

## Supporting Information

### **LEDGINs inhibit the post-integration stage of the HIV-1 replication by modulating integrase multimerization in the virions**

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#### **Legends of Supporting Figures**

**Figure S1. HIV-1 produced in the presence of CX05045 failed to replicate.** HIV-1 replication monitored in MT-4 cells (infected for 1 h and washed 3 times to remove as much carryover free compounds as possible) by p24 quantification for (A) NL4.3 and (B) HXB2D produced in the presence of DMSO, raltegravir, CX05045 or ritonavir in 293T cells. While ritonavir delayed breakthrough for a few days compared to DMSO and raltegravir, CX05045 apparently crippled the progeny virions and the p24 level rather goes down after splitting. Although cells were split on day 6 and 9 post infection to prevent overgrowth and cell death p24 sampling continued, all but CX05045-pretreated virus replicates. (C, D) Analysis of MOI dependency of the infectivity of (C) NL4.3 and (D) YU-2 viruses, we evaluated viral breakthrough with 5, 50 or 500 ng p24 equivalent inoculum in MT-4 cells and MDM, respectively. Virus produced in the presence of

21 CX05045 again did not replicate compared to the DMSO-, raltegravir- or ritonavir-pretreated  
22 viruses during the course of the experiment.

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24 **Figure S2. LEDGINs do not affect virus production or gRNA packaging**

25 HIV-1 (NL4.3, HXB2D and NL4.3<sub>A128T</sub>) were produced in the presence of CX05045 (5  $\mu$ M),  
26 raltegravir (0.03  $\mu$ M) or ritonavir (0.3 $\mu$ M). (A) Virus production and (B) gRNA packaging as  
27 quantified by p24 ELISA and RT-qPCR for each strains. Mean values  $\pm$  standard deviations are  
28 shown. \*\*\*p < 0.0001; 2-way ANOVA.

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30 **Figure S3. Nuclear import of HIV-1 PICs produced in the presence of CX05045.**

31 Representative images of HeLaP4 cells infected with HIV<sub>DMSO</sub> and HIV<sub>CX05045</sub>. 7 hpi with HIV-  
32 IN-eGFP (*green*), cells were fixed and immunostained with the nuclear lamina A/C primary  
33 antibody and Alexa Fluor 633 labeled secondary antibody (*blue*). Three-dimensional confocal z  
34 stacks were acquired with maximum projection and to acquire the images shown here only five  
35 stacks were overlaid centered in the middle of the nucleus. Cell shapes are outlined in white.  
36 White arrows indicate nuclear PICs.

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44 **Supporting Tables**

45 **Table S1. Estimates of the average delay of TOA of compounds and 95% CI for 50%**  
 46 **inhibition of single cycle HIV-1 replication and infectivity in MT-4 cells.**

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**Delay time estimates (h)<sup>a</sup>**

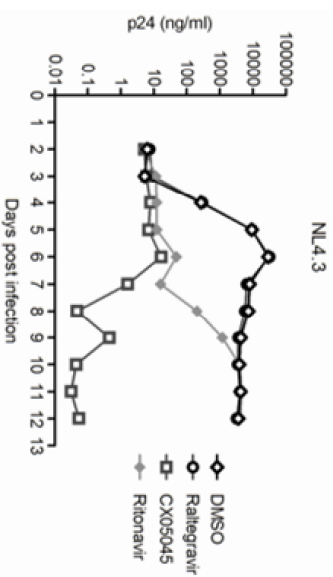
	<b>Parameter</b>	<b>AZT</b>	<b>Raltegravir</b>	<b>CX05045</b>	<b>Ritonavir</b>
	Time delay ( $t_{1/2}$ )	7.0	12.4	12.1	25.7
Single cycle replication inhibition <sup>b</sup>	95% CI for delay	6.6 – 7.4	12.2 – 12.5	11.7 – 12.5	25.5 – 26.0
	Goodness fit ( $R^2$ )	0.9112	0.9933	0.9167	0.7658
	Time delay ( $t_{1/2}$ )	4.0	11.3	24.5	20.6
Infectivity inhibition <sup>c</sup>	95% CI for delay	3.7 - 4.2	11.1 - 11.5	24.3 – 24.8	19.5-21.7
	Goodness fit ( $R^2$ )	0.9612	0.9862	0.8668	0.7203

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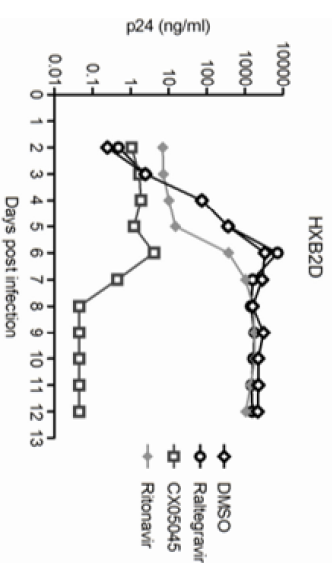
49 <sup>a</sup> Estimates of the time delays ( $t_{1/2}$ ; h) yielding 50% inhibition of HIV replication. <sup>b</sup> TOA was  
 50 performed to determine the estimated time delays for each compounds to corroborate with the  
 51 established target for each class of inhibitors. <sup>c</sup> The level of p24 protein in the supernatants of  
 52 MT-4 cells incubated with supernatants harvested from each time points of the TOA experiment  
 53 for each compound and relative replication capacity of the viruses was determined. The  
 54 calculation assumes the same replication rate across all compounds and all time points.

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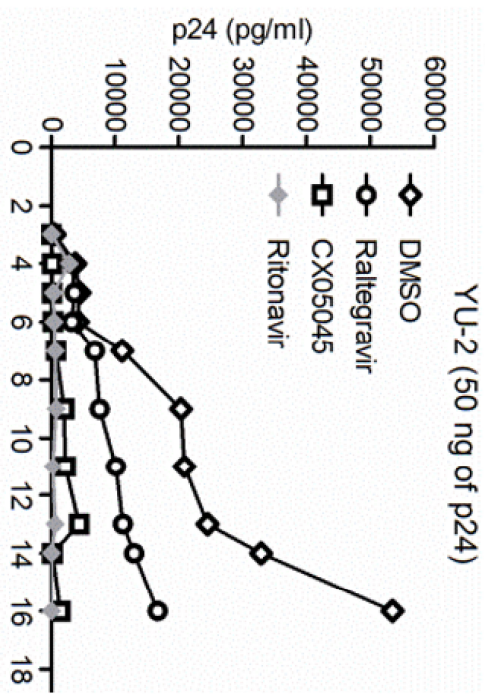
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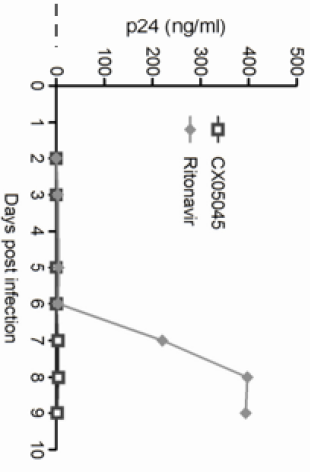
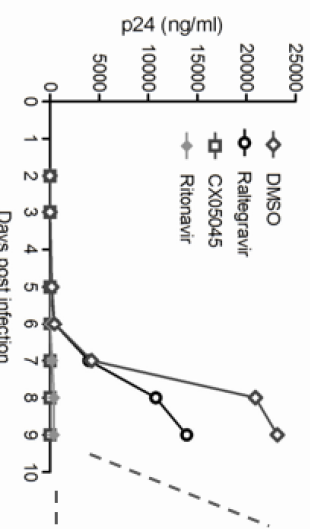
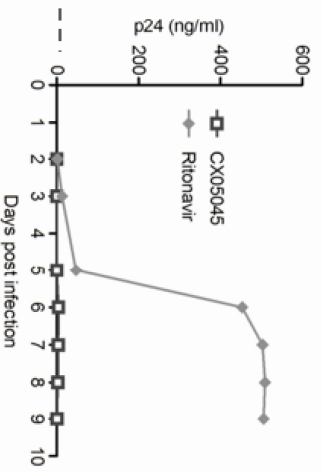
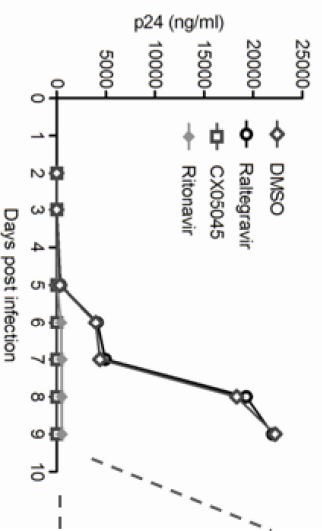
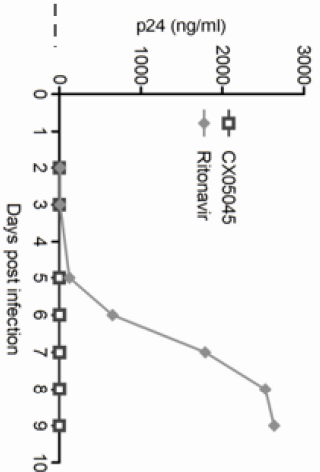
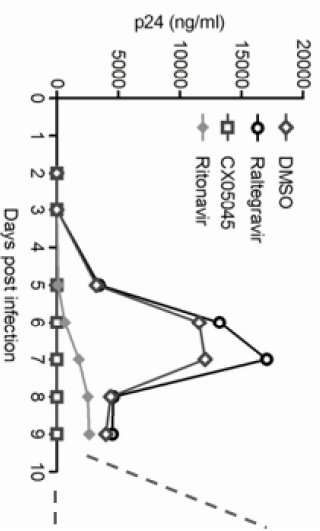
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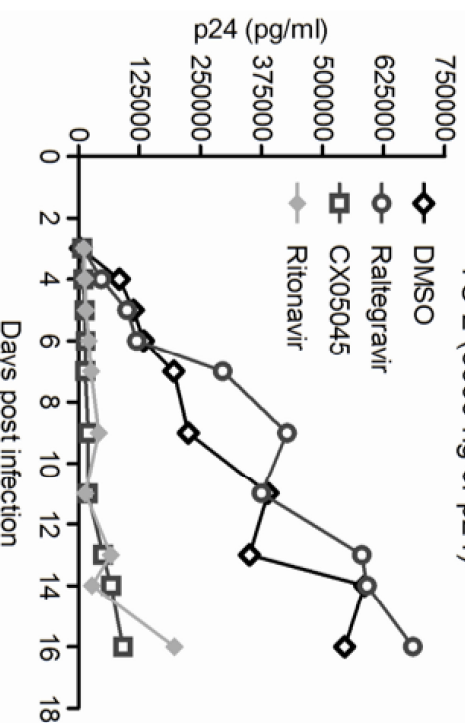
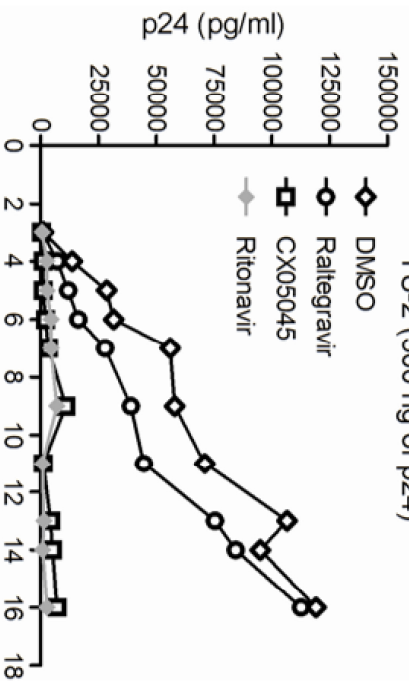
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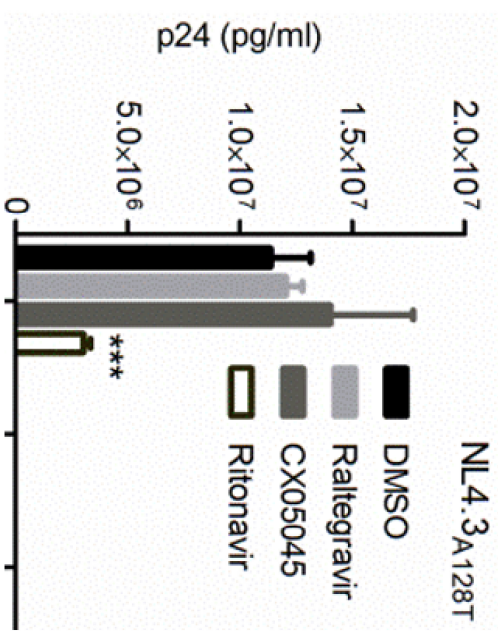
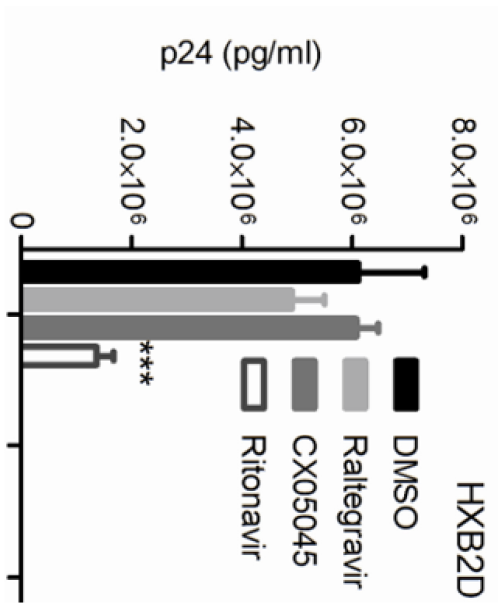
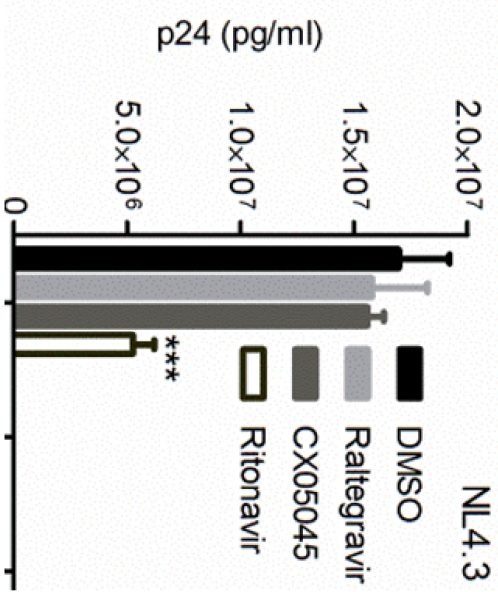
C



YU-2 (5000 ng of p24)



A



B

