

Immune Correlates of Anorectal HIV Shedding in Men on Antiretroviral Therapy

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Background

- Maintaining an undetectable viral load in the blood via antiretroviral therapy (ART) is an effective method of preventing sexual transmission of HIV
- However, several groups have detected HIV RNA in anogenital secretions – known as HIV shedding – among individuals on ART with suppressed viremia
- While several factors (such as reactivation of CMV, activated CD4+ T cells and inflammatory cytokines) have been associated with increased genital HIV shedding in ART-naïve individuals, little is known about correlates of HIV shedding in ART-treated individuals
- We previously reported low-level anorectal HIV shedding in 27.8% of participants from a cohort of 54 ART-treated men who have sex with men
- Anorectal HIV shedding was not associated with demographic and clinical parameters including the presence of HPV-associated high-grade anal intraepithelial neoplasia and the use of protease inhibitor-based ART regimen
- Here, we describe the mucosal correlates of anorectal HIV RNA shedding in ART-suppressed men

Methods

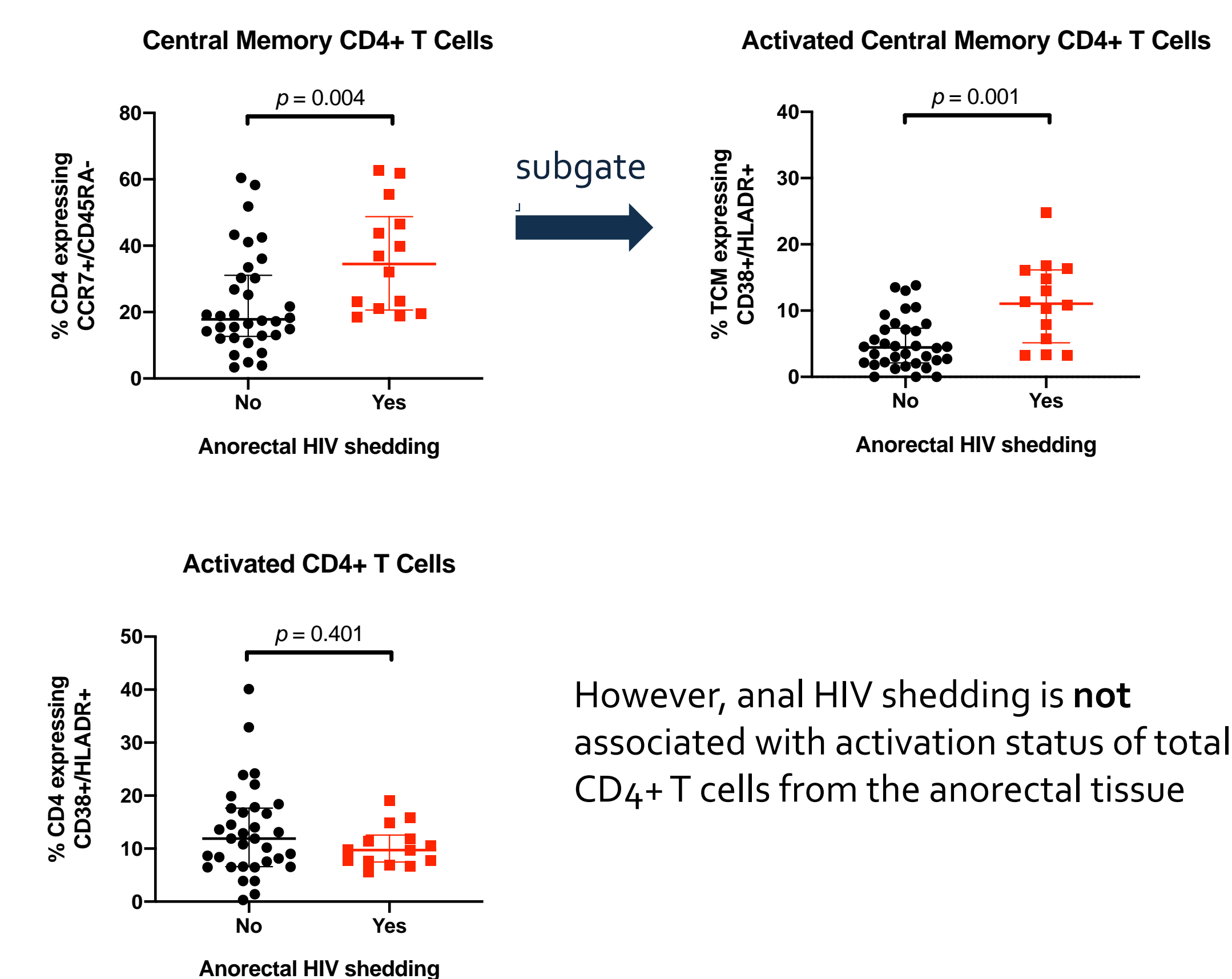
- Blood, anal swabs and behavioral data were collected, followed by anal biopsies during high-resolution anoscopy
- CD4+ T cells isolated from collagenase-treated biopsies were analyzed via multi-parameter flow cytometry
- Soluble factors were quantified by multiplex enzyme-linked immunosorbent assay
- Both tissue distribution of CD4+ T cells and epithelial integrity (claudin) were visualized using immunofluorescence microscopy
- Microbiome analysis was performed by qPCR and 16S rRNA gene sequencing

Table 1. Participant demographics

Variables	No HIV shedding (n=39)	HIV shedding (n=15)	P value
Demographics			
Age (y; (IQR))	55 (49 - 61)a	58 (52 - 64)	0.188
Years with HIV (y; (IQR))	21 (8 - 28)	28 (15 - 30)	0.082
Years on ART (y; (IQR))	17 (7.25 - 21.5)b	21 (10 - 25)	0.142
Caucasian (n (%))	31 (81.6)a	11 (84.6)b	1.000
Clinical characteristics (n (%))			
Detectable plasma viral load (> 40 copies/mL)	1 (2.6)	1 (6.7)	0.482
NNRTI-based ART	12 (32.4)b	4 (30.8)b	1.000
NRTI-based ART	35 (94.6)b	12 (92.3)b	1.000
II-based ART	30 (81.1)b	10 (76.9)b	0.707
PI-based ART	12 (32.4)b	5 (38.5)b	0.741
History of anal herpes	4 (11.1)c	3 (20.0)	0.406
STI in the past month	2 (5.1)b	1 (6.7)	1.000
HSV-1 positive serology	26 (66.7)	11 (78.6)a	0.510
HSV-2 positive serology	30 (76.9)	9 (60.0)	0.309
Blood CD4/CD8 ratio	0.765 (0.445 - 0.961)a	0.542 (0.326 - 0.859)	0.273
Behavioral characteristics (n (%))			
>100 Lifetime sexual partners	17 (47.2)c	11 (78.6)a	0.061
Rectal douching	21 (56.8)b	13 (86.7)	0.055
Receptive anal intercourse in the past week	7 (18.4)a	2 (13.3)	1.000
Smoking	8 (20.5)	2 (13.3)	0.708

a Missing one value; b Missing two values; c Missing three values

Figure 1. Anal HIV shedding is associated with number and activation status of central memory CD4+ T cells



Results

Figure 2. Density of CD4+ T cells within different tissue compartments (intraepithelial vs lamina propria) does not affect HIV shedding

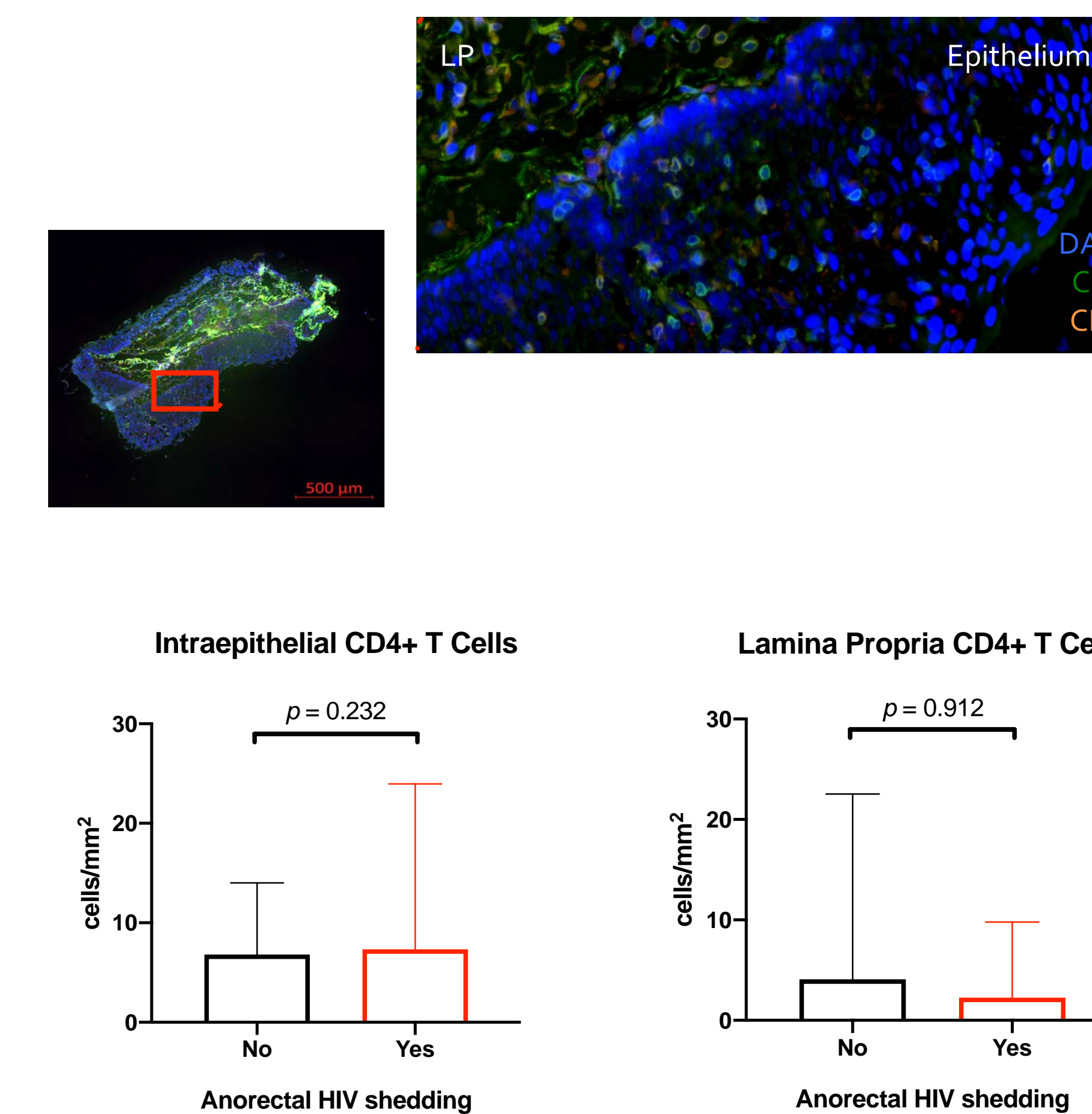


Figure 3. Anal HIV shedding is associated with the level of IL-7 and some inflammatory chemokines in anorectal secretions

Variables	No HIV shedding (n=39)	HIV shedding (n=15)	P value
Growth Factor (median (IQR))			
IL-7	13.2 (3.9 - 22.5)	17.2 (13.4 - 28.8)	0.028
Chemokines			
MIP-3a (CCL20)	55.3 (20.4 - 195.5)	392.3 (104.6 - 1026.7)	0.009
RANTES (CCL5)	383.4 (83.0 - 934.5)	1072.2 (260.3 - 2616.9)	0.033
IL-8	591.1 (177.5 - 3019.4)	906.2 (214.5 - 2712.3)	0.664
Cytokines			
IL-1b	50.8 (26.1 - 124.7)	56.6 (21.9 - 129.3)	0.832
IFN-g	12.4 (7.5 - 29.3)	21.2 (7.5 - 50.8)	0.591
IL-10	0.7 (0.2 - 2.5)	1.2 (0.6 - 3.9)	0.251

All reported values are measured in pg/mL

Figure 4. Anal HIV shedding is associated with a significant decrease in total bacterial load without perturbing bacterial diversity or relative abundances

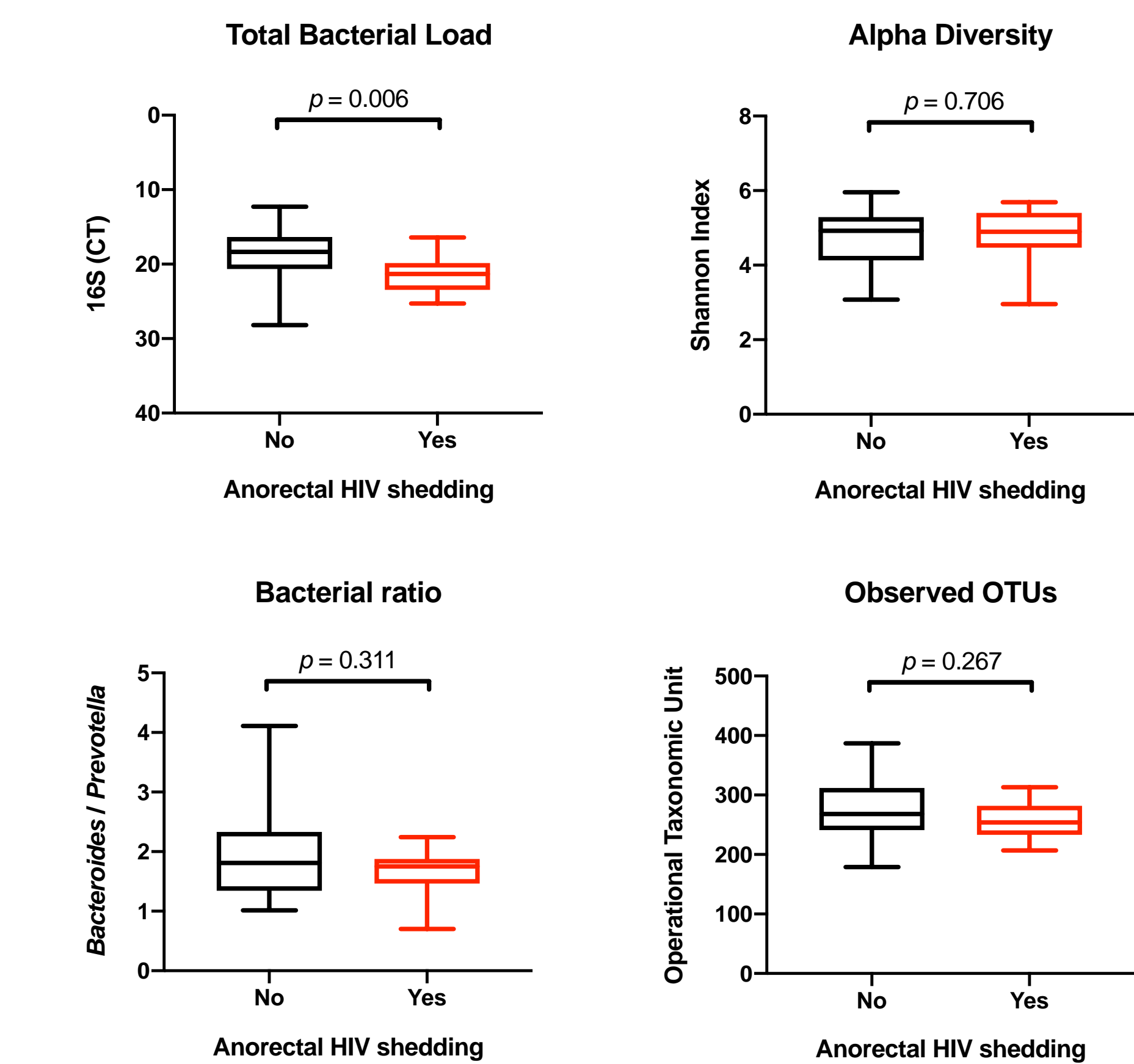
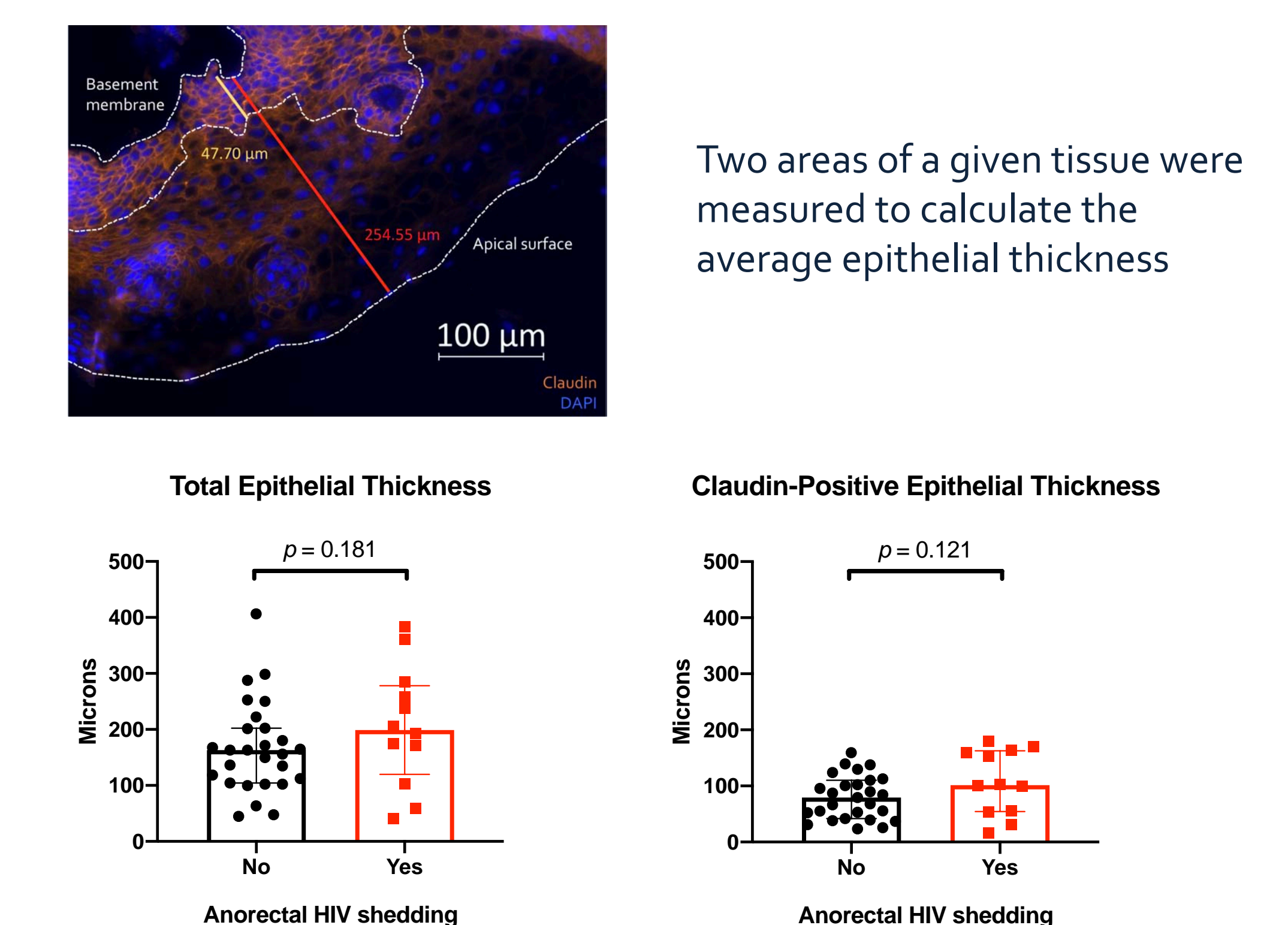


Figure 5. Neither epithelial thickness nor integrity were associated with anal HIV RNA shedding



Two areas of a given tissue were measured to calculate the average epithelial thickness

Conclusions

- Although this low-level HIV shedding is unlikely to lead to transmission (U=U), the association of HIV shedding with central memory CD4+ T cells suggests that a deeper understanding of this phenomenon may have relevance for the HIV eradication field
- Local inflammation and tissue injury are unlikely to be the drivers of HIV shedding in individuals with suppressed viremia

Acknowledgements

