with the WT pNL4-3 vector. The infectivity increased with increasing pNL4-3:pNL4-3 Gag-iNLuc ratio; at ratios above 2:1 the infectivity was at WT HIV-1 levels (Fig. 3C).

**HIV-1 Gag-iNLuc provides a robust tool for quantifying virus release.**

To examine the utility of the HIV-1 Gag-iNLuc vector in functional assays for virus release, we constructed versions of the vector that lacked the PTAP motif in the p6 domain of the HIV-1 Gag protein (HIV-1 Gag-iNLuc-PTAP-) and the vpu gene (HIV-1 Gag-iNLuc-delVpu) by cloning the iNLuc cassette into previously reported PTAP- and delVpu HIV-1 molecular clones (11, 12). The p6 domain of HIV-1 Gag is required for virus release (12, 13) because of its interaction with the ESCRT machinery (14-17). Vpu is also required for HIV-1 release in the presence of the restriction factor tetherin (also known as BST-2), which blocks release of virions by tethering them to the plasma membrane (18). Vpu counteracts tetherin by targeting it for proteasomal degradation (19). We transfected HEK293T cells with the WT, PTAP- and delVpu versions of the HIV-1 Gag-iNLuc vector with or without varying amounts of tetherin expression vector. At 48hrs post-transfection, we measured NLuc activity in the cell lysates and supernatants. We observed a 2-fold decrease in the NLuc activity in the supernatant of cells transfected with the PTAP- vs. the WT vector, but, as expected, no decrease in NLuc activity in the cell lysates. Likewise, co-transfection with a tetherin expression vector, but not an empty vector control, caused a 4- to 10-fold decrease in NLuc activity in the supernatant of cells transfected with the delVpu vector. The decrease in supernatant NLuc activity was proportional to the amount of tetherin vector transfected (Fig.4A and B). We performed western blot analysis of the cell lysates and the pelleted virions to analyze HIV-1 Gag expression. We observed that virus release measured by virion-associated p24 levels corresponded with the NLuc activity. Finally, we tested the utility of the HIV-1 Gag-iNLuc vector to detect impaired virus release induced by treatment of virus-producer cells
with amphotericin B methyl ester (AME), a compound that inhibits HIV-1 particle production (20). We transfected HEK293T cells with the HIV-1 Gag-iNLuc vector and at 24hrs post-transfection treated the cells with either vehicle or increasing amounts (5mM or 10mM) of AME. At 24hrs post-treatment, we collected the supernatant and measured NLuc activity. We observed a decrease in supernatant but not cell-associated NLuc activity in the presence of AME but not vehicle control. Again, the decrease in supernatant NLuc activity corresponded with reduced virion-associated p24 measured by western blot analysis. These results demonstrate that the Gag-iNLuc vector provides a highly sensitive and quantitative tool for measuring the effects of Gag mutations, host cell restriction factors, and small molecule inhibitors on HIV-1 particle assembly and release.

Methods

**Plasmids.**

The full-length HIV-1 molecular clone pNL4-3, a GFP-expressing HIV-1 molecular clone (pNL4-3Gag-iGFP), an HIV-1 molecular clone lacking the PTAP motif (pNL4-3PTAP-), a vpu-deleted HIV-1 molecular clone (pNL4-3delVpu) and an HA epitope-tagged tetherin expression plasmid (HA-Tetherin) were used in this study (5, 11, 12, 18, 21). The NanoLuc gene sequence was amplified by PCR from pUAS-NLuc (Addgene plasmid no. 87696) although the origin of the sequence is from pNL1.1 (Promega, Madison, WI) (22). The HIV-1 Gag-NLuc vectors were constructed by inserting the NLuc gene between the MA and CA domains of Gag flanked or not flanked by protease (PR) cleavage sites by overlap PCR. The PCR product generated encompassed the 5′UTR-MA-NLuc-CA with or without the PR cleavage sites SQNYPIVQ flanking the NLuc sequence. Where the SQNYPIVQ sequence was duplicated, synonymous changes were made on the DNA sequence to avoid duplicating the DNA sequence. The PCR product was then cloned in the HIV-1 molecular clone pNL4-3 via the BssHII and Spel restriction sites.

**Cells and Antibodies.**

HEK293T, HeLa and TZM-bl cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) with L-glutamine (Gibco), supplemented with 10% (vol/vol) fetal bovine serum (FBS), 100U/ml penicillin and
100mg/ml streptomycin at 37°C and 5% CO₂. TZM-bl are HeLa-derived indicator cells that express luciferase upon infection by HIV-1 (23). SupT1 CD4⁺ T cells were cultured in Rosewell Park Memorial Institute (RPMI) 1640 medium with L-glutamine (Corning, Corning, NY) supplemented with 10% (vol/vol) FBS, 100U/ml penicillin and 100mg/ml streptomycin. Pooled HIV-1 patient serum (HIV-Ig) was obtained from the NIH AIDS Reagent Program.

**Virus Release Assay.** HEK293T cells were plated in 12-well plates and transfected with proviral plasmids using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer’s protocol. At 48 hours post-transfection, virus particles were purified by filtering the supernatant through a 0.45mm filter and pelleted by ultracentrifugation. Cells and virion-containing pellets were resuspended in lysis buffer (30mM NaCl, 50mM Tris-HCl, pH 7.5, 0.5% Triton X-100, 10mM iodoacetamide, complete protease inhibitor cocktail (Roche Applied Science)), and cell- and virion-associated Gag proteins were analyzed by western blotting. The virus release efficiency was calculated as the amount of virion-associated Gag as a percentage of the total (cell- and virion-associated) Gag. Where the virus release inhibitor Amphotericin-B methyl ester (AME) (20) was used, HEK293T cells plated in a 12-well plate were transfected with the proviral plasmids as outlined above. The cell media were changed at 24 hours post-transfection and replaced with growth media containing AME and virus particles purified at 48 hours post-transfection.

**Virus infectivity Assay.** Viruses were generated by transfecting 2.5mg of proviral HIV-1 vectors into 0.5 x 10^6 HEK293T cells in 6-well plates. The virus-containing supernatants were collected at 48 hours post-transfection and assayed for reverse transcriptase (RT) activity as described (24). The supernatants were used to infect 1.0 x 10^4 TZM-bl cells per well in a 96-well plate, the volumes of the supernatants used for infection were normalized by RT activity. At 48 hours post-infection, the cells were lysed in Passive Lysis Buffer (Promega, Madison, WI), and the luciferase signal was measured using Britelite Plus (PerkinElmer, Waltham, MA). Infectivity is defined as the amount of luciferase
activity from the lysates of TZM-bl cells infected with RT-normalized virus supernatants (24).

**Spreading infection Assay.** 3.0 x 10^6 Jurkat cells were transfected with 3.0mg of HIV-1 proviral vector DNA. The cells and the DNA were mixed in 300ml of 0.8mg/ml DEAE-dextran solution and incubated for 15min at 37°C. The cells were washed in 4ml of STBS solution (25mM Tris-HCL [pH 7.4], 0.6mM Na$_2$HPO$_4$, 5mMKCL, 140mMNaCl, 0.7mM CaCl$_2$, 0.5mM MgCl$_2$), pelleted by centrifugation, resuspended in 3ml of RPMI media (with L-glutamine and supplemented with 10% FBS, 100U/ml penicillin and 100mg/ml streptomycin) and placed in culture. The transfected cells were split every other day starting from day 3 post-transfection and aliquots of the supernatants were collected for RT activity and Nano-Luciferase activity assays.

**Nano-Luciferase activity assay.** To measure Nano-Luciferase activity, 15ml of cell lysates and virus supernatant (passed through a 0.45mm filter) from cells transfected with the HIV-1 Gag-iNLuc vectors were diluted 1:2 in cell lysis buffer. Luciferase activity was measured using the Nano-Glo assay system (Promega, Madison, WI); the Nano-Glo luciferase assay substrate was mixed with the Nano-Glo luciferase assay buffer at a 1:50 ratio and equal volume of the mix was added to the samples. The samples were incubated at room temperature for 2-3 min and luminescence was detected in a plate reader (Perkin Elmer Wallac Microbeta 1450).

**HIV-1 p24 capture immunoassay.** The concentration of HIV-1 p24 in culture supernatant samples was determined by the AIDS and Cancer Virus Program using their in-house HIV-1 p24 antigen capture immunoassay.

**Discussion**

Almost four decades since the emergence of HIV-1 and the AIDS pandemic, the virus is still a global public health concern. Numerous technical innovations have led to detailed insights into the mechanism of HIV-1 replication. However, there still remain a number of knowledge gaps, particularly
regarding the late steps in the virus replication cycle, which are less amenable to high throughput analyses than the early steps. In this study, we set out to develop an HIV-1 reporter virus that would simplify and facilitate highly sensitive and quantitative measurements of the late stages of the virus replication cycle. We describe the generation of the HIV-1 Gag-iNLuc vector that expresses the NLuc reporter protein inserted into the viral Gag protein between the MA and CA domains. The Gag-NLuc fusion protein is expressed in the cell and released at similar levels to WT Gag, thereby enabling simple yet highly sensitive quantification of viral gene expression and virus particle production by measurement of the NLuc reporter protein bioluminescent activity in the cell lysates and supernatants.

The Gag-iNLuc vector reported here joins a number of other HIV-1 reporter vectors that are available to the field; these include Gag-fusion reporter vectors (4, 5, 8), nef-substituted reporter vectors (1-3, 8) and virus envelope/membrane-anchored reporter vectors (9). The Nef-substituted reporter vectors have the advantage of being replication competent, although with lesser efficiency than WT HIV-1. However, because the reporter protein is expressed only in target cells following infection and not packaged into virus particles, these vectors are suitable for detecting infection in target cells but not for measuring virus release directly from the supernatant. On the other hand, the Gag-fusion reporter vectors currently available use fluorescent reporter proteins which have low signal-to-background ratios making them less suitable for quantification of virus particles and studying virus release dynamics and so far have only been used to study intracellular Gag trafficking dynamics and as markers of infection in target cells. Because the Gag-iNLuc reporter vector encodes the bioluminescent NLuc protein, it is more suitable for quantification due to the high signal-to-background ratio of luminescence assays. Although, similar to the other Gag-fusion reporter vectors which are impaired in their infectivity, we demonstrated that the infectivity of the particles generated with the vector can be rescued by complementing it with the WT HIV-1 molecular clone. This is also true of other Gag-fusion reporters. Recently, an HIV-1 NLuc reporter virus vector developed by inserting the NLuc gene upstream of the Nef gene was reported by Ventura et al. (25). This vector generates replication-competent HIV-1 NLuc reporter virus and was used to study HIV-1 replication
dynamics in humanized mice during anti-retroviral therapy (ART) because it enables highly sensitive detection of the NLuc signal in the infected animals. Our NLuc vector generates virus particles that package the NLuc reporter, enabling the quantification of released particles directly from the supernatant, making it a suitable tool for studying virus release.

Compared to reporter viruses generated by co-packaging Vpr-fusion reporter proteins with HIV-1 Gag into virus particles, the Gag-iNLuc vector provides a higher sensitivity of detection because the NLuc reporter is packaged at higher amounts than a Vpr-fused reporter in the virus particles. This is because incorporation of NLuc protein into virus particles is at stoichiometrically equivalent amounts to the HIV-1 Gag protein which is ~ 2000-3000 Gag molecules per particle and ~ 150-200:1 Gag: Vpr molecules per virus particle (26-28). The high number of NLuc proteins that are packaged into each virus particle, coupled with the inherent brightness of the NLuc substrate, results in a high sensitivity of detection of virus particles in the supernatant. This allows the study of virus release dynamics at very low concentrations of the vector and thus minimizes possible cytotoxicity caused by expressing exogenous proteins and makes the Gag-iNLuc vector a suitable tool for functional and high-throughput assays measuring virus particle production. Besides the highly sensitive nature of NLuc detection, we hypothesize that the Gag-iNLuc virus will also be useful for measuring the binding of HIV-1 particles to target cells.

Conclusions

HIV-1 particle release is commonly measured by RT activity, p24 ELISA or p24 western blotting assays. These assays are relatively insensitive, laborious, expensive and time consuming, and they often involve multiple steps that can introduce errors that can compound at each step to significantly affect the final results. The HIV-1 Gag-iNLuc vector allows for a single-step assay to measure virus particle production and is significantly more sensitive and cheaper compared to other assays. Accordingly, this vector provides a valuable tool for studying the late stages of HIV replication, in particular in the context of high-throughput screening for late-acting cellular factors that facilitate or restrict virus release or for inhibitor screening. Previous studies have performed high-throughput screening for host factors affecting HIV-1 particle production by harvesting virus particles released
from the screening/producer cells to infect target cells and measuring particle infectivity (29-31).
While this approach has generated many significant findings, a limitation is that the assay readout is the infectivity of the released particles rather than the number of virus particles per se, even though the two can often be correlated. Effects on particle assembly/release therefore cannot be distinguished from effects on infectivity. The HIV-1 Gag-iNLuc reporter vector simplifies virus production screening by eliminating the infection step because measurement of particle production can be performed directly from the producer-cell supernatant. This reduces the number of steps in the screening protocol and minimizes opportunities for errors and experimental variability, therefore making the HIV-1 Gag-iNLuc reporter vector a valuable tool for functional studies of virus release and high-throughput screening for cellular factors and small-molecules that affect HIV-1 particle production.

Abbreviations
AIDS: Acquired immunodeficiency syndrome
AME: Amphotericin-B methyl ester
ART: Antiretroviral therapy
BST2: Bone marrow stromal antigen 2
CA: Capsid
CPM: Counts per minute
DNA: Deoxyribonucleic acid
ESCRT: Endosomal sorting complex required for transport
FBS: Fetal bovine serum
GFP: Green fluorescent protein
iGFP: Internal green fluorescent protein
HA: Hemmagglutinin
HEK: Human embryonic kidney
HIV-1: Human immunodeficiency virus type 1
MA: Matrix
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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
Not applicable

**Consent for publication**

Not applicable

**Competing Interests**

The authors declare that they have no competing interests.

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**Contributions**

Both authors conceptualized and designed the project. JK performed experiments and collected data. Both authors analysed data, wrote and approved the manuscript.

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Figures

Figure 1

Generation of HIV-1 Gag-iNLuc vectors. (A) A schematic representation of the HIV-1 Gag
polyprotein precursor indicating different domains (black rectangles) and PR cleavage sites (white lines). The PR cleavage site between the MA and CA domains of Gag (SQNYPIVQ) is shown with the dashed line. The NLuc gene is represented by the red rectangle. In the HIV-1 Gag-iNLuc vector, the cleavage site motif is duplicated to flank the NLuc gene on both sides. Synonymous changes made in the duplicated coding sequence are represented in bold and italics. Maturation products of the different vectors are indicated on the right. (B) Western blot analysis of HIV-1 Gag in lysates and supernatants from HEK293T cells transfected with the indicated HIV-1 vectors at 48hrs post-transfection. (C) NLuc activity of lysates and supernatants from HEK293T cells from (B) represented as Log10[RLUs]. (D) Relative virus release efficiency in HEK293T cells from (B). Error bars +/- SD, n=3 independent experiments for both panels C and D.
pNL4-3 Gag-iNLuc enables highly sensitive detection of virus release. (A) Western blot

Figure 2
analysis of HIV-1 Gag in lysates and supernatants from HEK293T cells transfected with 1.0µg pUAS-NLuc, 1.0µg pNL4-3, or varying amounts of pNL4-3 Gag-iNLuc (1.0µg, 0.5µg, 0.25µg, 0.125µg or 0.0625µg). (B) NLuc activity of lysates and supernatants from HEK293T cells transfected as in (A) at 48hrs post-transfection represented as Log10[RLUs]. (C) RT activity of supernatants from (B). (D) Relative amounts of p24 in pg/ml in supernatants from (B) (E) Correlation of supernatant NLuc-activity and RT activity from (B). (F) Correlation of supernatant NLuc activity and p24 abundance from (B). Error bars +/- SD, n=3 independent experiments for both panels B, C and D.
Infectivity of HIV-1 Gag-NLuc particles can be rescued by co-expression with WT Gag. (A) RT activity of virus particles produced in HEK293T cells transfected with either WT pNL4-3, pNL4-3 Gag-iGFP, or the indicated pNL4-3 Gag-iNLuc vectors. RT activity is shown relative to the WT HIV-1. (B). Infectivity of virus particles from (A) in the TZM-bl indicator cells at 48hrs post-infection. Viral inputs were normalized for RT activity. Infectivity is shown relative to WT HIV-1. (C) Infectivity in TZM-bl cells of virus produced in HEK293T cells transfected with either WT pNL4-3, pNL4-3 Gag-iNLuc, or both pNL4-3 and pNL4-3 Gag-iNLuc at indicated ratios of pNL4-3 to pNL4-3 Gag-iNLuc. Viral inputs were normalized for RT activity. Infectivity is shown relative to WT HIV-1(pNL4-3). Error bars +/- SD, n=3 independent experiments for all panels.
HIV-1 Gag-iNLuc vector recapitulates inhibition of HIV-1 particle release. (A) NLuc activity in lysates and supernatants from HEK293T cells transfected in 12-well plates with 1.0μg of either pNL4-3 Gag-iNLuc WT, pNL4-3 Gag-iNLuc PTAP- or pNL4-3 Gag-iNLuc delVpu and either 0, 0.1μg, 0.2μg or 0.3μg HA-tetherin (BST2). NLuc activity in lysates and supernatants was measured at 48 hours post-transfection and normalized to that of pNL4-3 Gag-iNLuc WT. (B) Western blot analysis of HIV-1 Gag (upper) in lysates and supernatant from (A) and HA-tetherin (lower) in cell lysates. (C) NLuc activity in lysates and supernatants from HEK293T cells transfected with 1.0μg of pNL4-3 Gag-iNLuc and treated with either 5μM or 10μM AME or vehicle at 48hrs post-transfection. AME treatment was done at 24hrs post-transfection following replacement of the growth media. (D) Western blot analysis of HIV-1 Gag in lysates and supernatant from (C). Error bars +/- SD, n=3 independent experiments for panels A and C.