

Supplemental methods

Ethics Statement

The Institutional Human Ethical committee approved the study and written consent was obtained from all study participants.

Study Participants and Sample Collection

HIV-1-infected individuals from the Punjab/Haryana region of North India were selected on a random basis for our studies. The samples were obtained from the Immunodeficiency Clinic of the Post Graduate Institute of Medical Research and Education, Chandigarh, India after obtaining all the required ethical clearances.

Amplification of the vpr Gene

Genomic DNA samples were obtained from the Immunodeficiency Clinic of the Post Graduate Institute of Medical Research and Education, Chandigarh, India. Sequences spanning vpr gene was amplified by PCR using gene specific primers (1). The HIV-1 genomic fragment encoding full-length vpr was amplified using High fidelity Taq DNA polymerase (Qiagen, Germany) using the following primer pair.

Forward: 5' GGCAGATCTTTATGGAACAAGCCCCAGAAGACC 3'

Reverse: 5' GCCTCGAGCTAGGATCTACTGGCTCCATTTTC 3'

The PCR conditions were as follows: one cycle of 5 min at 95°C for denaturation; 30 cycles of 10 sec at 94°C for denaturation; 30 sec at 60°C for annealing and 35 sec at 72°C for extension and a final extension cycle of 7 min at 72°C were carried out. In order to examine the genomic fragment of the major viral population in a sample, PCR products amplified at the end-point dilution of DNA templates were subjected to sequence analysis. The gel purified PCR products were cloned in pGEM-T Easy Vector System (Promega, USA) in between T7 and Sp6 promoters and also in the expression vector pCMV-Myc (Clontech). The cloning and sequencing was carried out at least twice on two separate occasion starting from genomic DNA to rule out the PCR generated mistakes in the sequence.

Phylogenetic Analysis, Genetic Subtyping and Sequence Alignment

These sequences were aligned with reference sequences of HIV- 1 strains of all subtypes (<http://www.hiv.lanl.gov>) using the Clustal W 1.83 program (2). The phylogenetic analysis was performed using the Neighbor Joining (NJ) method based on the Kimura two parameter distance matrix implemented in the MEGA 4.0 program (3-5). The multiple sequence alignment was done using CLC sequence server (CLC bio, Denmark).

Supplementary Figure 1

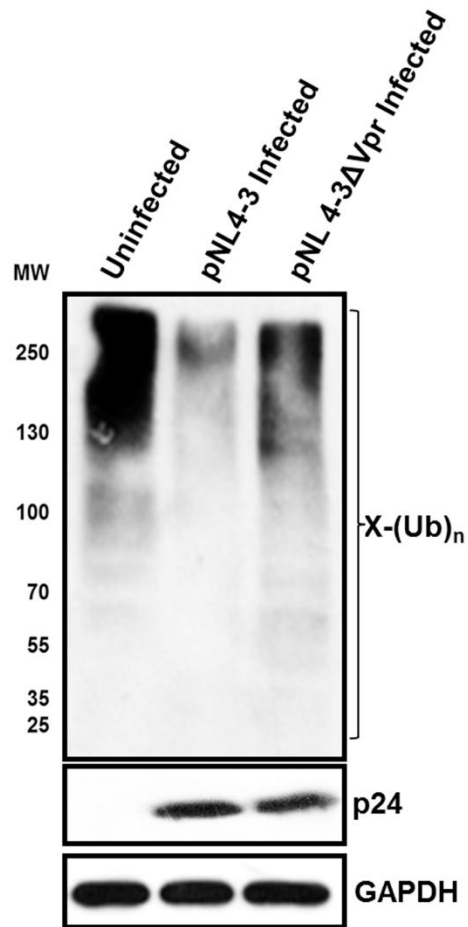


Figure S1: Whole cell ubiquitination profile in macrophages post HIV-1 infection.

Thp1 cells were infected with pNL4-3/ pNL4-3Δvpr viral supernatant for 4 hours and after 36 hours they were treated with MG132 for 8 hours. Cells lysed and whole cell ubiquitination was probed using anti-Ub antibody. GAPDH was used as loading control.

Supplementary Figure 2

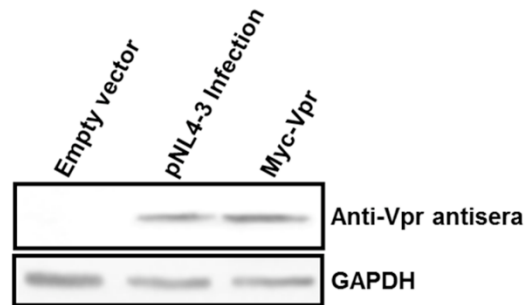


Figure S2: Expression levels of Vpr in infection and overexpression scenario.

HEK 293T cells were transfected with Myc-VprB/infected with pNL4-3. Post 48 hours cells were lysed and immunoblot analysis was done using Vpr antisera (NIH Cat No. 11836). GAPDH was used as a loading control.

Supplementary Figure 3

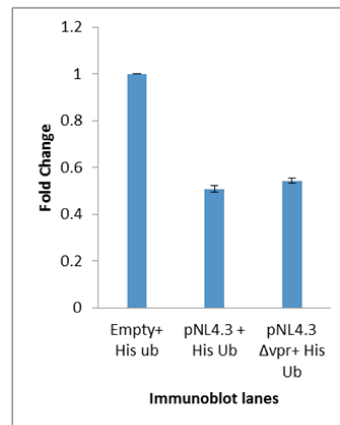


Figure S3: Quantitation of CD4 levels (input) in immunoblot 5B.

Densitometric analysis of input bands was done using Image J software. The levels were normalized with GAPDH.

Supplementary Figure 4

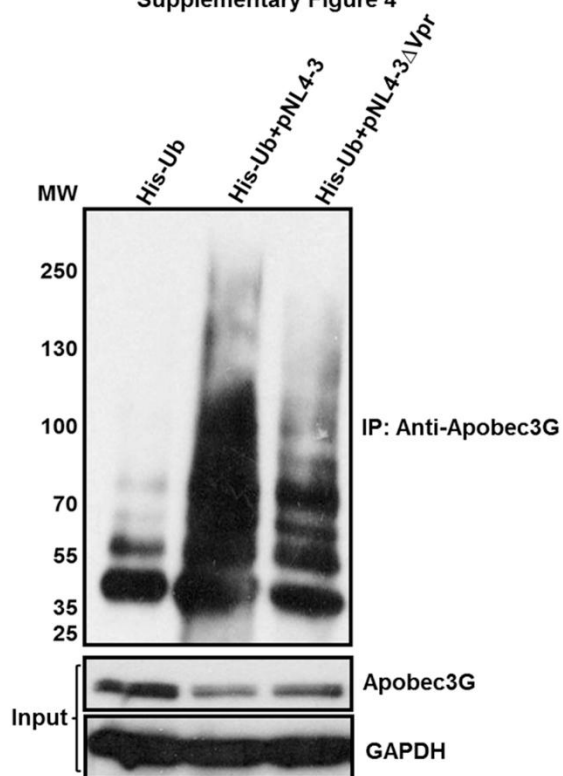


Figure S4: Ubiquitination of APOBEC3G in presence and absence of Vpr.

TZM-bl cells were co-transfected with His-Ub and pNL4-3/pNL4-3 Δ vpr. Post 36 hours, MG132 treatment was given for 8 hours and ubiquitinated proteins were enriched using Ni-NTA beads. Immunoblot analysis was done using anti-APOBEC3G (NIH Cat No. 9936) antibody. Levels of proteins are shown in the input (without MG132 treatment). GAPDH was used as a loading control.

Supplementary Figure 5

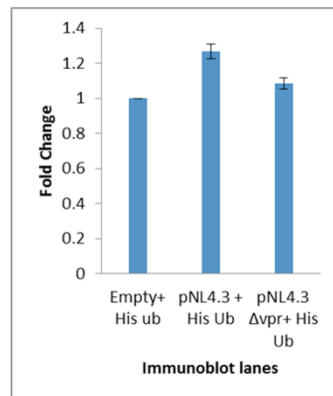


Figure S5: Quantitation of tubulin levels (input) in immunoblot 5E.

Densitometric analysis of input bands was done using Image J software. The levels were normalized with GAPDH.

Supplemental Reference

1. **Bano AS, Sood V, Neogi U, Goel N, Kuttia VS, Wanchu A, Banerjea AC.** 2009. Genetic and functional characterization of human immunodeficiency virus type 1 VprC variants from north India: presence of unique recombinants with mosaic genomes from B, C and D subtypes within the open reading frame of Vpr. *J Gen Virol* **90**:2768-2776.
2. **Thompson JD, Higgins DG, Gibson TJ.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**:4673-4680.
3. **Nabel G, Baltimore D.** 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature* **326**:711-713.
4. **Kimura M.** 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**:111-120.
5. **Tamura K, Dudley J, Nei M, Kumar S.** 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**:1596-1599.