Project Final Report (305267 HIT HIDDEN HIV)

Figures related to the final report and organized by WP

WP1: Development of a Non-human primate model for HIV latency (related figures)

Day post vial exposure	-7	0	4	7	10	14	21	28	56
Viral exposure		X							
Clinical examination, weight and temperature	X	X	X	X	X	X	X	X	X
CBC	X		X	X	X	X	X	X	X
T lymphocyte phenotype	X			X		X	X	X	X
SIV RNA in plasma	X		X	X	X	X	X	X	X
SIV DNA in PBMC	X					X	X	X	X
Anti-SIV antibodies	X							X	X
Cryopreserved buffy-coat	X			X		X	X	X	X
Cryopreserved PBMC	X			X		X	X	X	X
Cryopreserved LNMC	X				X				X

Table 1. Summary of the experimental procedure to assess infectivity and stability of SOCG and SORCG in vivo.

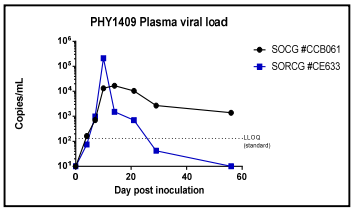


Figure 1. In vivo replication of SIVmac239-Nefopt-CMV-GFP (SOCG) and SIVmac239-Nefopt-RFP-CMV-GFP SORCG. The in vivo replication kinetic for both viruses displayed standard characteristics for SIVmac239 infection in macaque. However, GFP or GFP/RFP positive cells were not detected in PBMC and LN by FACS analysis.

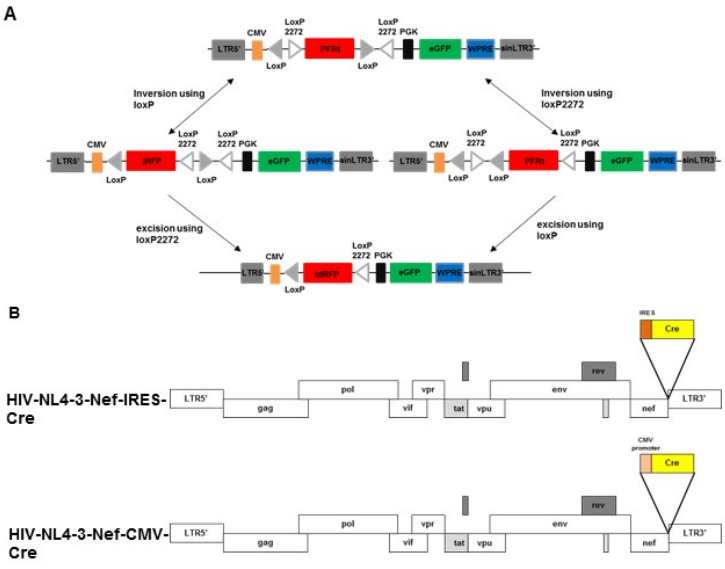


Figure 2: Vectors generated to establish a CRE-based HIV/Hu-mice latency model. (A) Schematic representation of the lentiviral vector expressing GFP and RFP (HR-4lox). Shown are the Cre induced recombination events required for the expression of the RFP. (B) Schematic representation of HIV-1 molecular clones expressing Cre recombinase. Cre is expressed together with Nef by the R5-HIV1-Nef-IRES-Cre construct via an internal ribosome entry site (IRES) or by R5-HIV1-CMV-CRE as an independent transcriptional unit under the control of CMV promoter.

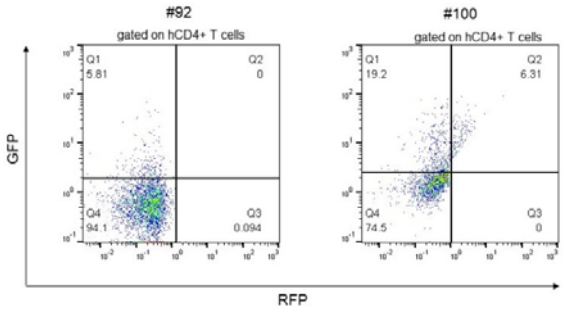


Figure 3: Facs analyses showing the presence of human CD45+, CD3+, CD4+ expressing GFP and RFP in the spleen of representative hu-mice immune reconstituted with CD34GFP+ cells and infected with HIV1CMVCRE (animal#100) or non-infected (animal#92). Animals were sacrificed 5 weeks after infection and viral RNA in plasma was 4.6x105copies/ml for the infected animal.

Identification of specific biomarkers of latently infected cells.

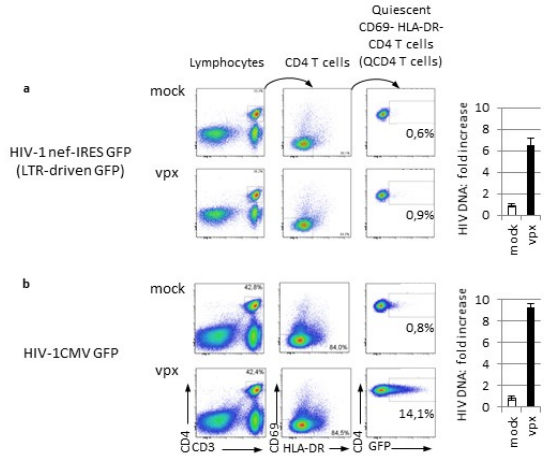
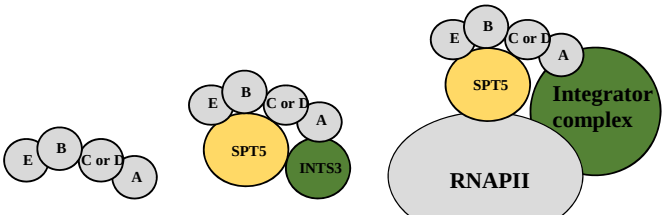


Figure 1: Depleting SAMHD1 in quiescent CD4 T cells allows HIV-1 reverse transcription and integration but not viral transcription. PBMCs from healthy donors were treated overnight by empty or Vpx containing Viral Like Particles (VLP-Vpx) and subsequently infected with (a) HIV-1 nef-IRES GFP in which GFP expression is driven by the viral LTR or (b) an HIV expressing GFP as an independent transcriptional unit at the end of nef gene (HIV-1CMV-GFP). Of note, unlike the HIV LTR, CMV promoter is active in quiescent CD4 T cells. The number of GFP positive quiescent CD4 T cells was assessed by FACS analysis at day 4 post infection. Bar graphs show the fold increase of total HIV-DNA measured by quantitative PCR in quiescent CD4 T cells at day 3 post infection.

WP2: Fundamental mechanisms of latency (related figures)



NELF <u>Core</u> Complex

<u>Small</u> NELF-SPT5-INTS3 Complex

<u>Large</u> NELF-SPT5-INTScom-RNAPII Complex

Figure 1. Schematic integration of IP/ReIP and glycerol gradient sedimentation analysis: NELF forms a small (SC) and a large (LC) complex together with subunits of the Integrator Complex. SC: NELF-A/B/(C/D)/E, SPT5 and INTS3. LC: NELF-A/B/C/D/E, SPT5, RNAPII, INTS1/3/13 and the catalytic Integrator subunit INTS11.

Objective 2: Identification of NELF inhibitors

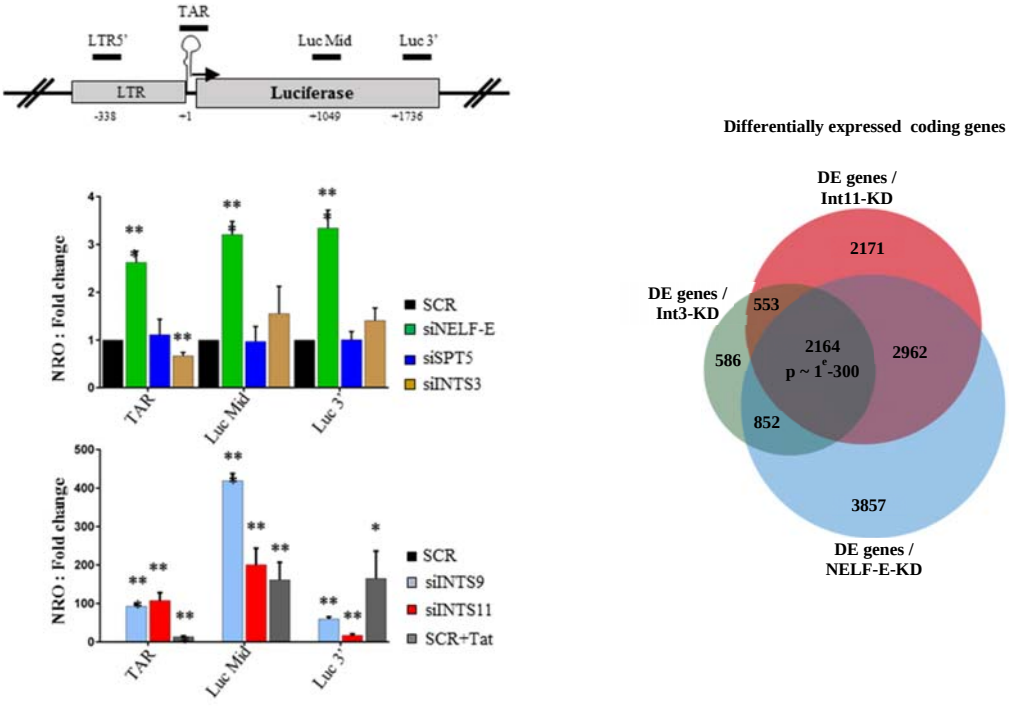


Figure 2. Integrator complex regulates transcription elongation at the HIV-1 LTR and at subset of cellular genes. *Schematic representation of the LTR-Luciferase locus in HeLa-LTR-Luciferase cells depicting positions of primers used in ChIP (Chromatin Immuno-Precipitation) and NRO (Nuclear Run-On) assays. NROs were performed using nuclei prepared from HeLa-LTR-Luc cells transfected with the indicated siRNAs. Results are presented as fold change over control condition SCR, the average profiles of the 4 normalizations are shown. Results are presented as fold change over control condition SCR. *= p-value < 0.05; **= p-value < 0.005; ***= p-value < 0.0005, no*=no significant p-value as measured by student's t-test. Error bars represent standard deviations (n=3). Integrator complex regulates NELF-mediated RNAPII pausing at coding genes. Venn diagram showing the intersection among differentially expressed (DE) genes identified by microarray analysis (n=3) upon depletion of -INTS11, -INTS3- or –NELF-E (p < 0.001).*