

Host factors influencing HIV-1 infection in the Male genital tract

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Summary of the global HIV epidemic, 2021

	People living with HIV in 2021	People acquiring HIV in 2021	People dying from HIV-related causes in 2021
 Total	38.4 million [33.9–43.8 million]	1.5 million [1.1–2.0 million]	650 000 [510 000–860 000]
 Adults (15+ years)	36.7 million [32.3–41.9 million]	1.3 million [990 000–1.8 million]	560 000 [430 000–740 000]
 Women (15+ years)	19.7 million [17.6–22.4 million]	640 000 [480 000–870 000]	240 000 [180 000–320 000]
 Men (15+ years)	16.9 million [14.6–19.7 million]	680 000 [500 000–920 000]	320 000 [250 000–430 000]
 Children (<15 years)	1.7 million [1.3–2.1 million]	160 000 [110 000–230 000]	98 000 [67 000–140 000]

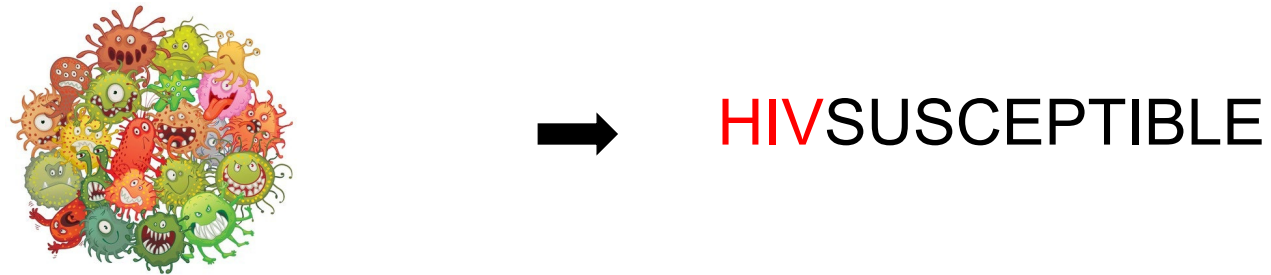
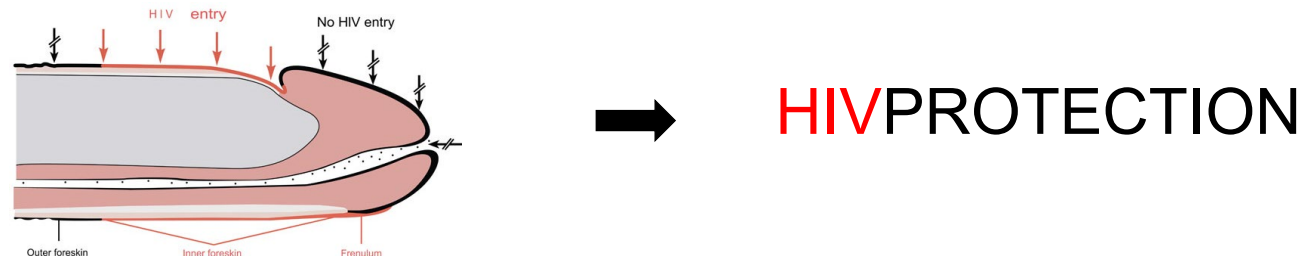
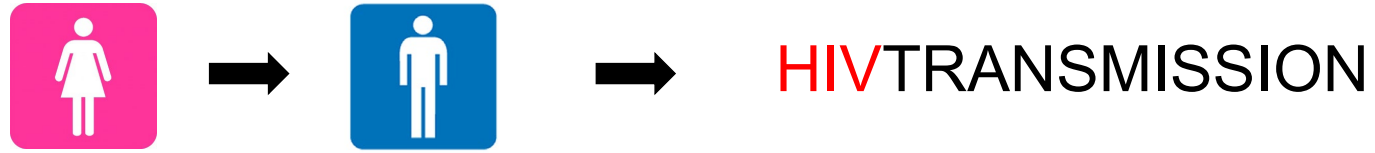
Source: UNAIDS/WHO estimates

Updated: July 2022

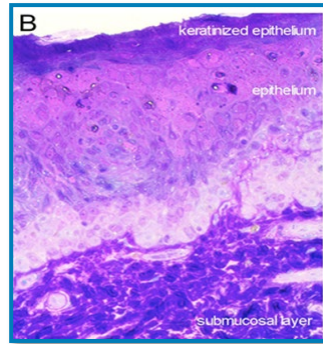


World Health
Organization

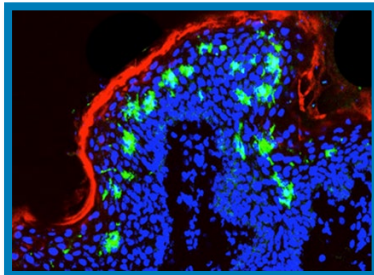
What we don't know



Molecular clues to susceptibility: Foreskin



Immunological
tissue

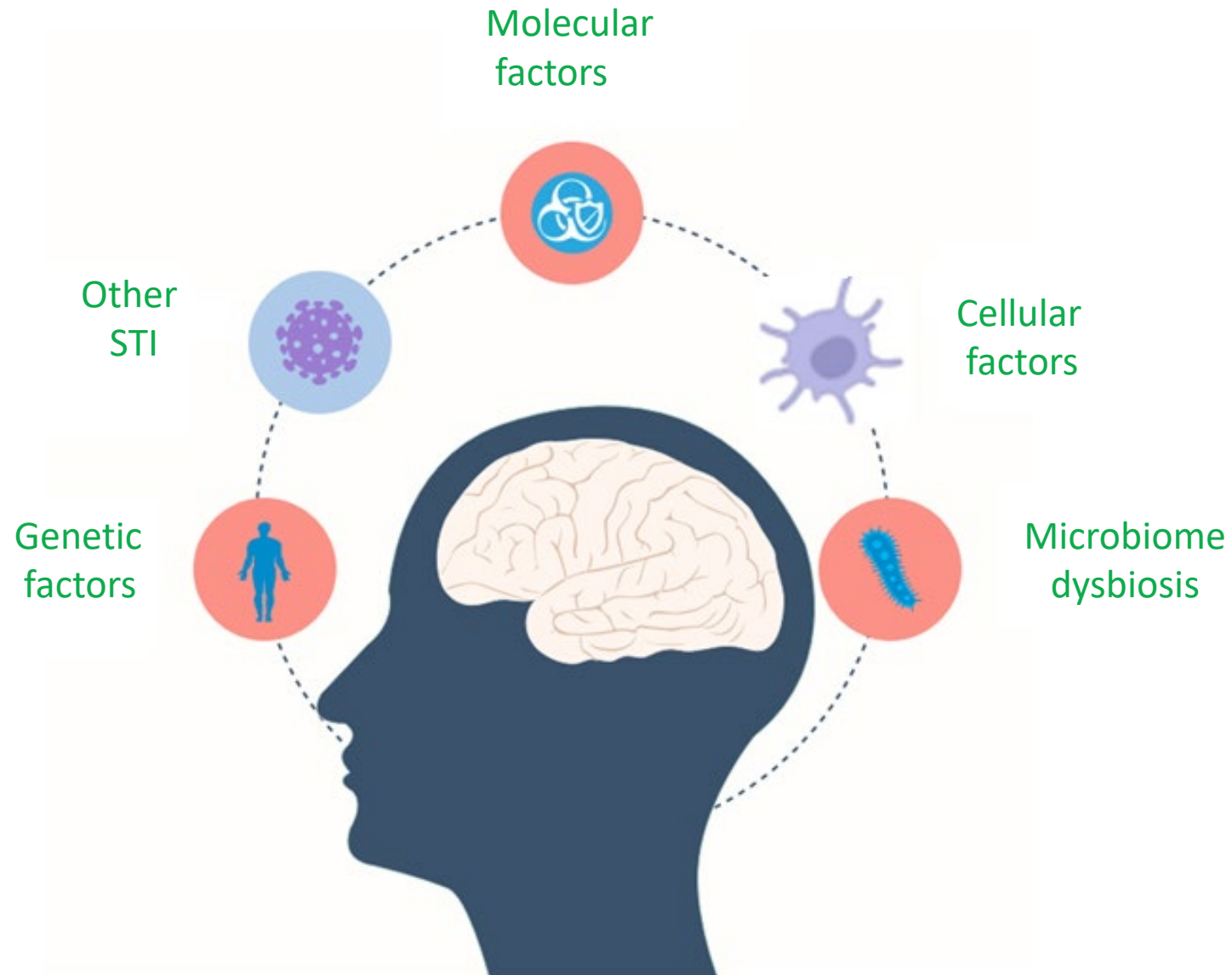


HIVSUSCEPTIBLE



HIVRISK
REDUCTION

Host factors influencing HIV-1 acquisition in tissue



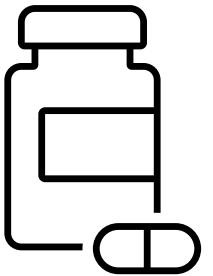
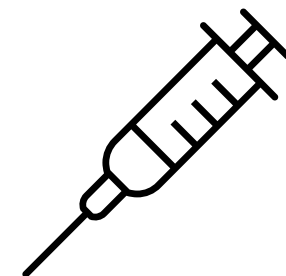
Understanding the yin yang of HIV susceptibility in the MGT

“Anti-viral”



HIV susceptible

1. Cell states and phenotypes
2. Impact of viral factors e.g tropism
3. Inducers of susceptibility e.g STI's



Investigation of Factors associated with HIV susceptibility in the MGT tissue

Host Factors

Impact of aSTI on molecular factors

Cellular factors associated with HIV susceptibility

Viral features

Viral tropism



Host Factors

Impact of aSTI on molecular factors

Cellular factors associated with HIV susceptibility

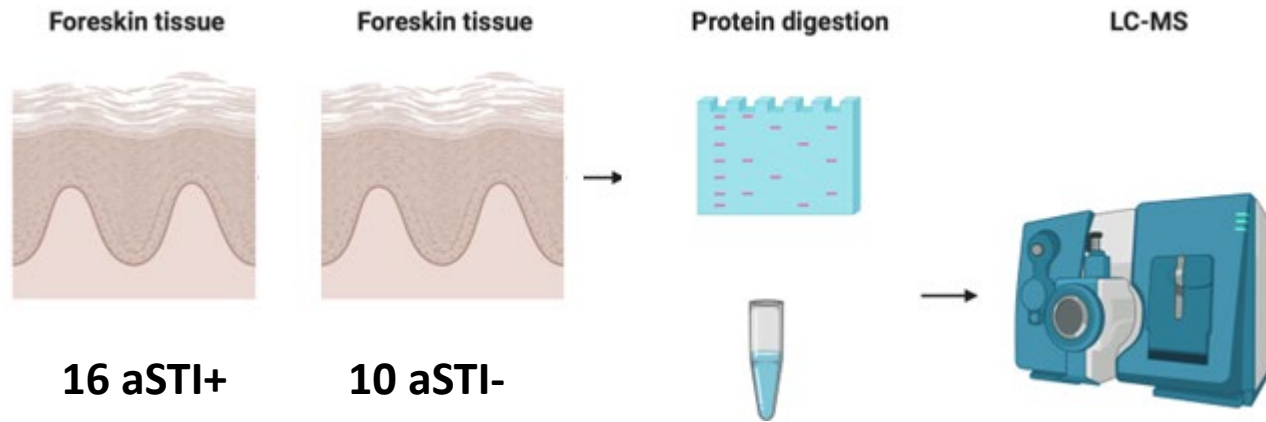
Viral features

Viral tropism

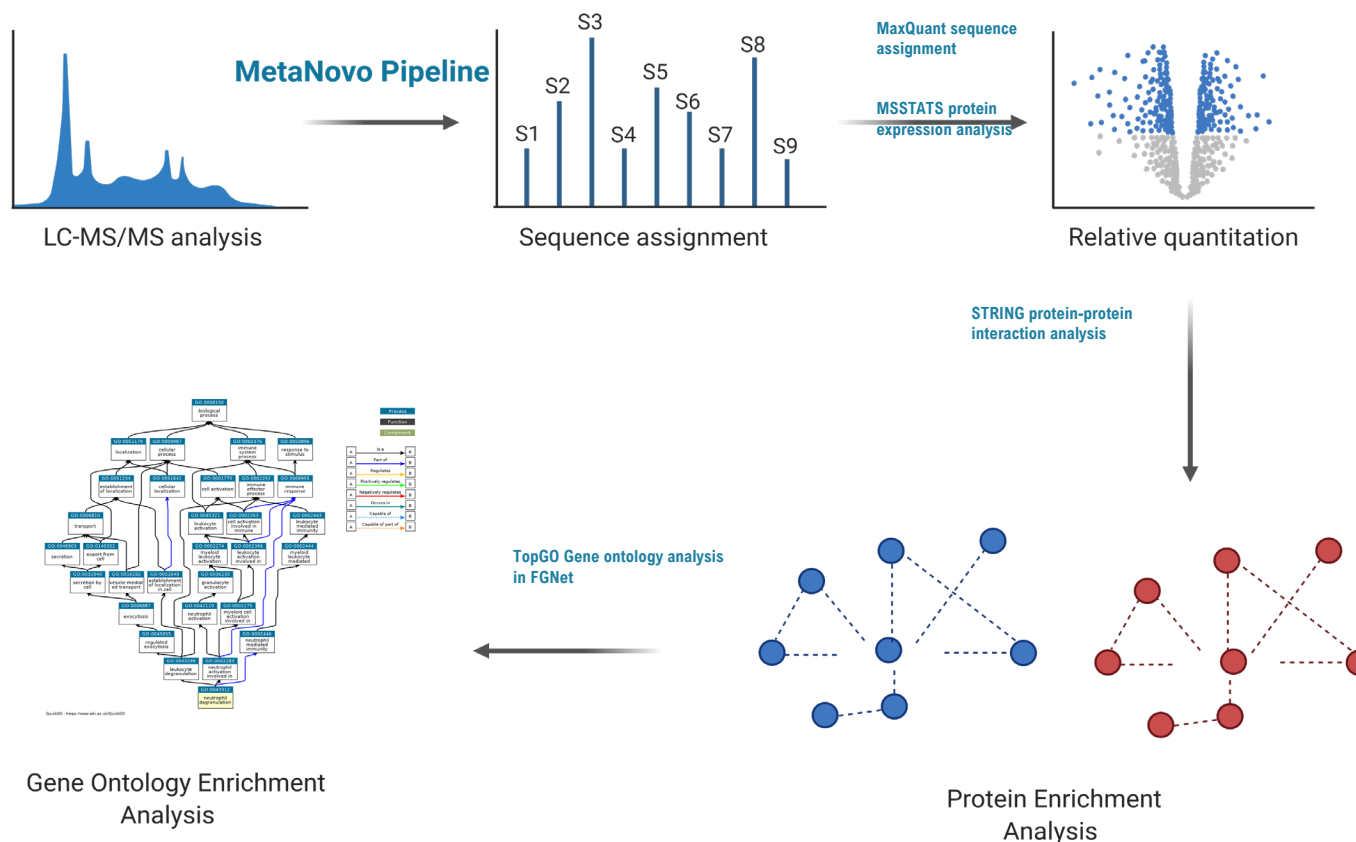
Effect of asymptomatic STI's on the foreskin proteome

What role do asymptomatic STI's play on HIV acquisition?

A



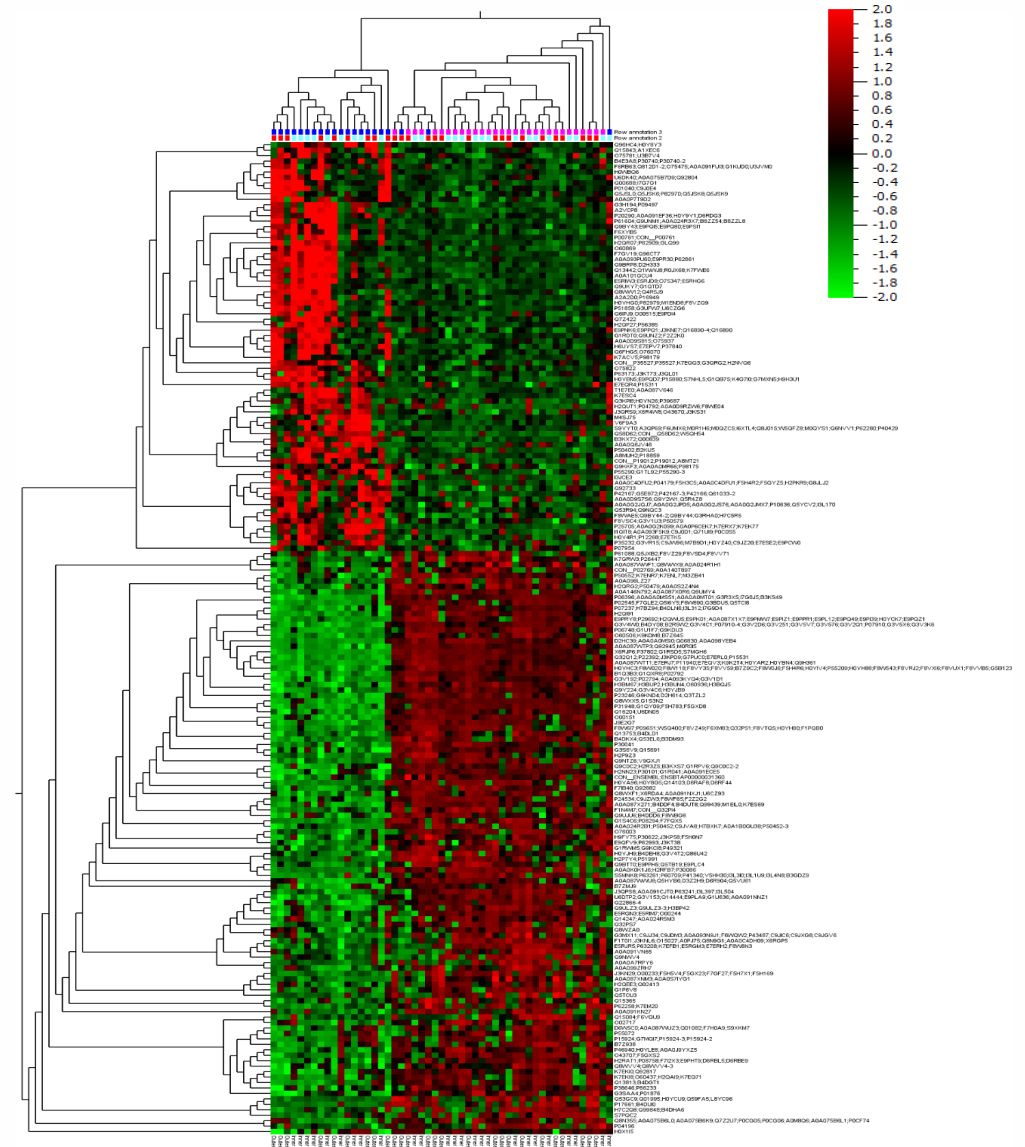
B



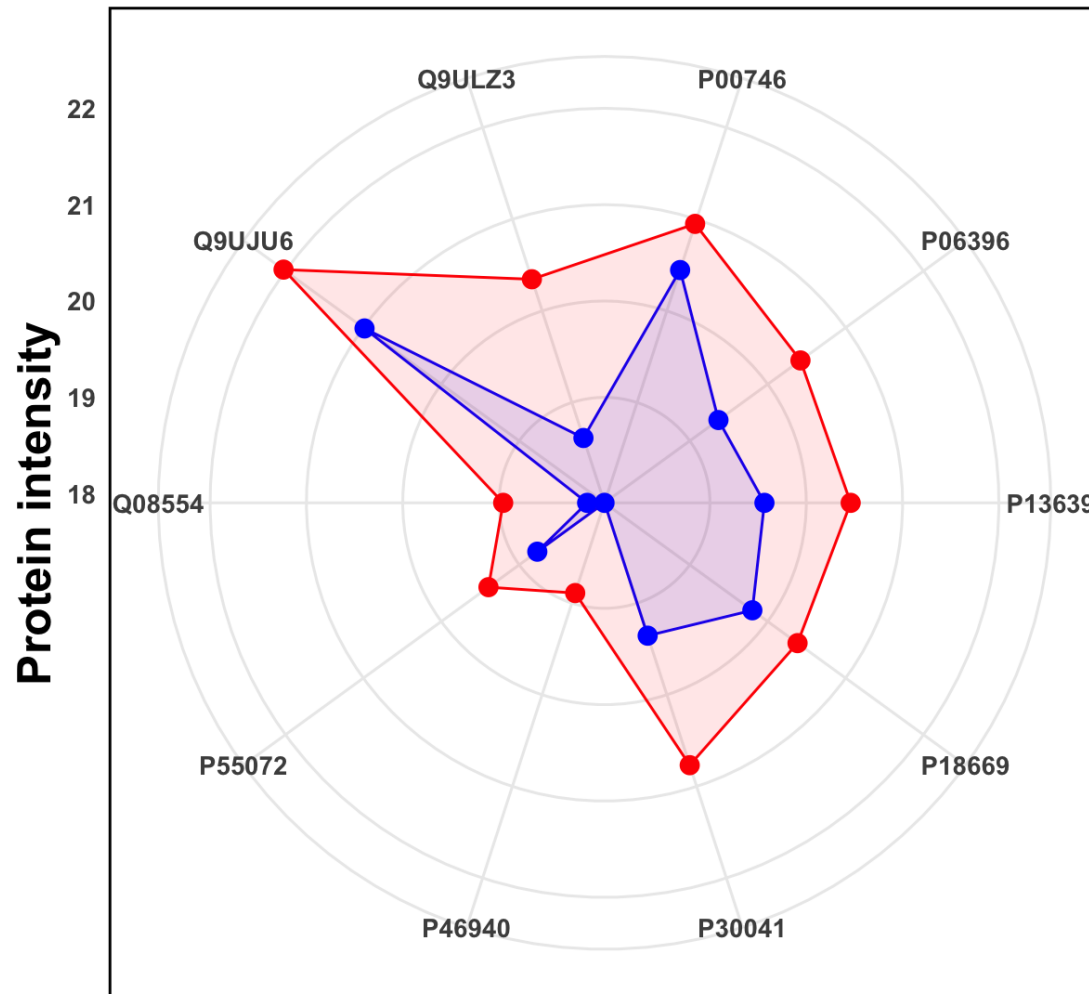
Metaproteomic analysis of foreskin tissue

aSTI significantly alter the FS proteome^B

- Host proteomes were significantly different between aSTI+ and aSTI-
- 400 host proteins were significantly differentially expressed with differences driven by aSTI



Functional enrichment of GO terms involving IL12 in aSTI proteomes



- Cellular response to interleukin-12
- Myeloid cell activation involved in immune response
- Interleukin-12-mediated signalling pathway
- Neutrophil degranulation and activation

● Control
● STI

Q9ULZ3: Protein Apoptosis-associated speck-like protein containing a CARD. PYCARD.

Q9UJU6: Drebrin-like protein

P19105: Myosin regulatory light chain 12A

P00746: Complement factor D

P46940 : Ras GTPase-activating-like protein

P30041: Peroxiredoxin-6

Expression of various proteins with within Myeloid cell activation involved in immune response GO term

aSTI driven pro-inflammatory signature mediated by IL12 in MGT tissue?

Asymptomatic STI

Recruit and activate



Dendritic cells

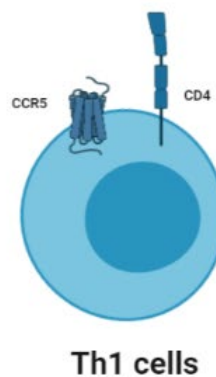
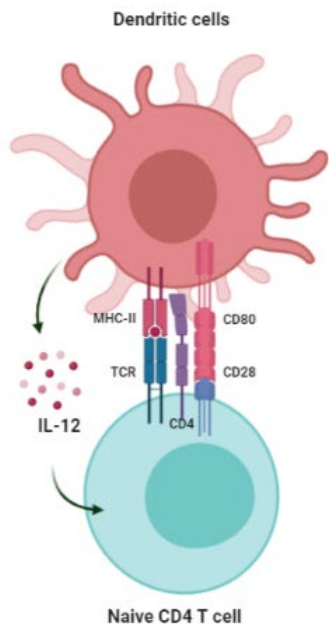


Neutrophils

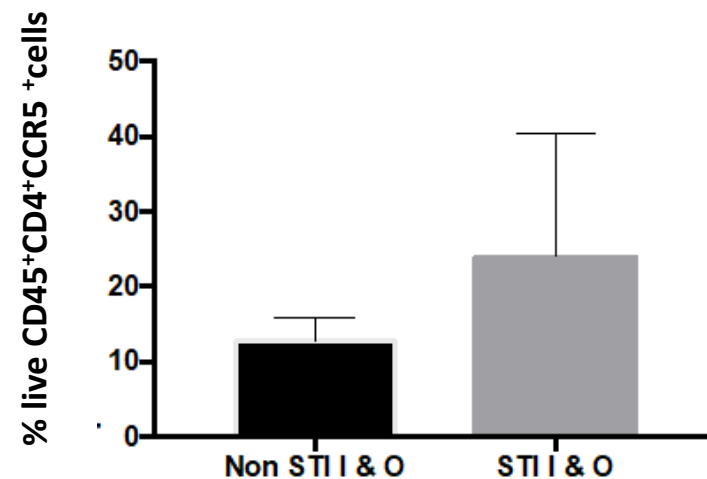
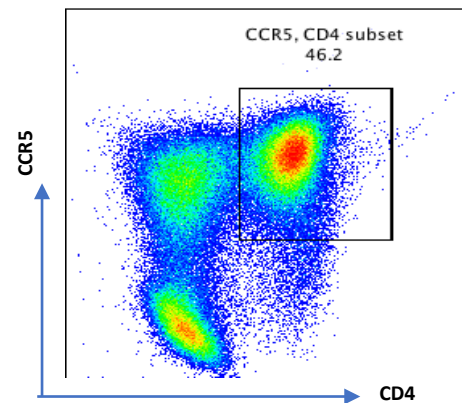


Macrophages

Interleukin 12

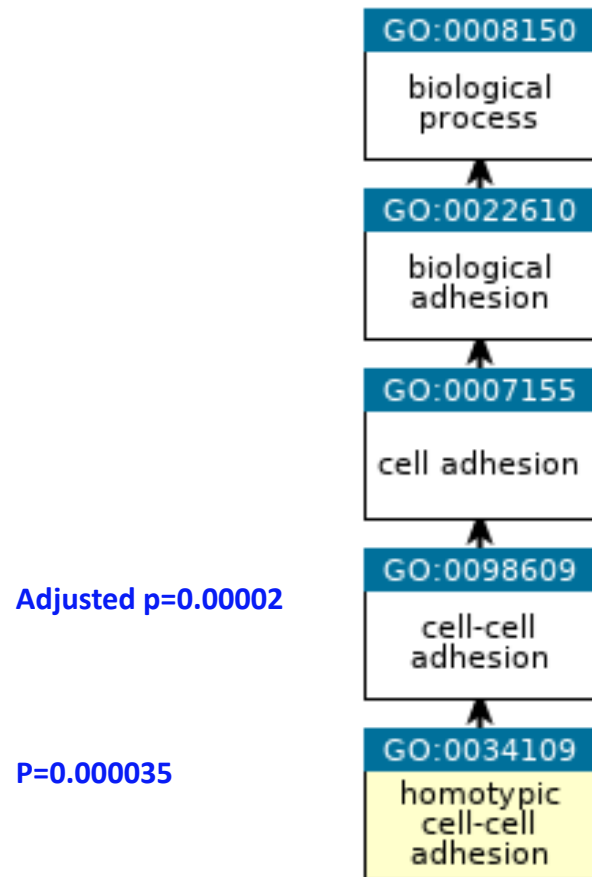


Th1 like cells elevated in aSTI



Enrichment of GO terms for barrier function in aSTI-proteomes

Homotypic cell-cell adhesion

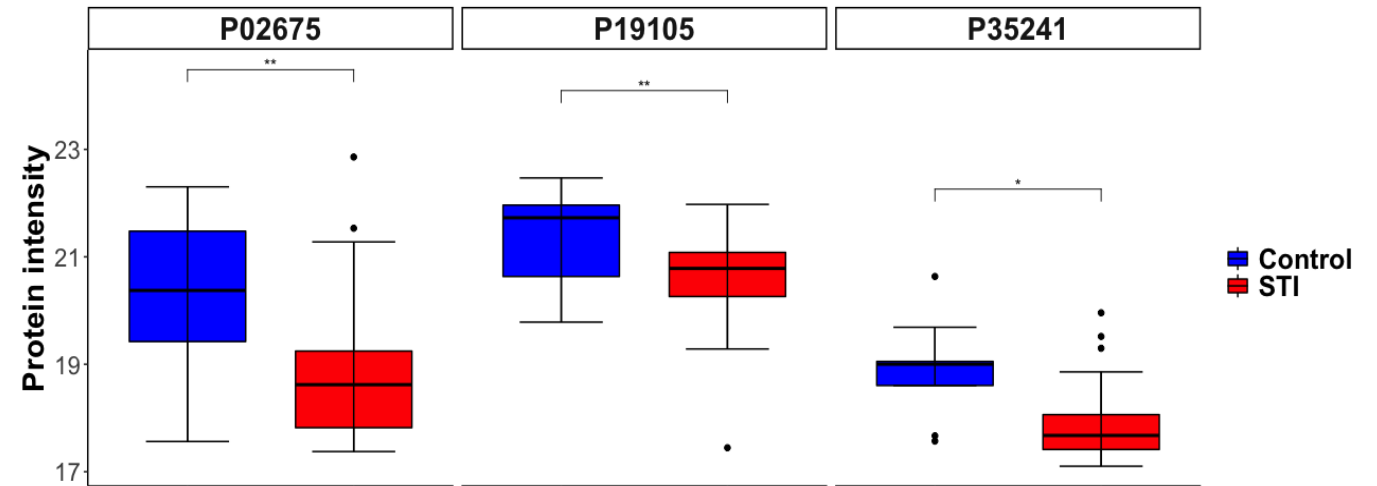


A

P02675:Fibrinogen beta chain

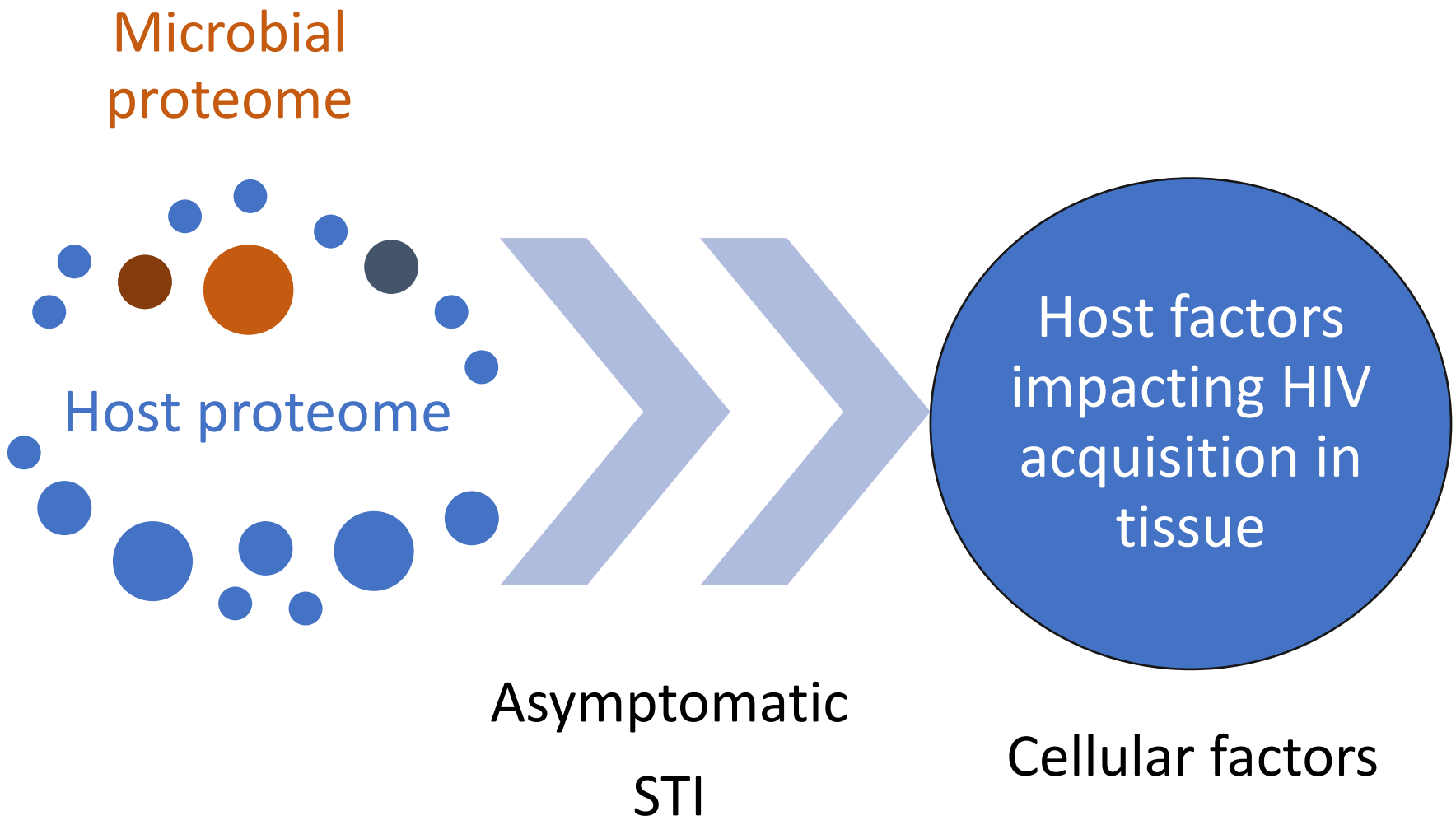
P35241:Radixin

P19105:Myosin regulatory light chain 12A



B

Multi-modal effects of aSTI on HIV susceptibility in MGT





Host Factors

Impact of aSTI on molecular factors

Cellular factors associated with HIV susceptibility

Viral features

Viral tropism

Cellular factors impacting HIV susceptibility in MGT tissue

What role do myeloid cell subsets play in HIV acquisition?

Cellular factors impacting HIV susceptibility in MGT tissue

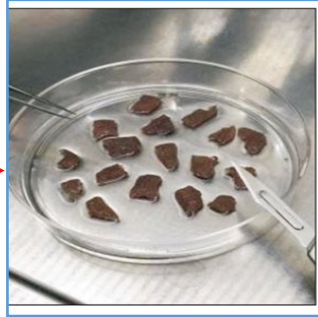
What role do myeloid cells play in HIV acquisition?

Infection of spontaneously migrating foreskin cells

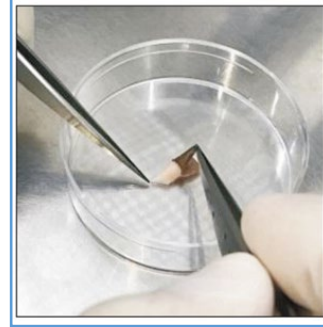
Inner & Outer Foreskin



1 cm x 0.5 cm blocks



Stripping



Cells harvested



Virus added at
MOI 0.01

HARVESTING

96 HRS

HARVESTING

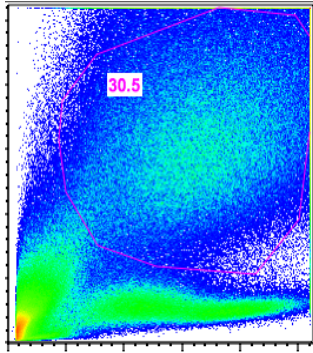
48 HRS

HARVESTING

18 HRS

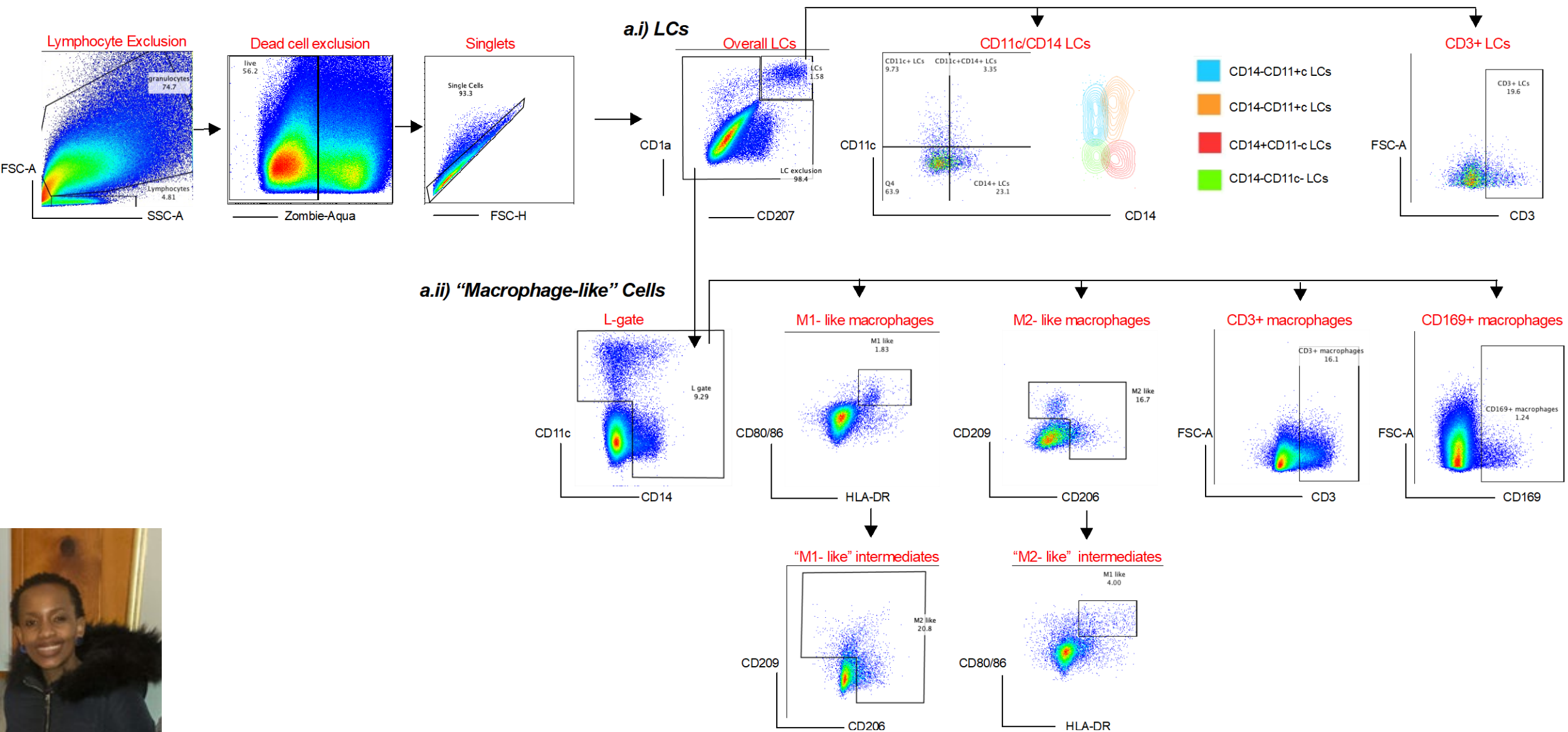
HARVESTING

5 HRS



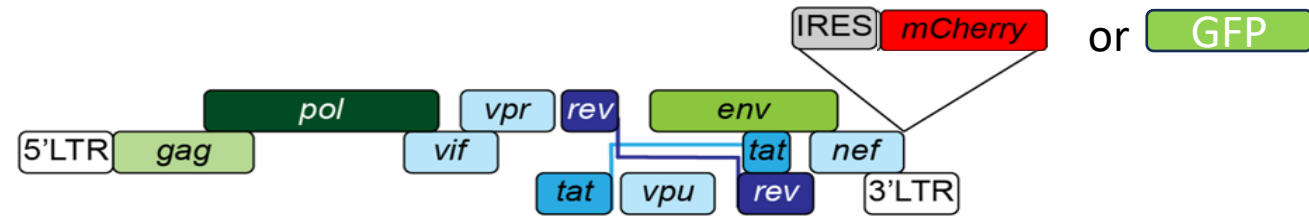
FIXING AND STAINING FOR
p24 FLOW CYTOMETRY

Myeloid cell characterization

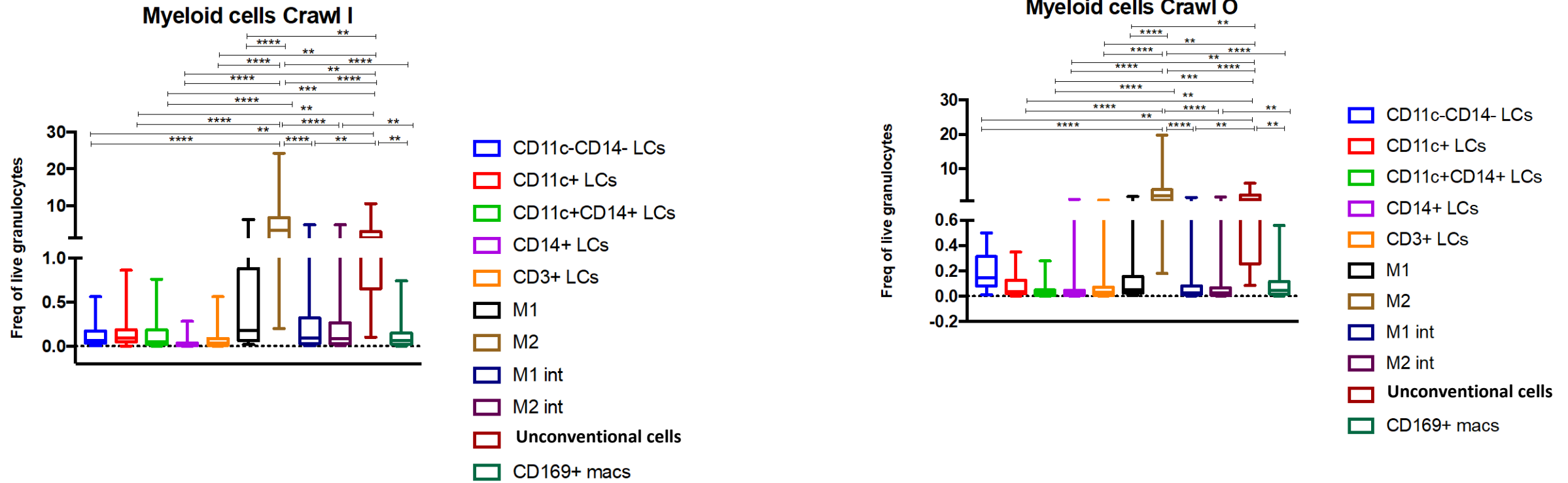


Infectious Molecular clones (IMCs)

Virus	Resistance	Code used
pBR_HIV-1 M NL4-3 (X4)	amp	CS105
pBR_HIV-1 M NL4-3 92TH14-12 (R5)	amp	CS108
pBR322HIV-1 M subtype B CH077.t (TF)	amp	CS380
pBR322HIV-1 M subtype B CH058.c (TF)	amp	CS377
pBR_HIV-1 M subtype C ZM246F-10 (TF)	amp	CS596
pCR-XL-TOPO_HIV-1 M subtype C CH198 (TF)	kana	CS769
pBR322HIV-1 M subtype B CH077.t (6 month)	amp	CS814
pUC57 HIV-1 M subtype C CH167 (CC)	amp	CS642
pBR322 HIV-1 M subtype B RHGA (CC)	amp	CS656



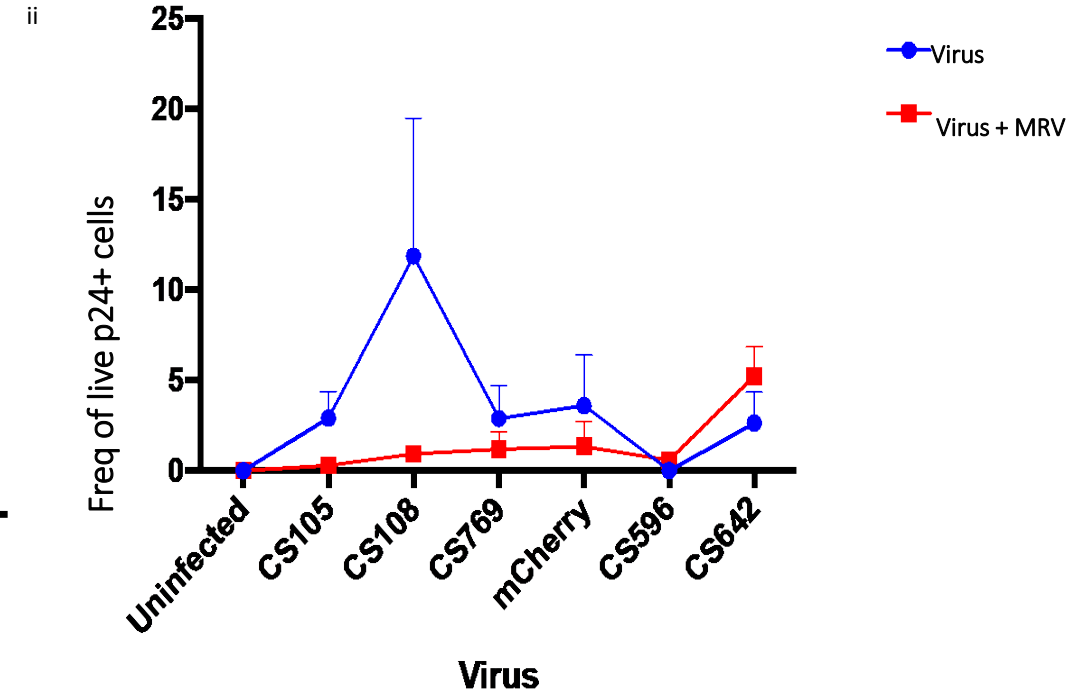
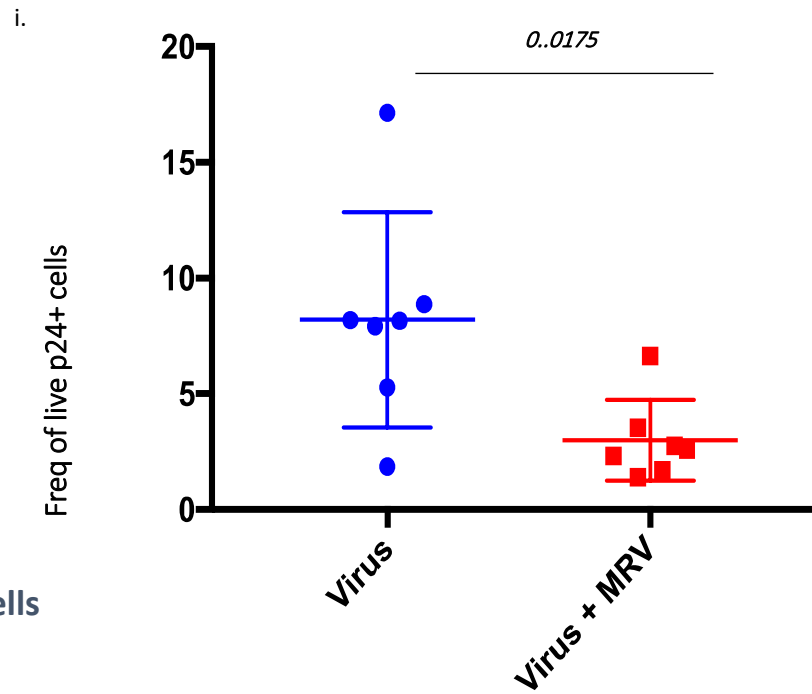
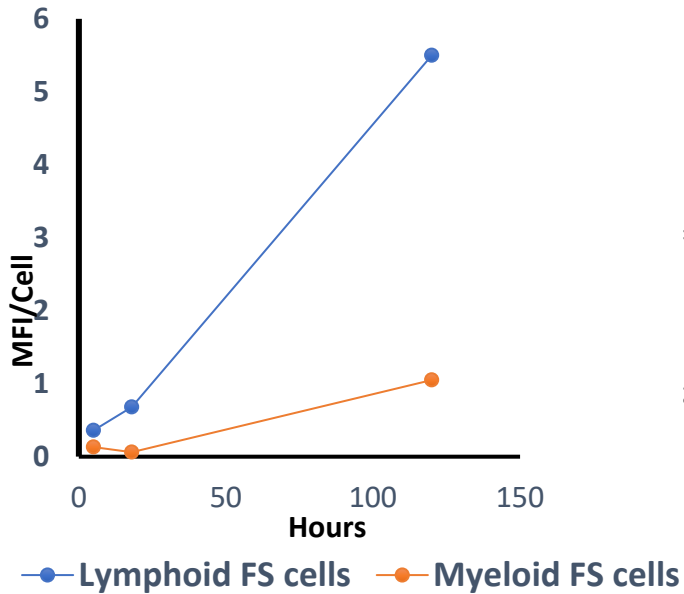
Quantification of FS Myeloid subsets in 25 HIV-aSTI- individuals



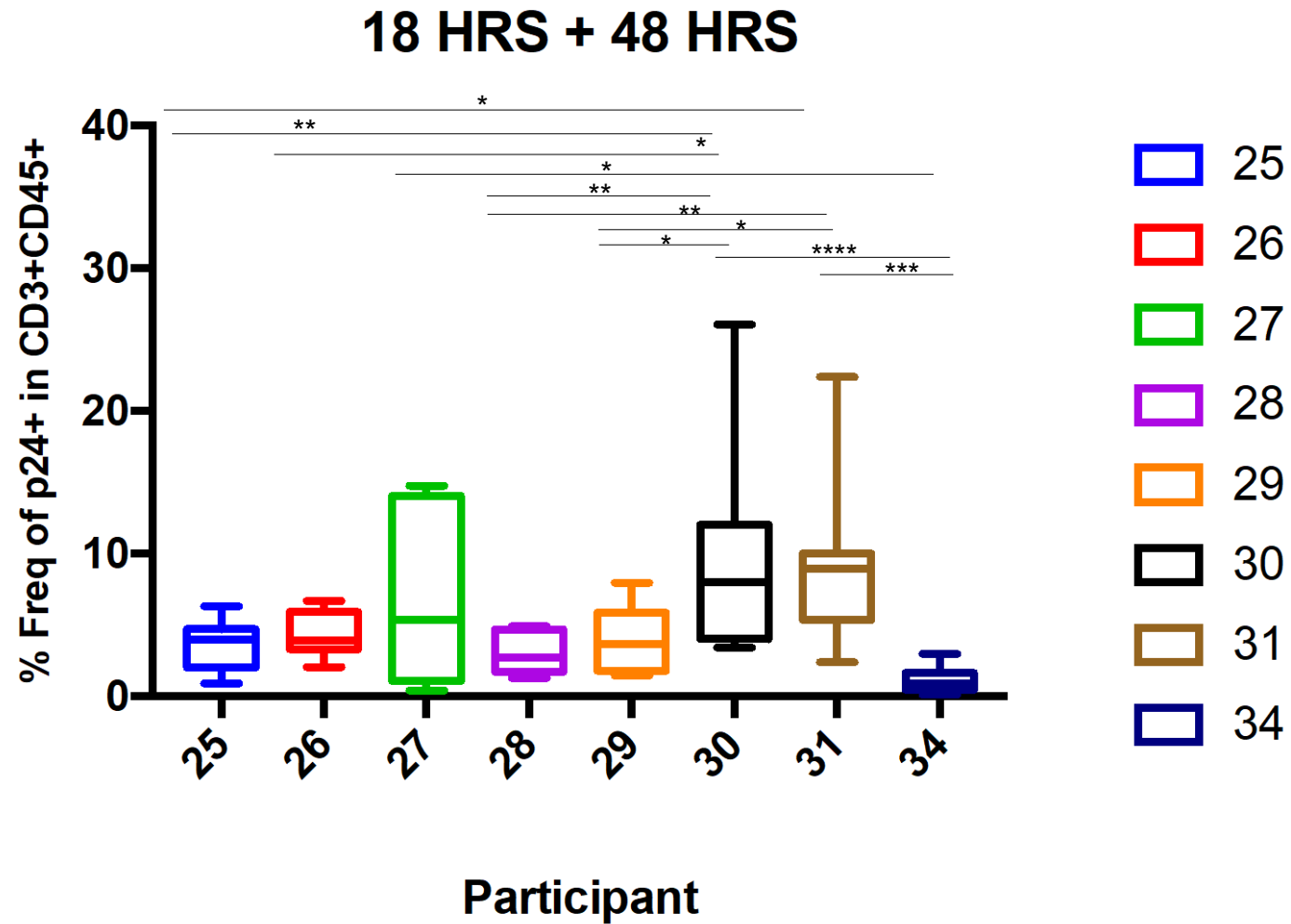
Statistics were generated two-way ANOVA using the Tukey's multiple comparisons test at 95 % CI of difference and adjusted p values. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001

Ex vivo HIV infection of FS cells incremental with time and inhibited by Maraviroc

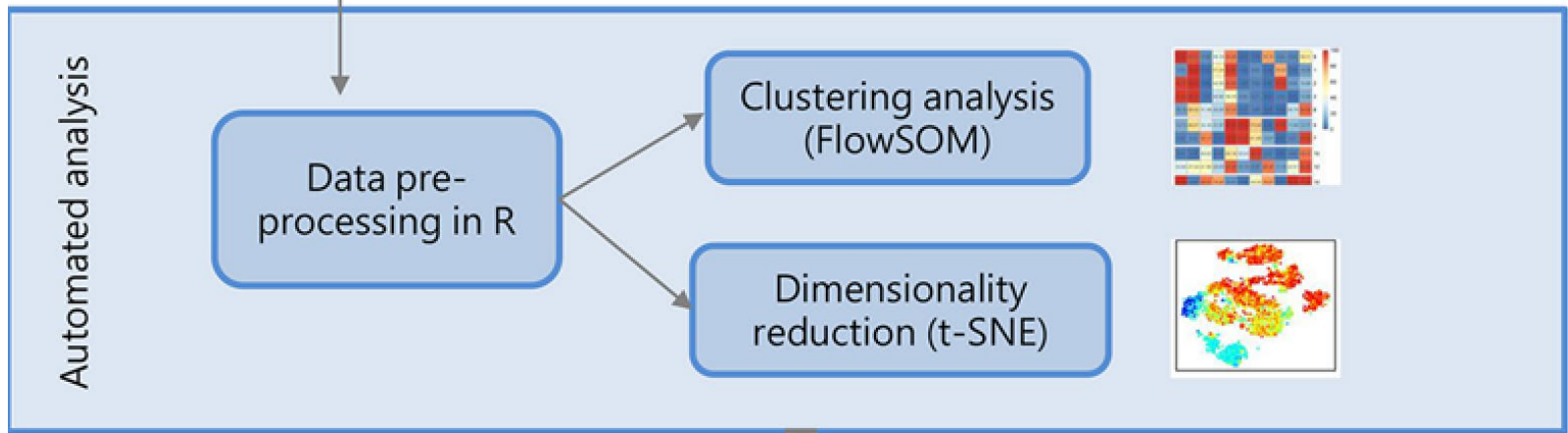
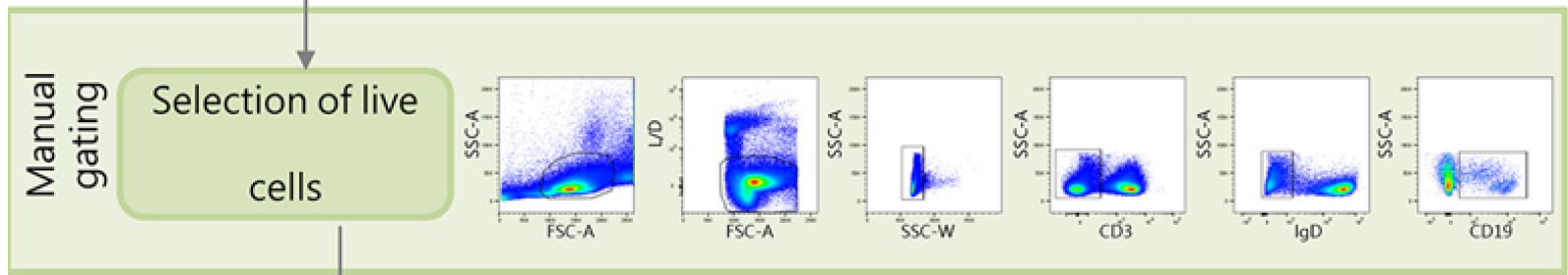
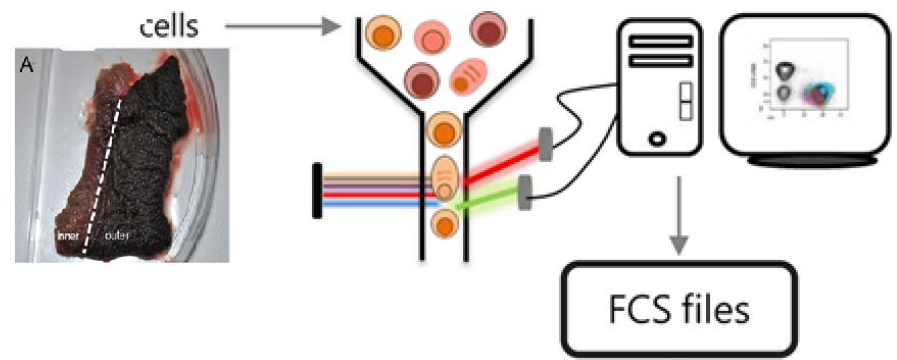
Maraviroc restricts p24 expression



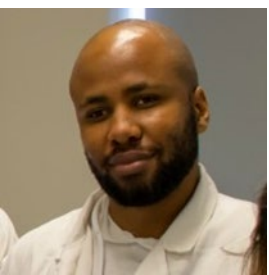
Infection variable in participants



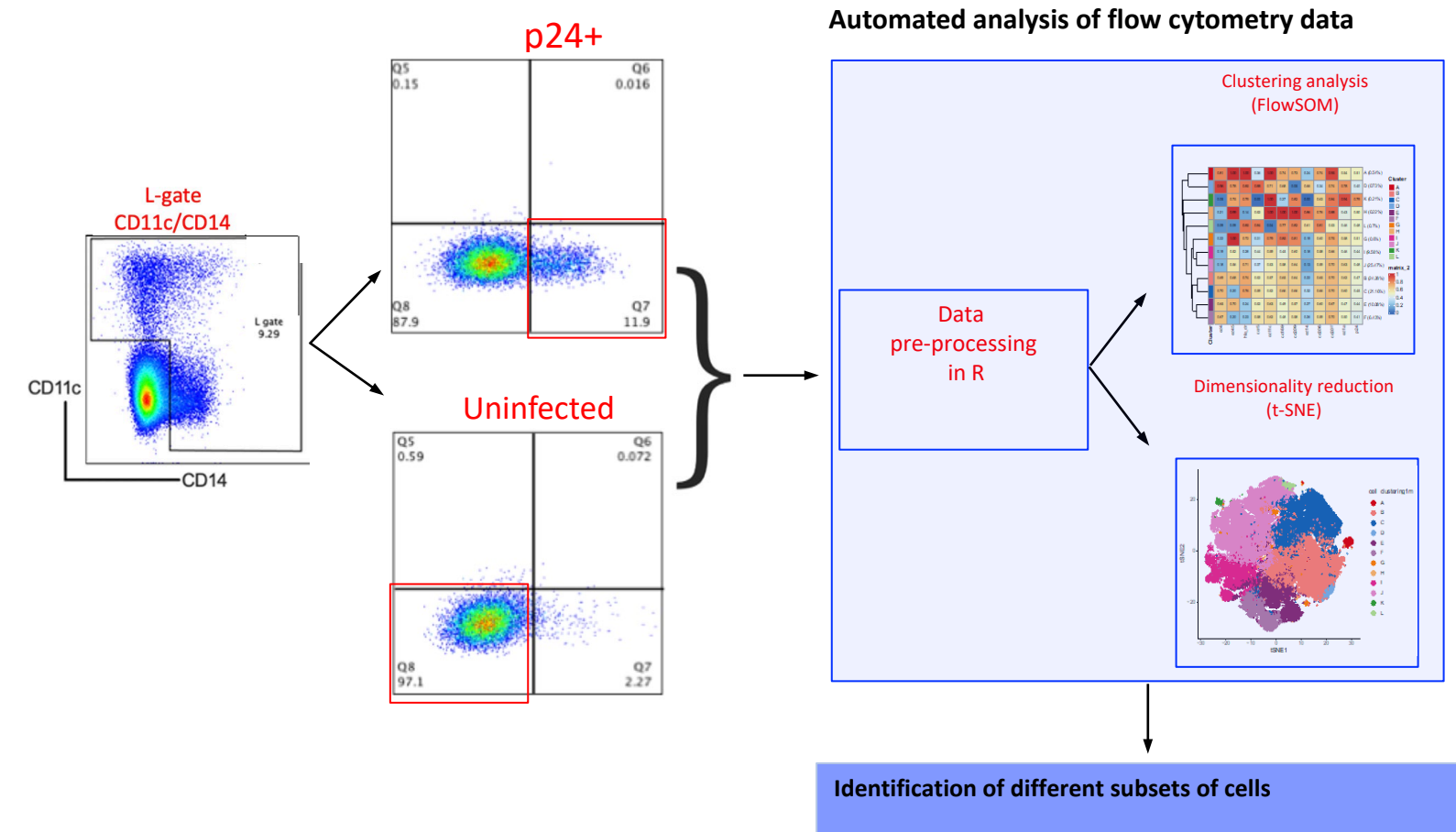
Computational Analysis of Multiparametric Flow cytometric Data



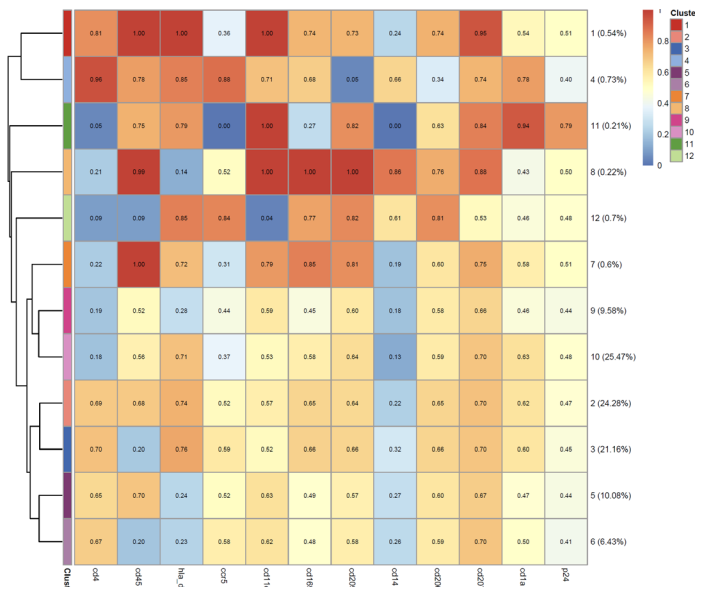
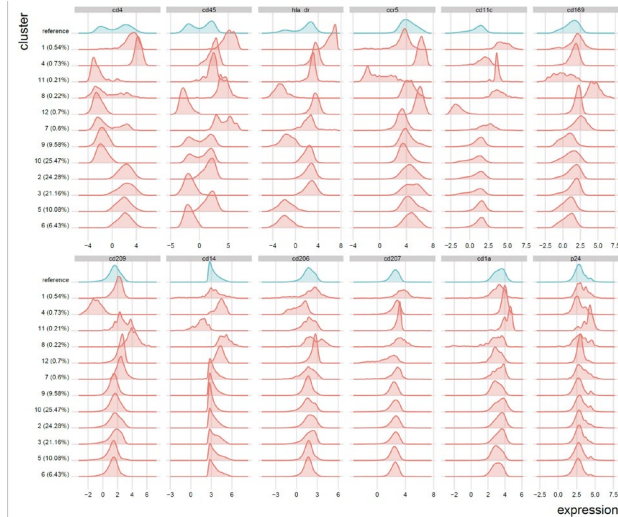
Identification of different subsets of cells



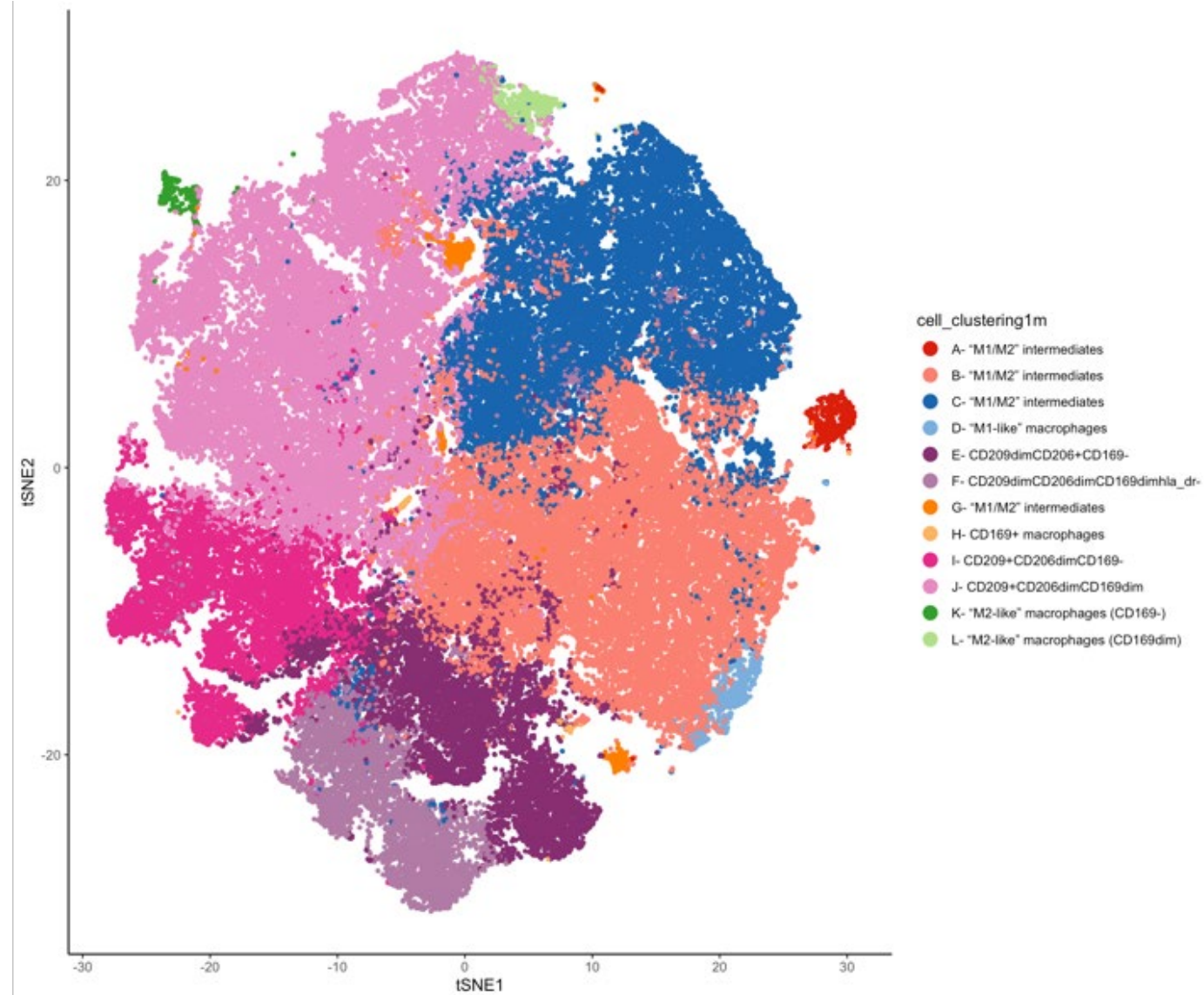
Ex vivo HIV infected macrophage-like cells computational analysis flow



Identification of unsupervised macrophage cell subsets

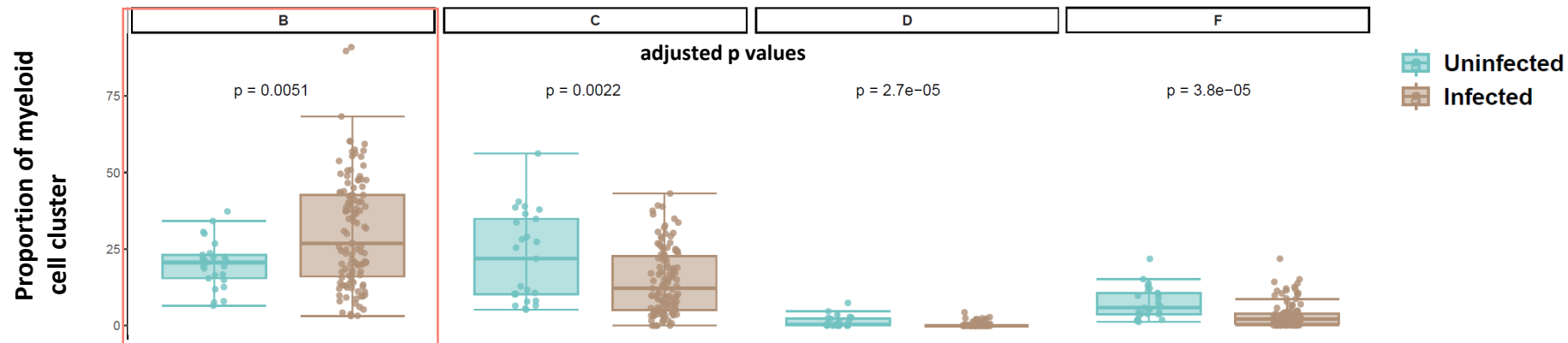
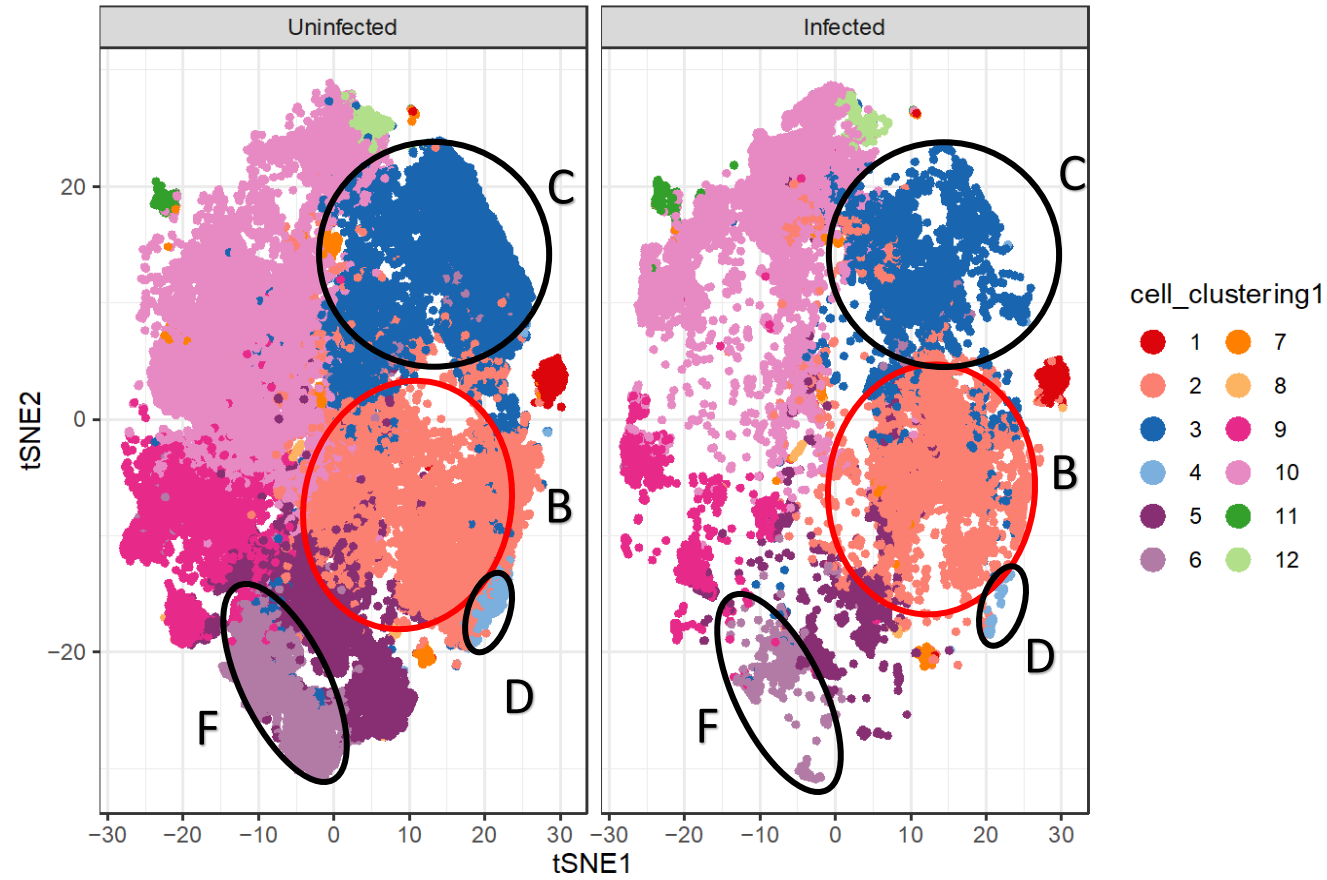


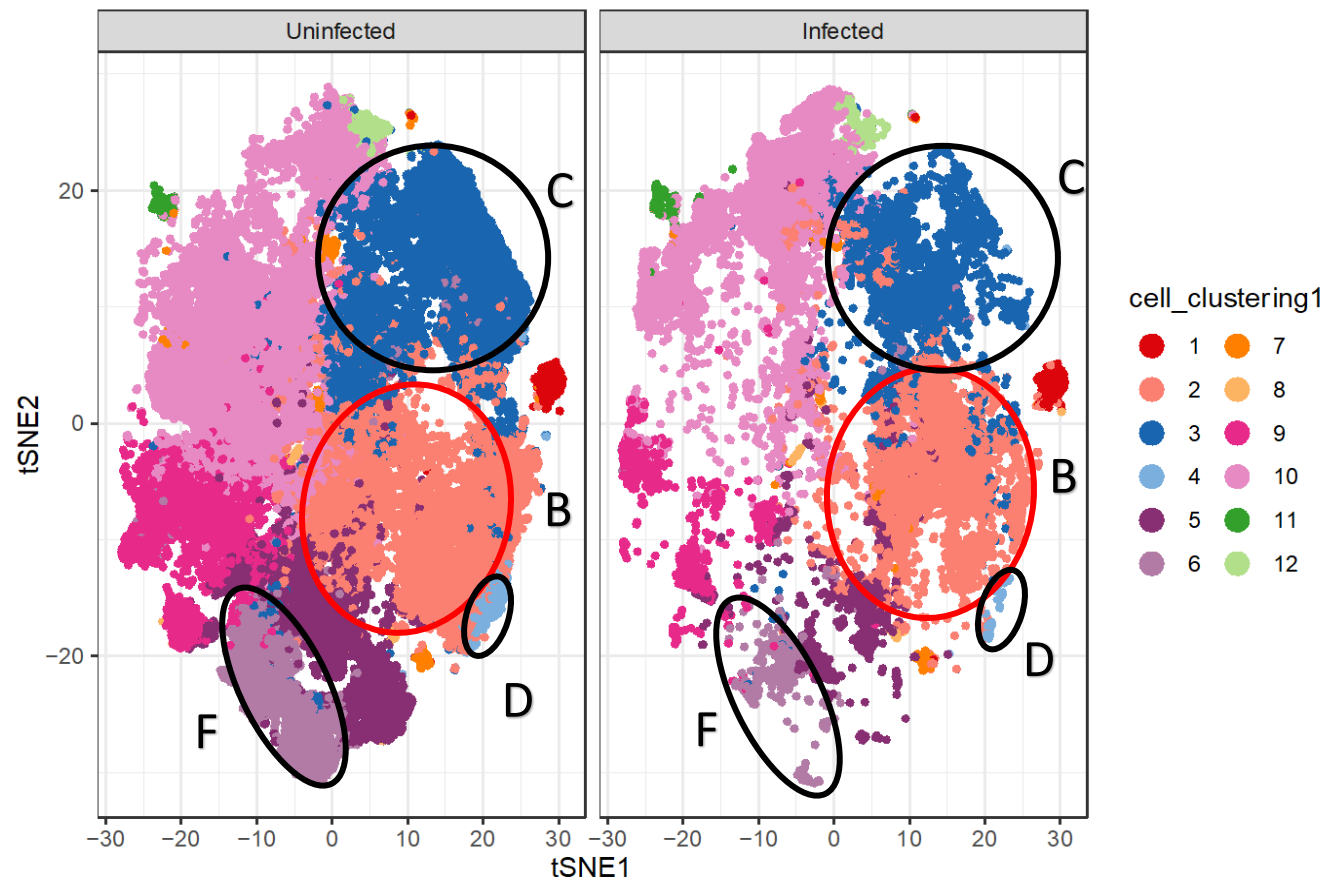
Computational analysis in R



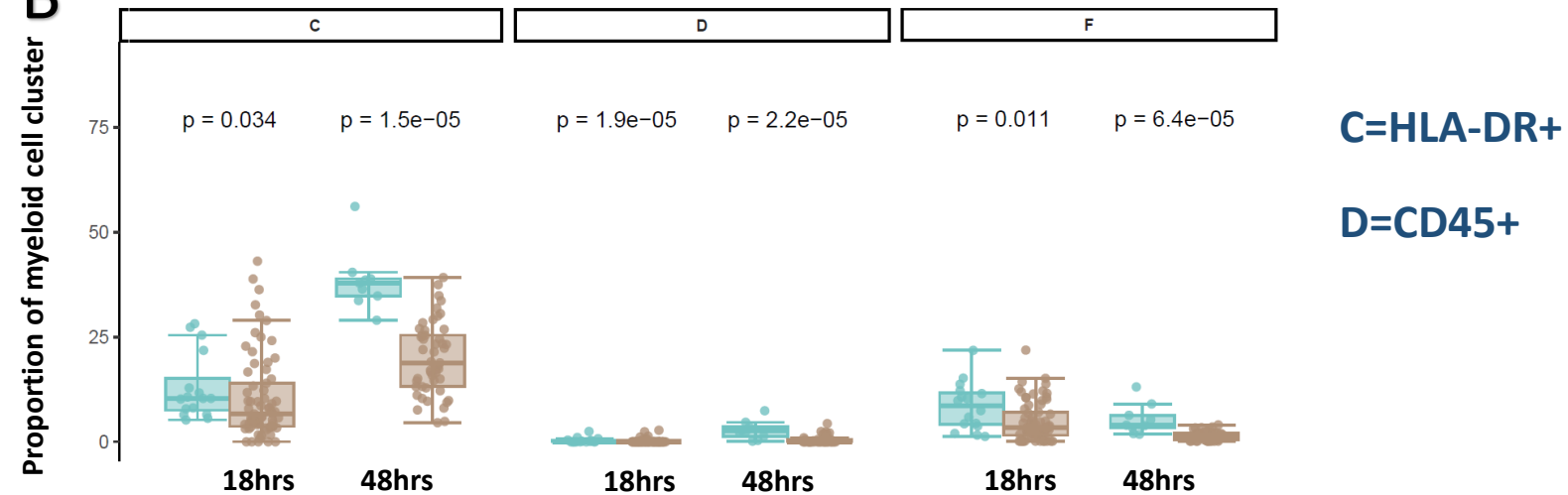
Macrophage subsets

Identification of differentially infected macrophage cell subsets

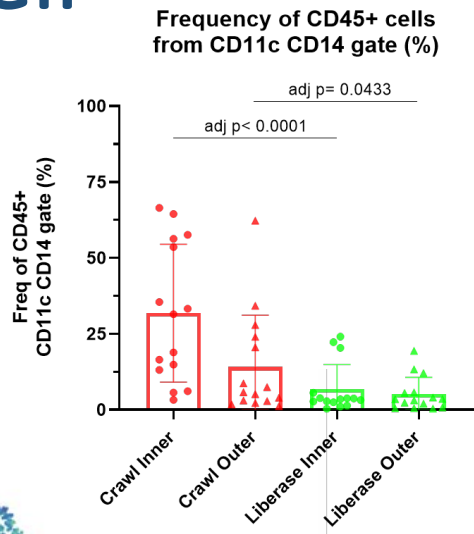
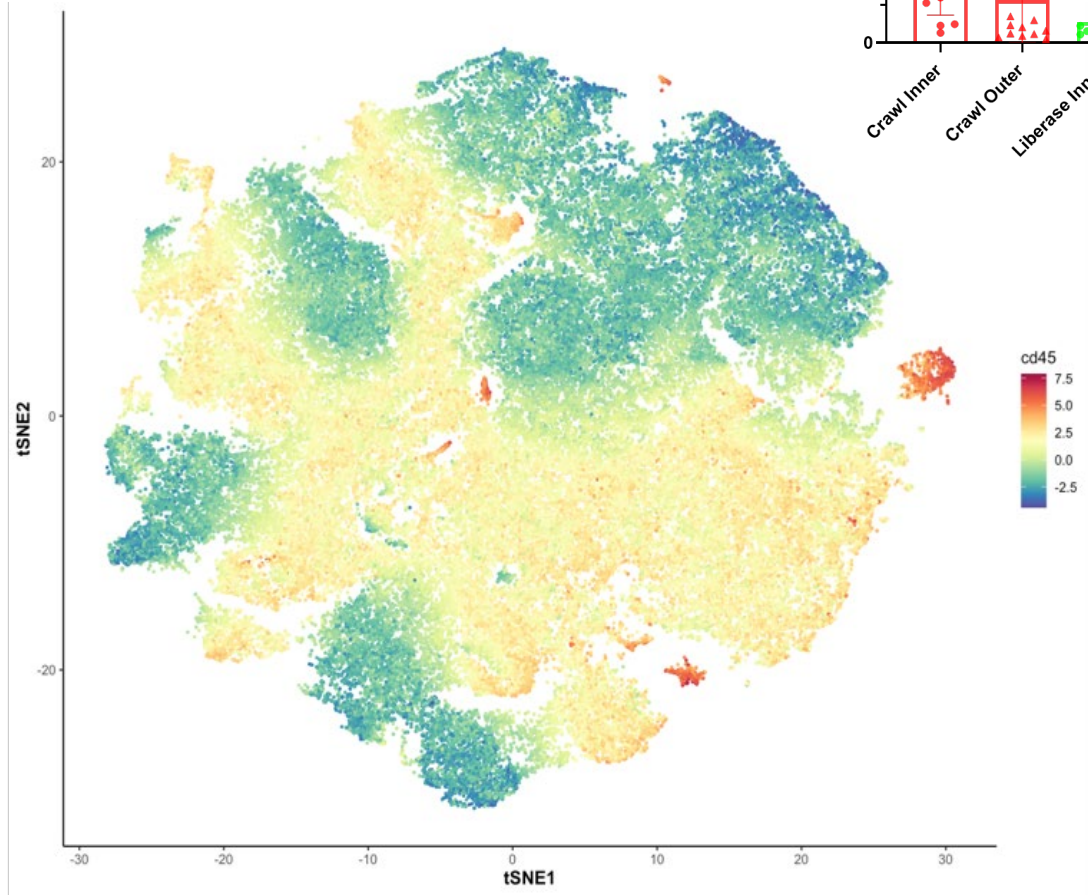
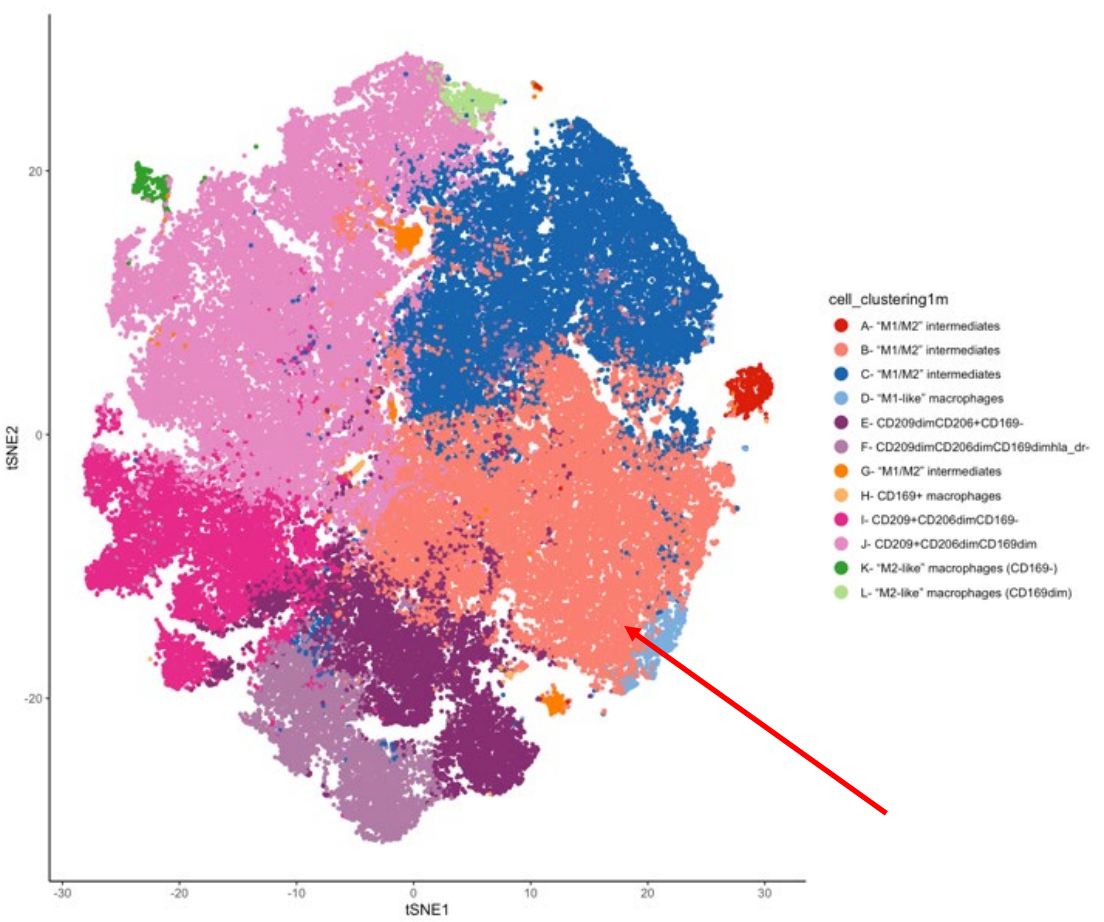


A

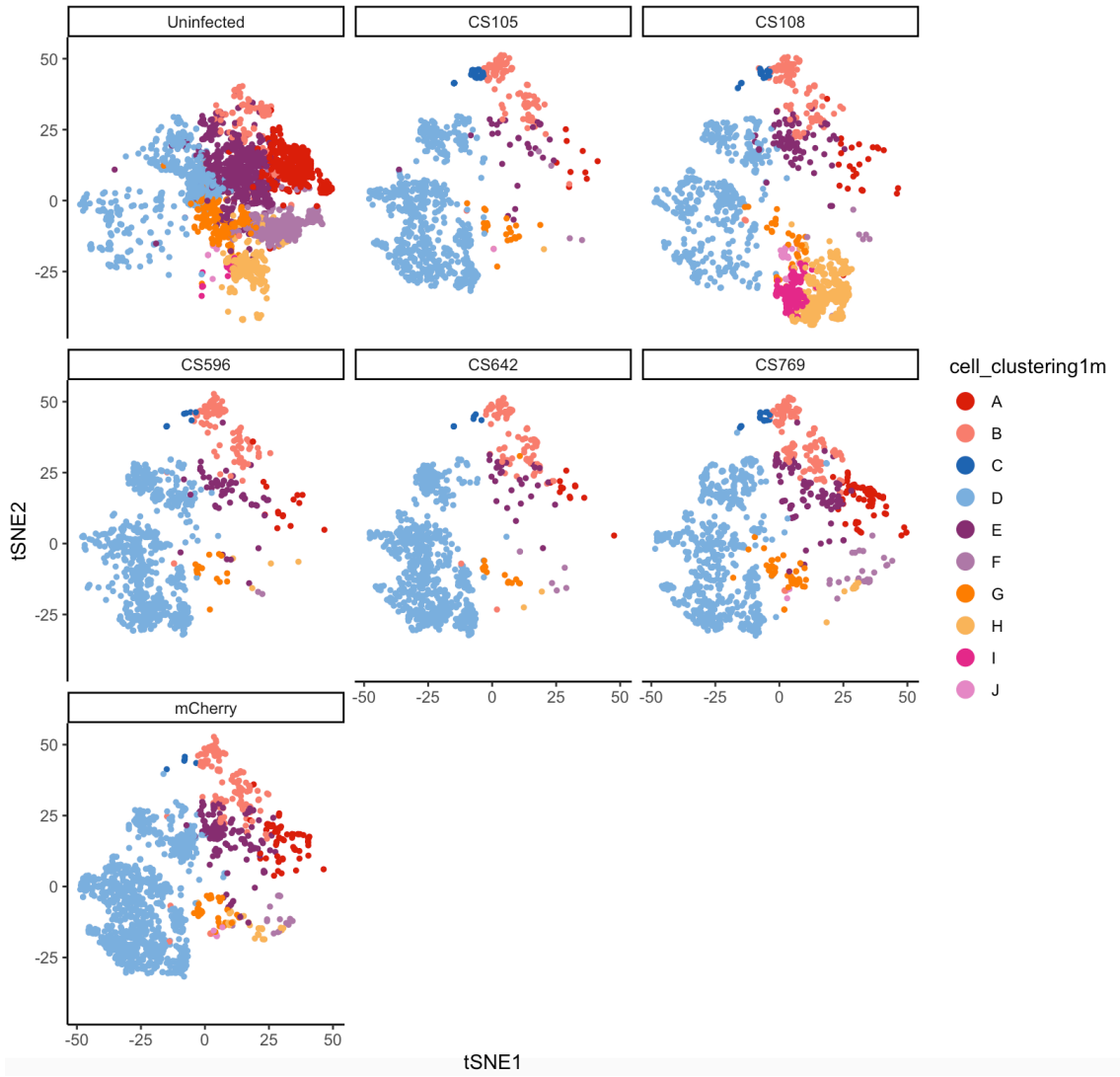
CD206⁺CD11c⁺CD14⁻
CD45⁻macrophage cell
subsets more frequent in
HIV uninfected
macrophages

B

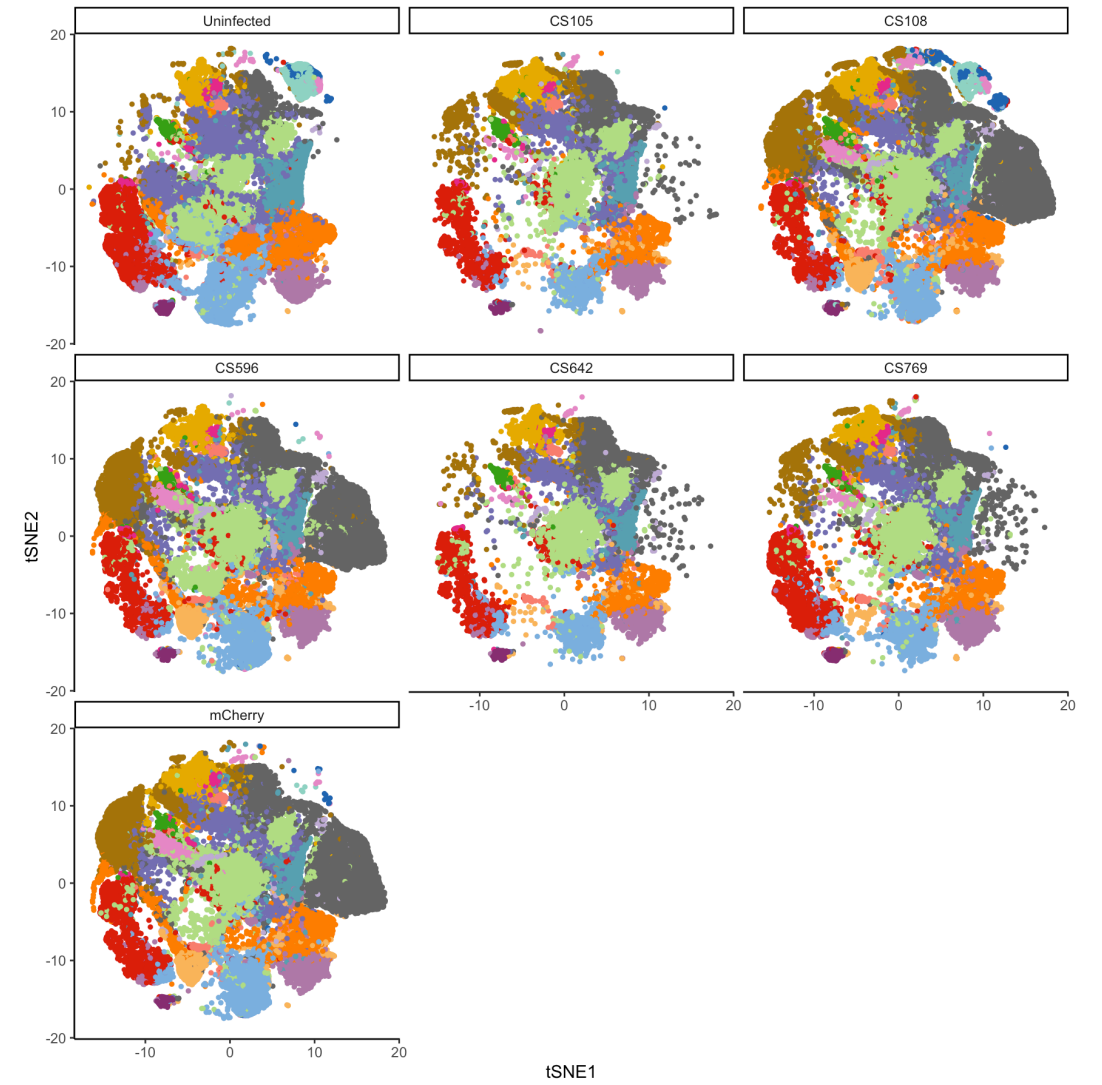
CD206⁺CD11c⁺CD14⁻CD45⁺ CD4⁺ macrophage cell subsets more frequent in HIV infected macrophages



HIV infection



LC's



Lymphoid cells



Summary

- Differential ex vivo HIV infection of subsets and cells states
- CD206⁺CD11c⁺CD14⁻CD45⁻



Host Factors

Impact of aSTI on molecular factors

Cellular factors associated with HIV susceptibility

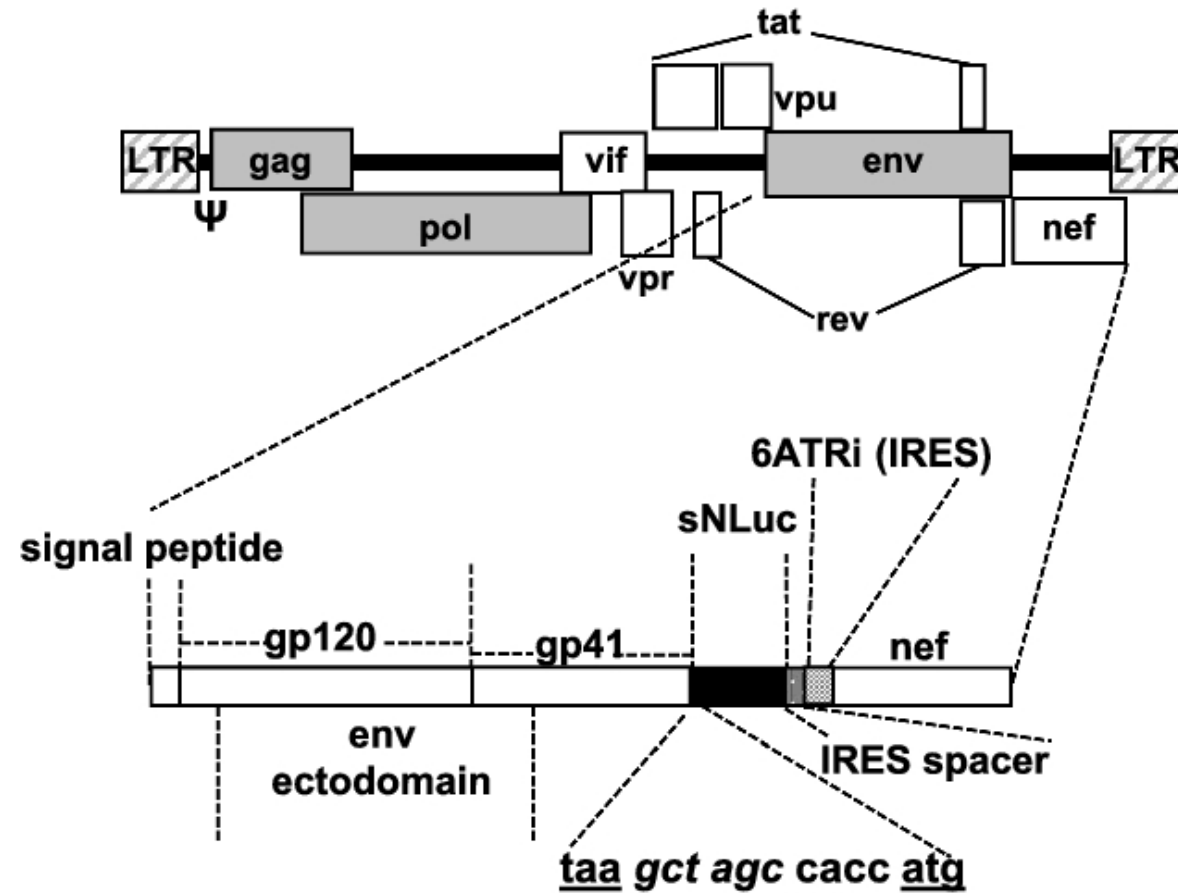
Viral features

Viral tropism

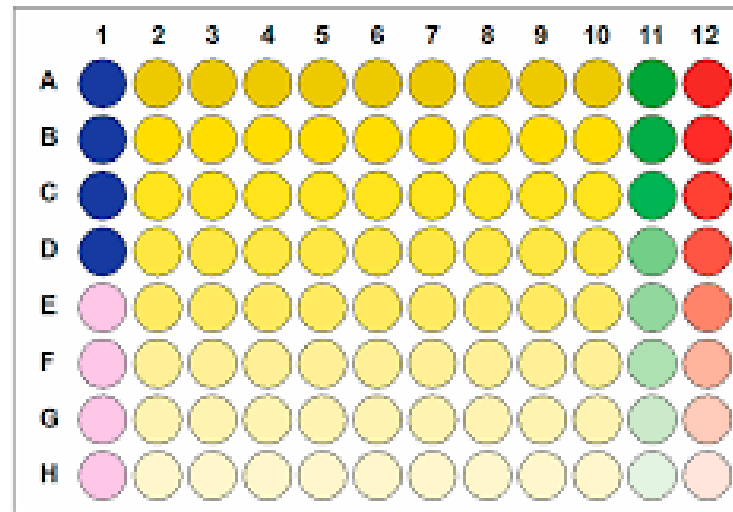
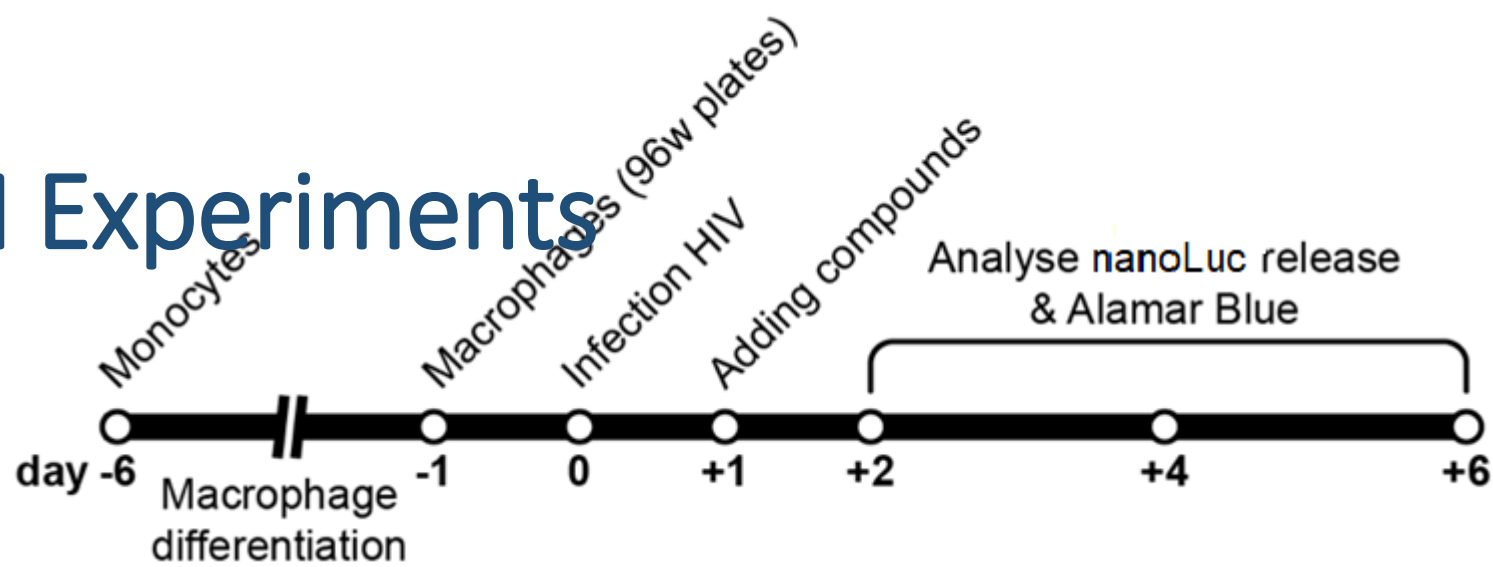
Viral features impacting HIV susceptibility in MGT tissue

What role does viral tropism play in different cellular FS subsets?

Secreted nanoLUC releasing virus constructs



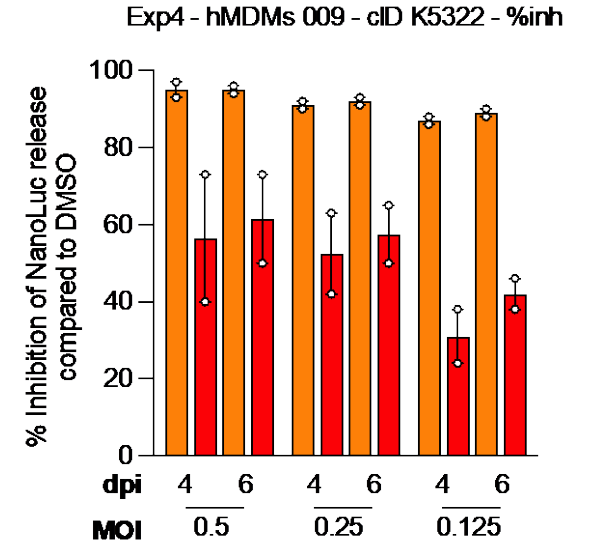
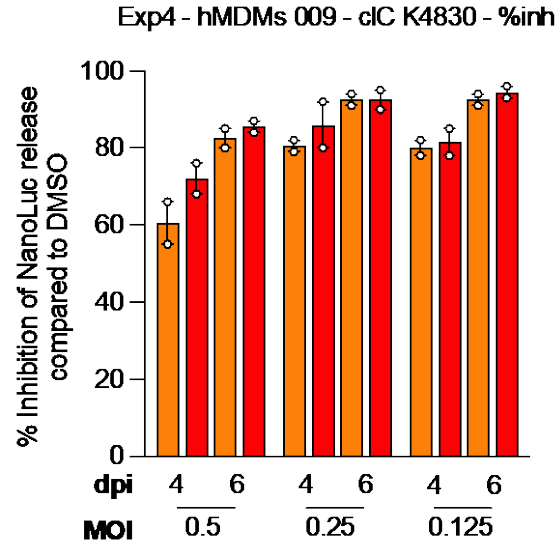
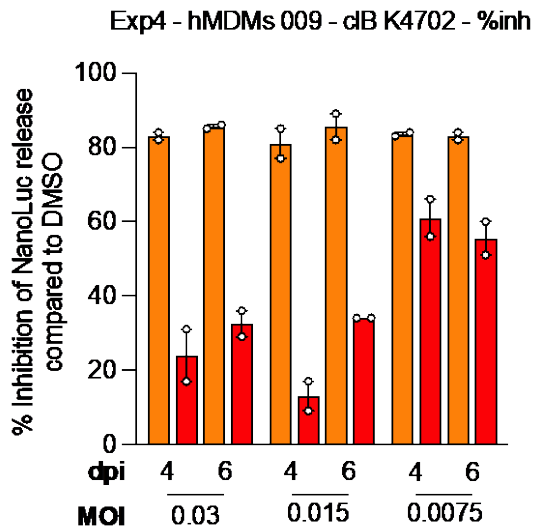
hMMDM Experiments



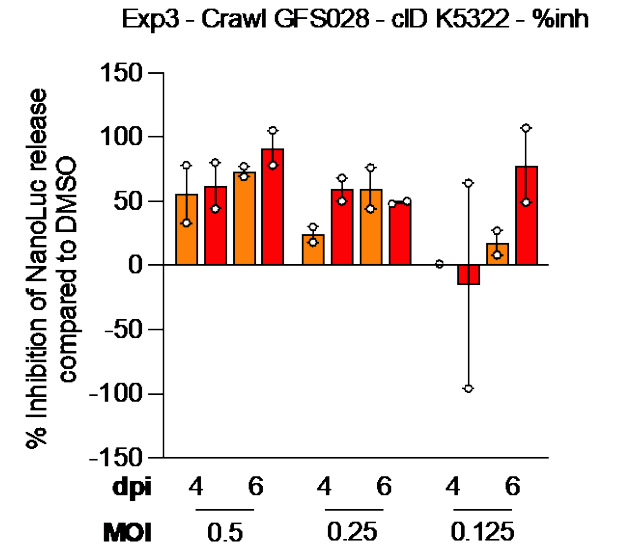
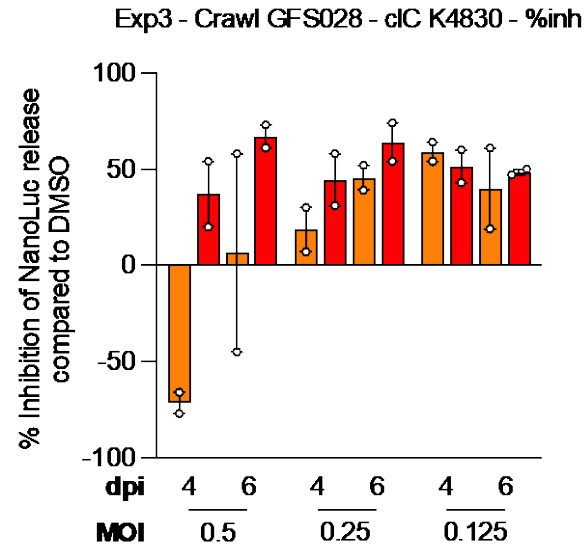
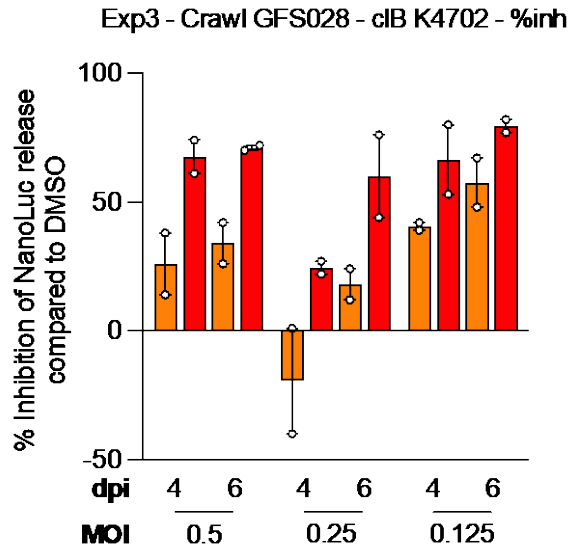
K4830	Env-IMC-(sec)NLuc.6ATRI	pNL-sNLuc.6ATRI-C.1086.B2.ecto	C
K4702	Env-IMC-(sec)NLuc.6ATRI	pNL-sNLuc.6ATRI-B.Bal.ecto	B
K5322	Env-IMC-(sec)NLuc.6ATRI	pNL-sNLuc.6ATRI-D.191882.env-minus+vsv-g	D
K5311	Env-IMC-(sec)NLuc.6ATRI	pNL-sNLuc.6ATRI-D.191882.env	D

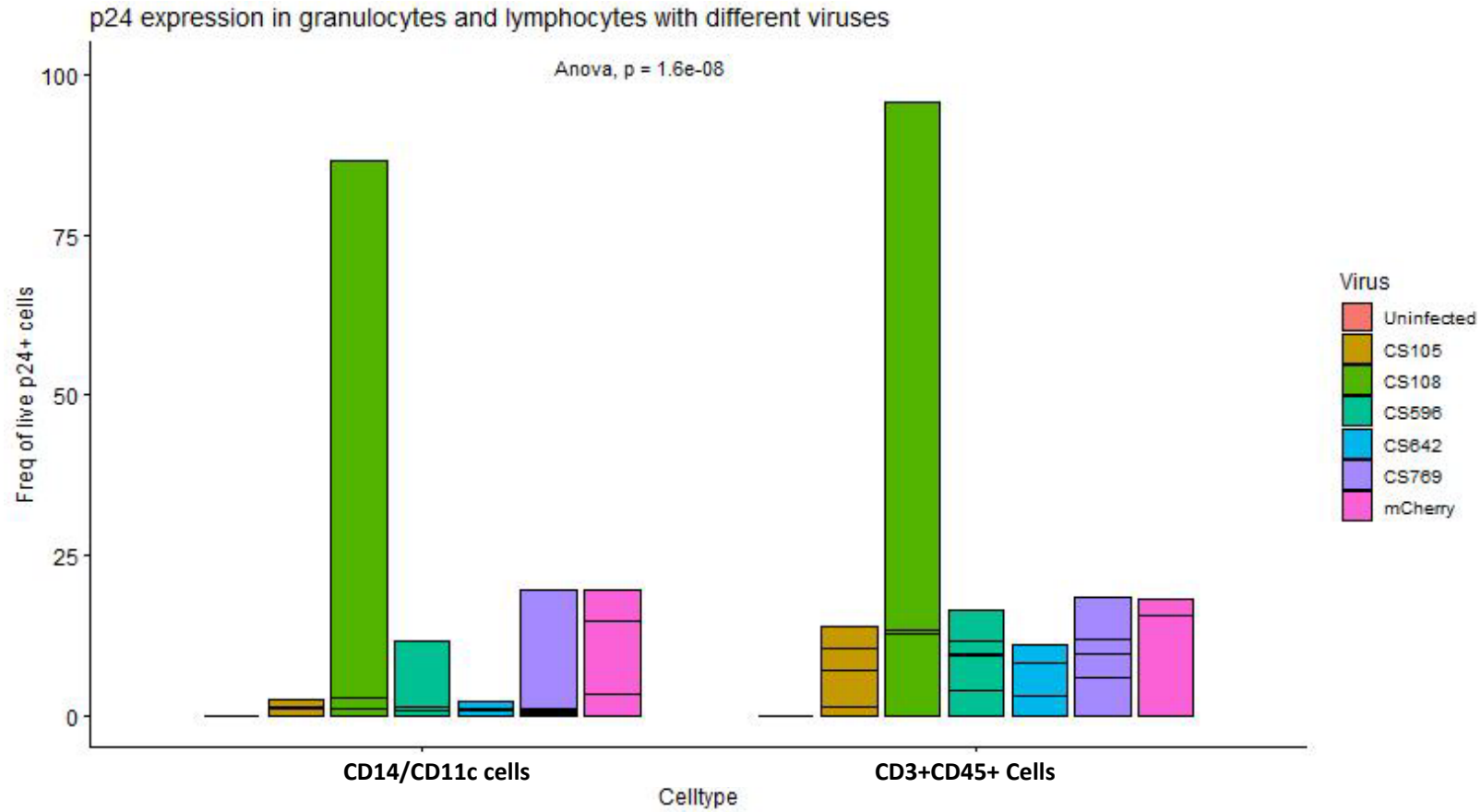
Uninfected
 DMSO
 EFV (5uM)
 RAL (10uM)

Human Monocyte derived Macrophages



Human Primary foreskin cells

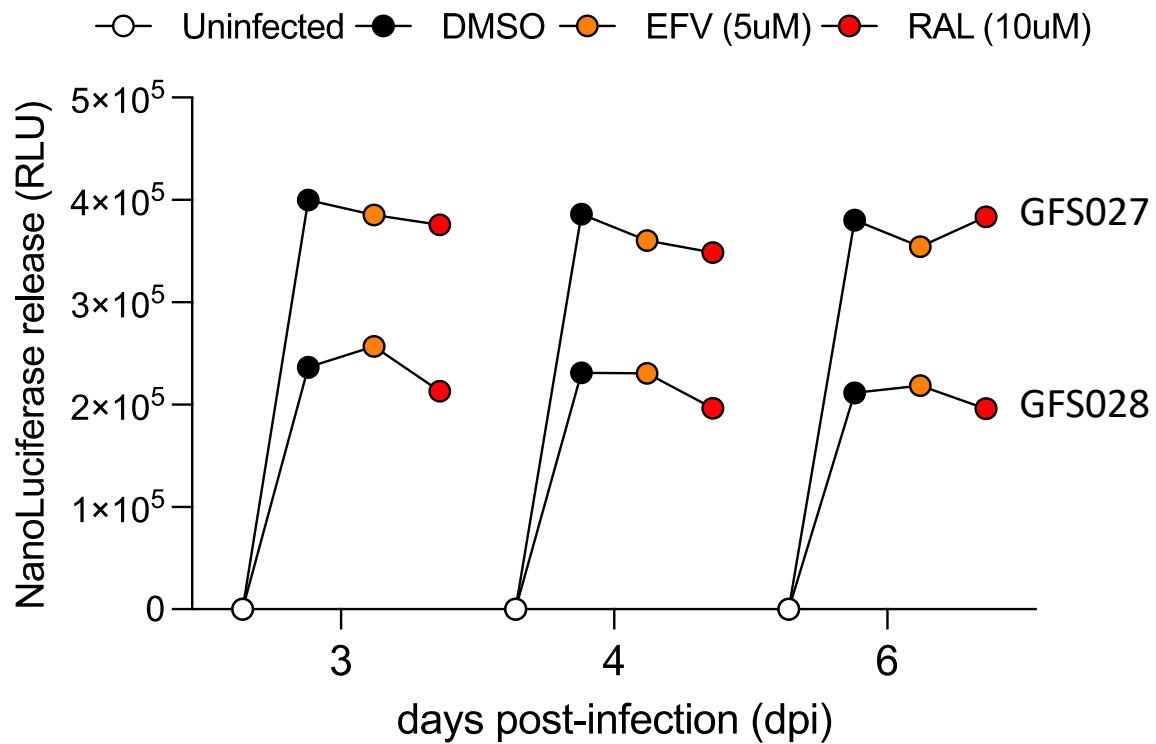




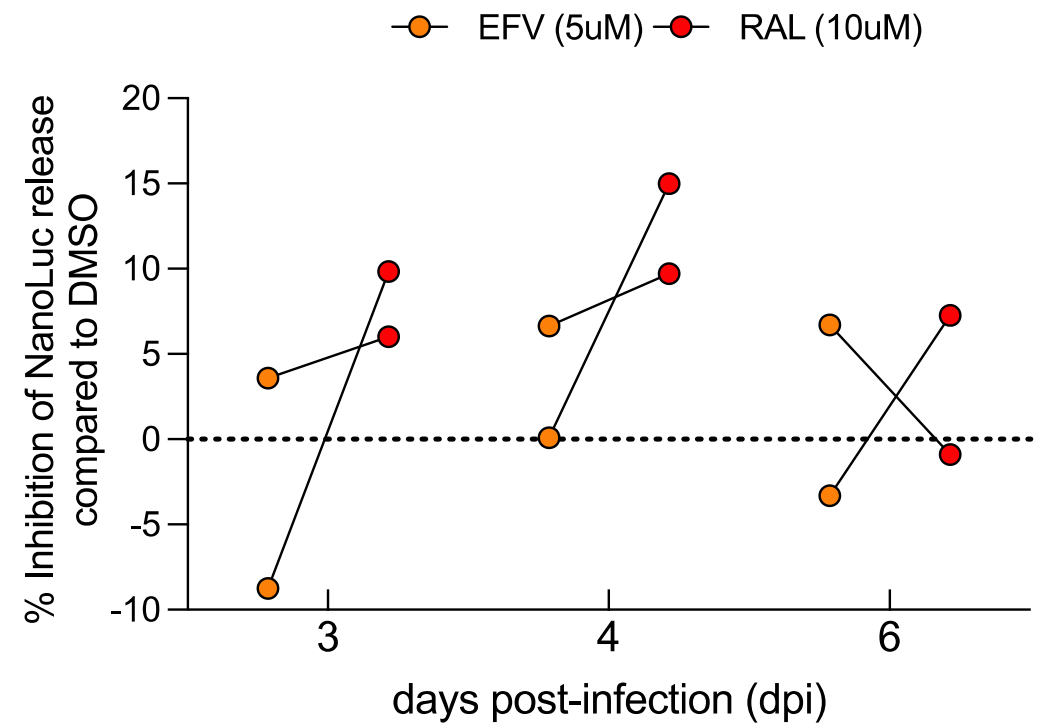
Proportions of p24+ lymphocytes and myeloid cells infected using different HIV strains. Foreskin lymphocytes and myeloid cells were infected using the cell suspension model with different viruses; NL4-3 viruses (X4-CS105, R5-CS108, mCherry), Subtype C TF viruses (CS596, CS769) and Subtype CC (CS642). Statistics were generated using ANOVA to compare HIV infection in foreskin lymphocytes against foreskin granulocytes.

Biopsies— HIV Clade B

Exp1 - Biopsies GFS027-28 - RLU - clade B K4702

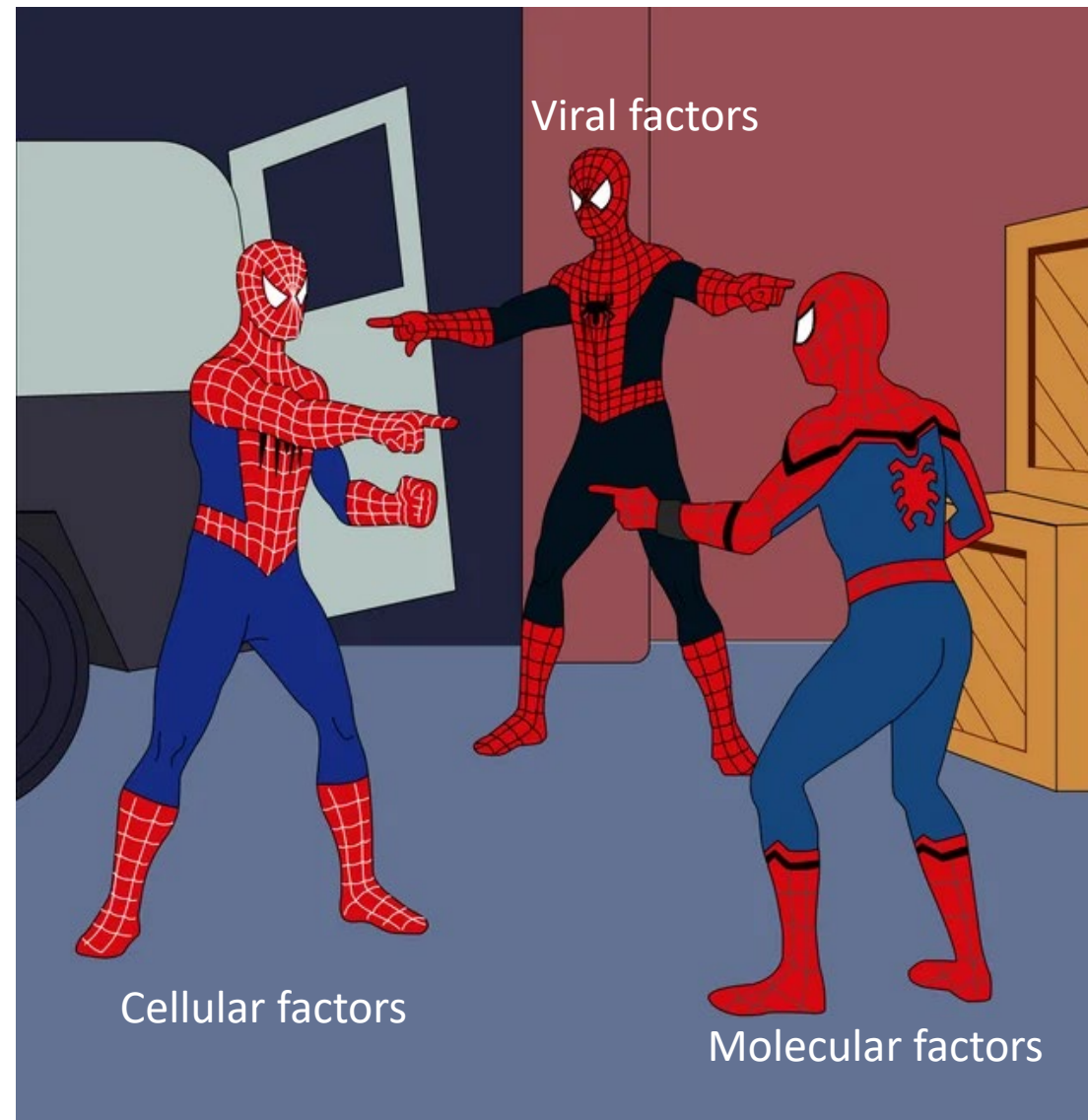


Exp1 - Biopsies GFS027-28 - %inh - clade B K4702





Interplay of Host factors associated with HIV susceptibility in the MGT



Summary

“Anti-viral”



HIV susceptible

1. aSTI
2. Myeloid cell activation and IL12 mediated immunology Th1?
3. Host proteins sequestered by HIV
4. HIV target cells

1. Barrier function proteins
2. Innate Immune proteins
3. Protective immune subsets

Acknowledgements

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3. Sonwabile Dzanibe
4. Clive Gray, David Russell, Frank Kirchhoff.
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6. Colleagues at the University of Cape Town

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