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A Novel HIV-1 Reporter Virus with a Membrane-Bound *Gaussia princeps* Luciferase

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Summary

HIV-1 reporter viruses are a critical tool for investigating HIV-1 infection. By having a reporter gene incorporated into the HIV-1 genome, the expressed reporter protein acts as a specific tag, thus enabling specific detection of HIV-1 infected cells. Currently existing HIV-1 reporter viruses utilize reporters for the detection of HIV-1 infected cells by a single assay. A reporter virus enabling the detection of viral particles as well as HIV-1 infected cells by two assays can be more versatile for many applications. In this report, a novel reporter HIV-1 was generated by introducing a membrane-anchored form of the *Gaussia princeps* luciferase gene (mGluc) upstream of the *nef* gene in the HIV-1_{NL4-3} genome using a picornaviral 2A-like sequence. The resulting HIV-1_{NL4-3mGluc} virus expresses *Gaussia princeps* luciferase efficiently on viral membrane and the cell surface of infected human T cell lines and primary peripheral blood mononuclear cells. This HIV-1 reporter is replication competent and the reporter gene mGluc is expressed during multiple rounds of infection. Importantly, viral particles can be detected by bioluminescence and infected cells can be detected simultaneously by bioluminescence and flow cytometric assays. With the versatility of two sensitive detection methods, this novel luciferase reporter has many applications such as cell-based screening for anti-HIV-1 agents or studies of HIV-1 pathogenicity.

Keywords

HIV-1; reporter virus; *Gaussia princeps* luciferase; flow cytometry; bioluminescence

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1. Introduction

Over the years, reporter viruses have been utilized as critical tools to investigate HIV-1 infection. Reporter genes incorporated into the HIV-1 genome enable sensitive detection of HIV-1 infected cells. Several examples of genes that have been used to construct HIV-1 reporter viruses include murine heat-stable antigen (HSA) (Jamieson and Zack, 1998; Marodon et al., 1999; Chiu et al., 2005; Ali and Yang, 2006; Imbeault et al., 2009), human placental alkaline phosphatase (PLAP) (He and Landau, 1995; Chen et al., 1996), and cytosolic fluorescence/bioluminescence reporters such as luciferases (Connor et al., 1995; Edmonds et al., 2010) and enhanced green fluorescence protein (EGFP) (Herbein et al., 1998; Kutsch et al., 2002; Rich et al., 2002; Brown et al., 2005). While these reporter genes are useful, they provide only a single method of either detecting the production of viral particles, or detecting the HIV-1 infected cells specific to each reporter. Therefore, a reporter virus enabling the detection of both viral particle and HIV-1 infected cells, and by two different highly sensitive assays, can be more versatile for many applications. Recently, a novel, membrane-anchored form of the *Gaussia princeps* luciferase (mGluc) reporter gene was engineered for *in vivo* bioluminescent T cell imaging (Santos et al., 2009). mGluc was created by a genetic modification of the native Gluc, adding the human CD8 leader sequence and the human CD8 transmembrane domain at the amino and the carboxy-termini, respectively (Santos et al., 2009). This modification allows the native, secreted Gluc to be retained at the cell membrane. Because of this cell surface expression, mGluc expressing cells can be detected by a mGluc specific monoclonal antibody and flow cytometry. Moreover, cell surface expressed mGluc emits a significantly higher bioluminescent signal than other luciferases, allowing sensitive detection by bioluminescent assay (Tannous et al., 2005; Santos et al., 2009; Tannous, 2009). The relatively small size (262 amino acids) mGluc has minimal impact on HIV-1 fitness when it is incorporated into the viral genome. Based on these advantageous features, we hypothesized that mGluc is an excellent gene to generate a sensitive and versatile HIV-1 reporter virus.

In this report, a new HIV-1 reporter was generated by incorporating the mGluc gene upstream of the *nef* gene of HIV-1_{NL4-3} genome with a picornaviral 2A-like (P2A) sequence. The HIV-1_{NL4-3} mGluc reporter HIV-1 is replication competent in human T cell lines and primary human T lymphocytes. Bioluminescent signal can be detected from both HIV-1_{NL4-3} mGluc particles and infected cells. Cell surface expressed mGluc can be detected in infected cells via mGluc specific monoclonal antibody staining and flow cytometry.

2. Materials and methods

2.1 Construction of the HIV-1_{NL4-3}mGluc plasmid DNA

A synthetic 1097-bp DNA fragment, containing a 138 bp of 3' portion of the HIV-1_{NL4-3} *env* gene, a human CD8 signal sequence, a codon-optimized *Gaussia princeps* luciferase gene, a CD8 transmembrane domain sequence, a P2A sequence, and a 106 bp of 5' portion of the HIV-1_{NL4-3} *nef* gene, were digested with *HpaI* and *XhoI* and ligated subsequently with *HpaI* (within the *env* gene) and *XhoI* (within the *nef* gene) digested pNL4-3 plasmid DNA (Adachi et al., 1986). The inserted DNA sequence in the HIV-1_{NL4-3}mGluc reporter virus plasmid DNA was confirmed to be correct by sequencing analysis.

2.2 HIV-1 based lentiviral vector for mGluc expression

To express the mGluc from a lentiviral vector, mGluc cDNA was inserted into the FG11F lentiviral vector (Qin et al., 2003). VSV-G pseudotyped lentiviral vectors were prepared by calcium phosphate plasmid DNA transfection in 293T cells as previously described (Qin et al., 2003).

2.3 Cell culture

CEMx174 cells (Salter et al., 1985) were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program. T1 lymphocytes (Salter et al., 1985) were provided by Dr. Ayub Ali (UCLA AIDS Institute). These cells were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal calf serum (FCS), L-glutamine (2mM), penicillin G (100 units/ml), and streptomycin (100 µg/ml) (GPS) at 37°C under 5% CO₂. Human embryonic kidney 293T cells were cultured in Iscove's modified Eagle medium supplemented with 10% FCS and GPS. Human peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood of healthy donors by Ficoll-Hypaque PLUS (GE Healthcare Life Sciences, Piscataway, NJ). PBMCs were depleted of CD8⁺ T cells using magnetic bead conjugated anti-human CD8 monoclonal antibodies (Dynabeads, Invitrogen, Carlsbad, CA), and cultured in RPMI-1640 with 20% FCS, GPS and 5 µg/ml of phytohemagglutinin-P (PHA-P; Sigma-Aldrich, St Louis, MO) for 2 days. Cells were then washed and cultured in RPMI-1640, 20% FCS, GPS with 10 U/ml recombinant human interleukin-2 (hIL-2; Roche, Indianapolis, IN).

2.4 Preparation of virus stocks

HIV-1_{NL4-3} and HIV-1_{NL4-3mGluc} were produced by calcium phosphate transfection of 293T cells with plasmid DNAs containing the full-length proviral HIV-1 DNA. Cell-free viral supernatants were filtered through 0.22 µm filter, aliquoted and stored at -80°C. The amount of p24 in viral stocks was determined by an enzyme-linked immunosorbent assay. All viral aliquots used for infection underwent only one freeze-thaw cycle.

2.5 HIV infection

Cells (4×10⁵) were infected with different levels of HIV-1 (1 to 1000 ng of p24 in 0.5 ml) for 2 hours. After infection, cells were washed 3 times with phosphate buffered saline (PBS, pH 7.4) or RPMI-1640 media and cultured.

2.6 Flow cytometry

Cells (1×10⁵) were washed with FACS buffer (PBS supplemented with 2% FCS) and stained with 1 µl of Gluc-specific mouse IgG_{2a} monoclonal antibody (Nanolight Technology) or mouse isotype control IgG_{2a} in 50 µl FACS buffer for 30 min. After a single wash, cells were labeled with 1 µl phycoerythrin (PE)-conjugated goat sera specific to mouse IgG (Invitrogen, Carlsbad, CA) in 100 µl of FACS buffer for 30 min. Cells were washed once and fixed in 1% formaldehyde in PBS overnight and analyzed on a cytofluorometer, Cytomics FC500 (Beckman Coulter, Fullerton, CA), or LSRII (Becton Dickinson, Franklin Lakes, NJ). Data were analyzed by FLOWJO (Tree Star, Ashland, OR) software. For CD4 and MHC-I downregulation studies, cells were stained with 1 µl of human CD4 specific

mouse IgG₁ monoclonal antibodies conjugated with PE-Cy7 (BD Biosciences, Franklin Lakes, NJ) and a 1 µl of human HLA-A*02 specific mouse IgG₂ monoclonal antibodies conjugated with Alexa Fluor 647 or mouse isotype control IgG₂-Alexa Fluor 647 (AbD Serotec, Raleigh, NC) in 100 µl of FACS buffer for 30 min.

2.7 Intracellular p24 staining

Cells were fixed for 1 hour in 2% formaldehyde in PBS and washed. Fixed cells were permeabilized with ice-cold methanol for 15 min, pelleted and treated with 0.5 ml PBS containing 0.1% NP-40 for 5 min on ice. Alternatively, Fix & Perm Cell Permeabilization Kit (Invitrogen, Carlsbad, CA) was used for permeabilizing and fixing cells. The cells were stained with a 1:100 dilution of anti-p24 antibody conjugated with PE (KC57-RD1, Coulter Immunology, Hialeah, FL) or mouse isotype control IgG₁ in 100 µl of FACS buffer for 30 min at room temperature. Following washes, the cells were fixed with 2% formaldehyde in PBS and analyzed by flow cytometry.

2.8 Bioluminescence assays

All bioluminescence assays were performed in a FLUOstar Optima microplate fluorometer (BMG Labtech, Cary, NC). A commercial luciferase assay kit was used and manufacturer's instruction was followed (Promega, Madison, WI). Briefly, concentrated virus or cells were lysed with the Mathews lysis buffer (0.25% Triton® X-100, 1 mg/ml porcine gelatin, 10% glycerol, 0.05% antifoam 289, 150 mM HEPES pH 8.0), then 20 µl of the lysate was diluted serially. The bioluminescence reaction was started by addition of 50 µl of 1× coelenterazine substrate solution. Bioluminescent signal (photons per 10 seconds) was measured and recorded using a luminometer.

2.9 Western blots

Infected CEMx174 cells (1×10^5) were washed with PBS and boiled in Laemmli's sample buffer (4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromphenol blue, 0.125 M Tris HCl, pH 6.8). Cellular proteins were resolved on 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to the polyvinylidene fluoride (PVDF) membrane. Immunoblotting against HIV-1 Nef protein was performed using a 1:1,000 dilution of polyclonal rabbit anti-Nef antisera (AIDS Reagent Program, catalog #2949) (Shugars et al., 1993). The blot was probed with a 1:5,000 dilution of horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA) and visualized with SuperSignal® West Pico chemiluminescent substrate for detection of HRP (Thermo Scientific, Reckford, IL). Equal loading of samples was confirmed by probing with a β -actin antibody. Mock infected cells were used as negative control. Immunoblotting against mGluc protein was performed similarly using a 1:1,000 dilution of polyclonal rabbit anti-Gluc sera (Nanolight Technology, Pinetop, AZ) and probed with a 1:5,000 dilution of HRP-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA).

3. Results

3.1 Construction of the membrane-bound *Gaussia princeps* luciferase (mGluc) reporter HIV-1

To engineer an infectious HIV-1 reporter expressing mGluc, the full-length genome of HIV-1_{NL4-3} was modified to incorporate mGluc gene, followed by a P2A sequence, inserted in between the *env* and *nef* genes (Fig. 1A). The resulted reporter virus (HIV-1_{NL4-3mGluc}) has an extra 852 nucleotides compared to the wild-type HIV-1_{NL4-3}. (Fig. 1B)

3.2 Bioluminescent signal in the HIV-1_{NL4-3mGluc} viral particles

Membrane anchored mGluc can be incorporated in the HIV-1_{NL4-3mGluc} viral membrane allowing viral particles to emit bioluminescent signal. To examine the bioluminescent signal from the viral particles, HIV-1_{NL4-3mGluc} were produced in 293T cells and purified by ultracentrifugation through a 20% sucrose cushion. The purified HIV-1_{NL4-3mGluc} viral particle fraction (1ug of p24), but not the wild-type HIV-1_{NL4-3} viral particle fraction emitted approximately 28,000 photons of light per 10 seconds (Fig. 2). These data demonstrate that cell-free HIV-1_{NL4-3mGluc} viral particles can be detected using a bioluminescent assay.

3.3. Viral replication and bioluminescence in the HIV-1_{NL4-3mGluc}-infected human lymphoid cell lines

To examine HIV-1_{NL4-3mGluc} infection, human CEMx174 cell lines were infected with either HIV-1_{NL4-3mGluc} or wild-type HIV-1_{NL4-3} at two levels (10 ng or 100 ng of p24). Infected cells were analyzed by intracellular p24 staining and flow cytometric analysis at 3, 6 and 9 days post infection (Fig. 3A). The level of p24 expression in HIV-1_{NL4-3mGluc} infected cells was similar to that of HIV-1_{NL4-3} infected cells during the 9 day culture period. The level of p24 expression in HIV-1_{NL4-3mGluc} infected cells was greater than that in HIV-1_{NL4-3} infected cells at 9 days post infection. Next, bioluminescent signal was monitored in the cell lysate of infected cells by bioluminescent assay at 3, 6 and 9 days post infection (Fig. 3B). Bioluminescent signal was detected starting at day 3 post-infection, increased at day 6 and remained high (10⁷ photons of light/10 seconds/10⁴ cells) up to 9 days post infection in HIV-1_{NL4-3mGluc} -infected cells. These results demonstrate that HIV-1_{NL4-3mGluc} efficiently replicates in human CEMx174 cell line and the infected cells emit a strong bioluminescent signal.

To examine the stability of mGluc expression during multiple rounds of viral replication, CEMx174 cells were infected sequentially and analyzed for the bioluminescence signal in three consecutive passages. For example, after 10 days of infection the cell lysate was analyzed for bioluminescent signal and the cell-free supernatant was used to infect fresh cells (denoted as 2nd round of infection). The bioluminescent signal was detected in all three rounds of HIV-1_{NL4-3mGluc} infected cells. These data suggest that HIV-1_{NL4-3mGluc} expresses mGluc over multiple rounds of replication in CEMx174 cell line (Fig. 3C).

3.5 Viral replication and bioluminescence in HIV-1_{NL4-3mGluc}-infected human primary CD4⁺ T cells

To examine HIV-1_{NL4-3mGluc} infection in human primary CD4⁺ T lymphocytes, human primary CD8⁺ cell-depleted, PHA/hIL-2-stimulated PBMCs were infected with either HIV-1_{NL4-3mGluc} or wild-type HIV-1_{NL4-3} at 4 different levels (1, 10, 100 or 1000 ng of p24). HIV-1_{NL4-3mGluc} required 10 fold higher virus to achieve similar levels of p24 positive cells relative to the wild-type HIV-1_{NL4-3} by flow cytometry (Fig. 4A: 1000 ng of p24 HIV-1_{NL4-3mGluc} vs. 100 ng HIV-1_{NL4-3}; 100 ng HIV-1_{NL4-3mGluc} vs. 10 ng HIV-1_{NL4-3}; or 10 ng HIV-1_{NL4-3mGluc} vs. 1 ng HIV-1_{NL4-3}). These data suggest HIV-1_{NL4-3mGluc} replication is approximately 10-fold slower than the wild-type HIV-1_{NL4-3} in PBMCs. The bioluminescent signal increased in the infected PBMCs at 3, 7, 10 and 13 days post infection (Fig. 4B). The level of bioluminescent signal correlated with the level of input virus (Fig. 4B). These results demonstrate that HIV-1_{NL4-3mGluc} replicates and expresses mGluc efficiently in human primary CD4⁺ T lymphocytes.

3.5 mGluc detection in HIV-1_{NL4-3mGluc} infected cells by flow cytometry

To detect mGluc expression by flow cytometry, HIV-1_{NL4-3mGluc} infected CEMx174 cells and human PBMCs were co-stained with anti-Gluc and anti-p24 monoclonal antibodies and analyzed by flow cytometry. The majority of mGluc expressing cells also expressed intracellular p24 in the infected CEMx174 cell line (Fig. 5A) and human PBMCs (Fig. 5B). These results demonstrate that mGluc expression is specific in HIV-1_{NL4-3mGluc} infected cells and staining for mGluc is a reliable assay for detection of infected cells. Furthermore, mGluc expression increased during the 9 day culture period by flow cytometry, confirming that the reporter HIV-1 is replication competent (Fig. 5C and 5D).

3.6 Assessment of HIV-1 Nef and mGluc expression by Western blot

Although a small P2A-like self-cleaving peptide was used to co-express mGluc and Nef proteins, the insertion of mGluc gene upstream of *nef* gene can affect expression of *nef*. Therefore, Nef and mGluc protein expression levels were determined by Western blot analysis. Nef protein expressed in HIV-1_{NL4-3mGluc} infected CEMx174 cells appeared as a single band of approximately 32 kDa in size; a slight shift (~5 kDa) in mobility relative to the Nef protein (27 kDa) expressed in the wild-type HIV-1_{NL4-3} infected cells (Fig. 6A). Three bands were detected by Western blot analysis using anti-Gluc antibodies, suggesting that mGluc proteins were expressed in multiple forms in HIV-1_{NL4-3mGluc} infected cells (Fig. 6A). Since the size of Nef protein is altered in the Western blot analysis, the functions of Nef in HIV-1_{NL4-3mGluc} was investigated by examining MHC class I downregulation in HIV-1_{NL4-3mGluc} infected cells (Schwartz et al., 1996; Collins et al., 1998; Cohen et al., 1999) (Salter et al., 1985). HLA-A*02 expression was downregulated in HIV-1_{NL4-3mGluc} infected (p24⁺) cells; however, compared to the wild-type HIV-1_{NL4-3} infected (p24⁺) cells, this downregulation was 2 fold attenuated (29% decrease vs. 63% decrease) (Fig. 6B). CD4 downregulation was also examined. CD4 downregulation is another function of Nef, although other HIV-1 proteins such as *vpu* and *env* also share this function (Geleziunas, Morin, and Wainberg, 1996; Lindwasser, Chaudhuri, and Bonifacino, 2007; Piguet et al., 1999). CD4 downregulation was efficient in the p24⁺ population in HIV-1_{NL4-3mGluc}

infected cells (Fig. 6C). In conclusion, these data suggest that Nef expression and function in HIV-1_{NL4-3mGluc} are affected by mGluc insertion when compared to the wild-type HIV-1_{NL4-3}.

4. Discussion

In this report, a novel HIV-1 reporter was created with an engineered membrane-bound form of Gluc (mGluc). The resulting HIV-1_{NL4-3mGluc} reporter virus is replication competent, with replication kinetics comparable to the wild-type HIV-1_{NL4-3} in human T cell lines, and 10-fold attenuated in human PBMCs. The mGluc is biologically active, as an intense bioluminescent signal can be measured easily and effectively upon addition of the coelenterazine substrate to viral particles as well as infected cells lysates. Furthermore, by having the membrane-anchored form of Gluc, the cells infected by the HIV-1_{NL4-3mGluc} reporter virus can be detected specifically by flow cytometry, thus enhancing the versatility of this novel reporter. mGluc has proved successful in *in vivo* real-time bioluminescent imaging, as it was shown capable of targeting specific T cell populations in a live mouse without having to kill the animal (Santos et al., 2009).

The size of reporter genes inserted into the HIV-1 genome has been a major concern as it may lead to a severe reduction or even complete inhibition of viral replication. Investigators have used an internal ribosome entry site (IRES) from encephalomyocarditis virus (EMCV) to initiate separately cap-independent protein synthesis of Nef (Levy et al., 2004; Imbeault et al., 2009; Stripecke et al., 2000; Zhu et al., 2001). However, IRES have several limitations, notably the increased insertion size (~500–700 bp) and attenuated expression of the downstream gene (Osborn et al., 2005; Holst et al., 2006). The use of small 2A-like sequences (18–22 amino acids in length) was considered preferentially to allow multicistronic and stoichiometric expression of both mGluc and Nef. This may prove useful for creating a more efficient HIV-1 reporter virus, as a smaller insertion into viral genome reduces the burden on the virus, thereby maintaining the reporter gene's genetic stability over multiple rounds of replication (Ali and Yang, 2006). However, the Nef protein in HIV-1_{NL4-3mGluc} cells was slightly larger than the Nef protein in wild-type HIV-1_{NL4-3} infected cells, suggesting the mGluc insertion with a 2A-like sequence affected Nef processing. A recent report on the 2A-like sequence has shown evidence of potential misfolding or misglycosylation of certain C-terminal proteins (de Felipe et al., 2010), where an interaction between the C-terminal region of certain nascent peptides and the translocon complex can affect the structure of the C-terminus of 2A within the peptidyltransferase center of the ribosome. This leads to inhibition of the 2A reaction and the production of 'uncleaved' fusion proteins (de Felipe et al., 2010). This implies that the Nef proteins may likely still have P2A peptide intact. Although the results from this present study suggest that the Nef protein found in the cells infected with HIV-1_{NL4-3mGluc} is altered, we believe that this replication competent HIV-1_{NL4-3mGluc} can be used effectively as a versatile reporter virus to tag viral particles and infected cells specifically. Infected cells can be detected simultaneously by bioluminescence and by flow cytometry.

5. Conclusions

A novel luciferase reporter HIV-1 was generated and shown to be replication competent in human T cell lines and primary PBMCs, and expresses mGluc efficiently on the cell surface. The viral particle can be detected by bioluminescence. The infected cells can be detected by bioluminescence as well as flow cytometry. This novel reporter HIV-1 can be used in cell-based high-throughput screenings for anti-HIV agents or studies of HIV pathogenicity.

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highlights

A new HIV-1 reporter virus is described that is replication competent in human T cell lines and primary cells

The virus efficiently expresses membrane-anchored *Gaussia princeps* luciferase (mGluc) on the cell surface, which can be stained by anti-Gluc monoclonal antibody

Viral particles can be detected by bioluminescence and infected cells can be detected simultaneously by bioluminescence and by flow cytometric assays

Fig. 1.

(A) Schematic representation of HIV-1_{NL4-3mGluc} reporter construct. mGluc and P2A

sequences were inserted between the *env* and the *nef* genes of the HIV-1_{NL4-3}. (B)

Nucleotide sequences of the mGluc and P2A in HIV-1_{NL4-3mGluc} DNA. *env*: envelope. CD8

LS: the human CD8 leader sequence. CD8 TM: the human CD8 transmembrane domain

sequence. Sites of restriction enzyme digestions are indicated.

Fig. 2.

Bioluminescent signal from concentrated HIV-1_{NL4-3mGluc} viral particles. HIV-1_{NL4-3mGluc} was produced by plasmid DNA transfection in 293T cells and concentrated 100 fold by ultracentrifugation through a 20% sucrose cushion. The bioluminescent signals (photons per 10 seconds) from three HIV-1_{NL4-3mGluc} viral concentrates were normalized to their p24 concentrations (1 μ g). Wild-type HIV-1_{NL4-3} was used as negative control.

Fig. 3.

HIV-1_{NL4-3mGluc} replication and bioluminescent signal in a human lymphoid cell line. (A) CEMx174 cell lines were infected with HIV-1_{NL4-3mGluc} or wild-type HIV-1_{NL4-3} at two viral inputs (10 or 100ng of p24). Viral replication was assessed by the intracellular p24 staining and flow cytometry at the indicated days post-infection. (B) Bioluminescent signal was monitored in infected cell lysates (1×10^4 cells). (C) Cells were infected with either HIV-1_{NL4-3mGluc} to assess mGluc expression over multiple rounds of viral replication. The culture supernatants were harvested 7–10 day post infection, filtered and used to infect

uninfected CEMx174 cells (denoted '2nd round infection'). The supernatants were harvested 7–10 day post 2nd round infection (denoted '3rd round infection'). Bioluminescent signals were detected in HIV-1_{NL4-3mGluc} infected cell lysates in the three consecutive rounds of infection. HIV-1_{NL4-3} was used as a negative control.

Fig. 4.

HIV-1_{NL4-3mGluc} replication and bioluminescent signal in PBMCs. (A) CD8 depleted, PHA/hIL-2-stimulated human PBMCs were infected with HIV-1_{NL4-3mGluc} or wild-type HIV-1_{NL4-3} at 4 different viral inputs (1 to 1000ng of p24). Viral replication was assessed by the intracellular p24 staining and flow cytometry at the indicated days post-infection. (B) Bioluminescent signal was detected in the HIV-1_{NL4-3mGluc} infected cell lysates. Each data point represents average value calculated from three independent replicates. Error bars are the standard deviation of each data set.

Fig. 5. mGluc detection by flow cytometry. A representative data showing mGluc and intracellular p24 coexpression in the HIV-1_{NL4-3mGluc} infected CEMx174 cell line (A) or human PBMCs (B). Quadrants were set based on the plots with mock and isotype antibody stained infected cells. (C) Kinetics of %mGluc expression in HIV-1_{NL4-3mGluc}-infected CEMx174 cells. (D) Kinetics of % mGluc expression in HIV-1_{NL4-3mGluc} PBMCs.

Fig. 6.

(A) Nef protein expression in HIV-1_{NL4-3mGluc} infected cells by Western blotting. T1 cell line were infected with either wild-type HIV-1_{NL4-3} (left), HIV-1_{NL4-3mGluc} (middle) or VSV-G pseudotyped HIV-1 based lentiviral vector expressing mGluc. Cell lysates from 4 days post-infection were immunoblotted with either anti-Nef antiserum (left panel) or anti-Gluc antiserum (right panel). (B and C) HLA-A*02 and CD4 expression by flow cytometric analysis. The HLA-A*02 down regulation (B) and CD4 downregulation (C) are calculated by the relative difference in mean fluorescence intensities between p24+ and p24- populations.