

Supplementary data

Materials and Methods:

HIV-1 CRF07_BC infectious clone

The CRF07_BC isolate was collected from injected drug users (IDUs) and propagated in fresh IL-2 stimulated PBMCs. The proviral DNA was extracted using the Qiagen blood DNA kit (Qiagen, Hilden, Germany) from infected PBMCs. Specific primers were designed to amplify the full-length HIV DNA provirus as referenced [14]. The two partial molecules of the entire CRF07_BC genome were amplified with the Platinum Taq DNA polymerase High Fidelity kit (Invitrogen, Carlsberg, USA). The 5' partial fragment and 3' partial fragment were amplified using the primers 5LTR-F/Pol-R and Pol-F/3LTR-R, respectively. Both 5' partial fragment and 3' partial fragment containing the overlapping region were used as templates to generate full length CRF07_BC genome carrying SphI and EcoRV restriction sites at the 5' and 3' terminal, respectively, with the primers 5LTR-F/3LTR-R. The CRF07_BC full length genome was further cloned into pUC-57 vector. The morphology and infectivity of this infectious clone were validated.

Primer	Sequence	Region
5LTR-F	5'-GATAT-GGATCCTGGAAGGGCTAATTCAGGAAAA-3'	5'LTR
Pol-R	5'GTAATTCGTTAGTTTGTATGTCTGTTGCTATTATATCTATT -3'	pol
Pol-F	5'-CAGTGGCTACATGGAAGCAGAGGTTATCCCAGCAG-5'	pol
3LTR-R	5'-GCATGC-GCGGCCGCACGCGTGGTCTGAGGGATCTC-3'	3'LTR

Virus titration

HIV-1 virus titers were determined with a flow cytometry-based HIV-1 titration assay using GHOST /X4/R5 cells (GHOST are human osteosarcoma cells expressing high levels of CD4 and CCR5 and carrying LTR-GFP). The percentage of GFP expression in infected cells was measured and the Infectious Units (IU) were calculated. The virus titer was calculated by the equation- [Infectious Units (IU)/ml= (cell number) × (% of GFP-positive cells) × (dilution factor)] (The dilution factor = 1,000 µl /viral input (µl)). After the virus stocks were quantified, the experimental cells were infected at a specific multiplicity of infection (MOI). The MOI was determined via the equation: MOI = [(virus stock IU/ml) × (volume of virus used)]/(number of cells in infection). Most assays were used 10µl of CRF07_BC viral stock (10⁶-10⁵ IU/ml) administering to 10⁵ cells/well (MOI=0.1-0.01).

ELISA

Purified HIV-1 CRF07_BC viruses (0.1 ug of HIV-1 p24/well) or glycosylated gp120 recombinant proteins (HIV-1 CRF07_BC) (0.1 ug/well) (Sino Biological, Cat. No. 11233-V08H) or recombinant human CD4 proteins (R&D Systems. Cat.No. 514-CD) were coated on 96 well plates in PBS buffer and held overnight at 4°C, blocked with 5% BSA in PBST (PBS buffer containing 0.05% Tween 20), and incubated for 1 hr at 37°C. Recombinant human galectin-3 or human galectin-3 CRD proteins (provided by GylcoCore, Institute of Biomedical Sciences, Academia Sinica, Taiwan) or recombinant gp120 proteins were added into the each well with/without co-treatment of lactose (50mM), sucrose (50mM) or B₂C₁₀ monoclonal antibody (5-10 µg) at 37°C for 2 hrs. After three washes with PBST, rabbit anti-galectin-3 polyclonal antibodies or rabbit anti-CRF07_BC gp120 polyclonal antibodies (Sino Biological, Cat. No. 11233-R011) (1:3000 dilutions) were added and incubated at 37°C for 1 hr. After three additional washes, horseradish peroxidase (HRP) conjugated sheep anti-rabbit IgG (1:3000 dilution) (Amersham Biosciences) was added prior to another 1 hr of incubation at 37°C. Finally, 200 µl of 0.015% o-phenylenediamine dihydrochloride substrate (Sigma-Aldrich, St. Louis, MO) was added before a final incubation at 37°C for

30 min. Reactions were stopped by 3N HCl and absorbances were measured with a spectrophotometer (490 nm).

Co-immunoprecipitation (Co-IP)

Jurkat-R5 cells were incubated with HIV-1 CRF07_BC viruses (200ng p24) at 37°C for 1hr in presence of rhGal3 (1µM). After three washes with PBS, cells were treated with DSP (8 µg/ml) crosslinker (3,3'-dithiodipropionic acid di[N-hydroxysuccinimide ester]) (SIGMA) at room temperature for 30 min, incubated with 1M Tris-HCl (pH 7.5) at room temperature for 15 min, and lysed in NP-40 buffer (150 mM NaCl, 25 mM Tris-HCl [pH 7.4], 1 mM EDTA, 0.5% NP-40, and 5% glycerol and containing protease inhibitor cocktail) (CALBIOCHEM). Lysates were centrifuged at 16,000 × g and supernatants were immunoprecipitated with rabbit anti-Gal3 polyclonal antibodies. Aliquots were analyzed by SDS-PAGE and immunoblotting, using antibodies against CD4, galectin-3 and CRF07_BC gp120.

Life images for monitoring galectin-3 binding with HIV-1 to T cells

HIV-1CRF07_BC viruses were labeled with Alexa-555 fluorescent dye on the envelope by using Alexa Fluor 555 carboxylic acid, succinimidyl ester (Life Technology). Recombinant galectin-3 was labeled with Alexa-488 by using Lightning-Link Conjugation kit (Innova Biosciences). Alexa-555 envelope protein labeled CRF07_BC viruses were mixed with Alexa-488 labeled galectin-3 recombinant proteins then subjected to Jurkat-R5 cells. The binding phenomenon of galectin-3 with CRF07_BC to Jurkat-R5 was observed by LSM 780 confocal microscopy.

Electron microscopy

The transmission electron microscope (TEM) was used to analyze the characteristics of the HIV-1 CRF07_BC virions and the Gal3 binding to HIV-1 CRF07_BC virions. Briefly, the concentrated and purified viral stocks were inactivated with 0.025% formaldehyde solution. One drop (~3 µL) of inactive virus in 2% glutaraldehyde buffer was applied to glow-discharged TEM copper carbon-coated grids (Electron Microscopy Sciences). To study the interaction of Gal3 with CRF07_BC virions, rGal3 (20 µg/ml) were incubated with 10ng p24 of CRF07_BC virus for 1 h at room temperature, followed by centrifugation to concentrate the complexes. The complexes were dropped onto the grids, fixed and stained with goat anti-galectin-3 (Gal3), mouse anti-gp120 (Env) or control IgG (IgG), followed by Donkey anti-goat-labeled 6-nm gold particles and Donkey anti-mouse-labeled 18-nm gold particles. Following 4% uranyl acetate staining, grids were examined by Hitachi H-7000 Transmission Electron Microscope.

Surface Plasmon Resonance (SPR)

The SPR chip used in this study was BK7 glass slide coated with a laminated Cr/Au (2/50 nm) metal layer. A mixed self-assembled monolayer (SAM) of dithiols, which consisted of 90% C₂₅H₄₄O₆S₂ and 10% C₃₃H₅₈O₁₁S₂ (SensoPath Technologies, Bozeman, MT) was used as a diluent to mix with Cr/Au (at 1:9 ratio), generating a binding matrix for the formation of the receptor monolayer (CRF07_BC gp120 protein) with the amine-coupling kit (Biacore, Inc. Uppsala, Sweden). The receptor was covalently immobilized to the SPR sensor chip according to the amine-coupling protocol. Finally, the ligands Gal3 in PBS solution were injected into the homemade flow-cell to interact with the immobilized receptor on the SPR sensor chip to form the receptor/ligand immunocomplex. To quantify the kinetic/affinity parameters between the receptor and the ligand, their interactions were measured by the surface plasmon resonance (SPR) biosensor which developed by Chou et al. [11].

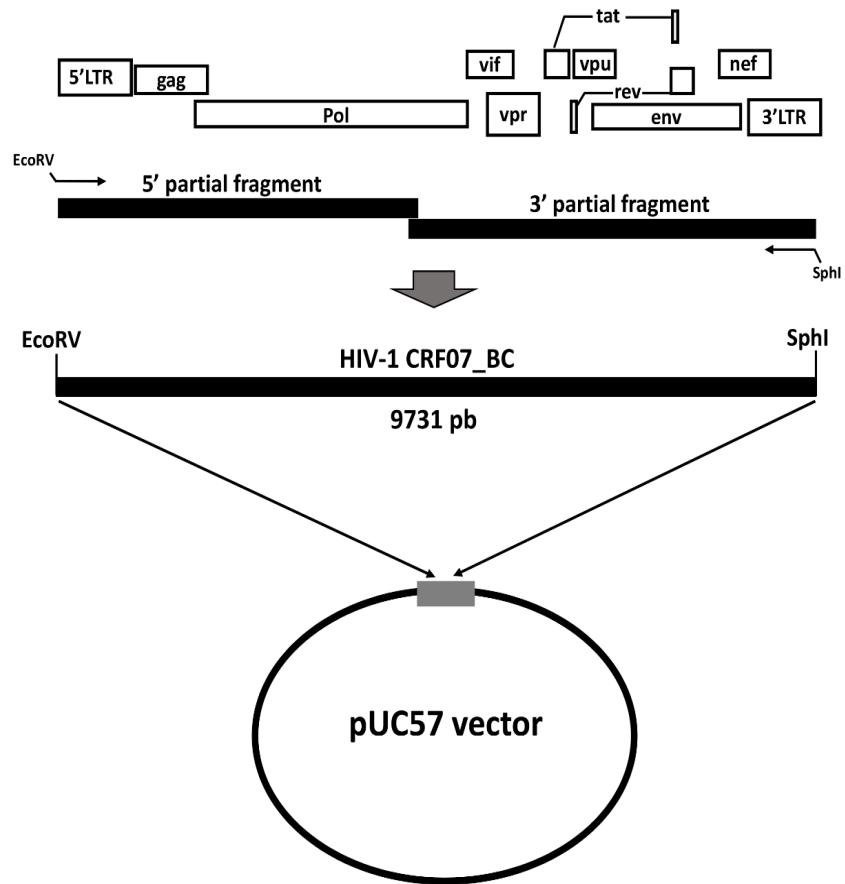


Figure S1: Generation of full-length molecular clones. Diagram of the full-length HIV-1 CRF07_BC cloning.

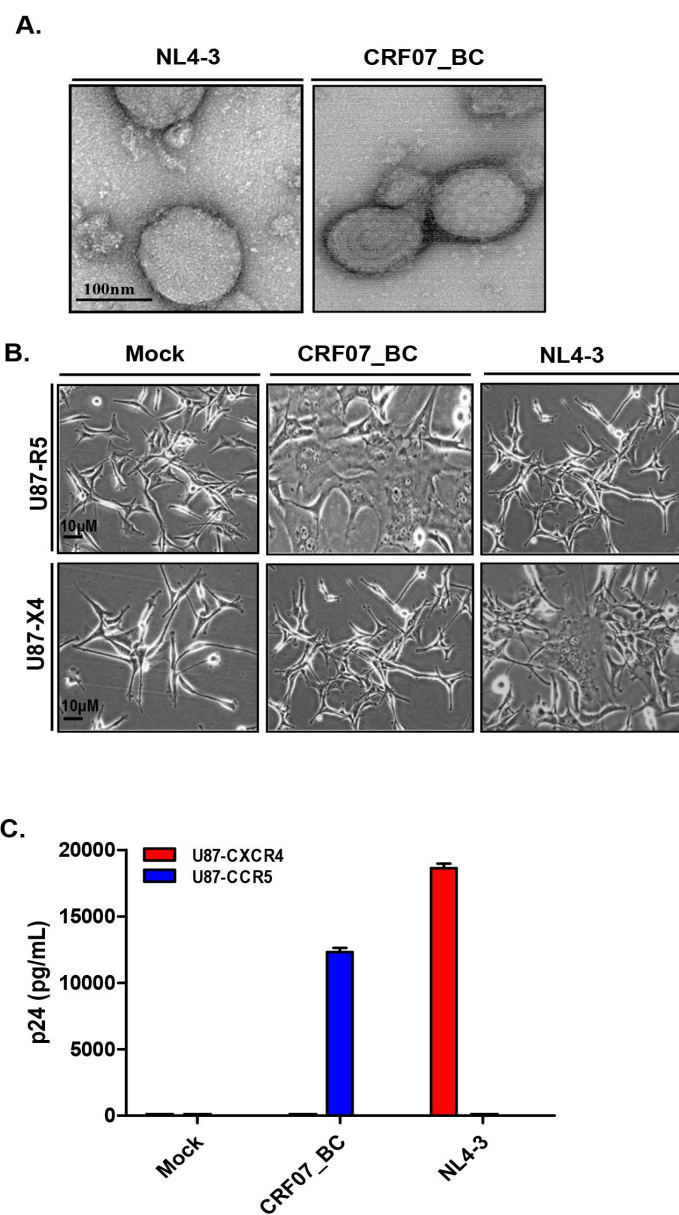


Figure S3. Determination of viral characteristics of HIV-1 CRF07_BC viruses. (A) The pNL4-3 and pCRF07_BC were transfected into HEK293T cells and the viral supernatants were subjected to TEM observation. (B) For determination of virus tropism, the HIV-1 CRF07_BC and subtype NL4-3 viruses were administered to U87-R5 (upper panel) and U87-X4 cells (lower panel), and the virus-induced CPE of the cells was observed. (C) The viral supernatants collected from (B) were subjected to HIV-1 p24 determination.

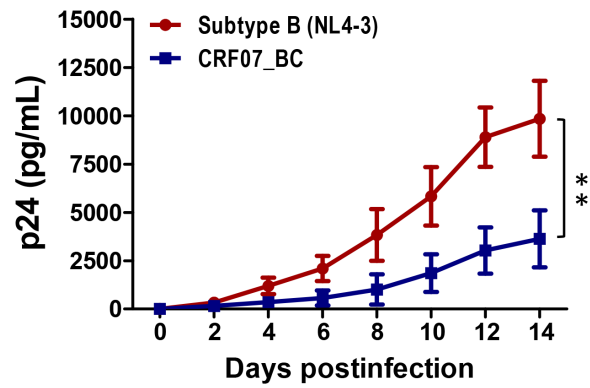


Figure S4. Viral characteristics of HIV-1 CRF07_BC. Viral growth kinetics was conducted using Jurkat-R5 cells (the Jurkat-CXCR4-CCR5 stably expressing cells) infected with B subtype NL4-3 and CRF07_BC viruses. The viral supernatants were collected at different time points and subjected to HIV-1 p24 determination. Quantitative data represent the mean \pm SD of results from three independent experiments (**P < 0.01).

Binding Constant	Galectin-3
Ka ($M^{-1} S^{-1}$)	4.567×10^{-4}
Kd (S^{-1})	3.223×10^{-4}
KD(M)	7.073×10^{-9}

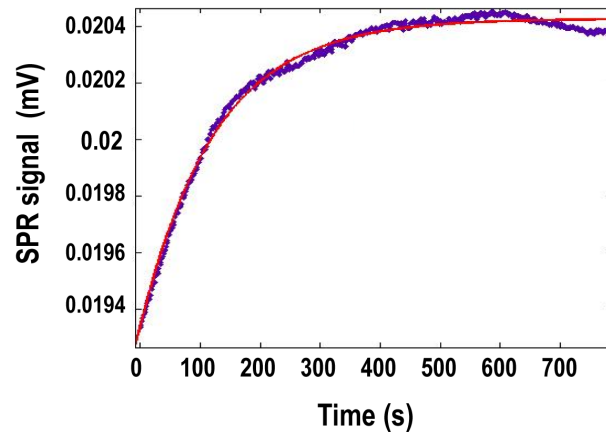


Figure S5: Determination of the binding affinities between galectin-3 and CRF07_BC gp120. The binding affinities between Gal3 and HIV-1 CRF07_BC gp120 were measured via SPR. The dissociation constant (KD) of galectin-3 was determined.

Table S1. The genotyping of enrolled HIV-1(+) IDUs

Sample ID	Genotyping		Consistency
	<i>Multiplex PCR</i>	<i>Sequencing & Phylogenetic analysis</i>	
TWKMU_IDU_001	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_002	CR07_BC	CR07_BC	Yes
TWKMU_IDU_003	B	B	Yes
TWKMU_IDU_004	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_005	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_006	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_007	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_008	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_009	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_010	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_011	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_012	B	B	Yes
TWKMU_IDU_013	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_014	B	B	Yes
TWKMU_IDU_015	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_016	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_017	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_018	CRF01_AE	CRF01_AE	Yes
TWKMU_IDU_019	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_020	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_021	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_022	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_023	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_024	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_025	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_026	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_027	CRF07_BC	CRF07_BC	Yes
TWKMU_IDU_028	CRF07_BC	CRF07_BC	Yes