

# HIV-1 reservoir size correlates to PD-1 expression in men, but not women, in Uganda.

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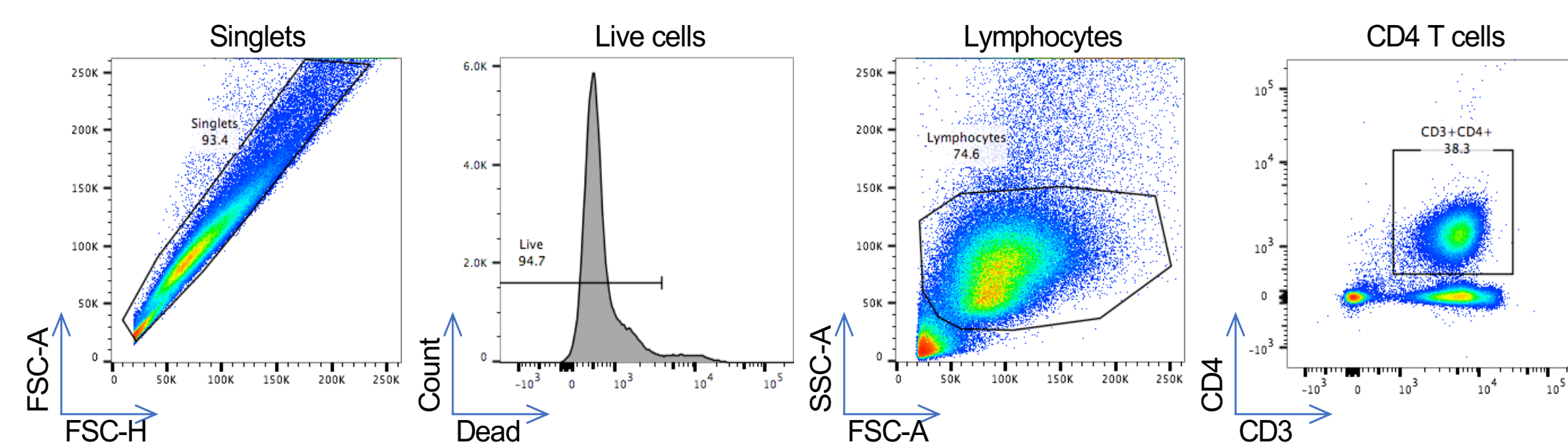
## Background

- 52% of people living with HIV-1 are females, yet they only represent ~11% of participants in HIV-1 cure studies.
- The latent viral reservoir, composed primarily of HIV-1 infected resting CD4<sup>+</sup> T cells (rCD4), is the barrier to achieving HIV-1 cure.
- Females differ in immune responses to HIV-1 infection, including elevated *in vivo* expression of IFN-stimulated genes, elevated T cell responses, and decreased markers of immune exhaustion.
- Current cure strategies are focused on (a) permanently silencing the latent viral reservoir, or (b) eradicating the reservoir through the combined use of latency reversal agents and PD-1 antagonists.

## Methods

- HIV-1 infected adults who were virally suppressed (<40 copies/mL) for over 1 year at time of enrollment (n = 90; female = 57, male = 33) were recruited from Rakai Health Sciences Program in Uganda.
- PBMCs were isolated from participant blood samples (180 mL) and used for the Quantitative viral outgrowth assay (rCD4 activation with PHA and  $\gamma$ -irradiated allogenic PBMC + limiting dilution culture) and total HIV DNA quantification (qPCR on a segment of the *gag* gene)
- Immune cell characterization (memory subsets, activation and exhaustion markers) was performed using flow cytometry (Figure 1).
- Soluble immune markers were quantified using an 8-plex custom Human Ultra-Sensitive kit (Meso Scale Discovery).
- Statistical analysis in R Version 3.5.2: Two sample comparison using Mann-Whitney's U-test, univariate regression analysis and stepwise regression using R's MASS package "stepAIC" function.

### CD4 T Cell Identification



### CD4 T Cell Memory Subsets

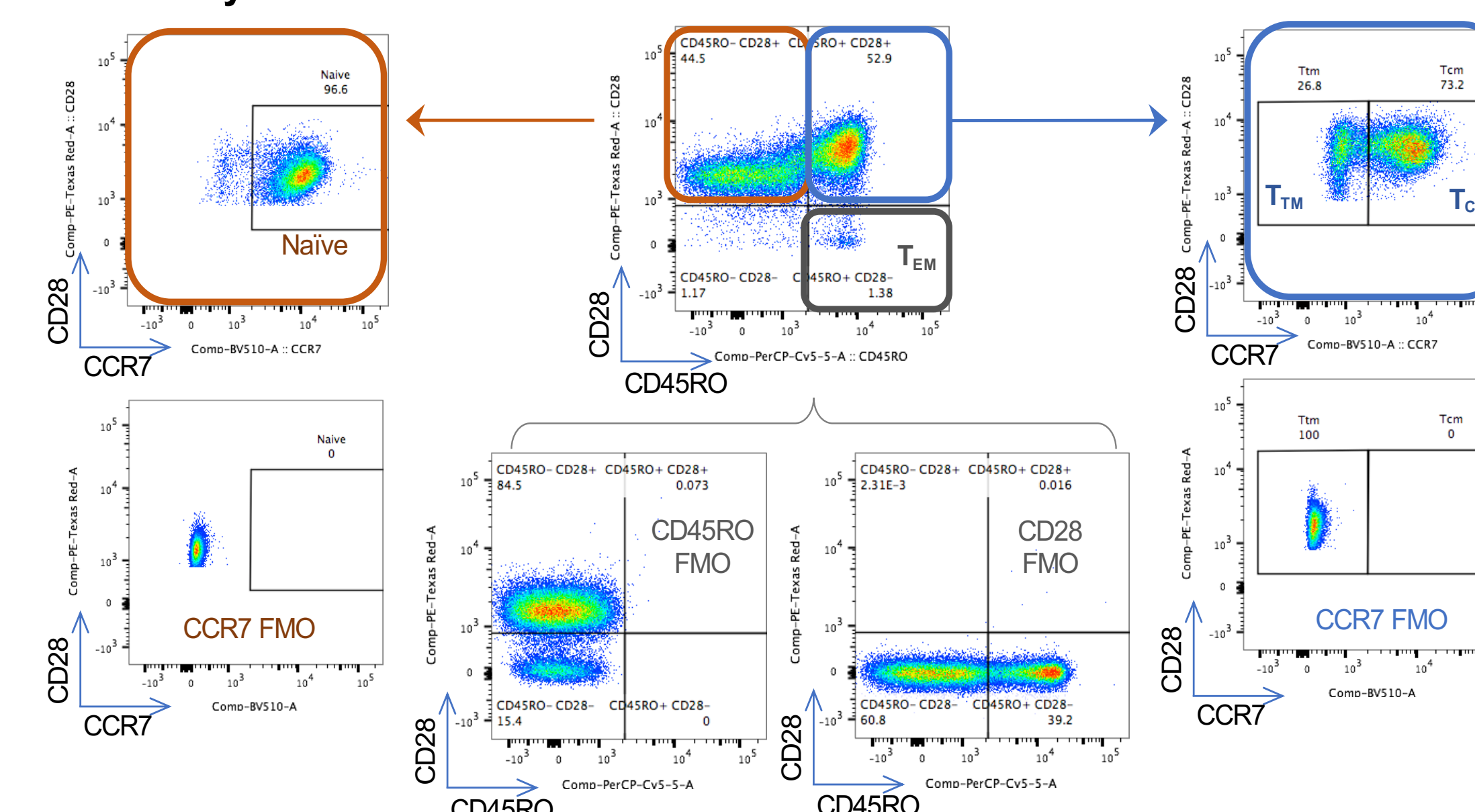


Figure 1. Flow cytometry characterization of CD4 T cell subsets.

## Results

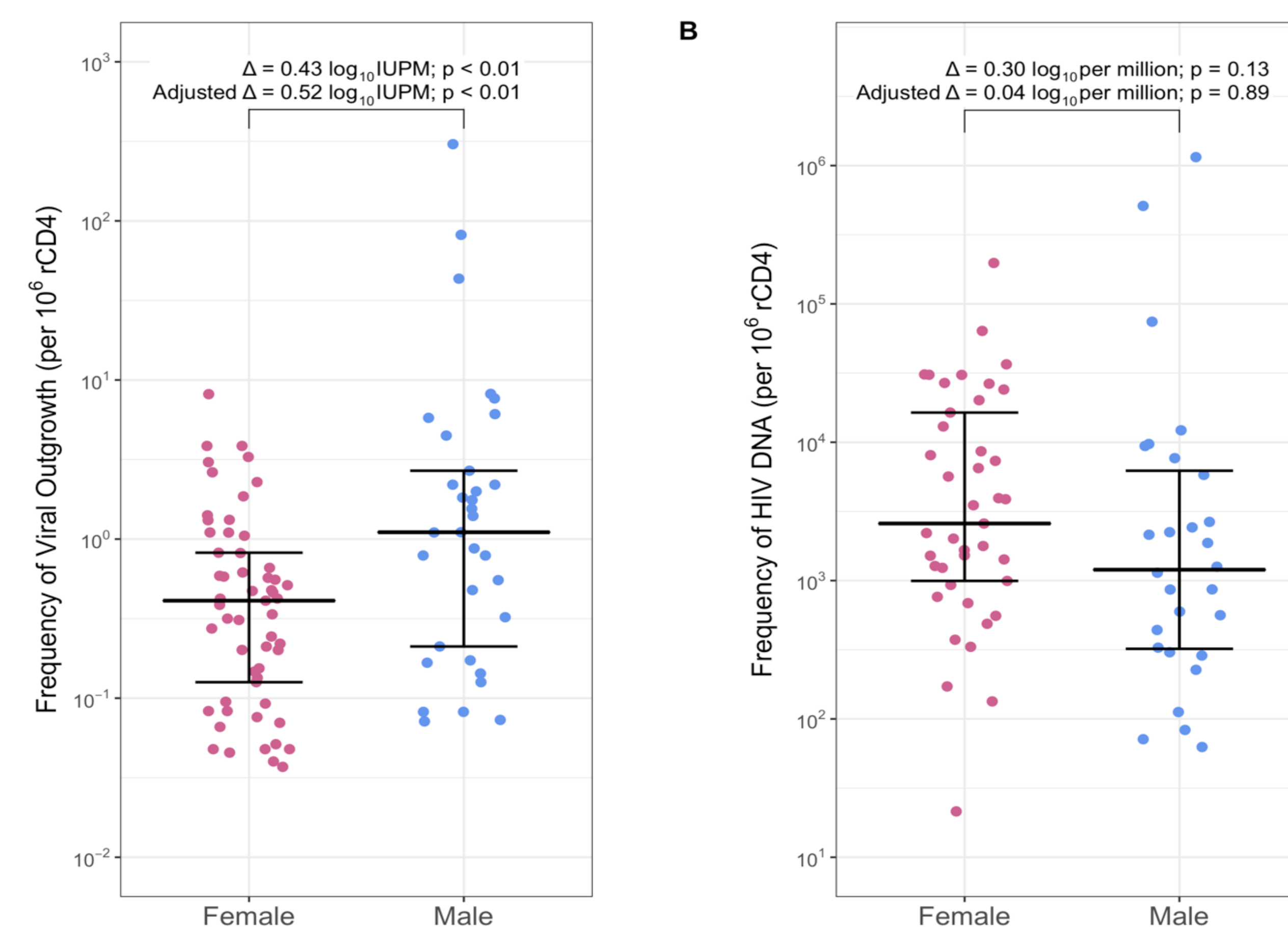


Figure 2. Differences in (A) frequency of rCD4 that produce replication competent virus when stimulated *ex vivo*, by the QVOA, and (B) frequency of rCD4 that contain HIV-1 gag DNA between females (n=57) and males (n=33). Adjusted analysis controls for age, pre-ART viral load, nadir CD4 T cell count, duration of ART, instances of transiently detectable viremia (proportion of on-ART viral loads >400 copies/ml), and CD4 T cell count at the time of QVOA.

Table 1. Clinical and immunological characteristics of HIV-1 positive Ugandans females and males.

Characteristic	Females (n = 57)	Males (n = 33)	P-value
Age (years)	41.1 (37.4, 47.2)	44.2 (40.3, 47.1)	0.15
Reservoir outgrowth (infectious units per million)	<b>0.41 (0.13, 0.81)</b>	<b>1.10 (0.21, 2.69)</b>	<b>&lt;0.01</b>
Total HIV DNA (log <sub>10</sub> per million)	3.38 (2.98, 4.19)	3.08 (2.51, 3.79)	0.13
Subtype, n (%)			
D	26 (45.6)	18 (54.5)	
C	9 (15.8)	6 (18.2)	
A	2 (3.5)	1 (3.0)	0.86
A/D	7 (12.3)	3 (9.1)	
A/F	1 (1.8)	0	
A/C	0	1 (3.0)	
Unknown	12 (21.1)	4 (12.1)	
Pre-ART viral load (log <sub>10</sub> copies/ml)	4.62 (3.88, 4.93)	4.72 (4.17, 5.22)	0.18
Nadir CD4 T cell count (cells/ $\mu$ l)	180 (109, 232)	168 (129, 238)	0.92
Time on ART (years)	7.0 (5.3, 8.5)	6.9 (3.3, 9.3)	0.86
Proportion of viral loads >400 copies/ml, mean (range)	0.005 (0, 0.118)	0.003 (0, 0.059)	0.93
Viremic time (years)	6.1 (4.2, 10.2)	5.6 (3.7, 7.8)	0.46
CD4 T cell count at QVOA (cells/ $\mu$ l)	<b>594 (461, 740)</b>	<b>458 (380, 559)</b>	<b>&lt;0.01</b>
CD4/CD8 T cell ratio at QVOA	<b>0.89 (0.65, 1.12)</b>	<b>0.63 (0.56, 0.84)</b>	<b>&lt;0.01</b>
D-dimer (ng/mL)	<b>273 (198, 534)</b>	<b>130 (80, 219)</b>	<b>&lt;0.01</b>
Effector memory CD4 T cells (proportion)	1.9 (1.1, 5.9)	3.4 (2.5, 6.1)	0.10
IL-2+ CD8 T cells (proportion)	1.9 (1.2, 3.9)	4.3 (1.6, 5.8)	0.08

Table 2. Coefficients and 95% confidence intervals of univariate and stepwise linear regression of reservoir outgrowth (IUPM) on clinical, and immunological covariates in females and males.

Characteristic	Univariate Regression		Stepwise Regression	
	Females (n = 57)	Males (n = 33)	Females (n = 29)	Males (n = 10)
Age (per years)	<b>-0.03*</b> (-0.05, -0.01)	0.01 (-0.04, 0.07)	-	-
Pre-ART viral load (per log <sub>10</sub> copy/ml)	<b>0.07</b> (-0.15, 0.29)	0.16 (-0.22, 0.53)	<b>0.29*</b> (0.03, 0.55)	0.47 (-0.42, 1.35)
Nadir CD4 T cell count (per 100 cells/ $\mu$ l)	<b>0.10*</b> (0.00, 0.21)	-0.10 (-0.35, 0.14)	-	-
Time on ART (per year)	<b>-0.09*</b> (-0.14, -0.04)	0.02 (-0.09, 0.13)	<b>-0.13*</b> (-0.24, -0.02)	-0.32 (-0.64, 0.01)
CD4 T cell count at QVOA (per 100 cells/ $\mu$ l)	-0.00 (-0.07, 0.07)	-0.10 (-0.34, 0.14)	-	-
CD4/CD8 T cell ratio at QVOA (per 0.1 proportion increase)	0.02 (-0.30, 0.74)	<b>-0.13*</b> (-0.23, -0.03)	-	-
PD-1+ CD4 T cells (per 1% PD-1+ CD4 T cells)	0.004 (-0.009, 0.016)	<b>0.040*</b> (0.004, 0.072)	-	<b>0.05*</b> (0.00, 0.09)
IL-2+ CD4 T cells (per 1% IL-2+ CD4 T cells)	<b>0.027*</b> (0.000, 0.054)	-0.036 (-0.106, 0.041)	-	-
TNF+ CD4 T cells (per 1% TNF+ CD4 T cells)	<b>0.014*</b> (0.002, 0.026)	-0.012 (-0.042, 0.019)	-	-
IL-2+ CD8 T cells (per 1% IL-2+ CD8 T cells)	<b>0.12*</b> (0.027, 0.205)	<b>-0.14*</b> (-0.241, -0.023)	-	-0.14 (-0.29, 0.01)
TNF+ CD8 T cells (per 1% TNF+ CD8 T cells)	<b>0.018*</b> (0.000, 0.035)	-0.018 (-0.052, 0.017)	0.01 (-0.01, 0.03)	-

Significant values are bolded and indicated by \* (p < 0.05)

## Conclusions

- Females have reduced viral outgrowth, but similar levels of total HIV DNA compared to males.
- This could be due to (1) a higher proportion of defective proviruses, and/or (2) a lower probability of provirus reactivation after *ex vivo* T cell stimulation in females compared to males
- Correlates of reservoir size differ between males and females:
  - In males, the most significant correlate of reservoir size is expression of PD-1 on CD4 T cells, as previous reported in predominantly male North American cohorts (i.e. Chomont Nat Med 2009).
  - In females, reservoir size correlated positively with pre-ART viral load and negatively with time on ART, but not PD-1 expression.
- Researchers should present HIV latency data stratified by sex and make a greater effort to increase participation of women in HIV-1 cure research.

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