

# **Supplementary Information**

## **Structural basis of lenacapavir-induced HIV-1 capsid defects during virion maturation**

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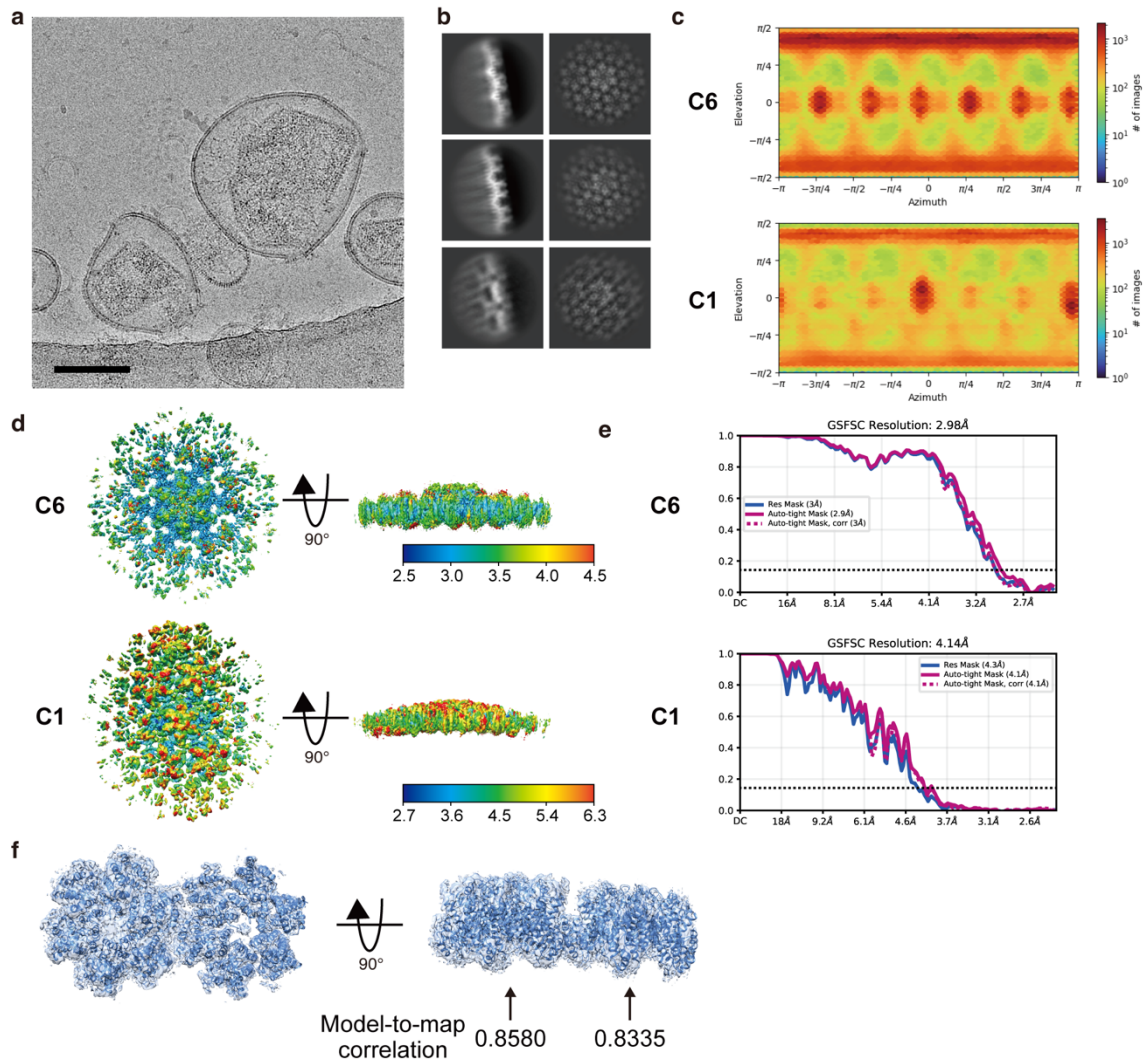
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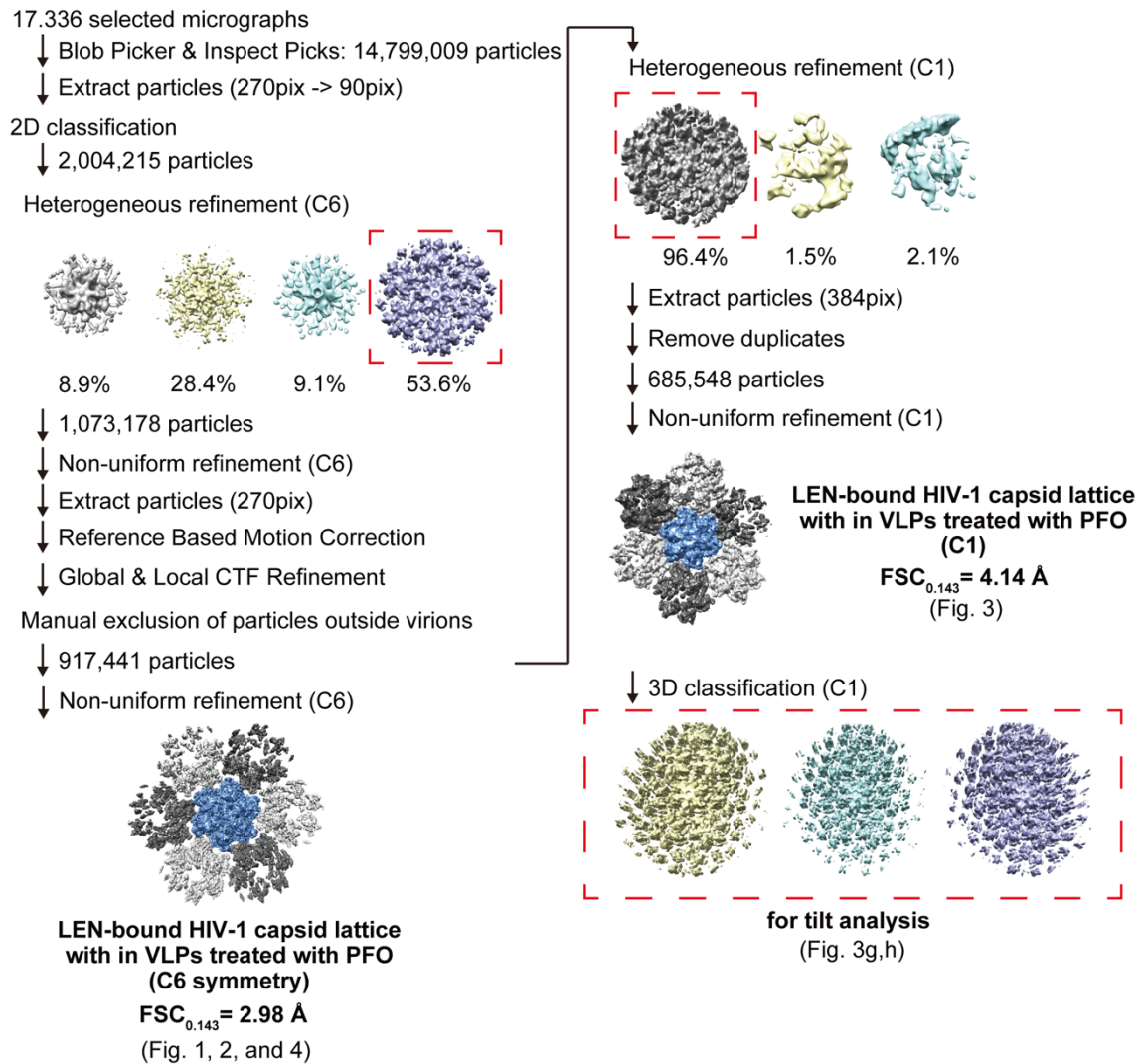
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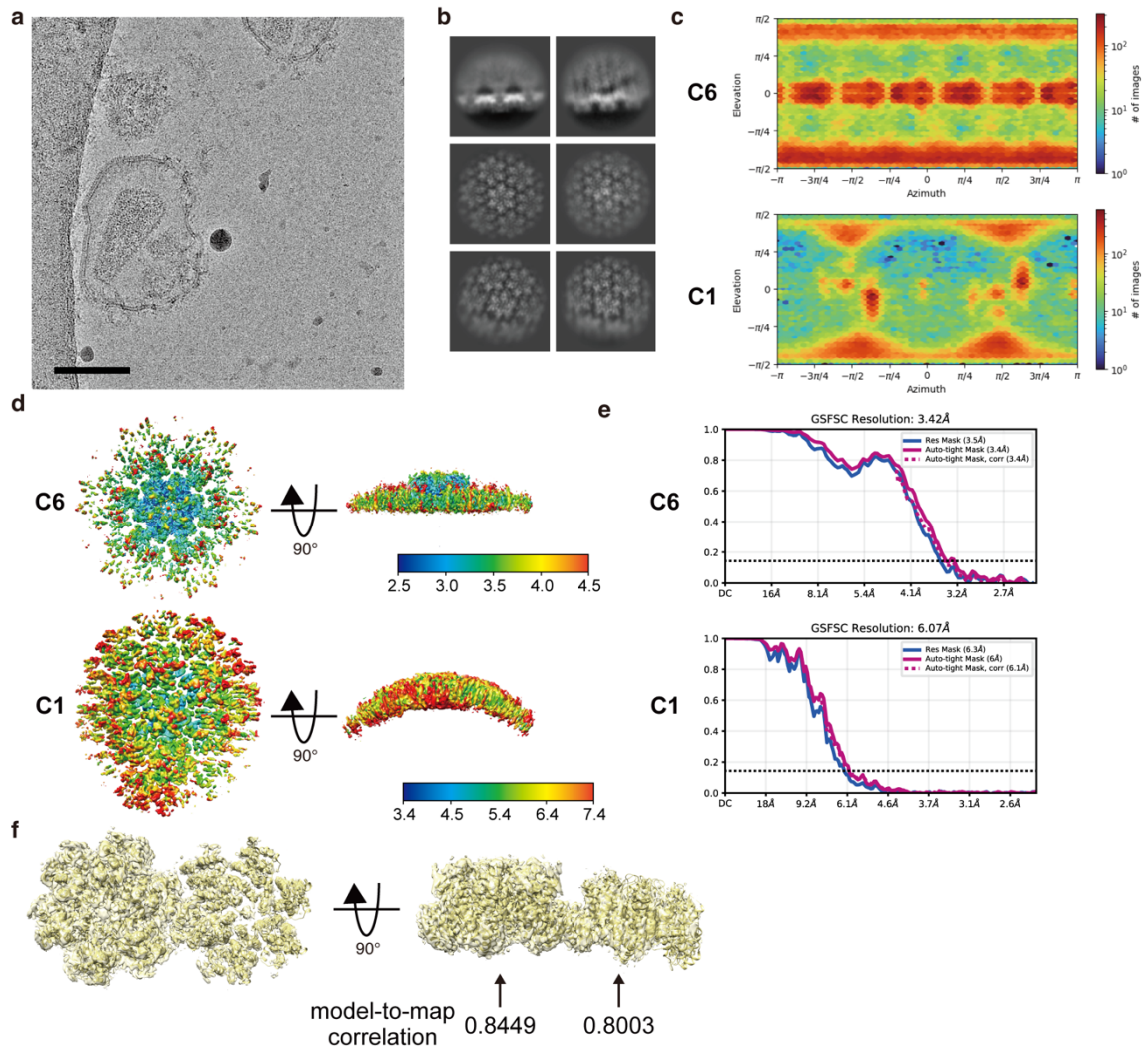
**Supplementary Fig. 1. Cryo-EM analysis of the LEN-bound HIV-1 capsid lattice within VLPs treated with PFO.**

**a**, Representative micrograph from the cryo-EM imaging dataset. Scale bar, 100 nm. **b**, Selected 2D class averages. **c**, Euler angle distribution plots of the particles of the LEN-bound CA lattice contributing to the final reconstruction. **d**, Local resolution map of the LEN-bound CA lattice. **e**, Fourier shell correlation (FSC) curve for the LEN-bound CA lattice. The dashed line indicates the FSC threshold of 0.143. **f**, Model-to-map fit of

adjacent CA hexamer models in the C6-symmetric LEN-bound CA lattice map. Model-to-map correlation values were calculated using “fit in map” tool in UCSF Chimera.



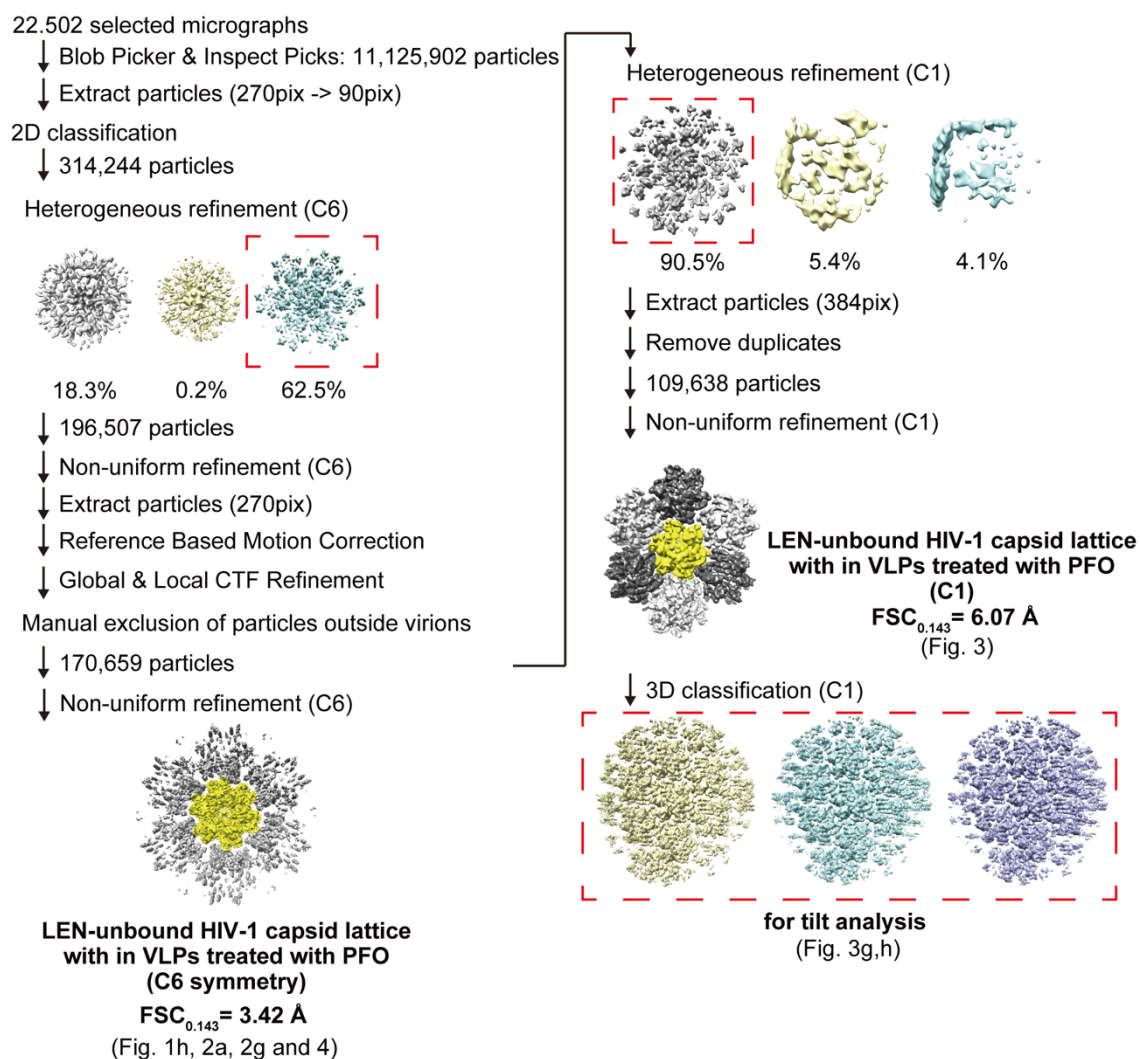
**Supplementary Fig. 2. Data processing workflow for the LEN-bound HIV-1 capsid lattice within VLPs treated with PFO.**



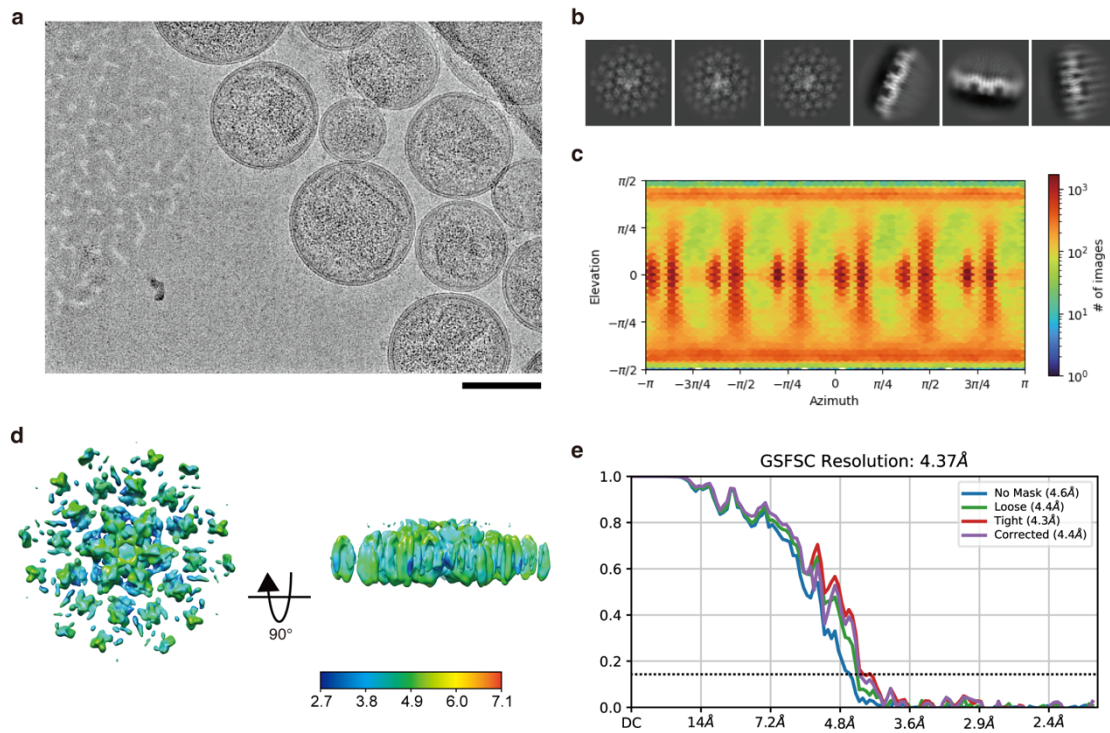
**Supplementary Fig. 3. Cryo-EM analysis of the LEN-unbound HIV-1 capsid lattice within VLPs treated with PFO.**

**a**, Representative micrograph from the cryo-EM imaging dataset. Scale bar, 100 nm. **b**, Selected 2D class averages. **c**, Euler angle distribution plots of the particles of the LEN-unbound CA lattice contributing to the final reconstruction. **d**, Local resolution map of the LEN-unbound CA lattice. **e**, Fourier shell correlation (FSC) curve for the LEN-unbound CA lattice. The dashed line indicates the FSC threshold of 0.143. **f**, Model-to-map fit of adjacent CA hexamer models in the C6-symmetric LEN-unbound CA lattice

map. Model-to-map correlation values were calculated using “fit in map” tool in UCSF Chimera.

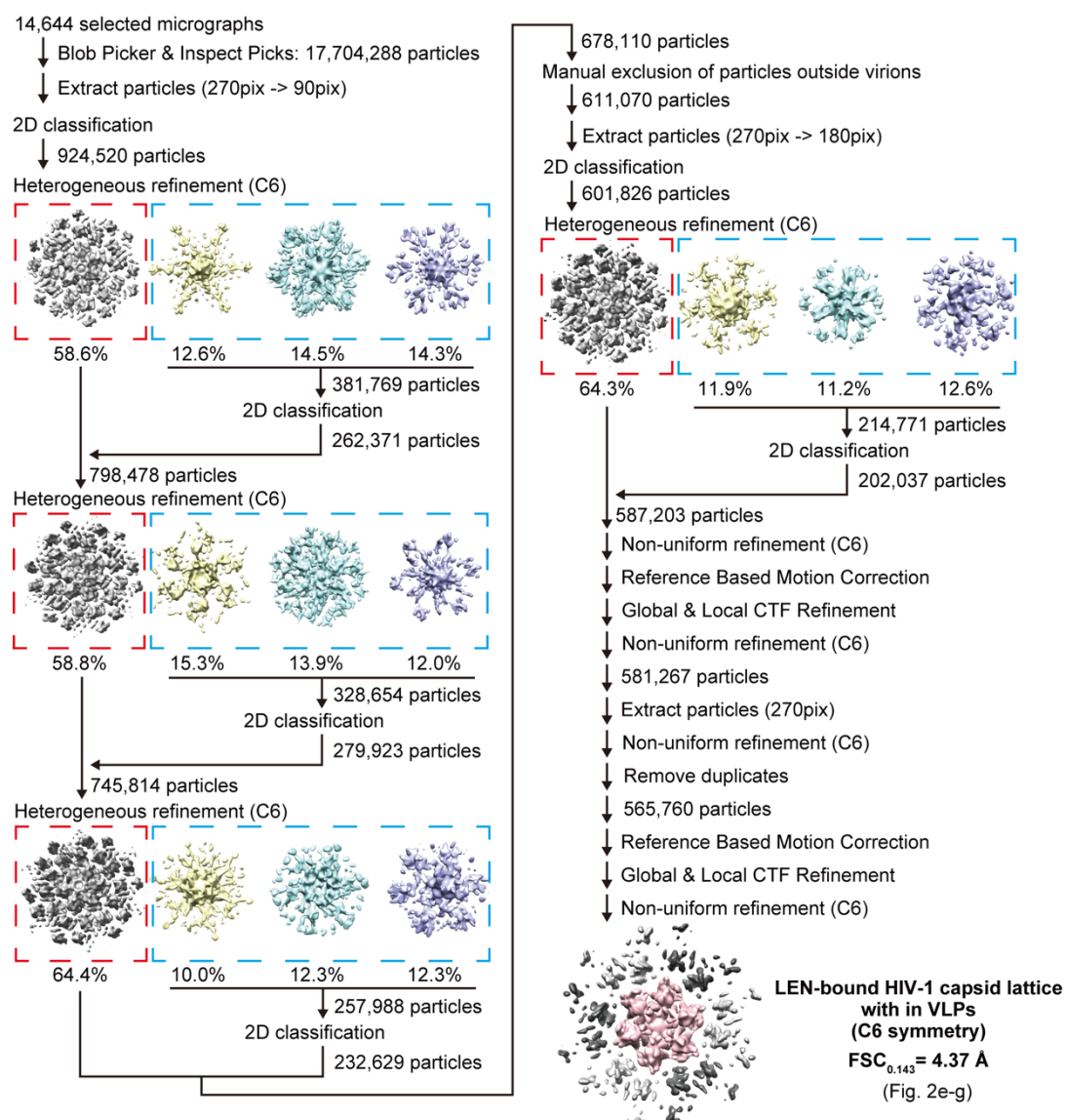


**Supplementary Fig. 4. Data processing workflow for the LEN-unbound HIV-1 capsid lattice within VLPs treated with PFO.**

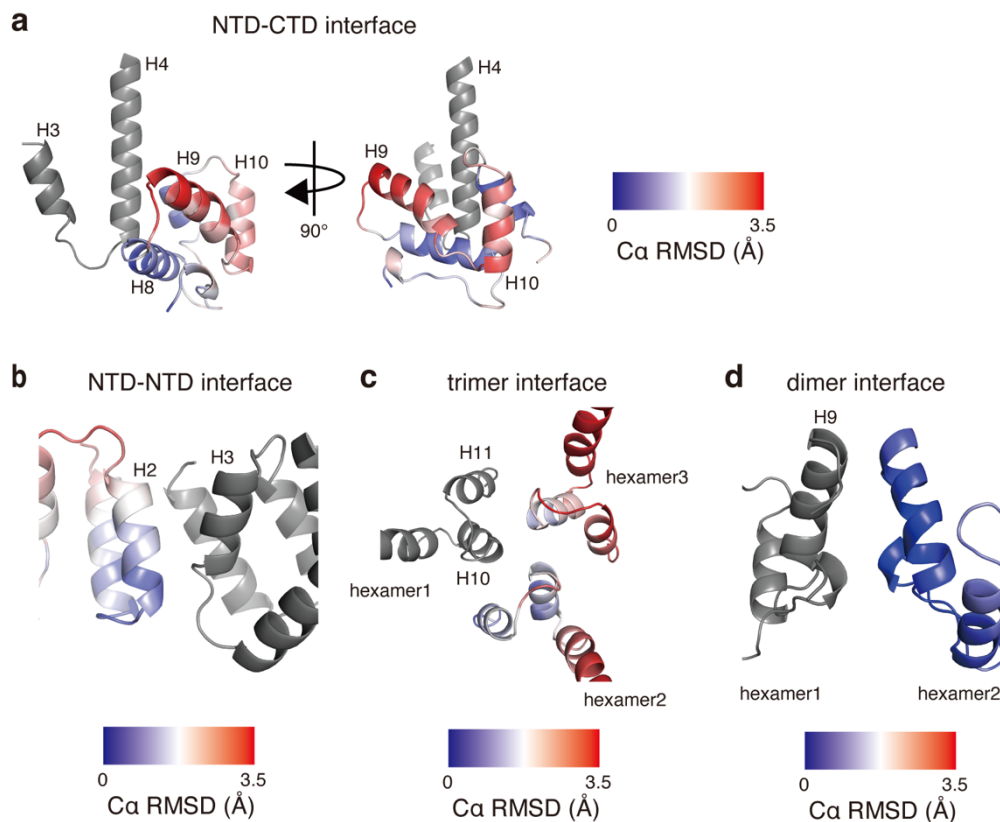


**Supplementary Fig. 5. Cryo-EM analysis of the LEN-bound HIV-1 capsid lattice within intact VLPs.**

**a**, Representative micrograph from the cryo-EM imaging dataset. Scale bar, 100 nm. **b**, Selected 2D class averages. **c**, Euler angle distribution plots of the particles of the LEN-bound CA lattice contributing to the final reconstruction. **d**, Local resolution map of the LEN-bound CA lattice. **e**, Fourier shell correlation (FSC) curve for the LEN-bound CA lattice. The dashed line indicates the FSC threshold of 0.143.

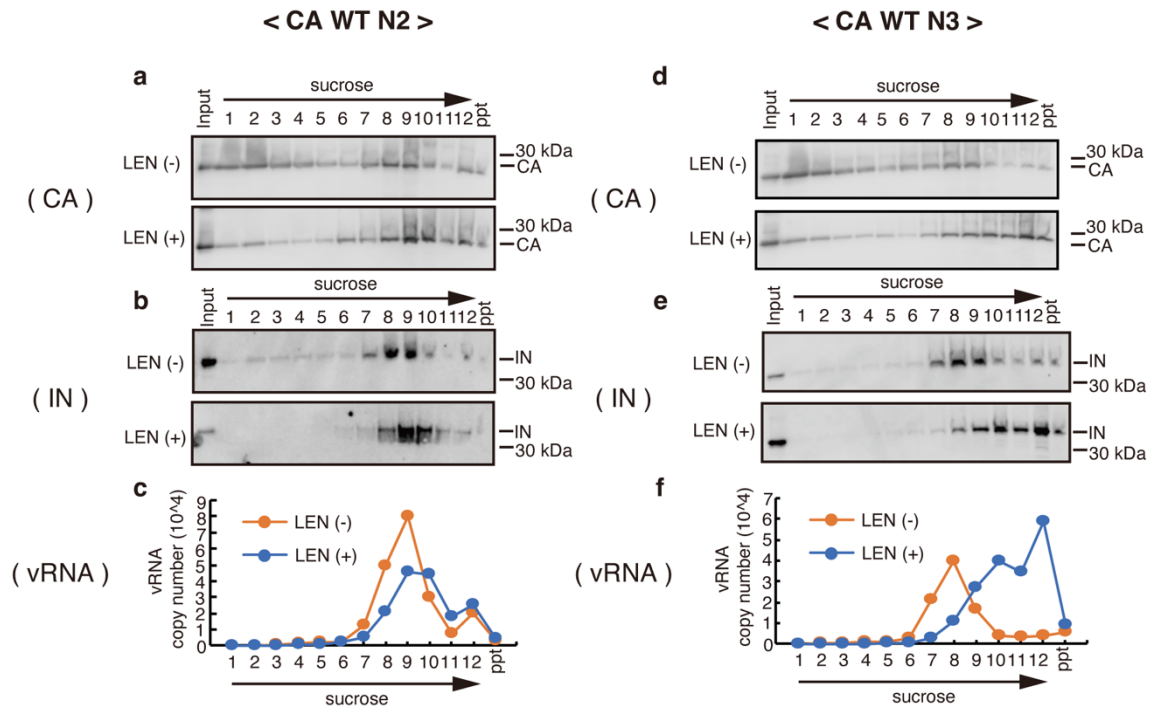


**Supplementary Fig. 6. Data processing workflow for the LEN-bound HIV-1 capsid lattice within intact VLPs.**



**Supplementary Fig. 7. RMSD-based comparison of intra- and interhexamer interfaces within the LEN-unbound and LEN-bound capsid lattices.**

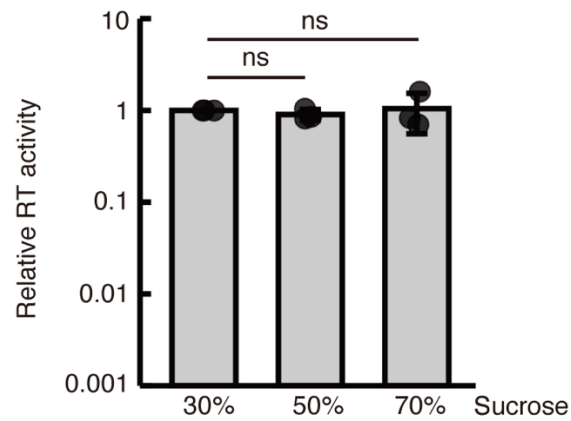
**a**, Comparison of the NTD–CTD interface between CA molecules in the region surrounding the FG pocket based on the cryo-EM structures of the LEN-bound and LEN-unbound CA lattices. This panel corresponds to Fig. 4b. **b**, Comparison of the NTD–NTD interface between CA molecules mediated by helices 2 and 3 based on the cryo-EM structures of the LEN-bound and LEN-unbound CA lattices. This panel corresponds to Fig. 4c. **c**, Comparison of the CTD-mediated trimer interface between CA molecules mediated by primary helix 10 based on the cryo-EM structures of the LEN-bound and LEN-unbound CA lattices. This panel corresponds to Fig. 4d. **d**, Comparison of the CTD–CTD dimer interface between CA molecules mediated by helix 9 based on the cryo-EM structures of the LEN-bound and LEN-unbound CA lattices. This panel corresponds to Fig. 4e. Models are coloured according to the RMSDs between the LEN-bound and LEN-unbound CA structures, from blue (0 Å RMSD) to red (3.5 Å RMSD).



**Supplementary Fig. 8. Reproducibility of sucrose gradient fractionation of WT**

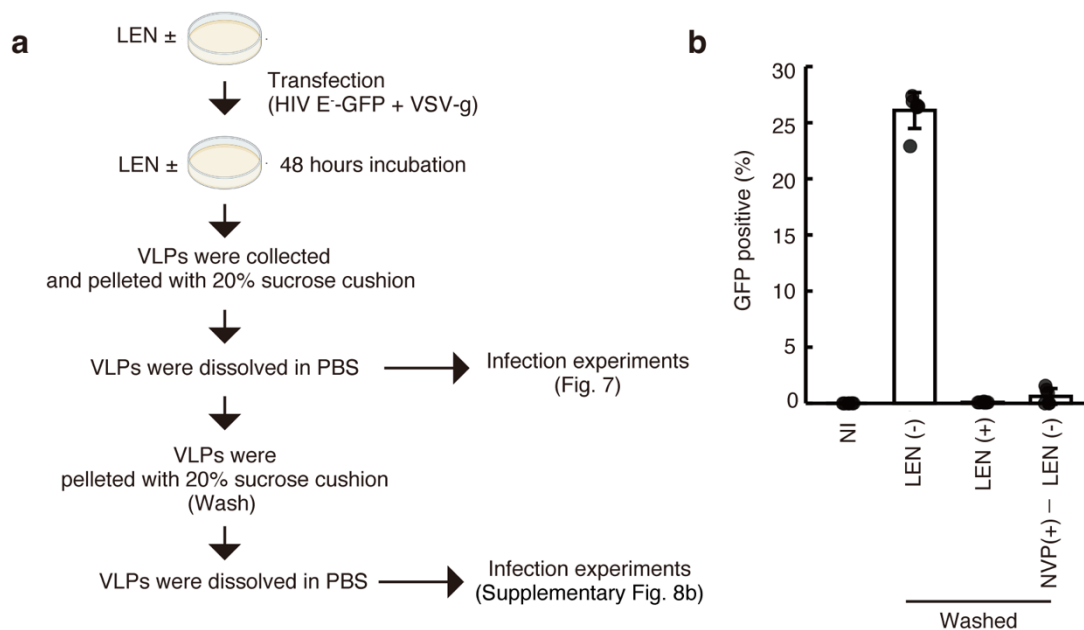
**HIV-GFP virions produced in the presence or absence of LEN.**

CA (a, d) and IN (b, e) levels were detected by western blotting. The amount of viral RNA in each fraction was quantified by RT-qPCR (c, f). The first experiment is shown as representative data in Fig. 5a–c, and the results of the two additional independent experiments are provided.



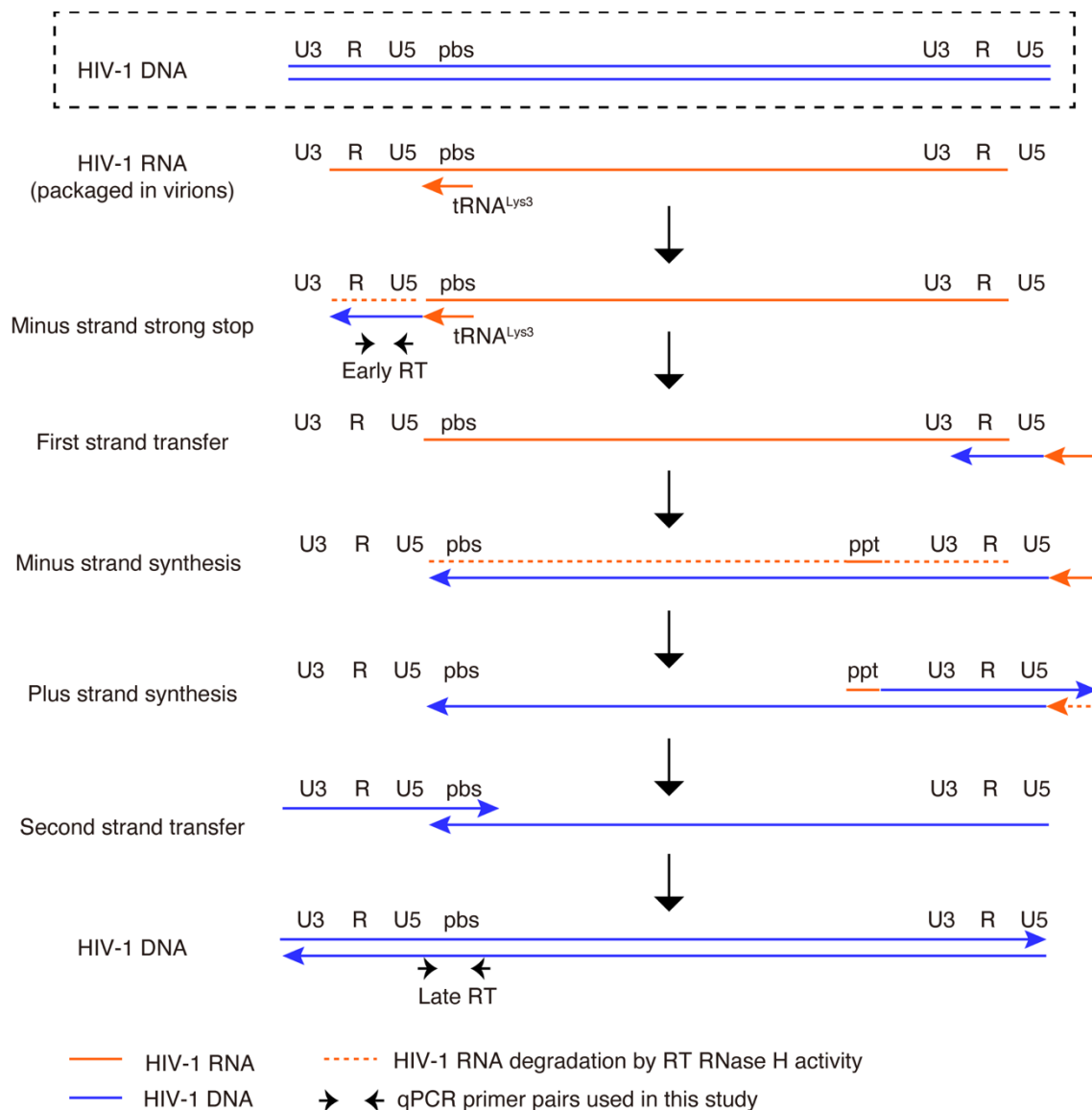
**Supplementary Fig. 9. Effect of sucrose concentration on reverse transcriptase activity.**

Reverse transcriptase (RT) activity was measured in the presence of 30%, 50%, and 70% sucrose. RT activity was normalized to that of the 30% sucrose sample. The data are shown as the means  $\pm$  s.d. ( $n = 3$  independent experiments). Statistical significance was assessed using a paired two-tailed Student's *t*-test. (30% vs 50%,  $P = 0.296$ ; 30% vs 70%,  $P = 0.883$ )



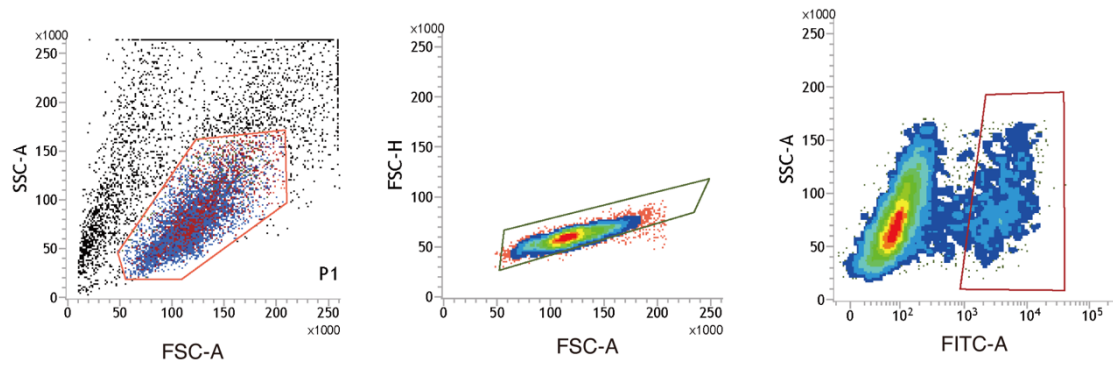
**Supplementary Fig. 10. HIV-1 produced in the presence of LEN exhibits reduced infectivity.**

**a.** Schematic of the experimental design. HIV-GFP virions were produced in the presence of DMSO or 50 nM LEN and harvested at 48 h post-transfection. Virions were pelleted through a 20% sucrose cushion to remove the residual free LEN before being used for the infectivity assay shown in Fig. 7. Virions were washed with PBS and pelleted again through a 20% sucrose cushion before being used for the infectivity assay shown in (b) to further minimize LEN carryover. **b.** Infectivity in Jurkat cells infected with HIV-GFP virions produced in the presence or absence of 50 nM LEN after an additional wash through a sucrose cushion. The data are presented as the means  $\pm$  s.d.; n = 6 independently treated cell cultures.



**Supplementary Fig. 11. Schematic of HIV-1 reverse transcription and the positions of the primers used for qPCR in this study.**

Early RT primers amplify minus-strand strong stop DNA (early reverse transcription products), whereas late RT primers amplify late reverse transcription products after plus-strand synthesis. Red and blue lines indicate HIV-1 RNA and DNA, respectively. Dashed orange lines indicate RNA degraded by the RNase H activity of reverse transcriptase. The U3, R, and U5 regions in the LTRs and the ppt (polypurine tract) are indicated. The schematic was adapted from Christensen *et al.*<sup>45</sup>.



**Supplementary Fig. 12. Flow cytometry gating strategy.**

The first gate (FSC-A versus SSC-A) selects cells and excludes debris. The second gate (FSC-A versus FSC-H) selects singlets. The third gate (FITC-A versus SSC-A) identifies GFP-positive cells.

**Supplementary Table 1. Median and mean areas of virus-like particles produced in the presence or absence of LEN.**

|                           | DMSO  | 50 nM LEN |
|---------------------------|-------|-----------|
| Median (nm <sup>2</sup> ) | 13525 | 15415     |
| Mean (nm <sup>2</sup> )   | 13607 | 20188     |

**Supplementary Table 2. Cryo-EM data collection, refinement and validation**

statistics.

|                                  | LEN-bound<br>CA lattice<br>within VLPs<br>treated with<br>PFO<br>(C6 sym.) | LEN-bound<br>CA lattice<br>within VLPs<br>treated with<br>PFO<br>(C1) | LEN-<br>unbound<br>CA lattice<br>within VLPs<br>treated with<br>PFO<br>(C6 sym.) | LEN-<br>unbound<br>CA lattice<br>within VLPs<br>treated with<br>PFO<br>(C1) | LEN-bound<br>CA lattice<br>within VLPs<br>(C6 sym.) |
|----------------------------------|----------------------------------------------------------------------------|-----------------------------------------------------------------------|----------------------------------------------------------------------------------|-----------------------------------------------------------------------------|-----------------------------------------------------|
| Data collection                  |                                                                            |                                                                       |                                                                                  |                                                                             |                                                     |
| Microscope                       | Krios G4                                                                   |                                                                       | Krios G4                                                                         |                                                                             | Krios G4                                            |
| Source                           | E-CFEG                                                                     |                                                                       | E-CFEG                                                                           |                                                                             | X-FEG                                               |
| Detector                         | Falcon 4i                                                                  |                                                                       | Falcon 4i                                                                        |                                                                             | K3                                                  |
| Energy Filter                    | Selectris                                                                  |                                                                       | Selectris                                                                        |                                                                             | BioContinuum                                        |
| Slit Width (eV)                  | 10                                                                         |                                                                       | 10                                                                               |                                                                             | 20                                                  |
| Magnification                    | 105,000                                                                    |                                                                       | 105,000                                                                          |                                                                             | 81,000                                              |
| Voltage (kV)                     | 300                                                                        |                                                                       | 300                                                                              |                                                                             | 300                                                 |
| Total dose (e-/Å²)               | 53.2                                                                       |                                                                       | 48.4                                                                             |                                                                             | 50.8                                                |
| Defocus range (µm)               | -0.8 to -2.4                                                               |                                                                       | -0.8 to -2.4                                                                     |                                                                             | -0.8 to -2.4                                        |
| Pixel size (Å)                   | 1.2                                                                        |                                                                       | 1.2                                                                              |                                                                             | 1.06                                                |
| Data processing                  |                                                                            |                                                                       |                                                                                  |                                                                             |                                                     |
| Final particle images<br>(no.)   | 917,441                                                                    | 685,548                                                               | 170,659                                                                          | 109,638                                                                     | 565,760                                             |
| Map resolution (Å)               | 2.98                                                                       | 4.14                                                                  | 3.42                                                                             | 6.07                                                                        | 4.37                                                |
| FSC threshold                    | (FSC=0.143)                                                                | (FSC=0.143)                                                           | (FSC=0.143)                                                                      | (FSC=0.143)                                                                 | (FSC=0.143)                                         |
| Symmetry imposed                 | C6                                                                         | C1                                                                    | C6                                                                               | C1                                                                          | C6                                                  |
| EMDB ID                          | 81945                                                                      | 81946                                                                 | 81947                                                                            | 81948                                                                       | 67116                                               |
| Refinement                       |                                                                            |                                                                       |                                                                                  |                                                                             |                                                     |
| Initial model used<br>(PDB code) | 4XFX                                                                       |                                                                       | 4XFX                                                                             |                                                                             | 4XFX                                                |
| Model composition                |                                                                            |                                                                       |                                                                                  |                                                                             |                                                     |
| Non-hydrogen atoms               | 9744                                                                       |                                                                       | 9744                                                                             |                                                                             | 4992                                                |
| Protein residues                 | 1248                                                                       |                                                                       | 1248                                                                             |                                                                             | 1248                                                |
| Ligands                          | 0                                                                          |                                                                       | 0                                                                                |                                                                             | 0                                                   |
| R.m.s. deviations                |                                                                            |                                                                       |                                                                                  |                                                                             |                                                     |
| Bond lengths (Å)                 | 0.005                                                                      |                                                                       | 0.004                                                                            |                                                                             | 0.005                                               |
| Bond angles (°)                  | 0.945                                                                      |                                                                       | 0.925                                                                            |                                                                             | 1.143                                               |
| Validation                       |                                                                            |                                                                       |                                                                                  |                                                                             |                                                     |
| MolProbity score                 | 1.66                                                                       |                                                                       | 1.75                                                                             |                                                                             | 0.89                                                |
| Clashscore                       | 11.37                                                                      |                                                                       | 14.09                                                                            |                                                                             | 0.95                                                |
| Poor rotamers (%)                | 0                                                                          |                                                                       | 0                                                                                |                                                                             | 0                                                   |
| Ramachandran plot                |                                                                            |                                                                       |                                                                                  |                                                                             |                                                     |
| Favored (%)                      | 97.55                                                                      |                                                                       | 97.55                                                                            |                                                                             | 97.47                                               |
| Allowed (%)                      | 2.45                                                                       |                                                                       | 2.45                                                                             |                                                                             | 2.53                                                |
| Disallowed (%)                   | 0                                                                          |                                                                       | 0                                                                                |                                                                             | 0                                                   |
| PDB ID                           | 43KN                                                                       |                                                                       | 43KO                                                                             |                                                                             | 9XQH                                                |

**Supplementary Table 3. Median and mean areas of HIV-1 virions produced in the presence or absence of LEN.**

|                           | DMSO  | 2 nM LEN | 5 nM LEN | 10 nM LEN | 50 nM LEN |
|---------------------------|-------|----------|----------|-----------|-----------|
| Median (nm <sup>2</sup> ) | 12035 | 11844    | 12081    | 12815     | 13461     |
| Mean (nm <sup>2</sup> )   | 12514 | 12330    | 13046    | 14092     | 17014     |

**Supplementary Table 4. Primers used for the quantification of HIV DNA.**

| Name       | Sequence (5'–3')                               | Target                                            | References              |
|------------|------------------------------------------------|---------------------------------------------------|-------------------------|
| Early RT_F | GGCTAACTAGGGAACCCACTG<br>C                     | Early RT product                                  |                         |
| Early RT_R | CAACAGACGGGCACACACTAC<br>T                     | Early RT product                                  |                         |
| Late RT_F  | TGTGTGCCCCGTCTGTTGTGT                          | Late RT product                                   | ( <sup>45</sup> )       |
| Late RT_R  | CTTCAGCAAGCCGAGTCCTG                           | Late RT product                                   | ( <sup>45</sup> )       |
| L-M667     | ATGCCACGTAAGCGAAACTCT<br>GGCTAACTAGGGAACCCACTG | Integrated HIV<br>(Alu-PCR (1st round of<br>PCR)) | ( <sup>44,46,47</sup> ) |
| Alu 1      | TCCCAGCTACTGGGGAGGCT<br>GAGG                   | Integrated HIV<br>(Alu-PCR (1st round of<br>PCR)) | ( <sup>44,46,47</sup> ) |
| Alu 2      | GCCTCCCAAAGTGCTGGGATT<br>ACAG                  | Integrated HIV<br>(Alu-PCR (1st round of<br>PCR)) | ( <sup>44,46,47</sup> ) |
| Lambda T   | ATGCCACGTAAGCGAAACT                            | Integrated HIV<br>(Alu-PCR (2nd round of<br>PCR)) | ( <sup>44,46,47</sup> ) |
| AA55M      | GCTAGAGATTTTCCCACTGA<br>CTAA                   | Integrated HIV<br>(Alu-PCR (2nd round of<br>PCR)) | ( <sup>44,46,47</sup> ) |
| β-globin-F | CCCTTGGAACCCAGAGTTCT                           | β-globin                                          | ( <sup>44,46,47</sup> ) |
| β-globin-R | CGAGCACTTTCTTGCCATGA                           | β-globin                                          | ( <sup>44,46,47</sup> ) |