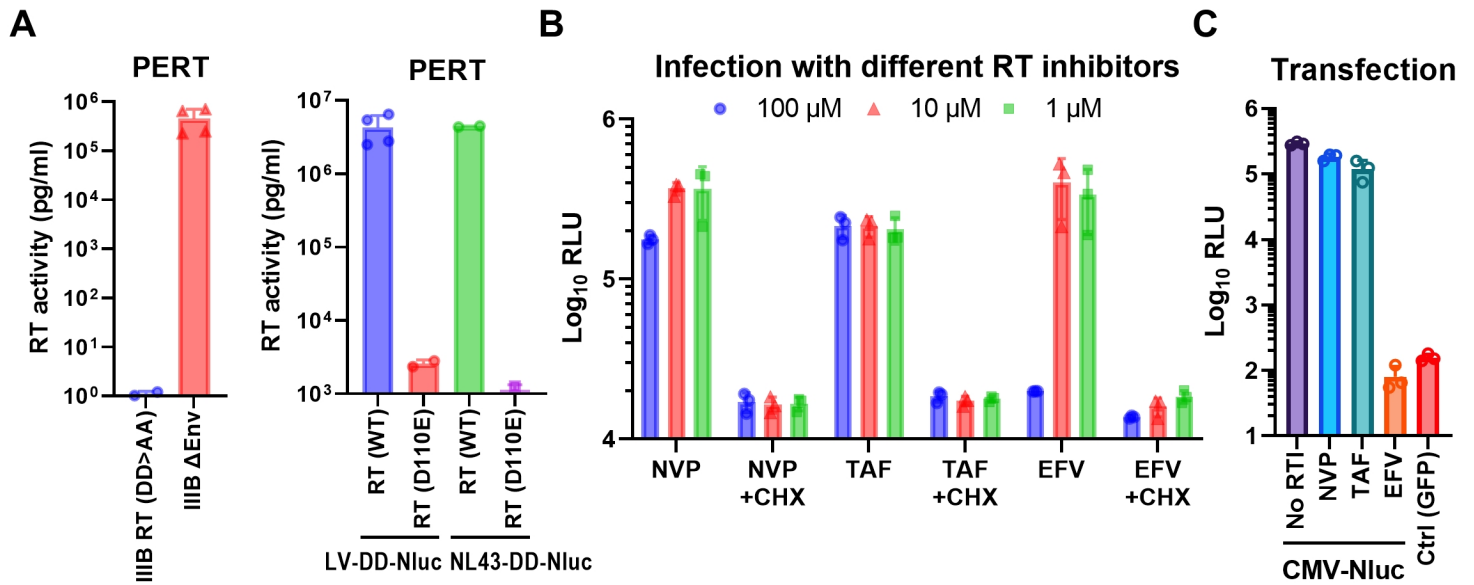


## **Supplementary information**

### **Direct translation of incoming retroviral genomes**

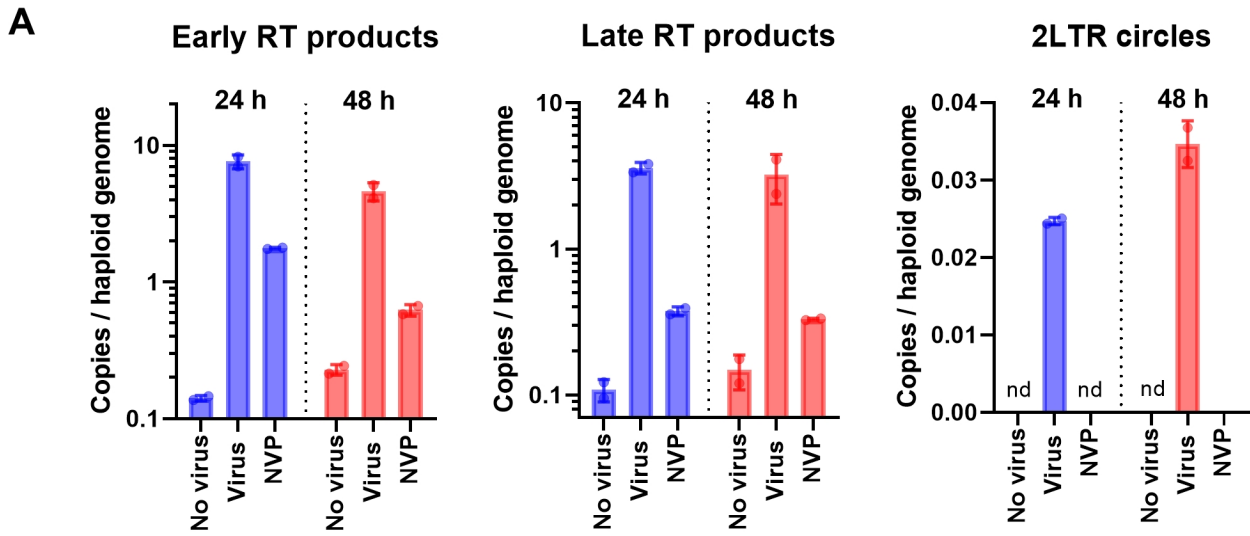
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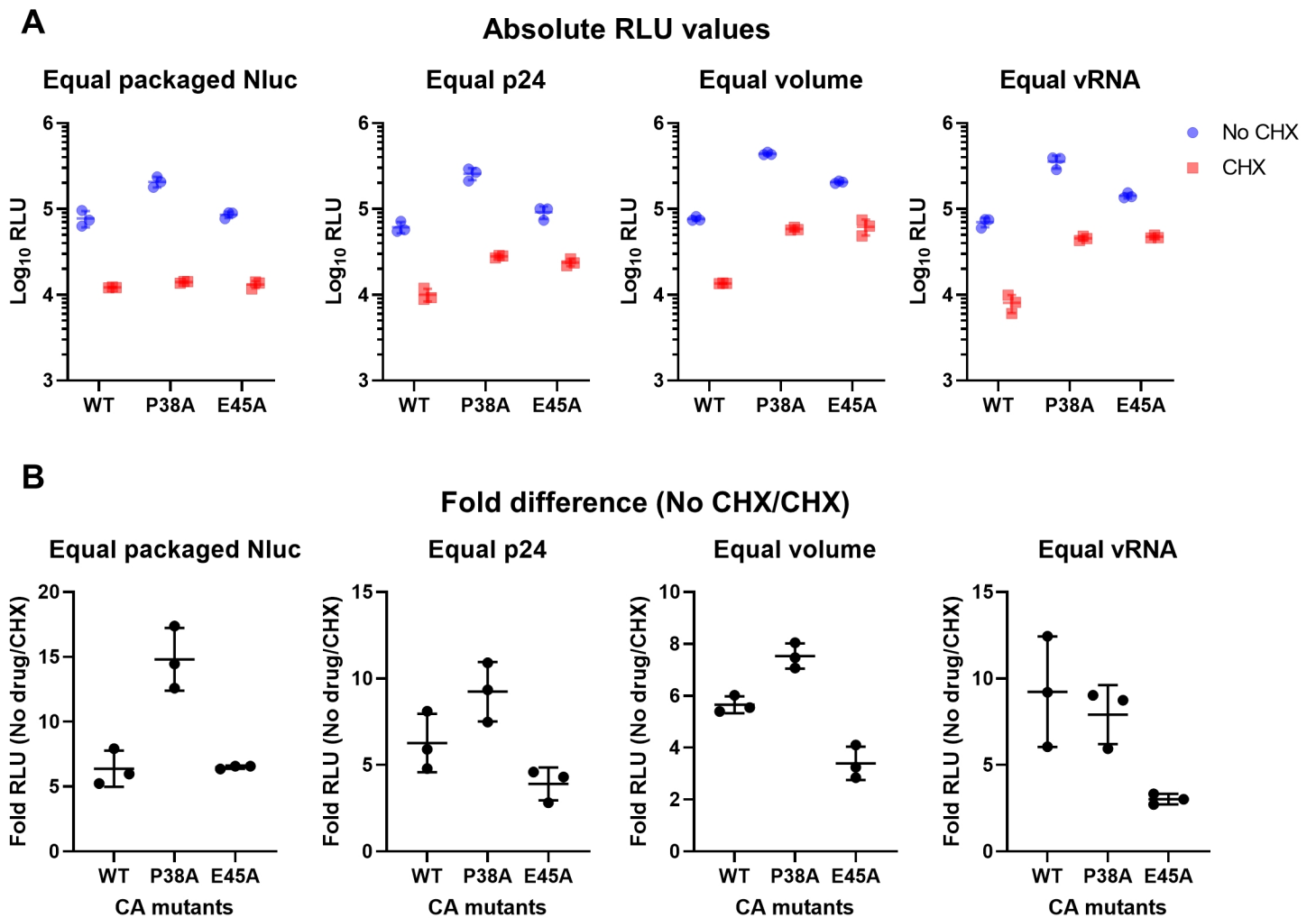


**Supplementary Figure 1. Loss of RT activity by catalytic mutations or the use of different RT inhibitors.**

(A) PERT assay on RT catalytic mutant virus HIV-1 IIIB (DD185/186AA) used in polysome fractionation, or the LV-DD-Nluc and NL43-DD-Nluc viruses produced with the RT catalytic mutant packaging vector (D110E) used in reporter assays. (B) Luciferase assay on cells transduced with LV-DD-Nluc virus in the presence of 1-100 μM of the RT inhibitors, with or without CHX. (C) Cells were transfected with a CMV-driven Nluc construct in the presence of 100 μM RT inhibitors. Toxicity was measured by luciferase assay 6 hours after transfection. NVP: Nevirapine, EFV: Efavirenz, TDF: Tenofovir disoproxil fumarate. Data represent mean +/- SD. Source data are provided as a Source Data file.



**Supplementary Figure 2. Quantification of post-entry events after virus challenge.** (A) 293T cells were infected with NL43-Firefly virus (40 ng/100K cells) and DNA was isolated at 24 and 48 hours post-infection. Early and late RT products, as well as 2-LTR circles were quantified by qPCR. Source data are provided as a Source Data file.



**Supplementary Figure 3. Capsid stability mutations alter the translation potential of incoming retroviral genomes.**

(A) Cells were infected with LV-DD-Nluc viruses containing WT, P38A or E45A capsids in the presence of NVP and Shield1, with or without CHX. Virus stocks were normalized based on either p24, viral RNA, virion-packaged Nluc or simple volume. (B) Fold difference between CHX-treated and untreated samples based on the data in (A). Data represent mean +/- SD. Source data are provided as a Source Data file.

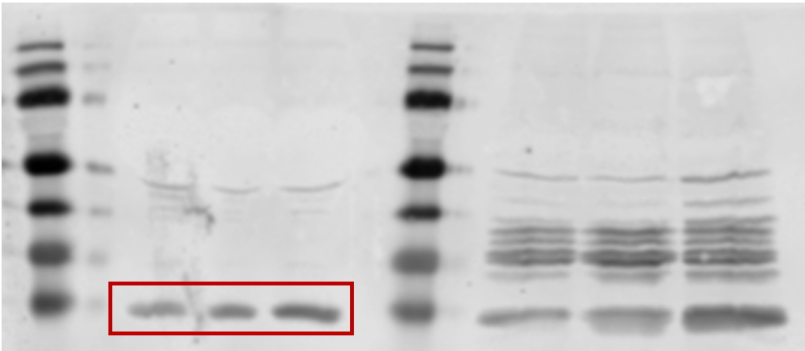
Fig. 3B



Fig. 4C



Fig. 5D



IB:  $\alpha$ -p24