HIV-1 Vpr Degrades the Polycomb Complex Component BCOR to Counteract Provirus Transcriptional Silencing

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Introduction

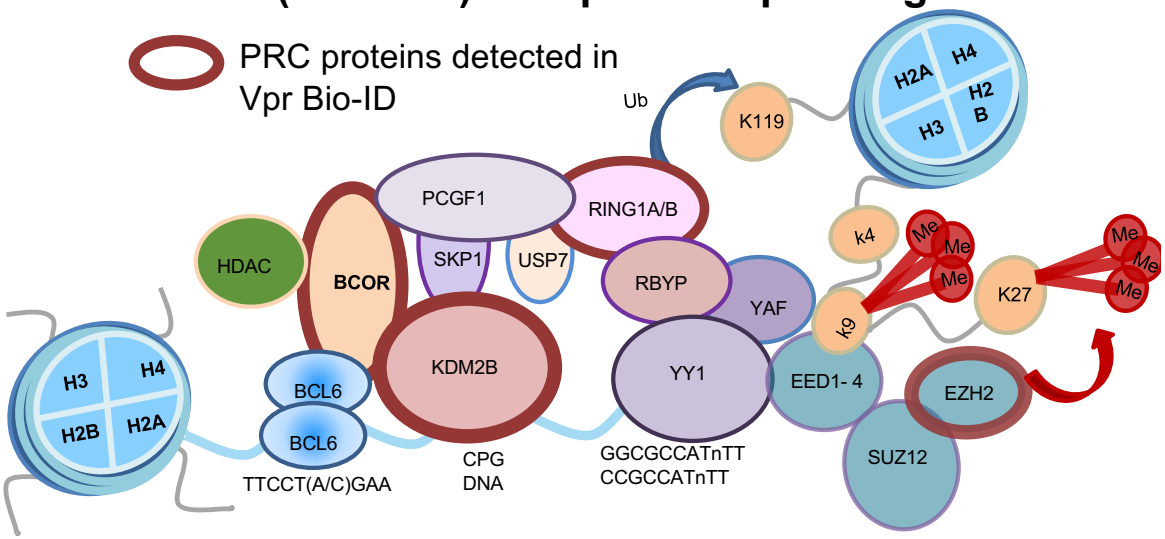
Previously, in a Vpr BioID, we identified multiple components of the Polycomb complex, including BCL6 co-repressor (BCOR).

The BCL6/BCOR complex (one of six PRC1 complexes) is well characterized to silence genes based on the BCL6 consensus sequence and CPG rich regions in cooperation with polycomb complex 2 (PRC2). Silencing is usually achieved by methylating lysine 27 (His3K27me3) (group 2 [Enhancer of Zeste Homolog 2 {EZH2}]), demethylating lysine 36 (His3K36me3) on histone 3 (group 1 [K-specific Demethylase 2B {KDM2B}]), and ubiquitinating lysine 119 on histone 2 (His2K119ub) (group 1 [Ring Finger Protein 1B {RING1B}]) as well as the recruitment of various HDACs. Recently, BCOR has been shown to function in the absence of BCL6. Indeed the BCOR complex (ncPR1.1) is a very dynamic gene repressor needing very few proteins to stabilize it, sometimes recruiting PRC2 instead of being recruited by it, and able to cooperate with other PRC1 complexes.

Conclusions

- BCOR is the only PRC component detected in our Vpr Bio-ID that is depleted by Vpr.
- Vpr is essential for BCOR depletion during HIV infection.
- BCOR depletion is not dependent on G2/M cell cycle arrest.
- Association with DCAF-1 is essential for BCOR depletion.
- Vpr degrades BCOR via the proteasome not lysosome.
- BCOR expression is higher in latently vs productively infected cells.
- BCOR shRNA knockdown in the latently infected CD4 T cell line, 2D10, is sufficient to increase HIV-1 expression.
- BCOR occupies the HIV-1 LTR in latently infected Jurkat T cells and this occupation is reduced during productive infection.

The BCOR (ncPR1.1) Complex Cooperating with PRC2



Future Directions

- Characterize the effects of Vpr-dependent BCOR degradation on HIV-1 expression.
- Characterize the contribution of BCOR repression to latency in human tissue (human blood and/or lymphoid tissue) derived CD4 T cells subpopulations, such as TFH.

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CanCURE
Together, we Can CURE HIV

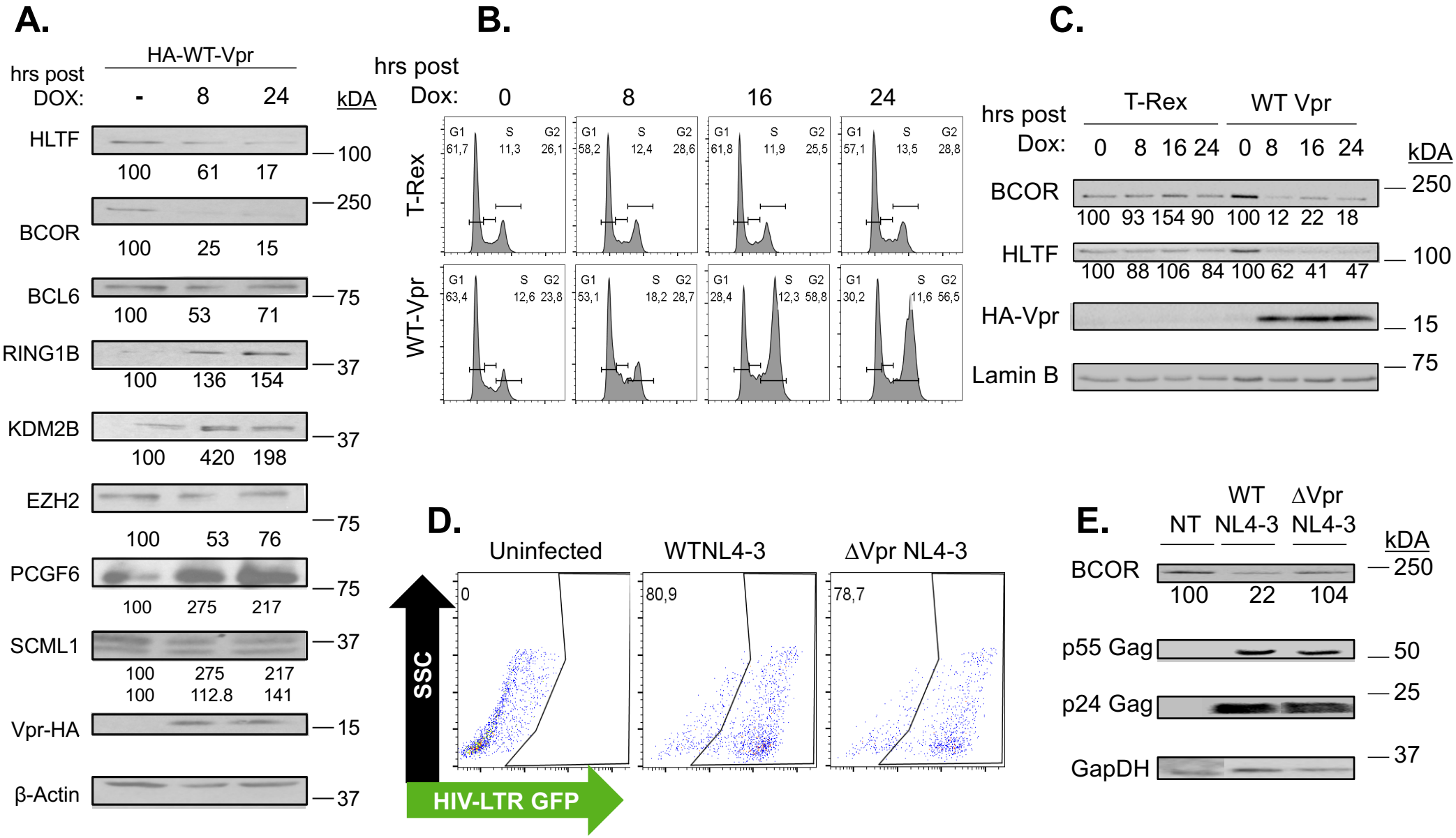

Figure 1. **A**) A doxycycline (Dox) inducible HA tagged Vpr HeLa T-Rex cell line was blotted for proteins detected in proximity to Vpr in our Vpr-Bio-ID. Cells were induced for 8 or 24 hrs. HLTF was used as a positive control for Vpr-dependent depletion. BCOR is the only protein detected in the Bio-ID that is consistently and efficiently depleted upon Vpr expression (N=3). **B**) To test if Vpr-dependent G2-M Cell cycle arrest precedes BCOR depletion, dox-induced cells (0.5 µg/mL) were harvested at 0, 8, 16, or 24 hrs and stained with propidium iodide to test what cell cycle phase the cells are at **C**) and also blotted for BCOR. BCOR depletion can be seen as early as 8 hrs post HA-WT Vpr induction when there is no significant increase in the percentage of cells in G2-cell cycle arrest (N=3). **D**) MT4 CD4 T cells were infected (MOI of 0.75) with WT or ΔVpr NL4-3 GFP for 40 hrs. Percent of infected cells was monitored by FACS analysis and **E**) cells were blotted for BCOR expression to test if BCOR is depleted during infection (N=2). These results show that Vpr-dependent BCOR depletion precedes G2-cell cycle arrest, and that Vpr is essential for BCOR depletion during infection

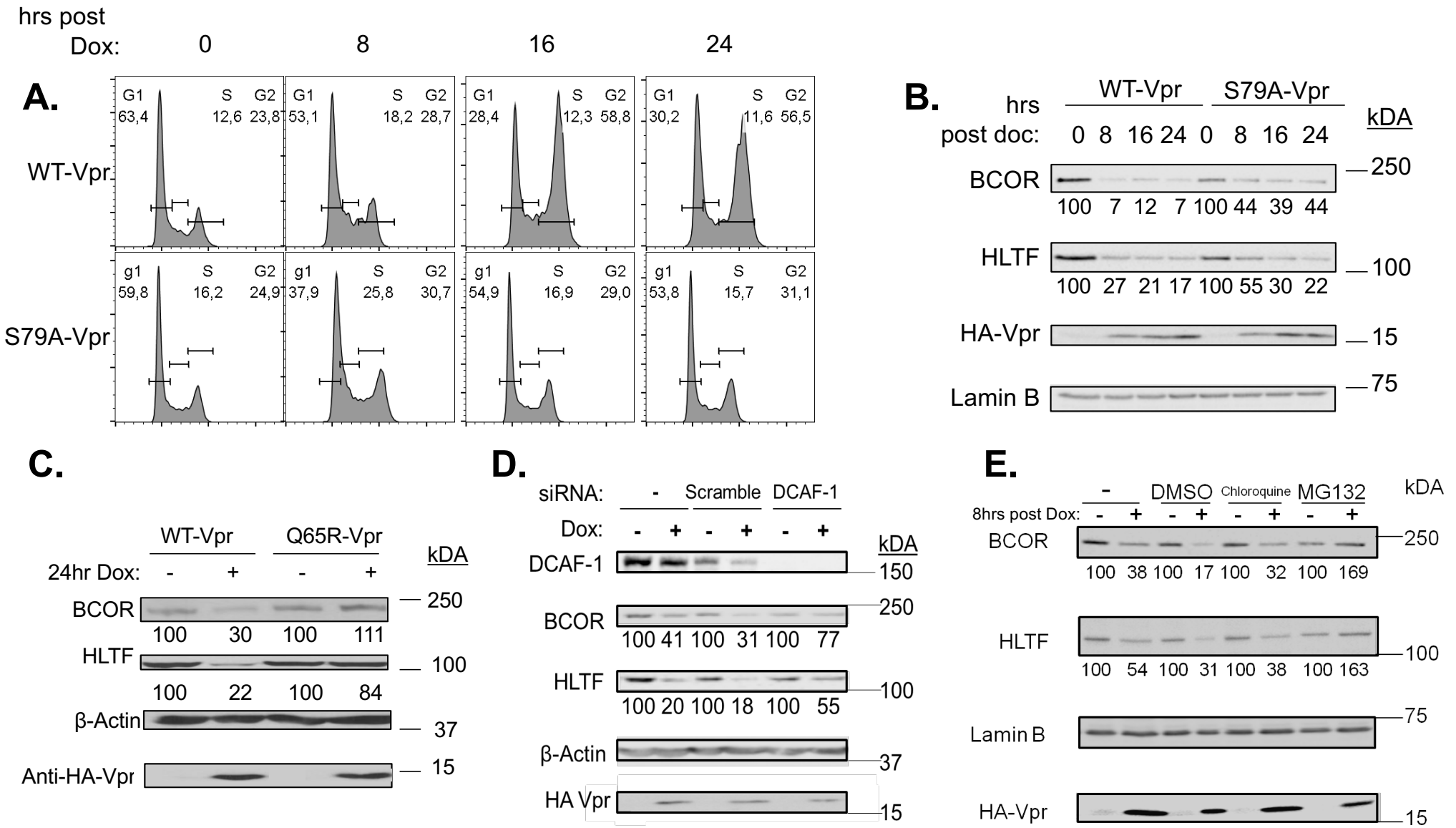

Figure 2. **A**) WT-Vpr and S79A HeLa T-Rex dox-inducible cell lines were stained with propidium iodide to compare G2-cell cycle arrest and **B**) blotted for BCOR expression to test if Vpr-dependent G2 cell cycle arrest is necessary for BCOR depletion. Despite the inability of S79A to induce G2 cell cycle arrest, both, WT and S79-Vpr showed significant BCOR depletion upon Dox induction (N=2). **C**) Q65R- vs WT-Vpr HeLa T-Rex cells were induced with Dox to test if DCAF-1 binding by Vpr is essential for BCOR depletion. Significant BCOR depletion was only observed with the WT-Vpr inducible cells and not with the DCAF-1 binding impaired mutant Q65R (N=3). **D**) WT-Vpr HeLa T-Rex cells were transfected with either scramble or siRNA targeting DCAF-1 to test if DCAF-1 is essential for Vpr-dependent BCOR depletion. Depletion of BCOR was impaired upon knock-down of DCAF-1 24 hrs post Vpr induction (N=3). **E**) To test if Vpr-dependent BCOR depletion occurs via the lysosome or the proteasome, induced (8hrs) WT-HeLa T-Rex were treated with DMSO, Chloroquine (lysosomal inhibitor (100 μM)), or MG132 (10 μM) for 7 hrs. MG132, but not Chrloroquine or DMSO rescued BCOR from Vpr-dependent depletion. These results show that BCOR is degraded by Vpr via DCAF-1 binding and proteasomal dependent degradation but independently of G2 cell cycle arrest.

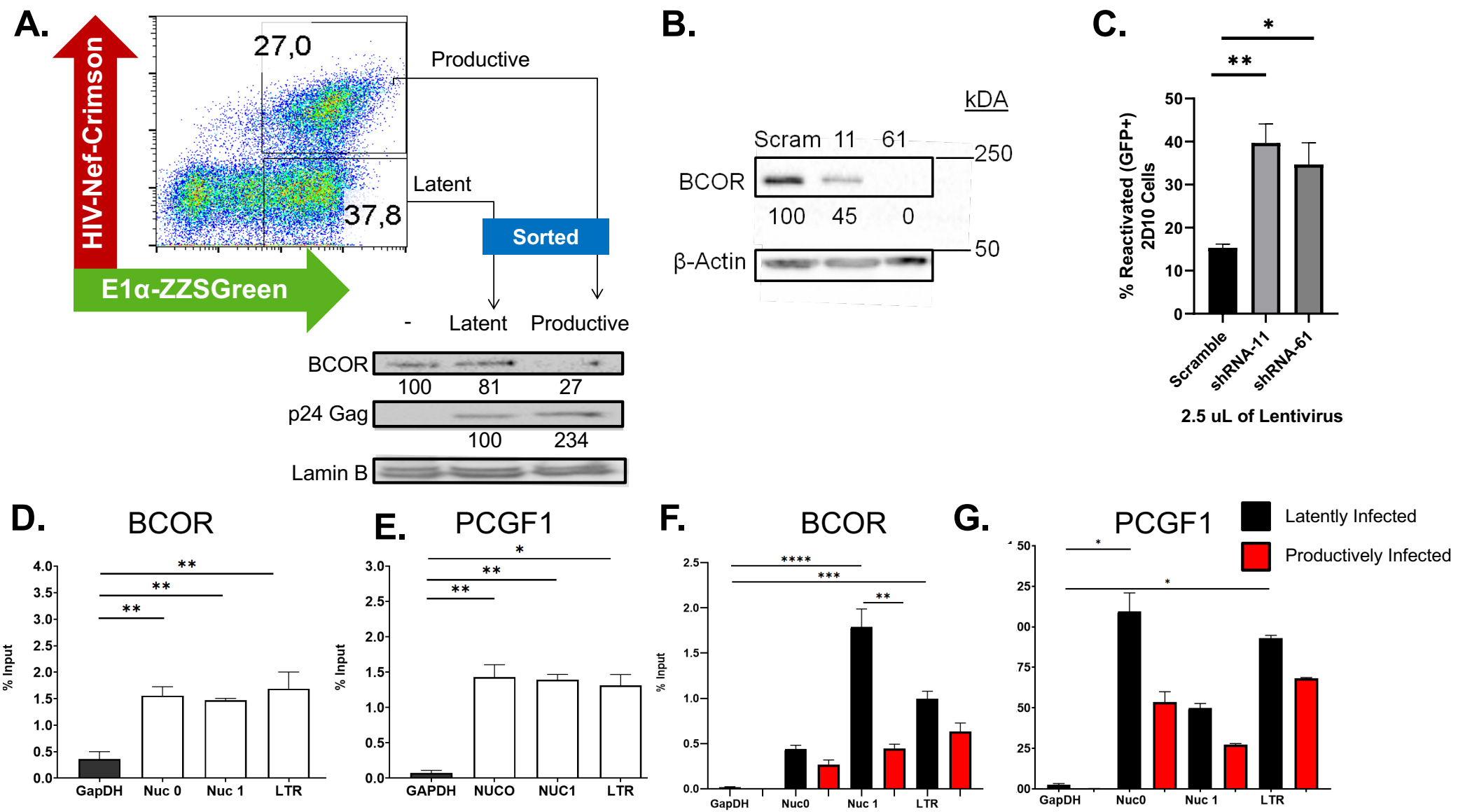

Figure 3. **A**) Jurkat CD4 T cells were infected (MOI of 1) with the dual reporter Crimson/ZS Green NL4-3 virus. Latently infected (Crimson- ZSGreen+) were sorted and blotted for expression of BCOR. BCOR expression was decreased in productively but not latently infected cells implying that BCOR may be repressing HIV expression (N=2). **B**) To test if BCOR can repress HIV-1 expression the latently infected CD4 Jurkat T cell line, 2D10, was transduced with shRNAs targeting BCOR or scramble. Knock down efficiency was compared by blotting for BCOR. **C**) Despite some background with the scramble, both of the BCOR targeting shRNA resulted in significant reactivation vs scramble implying that BCOR can repress HIV-1 expression in latently infected CD4 T cells. (N=3) **D**) To test if BCOR represses HIV expression in 2D10 cells by occupying the HIV-1 LTR and recruiting the PRC1.1 complex, 2D10 cells were analyzed by ChIP. Significant BCOR **(D)** and PCGF1 (**E**) was observed in the HIV-1 LTR vs the GAPDH promoter (N=3). **F**) Jurkat CD4 T cells were infected with Crimson/ZS-Green NL4-3 and then the populations were sorted as in (**A**) To test if the BCOR complex can occupy the LTR of latently vs productively infected cells. Both BCOR (**F**) and PCGF-1 (**G**) significantly occupied the HIV LTR in latently infected vs productively infected and vs GAPDH promoter (N=1). These results imply that BCOR represses HIV-1 expression by occupying the LTR of HIV-1 and recruiting PRC1.1 complex.