

Legends to Supplementary Figures

Figure S1. Effect of tCDK inhibition on cell viability. A – C: JLat10.6 cells were pre-treated 30 minutes with the indicated concentration of YKL-5-124 (CDK7i) (A), LDC000067 (CDK9i) (B), or Senexin A (CDK8/19i) (C) and were subsequently stimulated with 5 nM PMA/ 1 μ M ionomycin. Following 20 hours, cell viability was assessed and the results are normalized to untreated cells ($n = 2$, mean \pm SD). D – F: As in (A – C), but JLat9.2 cells were assessed ($n = 2$, mean \pm SD).

Figure S2. Effect of tCDK inhibitor combinations on cell viability. A – C: JLat10.6 cells were pre-treated for 30 minutes with 100 nM YKL-5-124 (CDK7i) and the indicated concentration of LDC000067 (CDK9i) (A) or Senexin A (CDK8/19i) (B), or were pre-treated 30 minutes with 1 μ M LDC000067 and the indicated concentration of Senexin A (C). Subsequently, cells were incubated with 5 nM PMA/ 1 μ M ionomycin for 20 hours. Following incubation, cell viability was determined and normalized to untreated cells ($n = 2$, mean \pm SD).

Figure S3. IACS-9571 causes synergistic activation of latent HIV-1 in combination with PEP005. JLat10.6 cells were left untreated, or incubated with 5 nM PEP005, 20 μ M IACS-9571, or 5 nM PEP005/ 20 μ M IACS-9571. GFP expression was measured 20 hours later by flow cytometry (Fig. 4A). Calculation of synergy between the indicated treatments was performed using Bliss Independence Modeling. Data are presented as the difference between the

predicted and observed fractional HIV-1 expression response to the given drug combination ($n = 2$, mean \pm SD). See Materials and Methods for details.

Figure S4. Effect of YKL-5-124 on background fluorescence. **A:** Representative flow cytometry scatter plots of wildtype Jurkat cells that were left untreated or were pre-treated for 30 minutes with 100 nM YKL-5-124 and subsequently incubated with 5 nM PMA and 1 μ M ionomycin for 20 hours. **B, C:** Wildtype Jurkat cells treated as in (**A**) were analyzed by flow cytometry with GFP expression reported as the percent of GFP positive cells (**B**) and the GFP Mean Fluorescence Intensity (MFI) (**C**) ($n = 3$, mean \pm SD).

Figure S5. Requirement of Tat for tCDK dependent HIV-1 expression. **A:** Representative flow cytometry scatter plots of GXR-5 CEM cells (-Tat) pre-treated for 30 minutes with a vehicle control (DMSO), 100 nM YKL-5-124 (CDK7i), 1 μ M LDC000067 (CDK9i), or 50 μ M Senexin A (CDK8/19i) and subsequently incubated with 5 nM PMA and 1 μ M ionomycin for 20 hours. **B, C:** GXR-5 cells were untreated or pre-treated for 30 minutes with a vehicle control (DMSO), 100 nM YKL-5-124, 1 μ M LDC000067, or 50 μ M Senexin A prior to incubation with 5 nM PMA/ 1 μ M ionomycin. Following 20 hours, flow cytometry was performed with viral expression reported as the percent of GFP positive cells (**B**) and the delta GFP Mean Fluorescence Intensity (MFI) (**C**) ($n = 2$, mean \pm SD).

Figure S6. Effect of tCDK inhibitors on cell viability during HIV-1 infection. Jurkat human T cells were left untreated or were pre-treated for 30 minutes with 100 nM YKL-5-124, 1 μ M

LDC000067, or 10 μ M Senexin A. Cells were subsequently infected with RGH and 1-day post-infection, media was refreshed and cells were either washed of the drug or maintained with the same concentration of inhibitor. 4 days post-infection, viability was determined and normalized to untreated cells ($n = 3$, mean \pm SD).

Figure S7. Effect of CDK9 and/or CDK8/19 inhibition on mdHIV #110 proviral latency. A:

Simplified depiction of the replication incompetent mini-dual HIV-1 reporter virus that is chromosomally integrated in the mdHIV #110 Jurkat Tat clonal T cell line (51), where dsRed is expressed from the 5' LTR as a fusion with p24 Gag while an internal EF1 α promoter expresses eGFP. All other viral proteins including Tat are not expressed from the mini virus. **B:** mdHIV #110 cells were incubated with a vehicle control (DMSO), 1 μ M LDC000067, 10 μ M Senexin A, or 1 μ M LDC000067 and 10 μ M Senexin A for 14 days, after which the drug was removed from the media by washing and viral expression was assessed by flow cytometry for 10 more days. **C:** Representative flow cytometry scatter plots following 4 days of drug treatment and 4 days following drug withdrawal. All "dots" are indicative of a single HIV-1 infected cell with Q2 (dsRed+/eGFP+) possessing cells with transcriptionally active provirus. **D:** mdHIV #110 cells were treated as in (B) with viral expression assessed on the indicated day by flow cytometry ($n = 2$, mean \pm SD). **E:** mdHIV #110 cells treated as indicated in (B) were examined for viability, with values normalized to the vehicle control ($n = 2$, mean \pm SD).

Figure S8. Effect of structurally unrelated CDK8/19 inhibitors on HIV-1 latency. A:

mdHIV #77 cells were incubated with a vehicle control (DMSO), 10 μ M Senexin A, or 10 μ M

66 BRD6989 for 7 days. Subsequently, media was exchanged by washing to remove the drug and
67 viral expression was assessed for the next 10 days by flow cytometry. **B:** Representative flow
68 cytometry scatter plots following 4 days of drug treatment and 4 days following the removal of
69 drug. All “dots” are indicative of a single HIV-1 infected cell with Q2 (dsRed+/eGFP+)
70 possessing cells with transcriptionally active provirus. **C:** mdHIV #77 cells were treated as in
71 (A) with viral expression assessed on the indicated day by flow cytometry ($n = 2$, mean \pm SD).
72 **D:** mdHIV #77 cells treated as indicated in (A) were examined for viability, with values
73 normalized to cells treated with a vehicle control ($n = 2$, mean \pm SD).

Figure S1

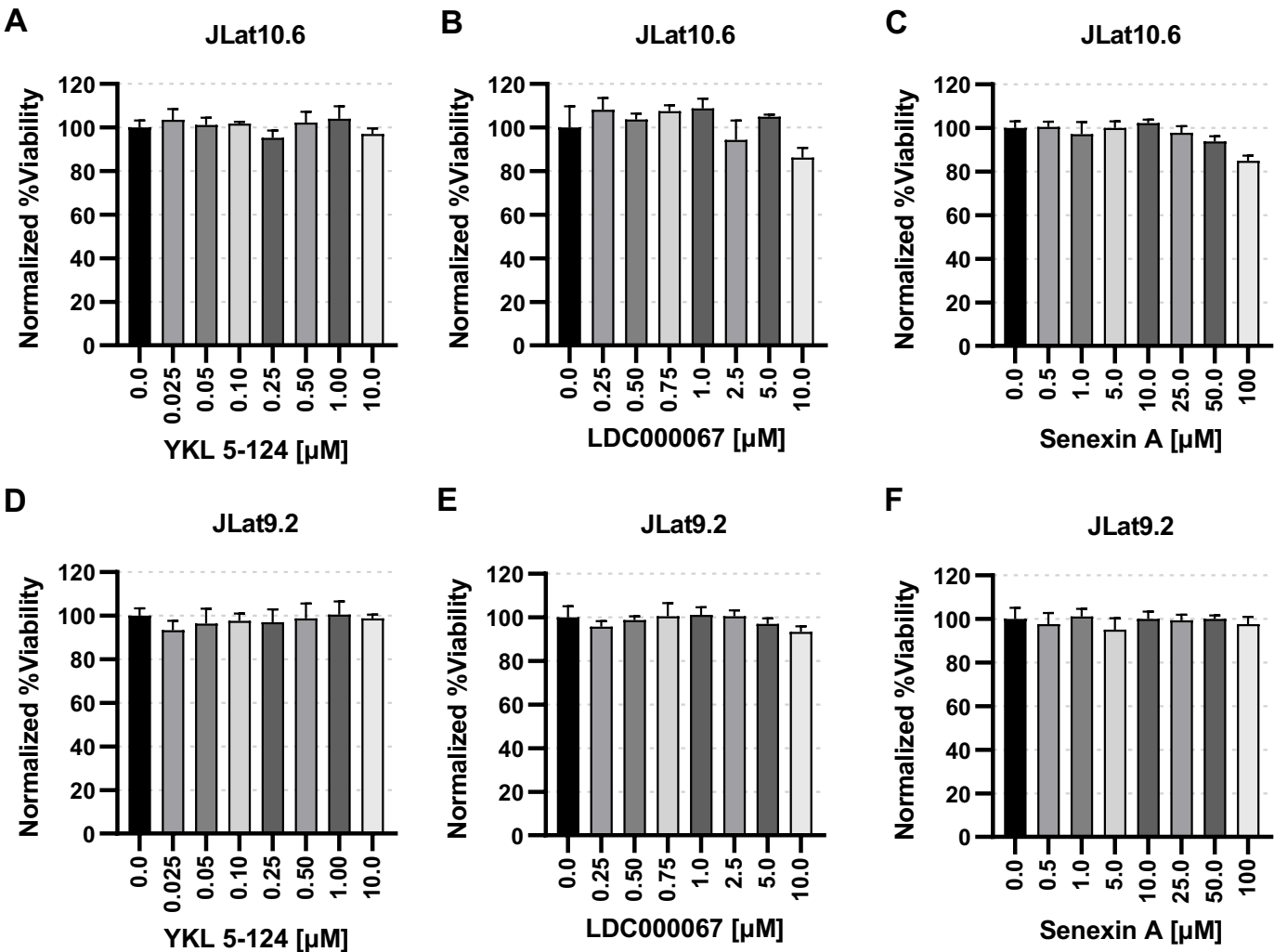
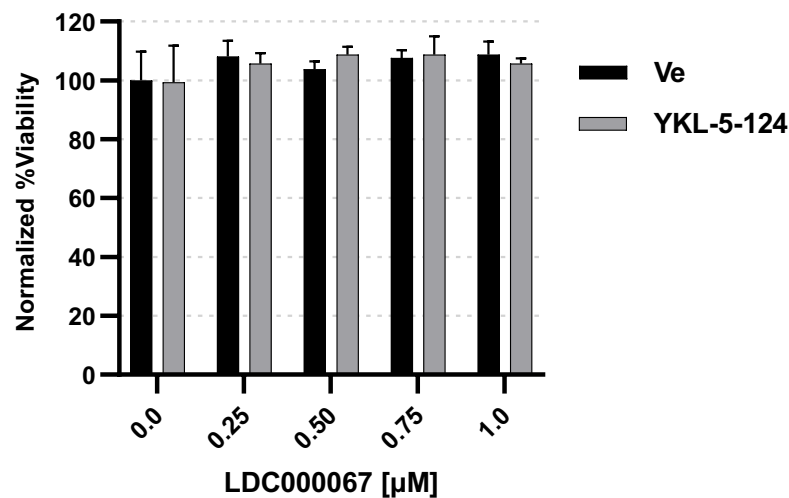
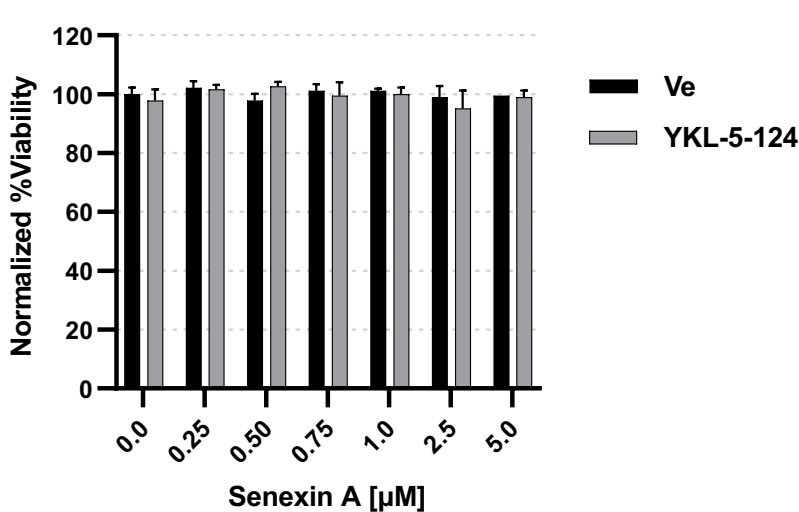


Figure S2

A



B



C

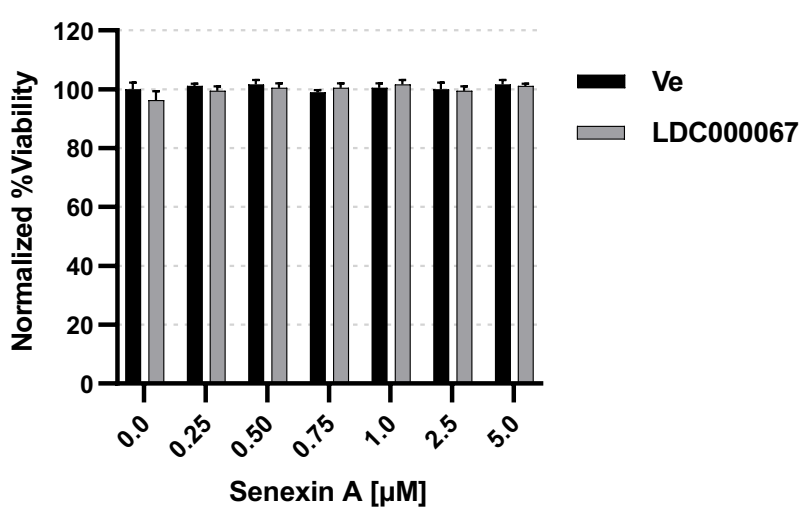


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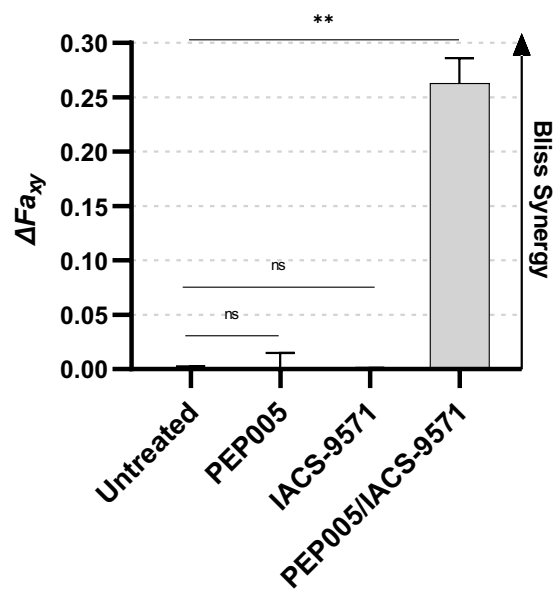


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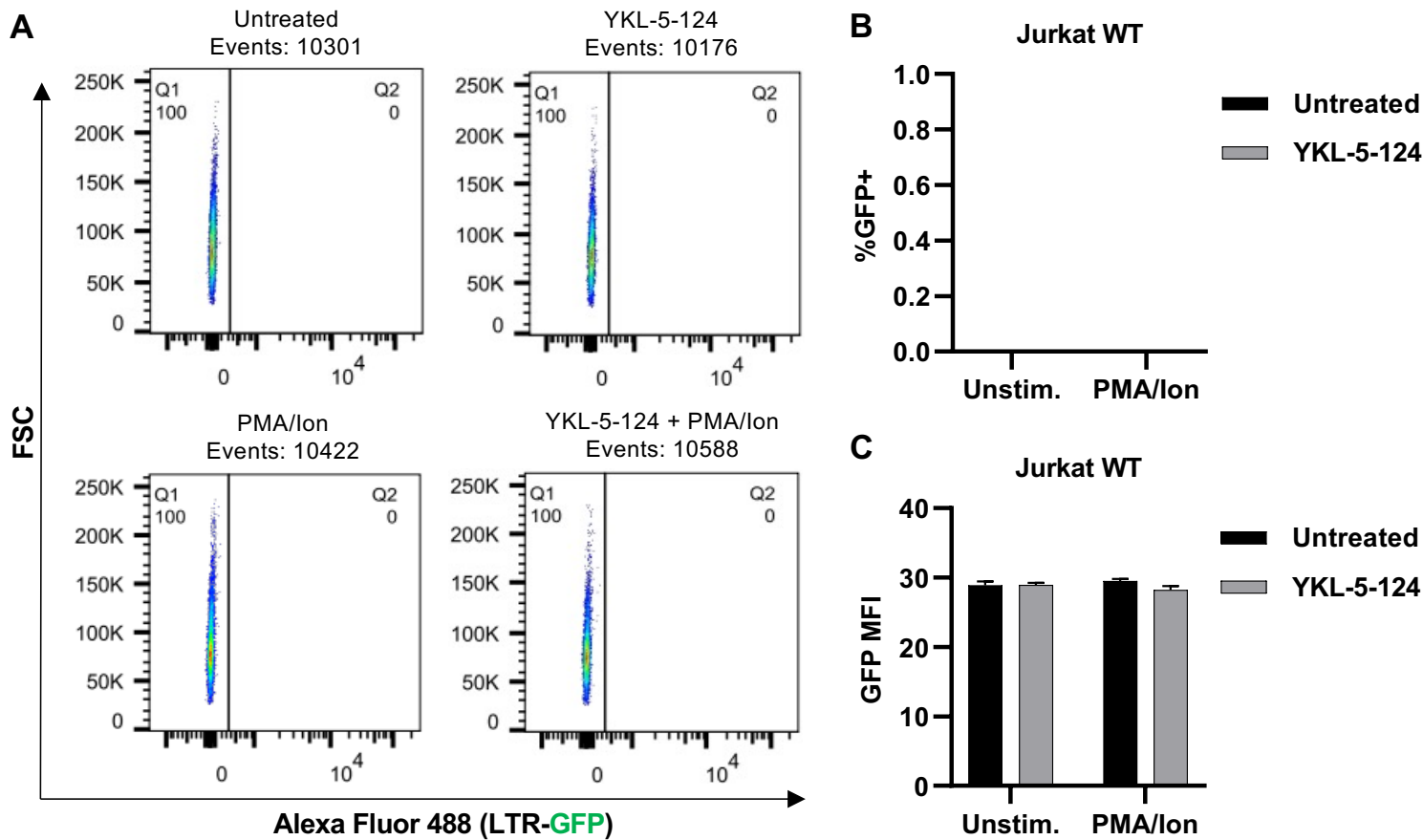


Figure S5

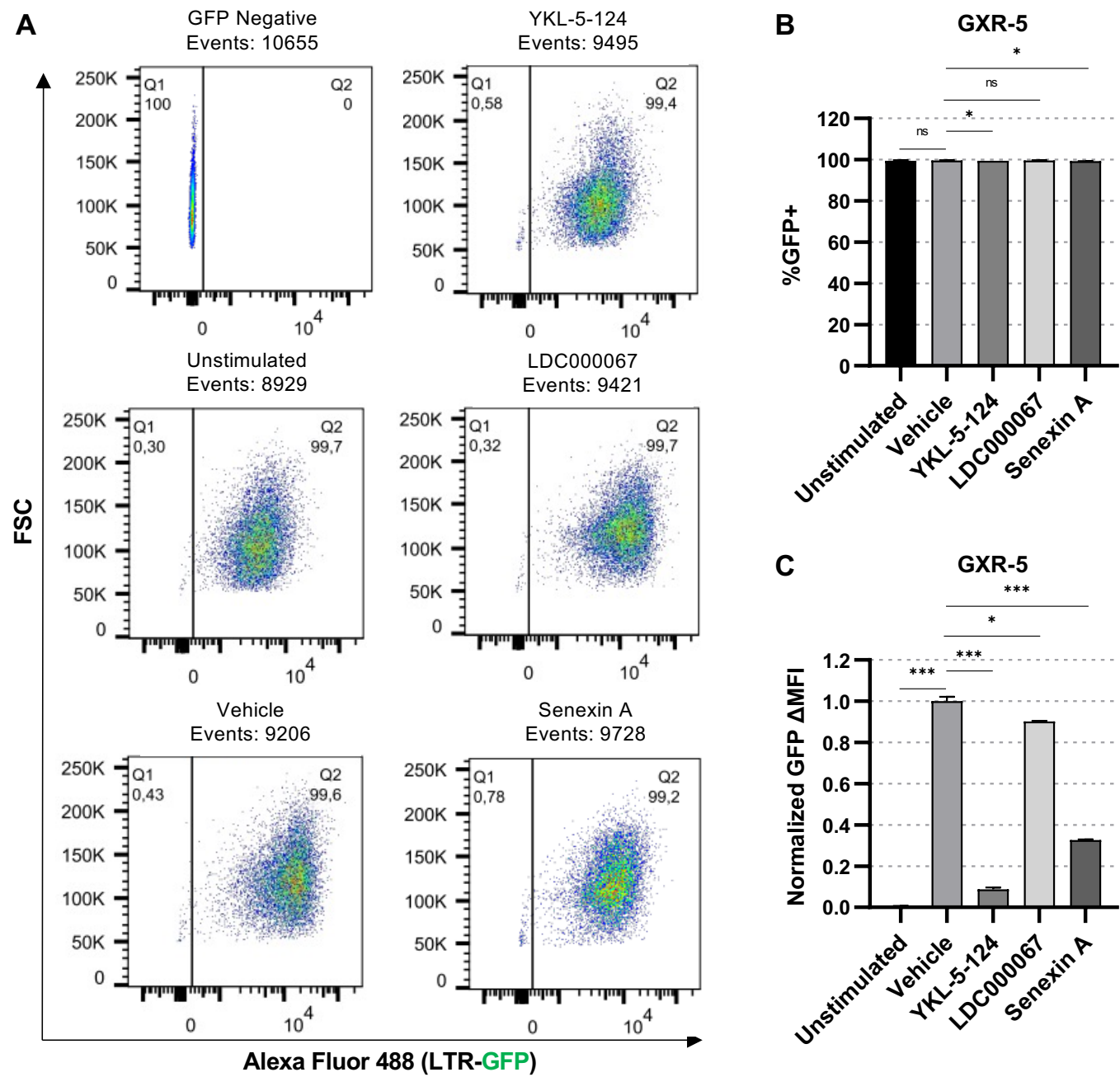


Figure S6

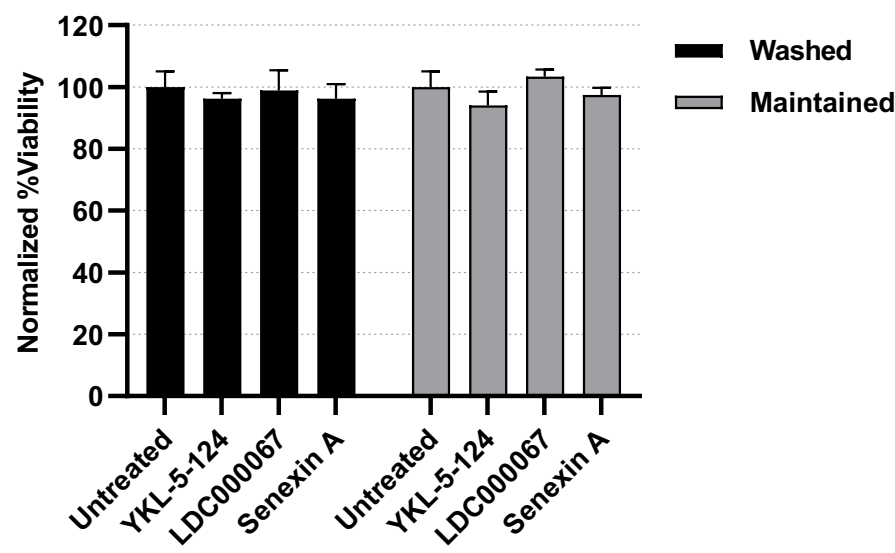


Figure S7

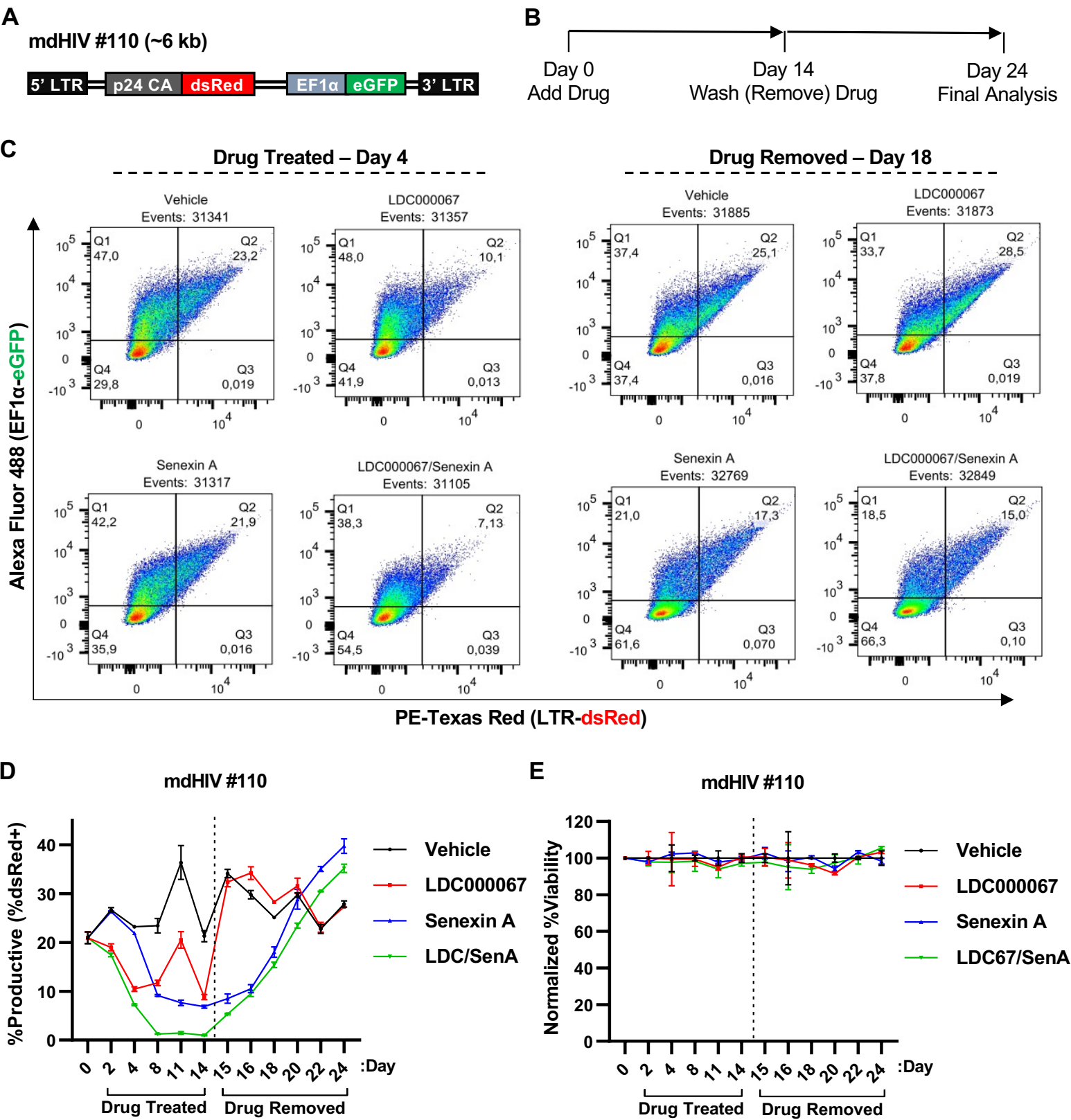


Figure S8

