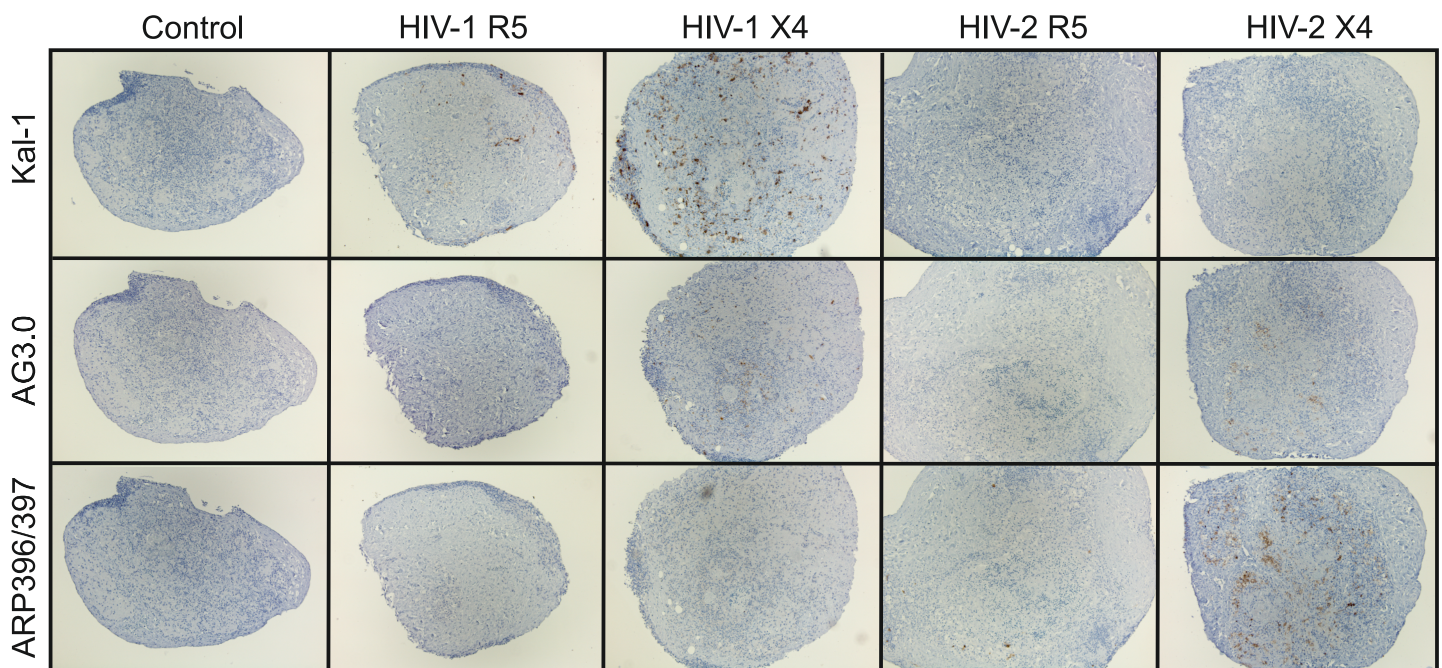
Supplementary Table 1: Primer and probe sequences.

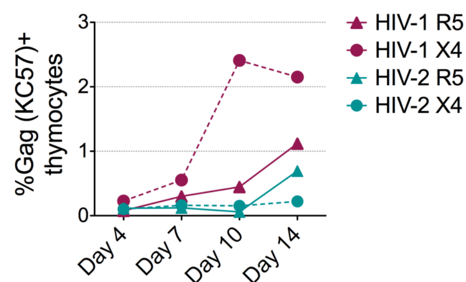
Primer or Probe		Sequence
HIV-1 Gag	Forward primer	5'-CGA GAG CGT CGG TAT TAA GC-3'
	Reverse primer	5'-AAC AGG CCA GGAT TAA GTG C-3'
	Probe	5'-FAM-CCC TGG CCT TAA CCG AAT T-MGB
HIV-2 Gag	Forward primer	5'-CGC GAG AAA CTC CGT CTT G-3'
	Reverse primer	5'-GCT GCC CAC ACA ATA TGT TTT A-3'
	Probe	5'- FAM-CCG GGC CGT AAC CT-MGB
CD3[a]	Forward primer	5'-AGG GCA AAA TGG AGG CTC TTA-3'
	Reverse primer	5'-TCT CCT CCA TGG GAC ACT GTT-3'
	Probe	5'- VIC-CTC TCT AGC AGA GAA GAG T-MGB
GAPDH[b]	Human GAPD (GAPDH) Endogenous Control (Applied Biosystems)	

[a]Reference gene for total HIV DNA quantification; [b]Reference gene for *gag* mRNA quantification.

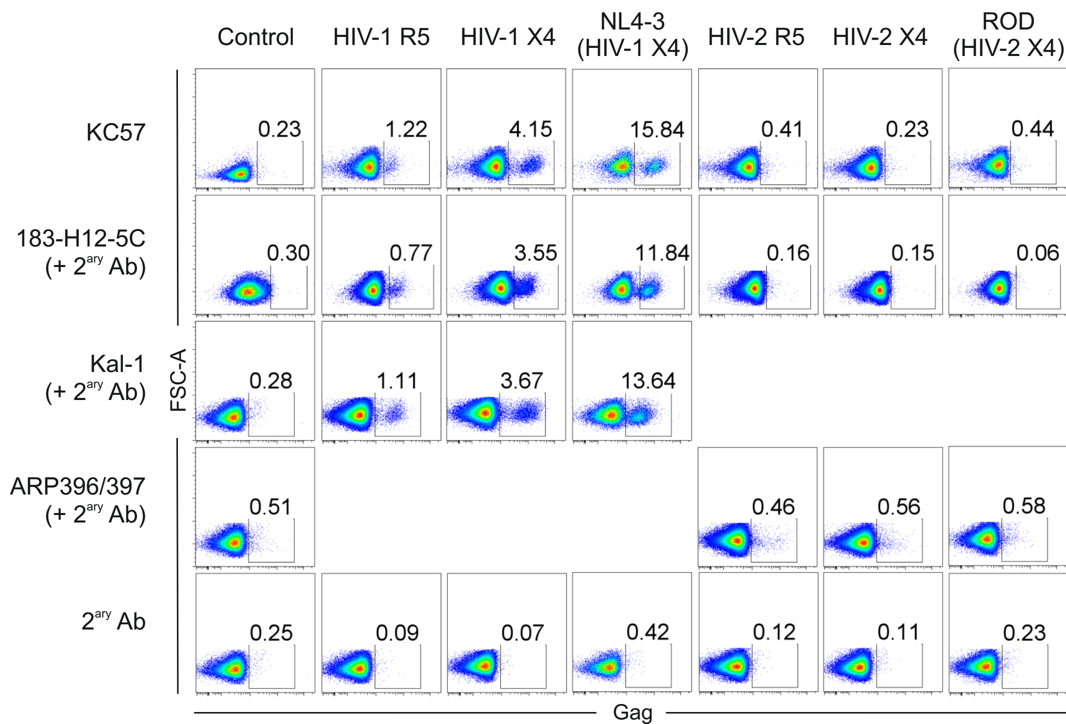


Supplementary Figure 1: Comparison of anti-Gag antibodies for the detection of HIV-2 and HIV-1 infection by immunohistochemistry. Comparison of Gag detection (brown staining) by immunohistochemistry using the following anti-Gag mAb: AG3.0 (anti-p24 from NIH); Kal-1 (anti-p24 from DAKO); ARP396/397 (anti-p27 from MRC). HIV-2 and HIV-1 detection were most efficient using ARP396/397 and Kal-1 antibodies, respectively. No staining was observed in negative controls, which included staining of uninfected tissue with anti-Gag antibodies (control condition presented in the first column) or absence of primary antibodies (not shown).

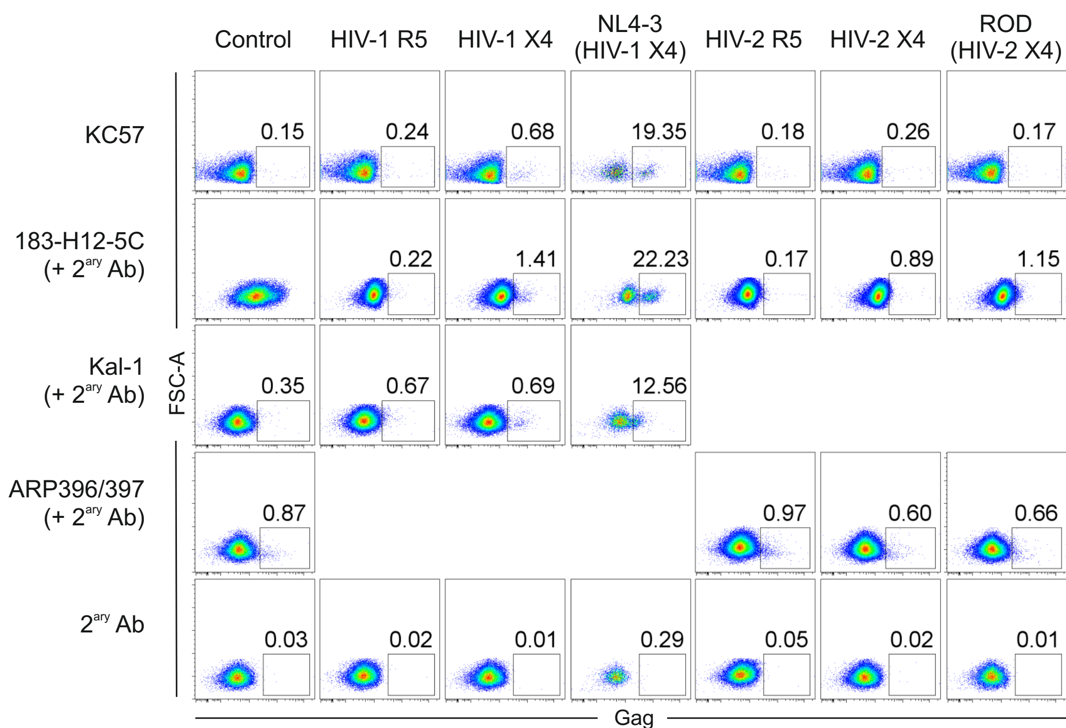
A.



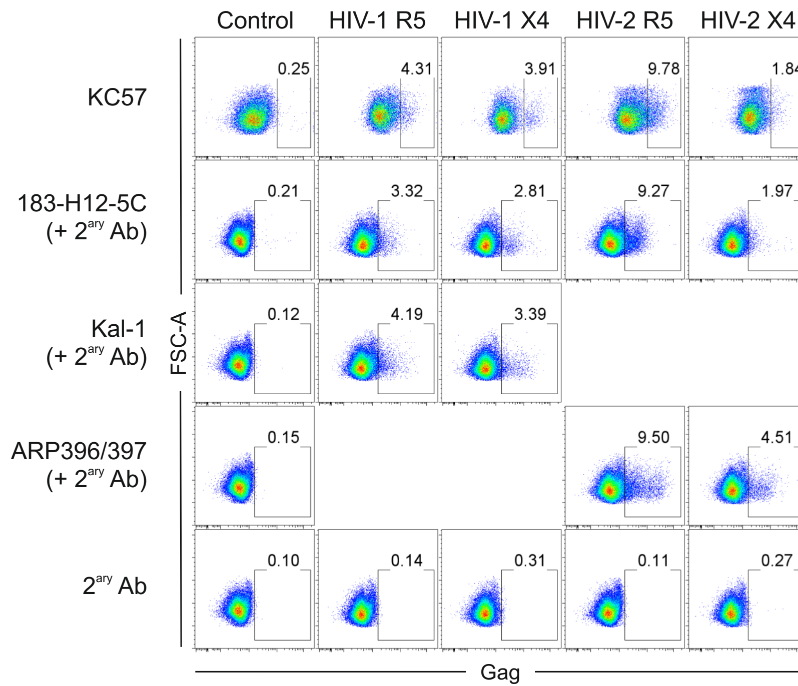
B. Thymocytes

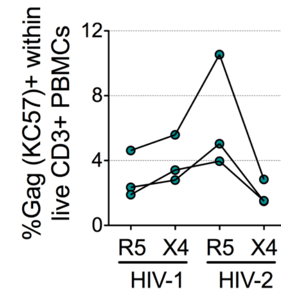


C. CD4SP thymocytes



Supplementary Figure 2: Low to undetectable intracellular Gag protein expression at the single-cell level in HIV-2-infected thymocytes. (A) Kinetics of intracellular Gag expression, as assessed by KC57 staining, in HIV-2 and HIV-1-infected thymocytes, confirming lower levels of intracellular Gag in HIV-2-infected thymocytes at all time-points analyzed. Intracellular Gag staining of HIV-2- and HIV-1-infected thymocytes (B) and CD4SP thymocytes (C) at day 10 post-infection with the viruses indicated, using validated anti-Gag mAbs (see Supplemental Figure 3) and secondary detection (when appropriate). Stainings using the secondary antibody alone are presented in bottom rows. Flow cytometry analysis was performed after exclusion of dead cells and aggregates.





Supplementary Figure 3: Validation of KC57 (anti-Gag) antibody for HIV-2 detection by flow cytometry. PHA-stimulated PBMCs (from 3 buffy coats) were infected with R5- or X4-tropic HIV-2 or HIV-1 primary isolates and cultured for 10 days. Intracellular Gag protein was analyzed using KC57 or other available anti-Gag mAb. (A) Illustrative dot-plots of intracellular Gag staining within total PBMCs for each of the viruses used. KC57 staining was comparable to the staining of other anti-Gag mAb. Detection of the unlabeled anti-Gag mAb was performed using secondary detection. Stainings using the secondary antibody alone are presented in the bottom row. Flow cytometry analysis was performed after exclusion of dead cells and aggregates. (B) Frequency of Gag(KC57)+ cells within live CD3+ PBMCs for each of the viruses used at day 10 post-infection. Each line connects dots from the same experiment (different buffy coats used in each experiment).