

Foreskin immune associations of asymptomatic Herpes Simplex Virus-2 infection

Jessica L. Prodger, R Gray, G Kigozi, F Nalugoda, R Galiwango, K Nehemiah, M Kakanga, T Hirbod, MJ Wawer, N Sewankambo, D Serwadda, R Kaul and the Rakai Genital Immunology Research Group.



Adult circumcision and STIs

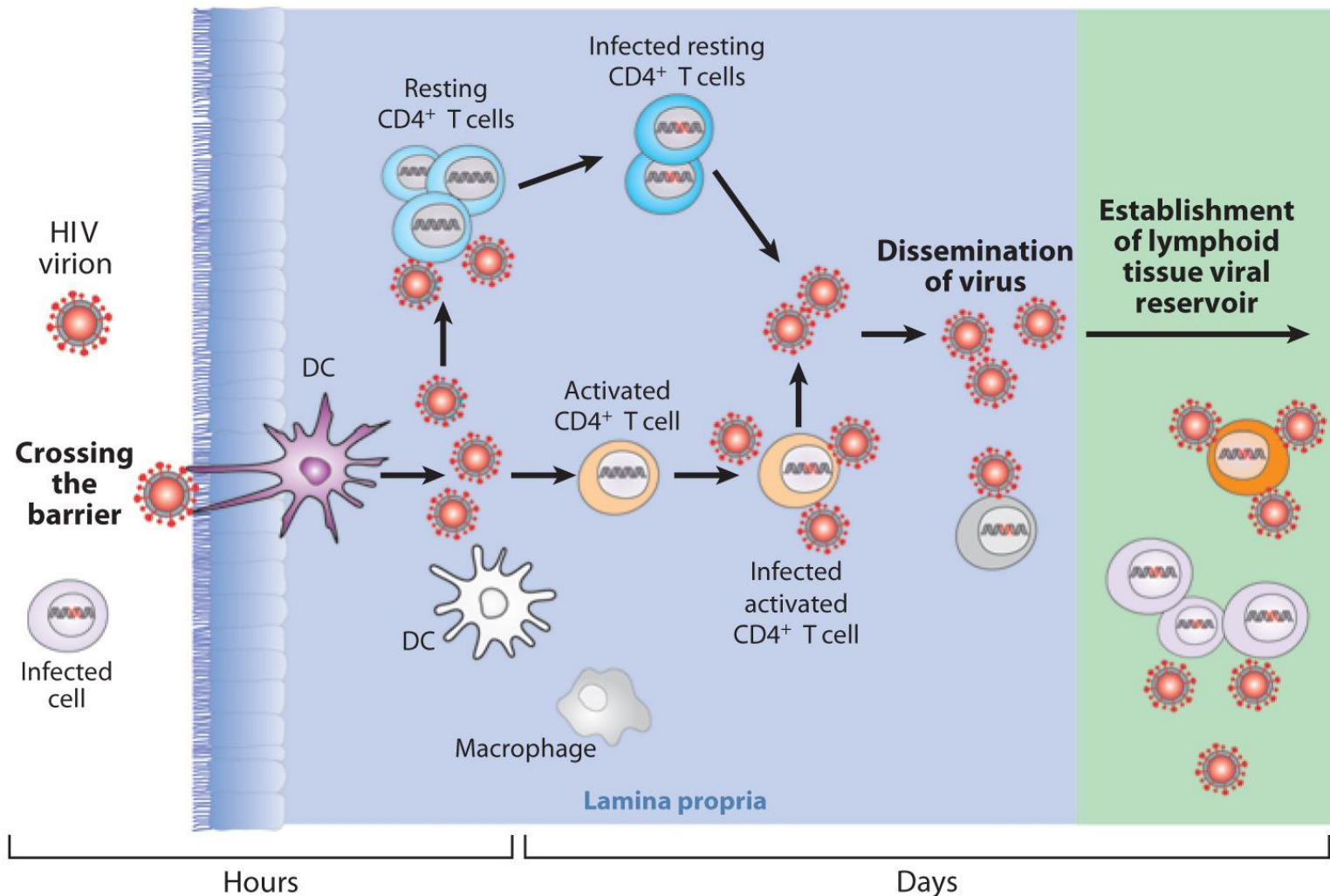
- HIV ↓ by 50-60%
 - ▣ 3 independent, well-controlled clinical trials agree
- HSV-2 ↓ 28-34%
- Genital ulcers ↓ 47%
- Oncogenic HPV ↓ 32-35%
- Bacterial infections (gonorrhea, syphilis, chlamydia) reduced in some studies but not others



Other than vaginal insertive sex?

- Anal sex
 - ... maybe?
 - Meta-analysis of >50,000 MSM shows no difference
 - If exclusively insertive may have protective effect
- Transmission rates to receptive partner
 - Protective in some observational studies
 - Clinical trials confounded: increased if sex before complete wound healing (3-6 weeks)
 - Indirectly protects women against co-infections
 - ↓ HPV by 28%; BV by 40%; TV by 48%; GUD by 22%

HIV infection at the mucosa



Not all CD4 T cells are equal



- At mucosa, HIV infects CD4 cells that express CCR5
 - ▣ Despite ample CXCR4⁺ CD4 T cells
- Certain subsets of CCR5⁺ CD4 T cells may be more permissive to infection
 - ▣ Th17 cells
- Virus replicates in activated CCR5⁺ CD4 T cells
 - ▣ Pro-inflammatory cytokines (TNF α and IFN γ)
 - ▣ Immunomodulatory Tregs – possibly protective?

HSV-2 and HIV



- Chronic HSV-2 infection = ~3x more HIV
 - ▣ Physical breaks in epithelium
 - ▣ BUT most HSV-2 is asymptomatic
- Acyclovir treatment does not reduce HIV acquisition in HSV-2 positive individuals
 - ▣ Even though it does reduce ulcers
- Suggests increased HIV susceptibility is not solely due to compromised epithelial integrity



Asymptomatic HSV-2

- Subclinical HSV-2 alters the immune environment
 - Density of CCR5⁺ CD4 T cells is increased at healed ulcer site, even after 20 weeks with no recurrence
 - Women with no prior symptoms have more CCR5⁺ CD4 T cells in the cervix
 - Studies from our group have shown increased CD4 T cell density in the foreskin
 - Functional characteristics of these cells might also be altered

Hypothesis



Asymptomatic HSV-2 infection is associated with increased CD4 T cell CCR5 expression, enhanced inflammatory cytokine production, and more Th17 cells within the foreskin.

Study Population



- Rakai Health Science Program- Uganda
 - ▣ Site of one of three circumcision trials in 2004
 - ▣ Currently circumcising ~80 men/week

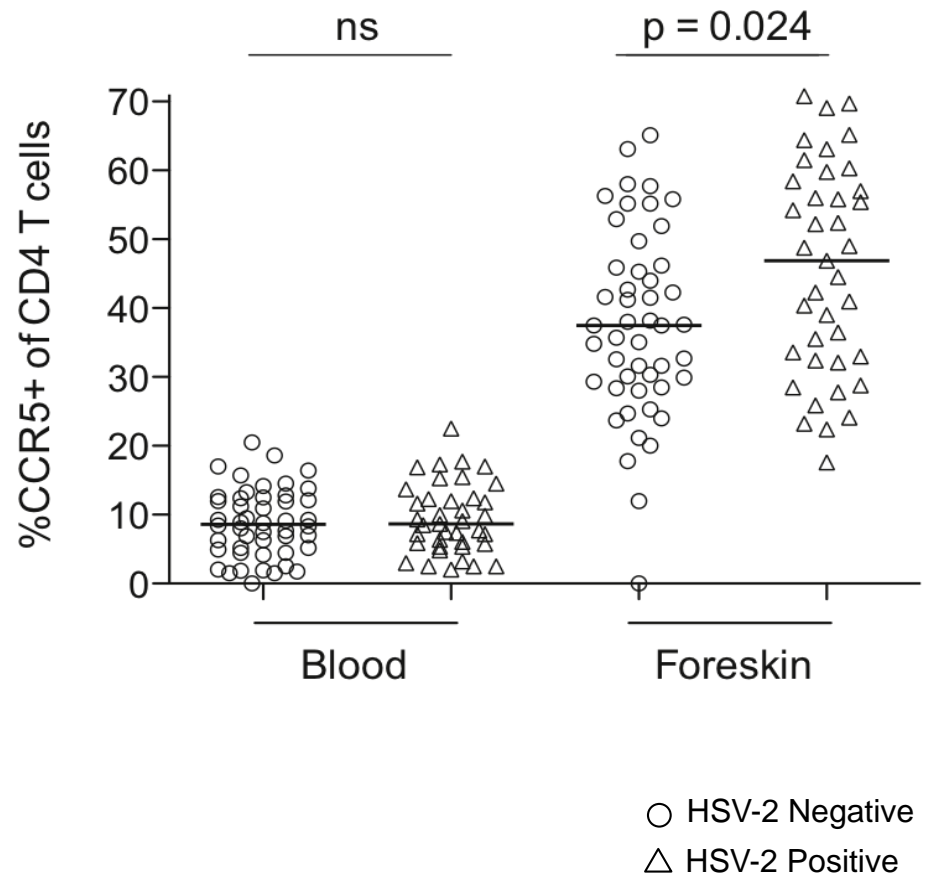
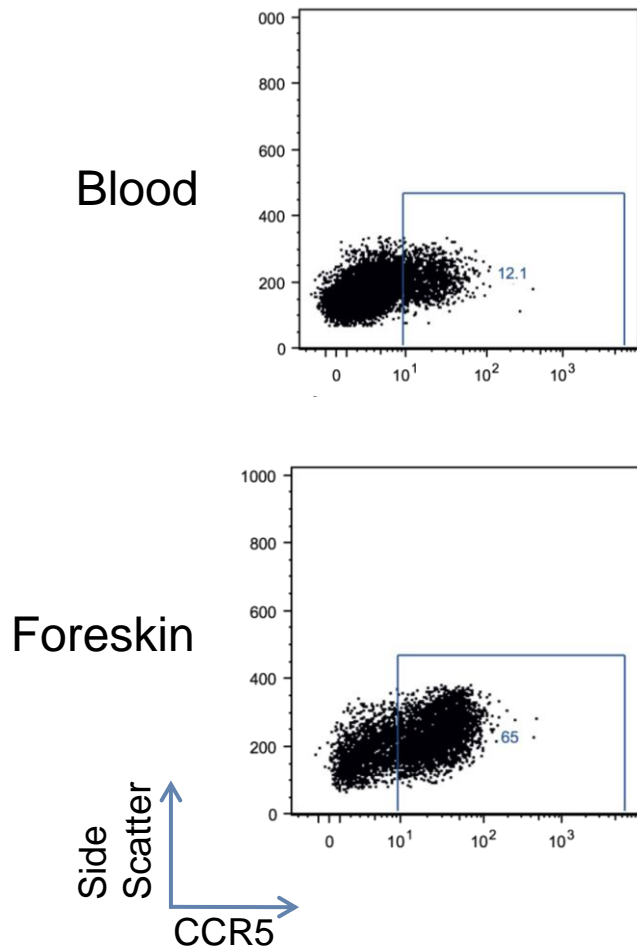
- Enrolled 87 HIV negative men, 39 HSV-2 positive
 - ▣ Confirmed to be HIV negative day of surgery
 - ▣ HSV-2 serology by Kalon ELISA
 - Previously validated in Rakai
 - ▣ Median age = 35.2 years

Methods



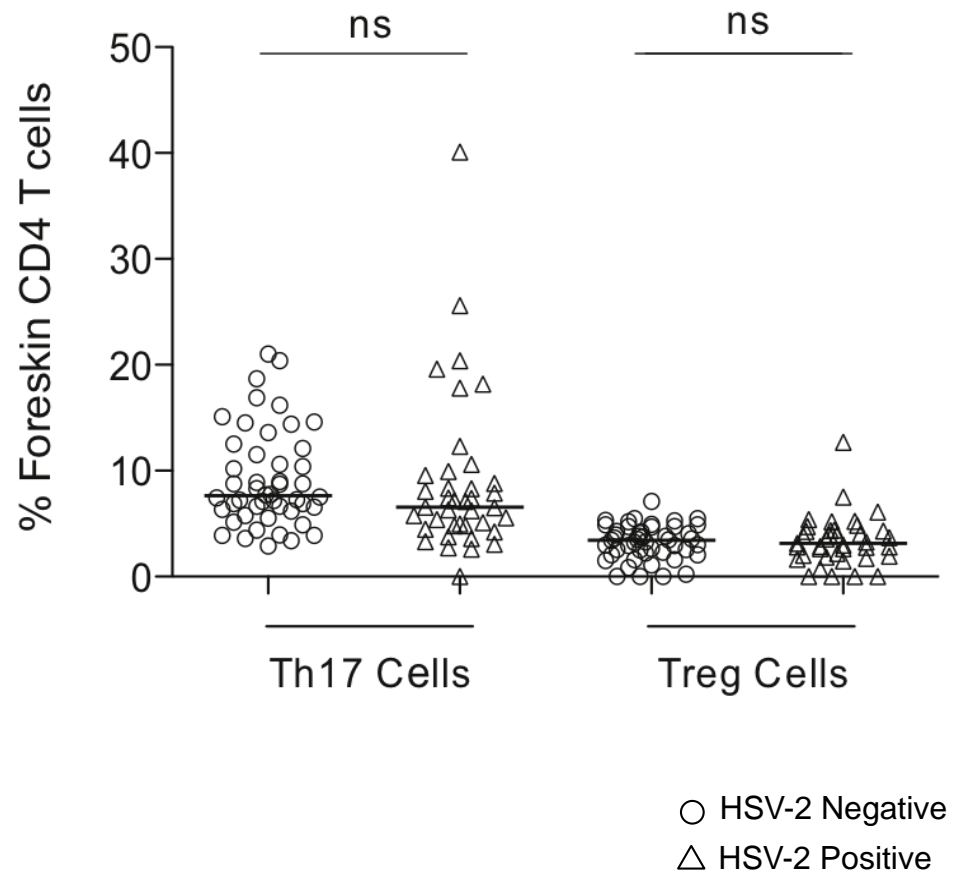
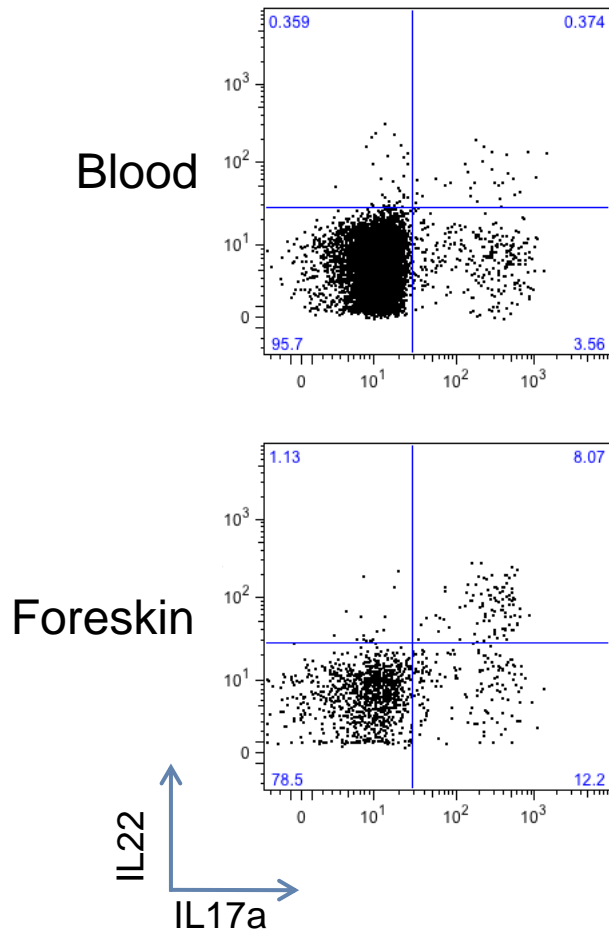
- Foreskin and blood cells isolated from each patient
- Cells stimulated, permeabilized, and stained for surface markers and cytokine production
 1. HIV “Target” Cells
 - CD3/CD4/CCR5⁺
 2. Tregs
 - CD3/CD4/CD25/FoxP3⁺
 3. Th17 cells
 - CD3/CD4⁺ & producing IL17 α
 4. CTL
 - CD3/CD8⁺ production of TNF α and/or IFN γ

CCR5 expression on CD4 T cells



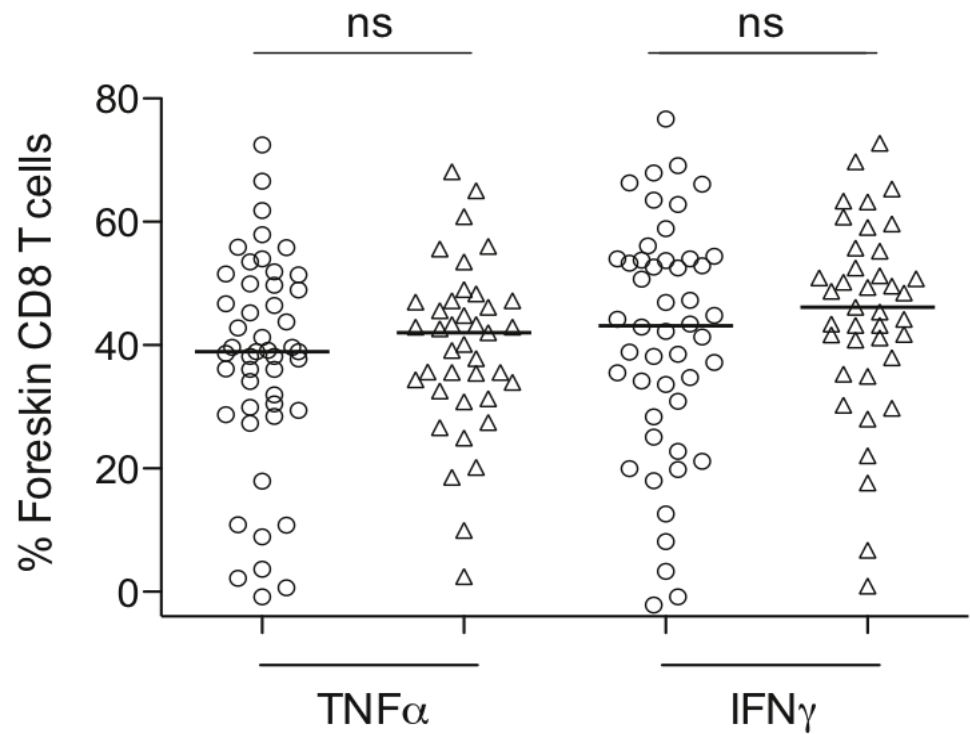
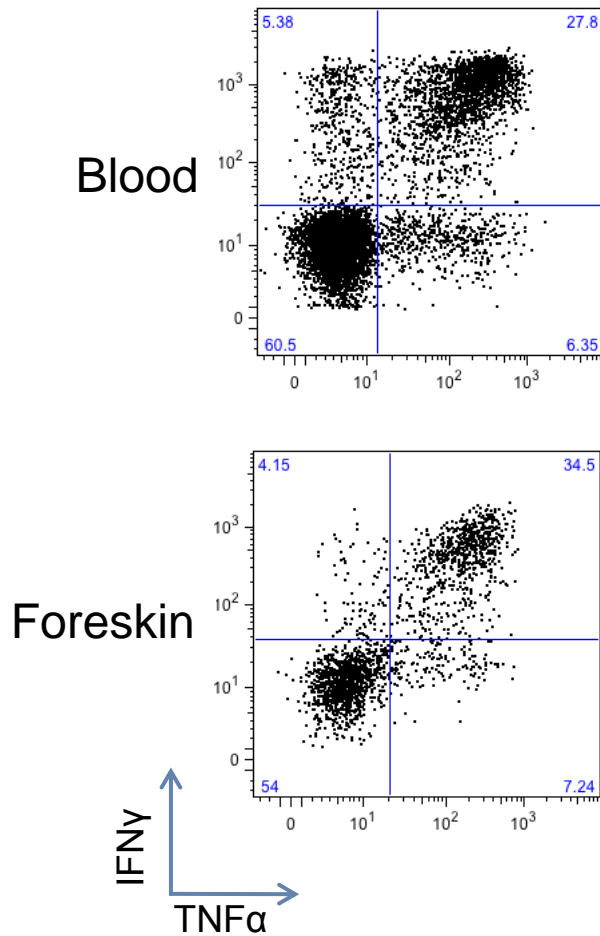


Th17 and Tregs





Pro-Inflammatory Cytokines



○ HSV-2 Negative
△ HSV-2 Positive

Conclusions



- Major immune association of asymptomatic HSV-2 infection in the foreskin was increased CCR5 expression on CD4 T cells
 - In addition to increased density of CD4 T cells in the foreskin shown previously
- Compartmentalized increase of CCR5⁺ CD4 T cells in the foreskin may contribute to HSV-2 associated increases in HIV susceptibility, even in the absence of any clinical symptoms.

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Rakai Genital Immunology Research Group Members:



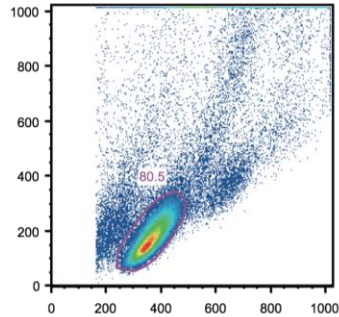
- Anyokorit Margaret
- Male Deo
- Kayiwa Dan
- Kalibbala Sarah
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- Otobi Ouma Joseph
- Kakanga Moses
- Okech John Baptist
- Okello Grace
- Aluma Gerald
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- Tumuramyé Denis
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- Isabirye Yahaya
- Mulema Patrick
- Teba James
- Atukunda Boru
- Mayengo Herbert
- Nakafeero Mary
- Mugamba Stephen
- Nakyeyune Mary

Gating

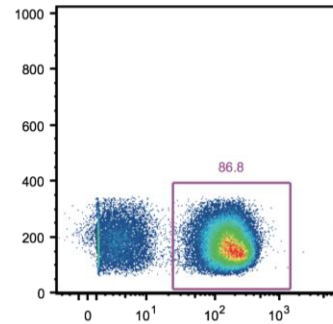


PBMC

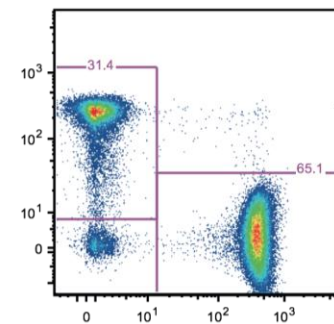
Forward/Side Scatter



CD3

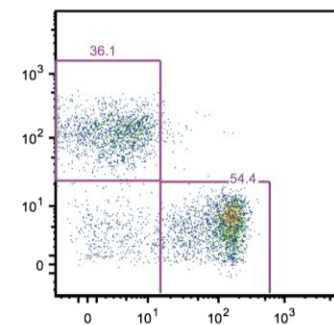
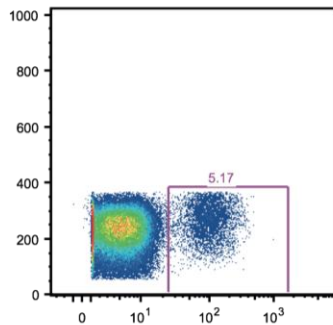
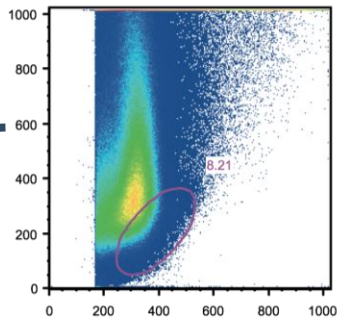


CD8 vs. CD4

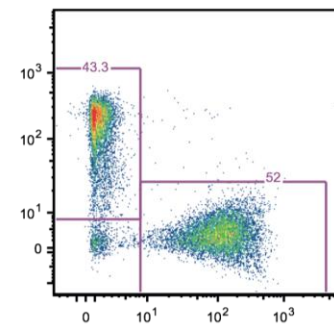
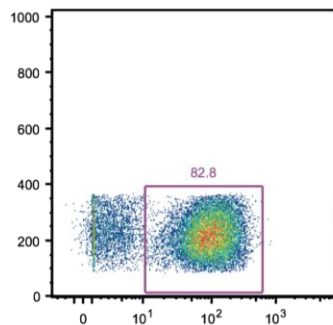
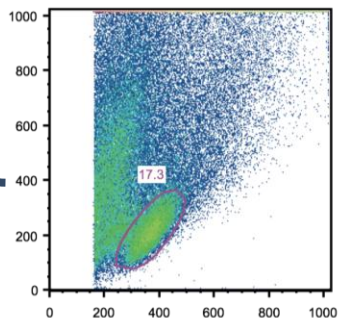


100,000 events

Foreskin Cells



Before permeabilization:
1,000,000 events



After permeabilization:
100,000 events