UNDERSTANDING INFLAMMATORY MONONUCLEAR PHAGOCYTE HETEROGENEITY IN HUMAN ANOGENITAL MUCOSA

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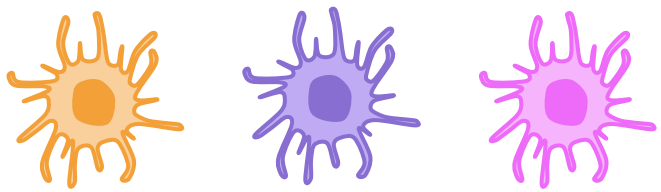
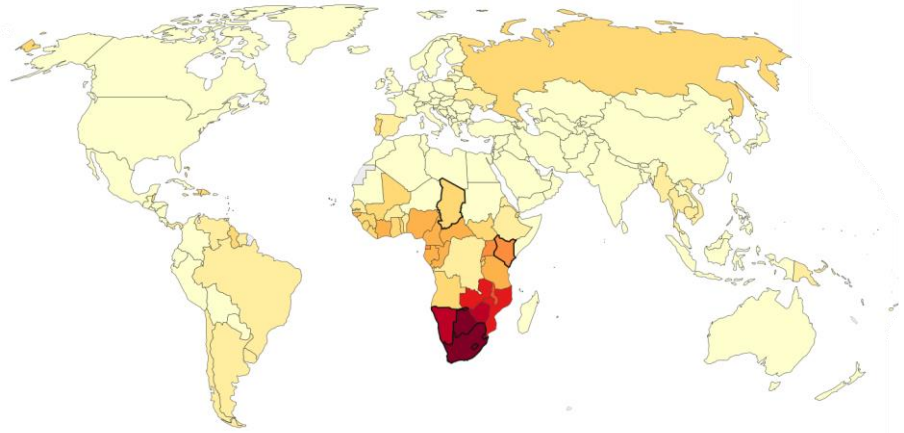


HIV current standing

- HIV infection rates are disproportionately high in sub-Saharan Africa.

Anogenital inflammation = ↑ **HIV risk**



nature medicine

- Pre-exposure prophylaxis (PrEP) can be rendered ineffective in inflammation.
- To develop effective treatments, need to better understand HIV interactions with target cells in inflammatory environment.

Genital inflammation undermines the effectiveness of tenofovir gel in preventing HIV acquisition in women

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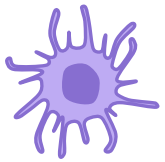

Mononuclear phagocytes

CD4 T cell

Mononuclear Phagocytes (MNPs) in human tissue

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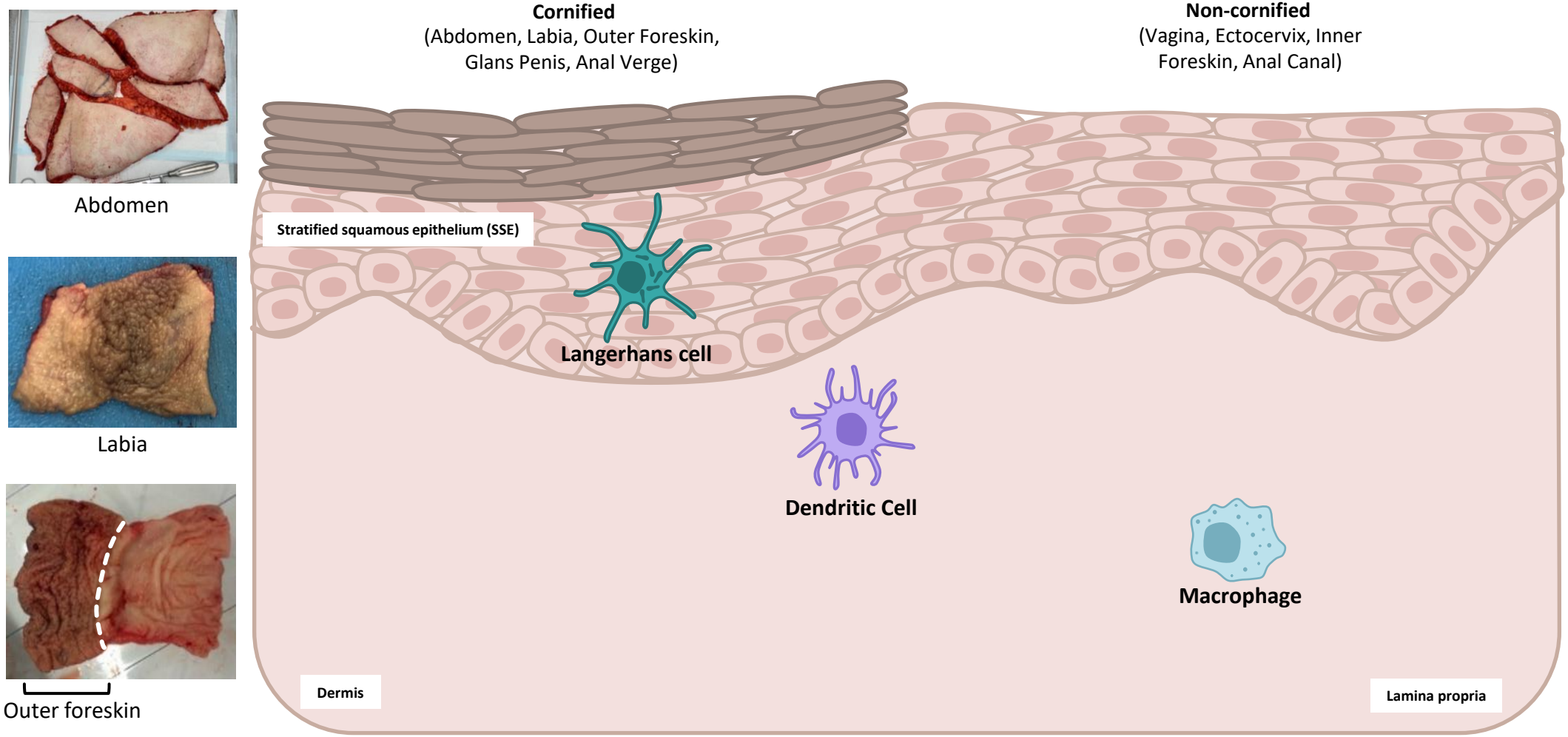
Skin

Cornified
(Abdomen, Labia, Outer Foreskin, Glans Penis, Anal Verge)

Type II Mucosa

Non-cornified
(Vagina, Ectocervix, Inner Foreskin, Anal Canal)

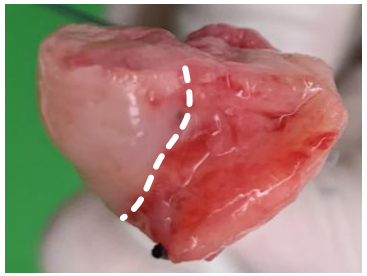


Abdomen

Labia

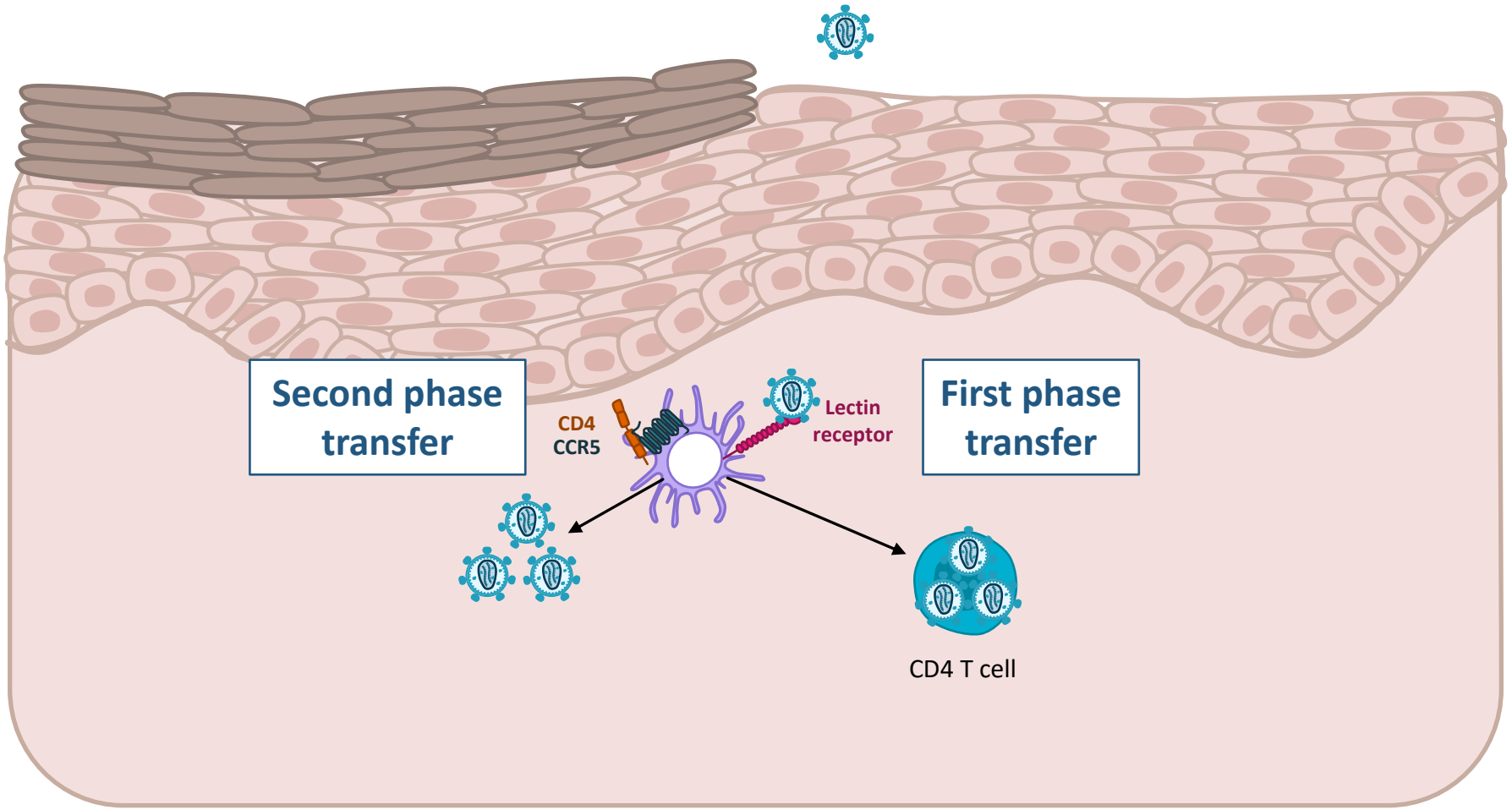
Outer foreskin


Vagina

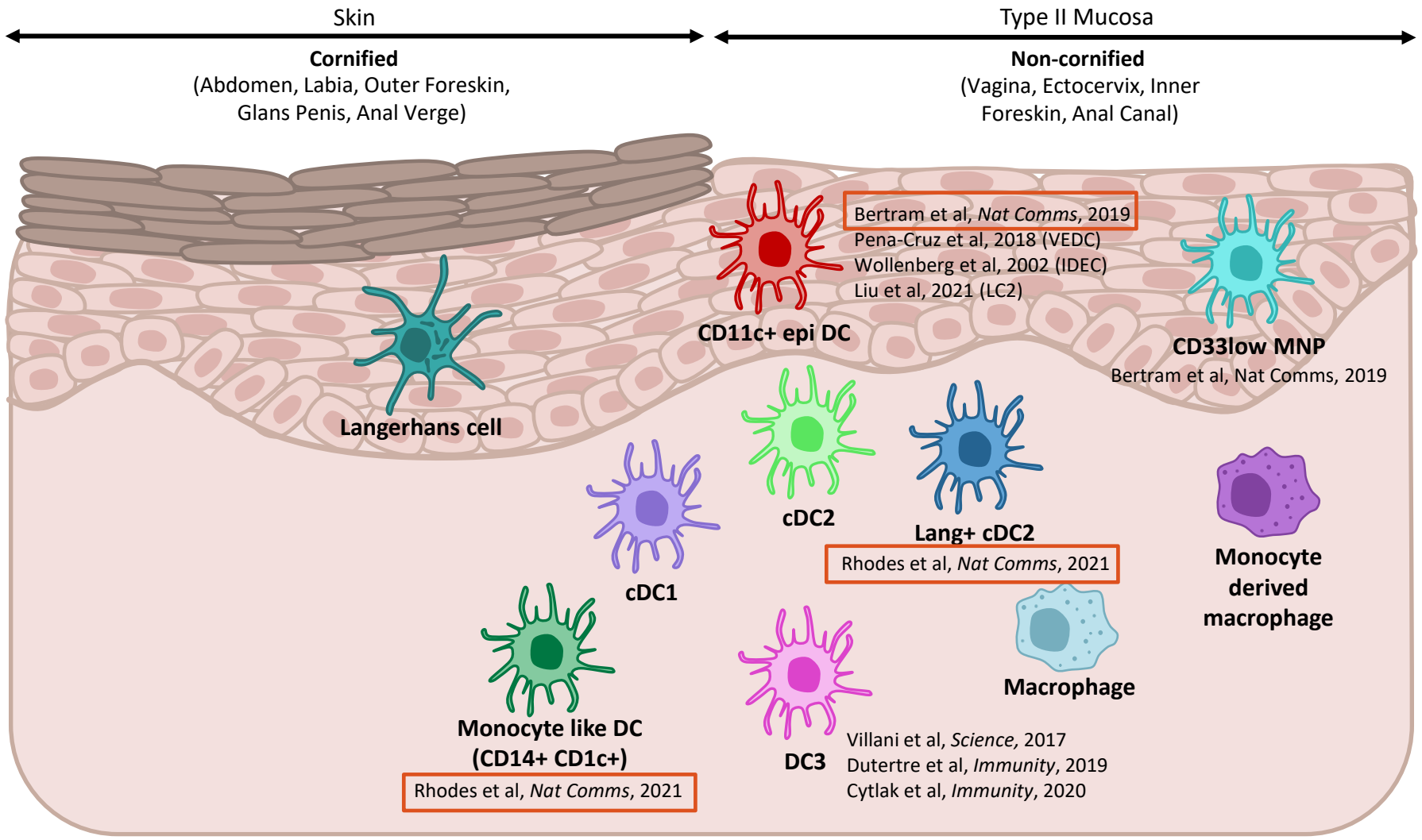

Ectocervix


Inner foreskin

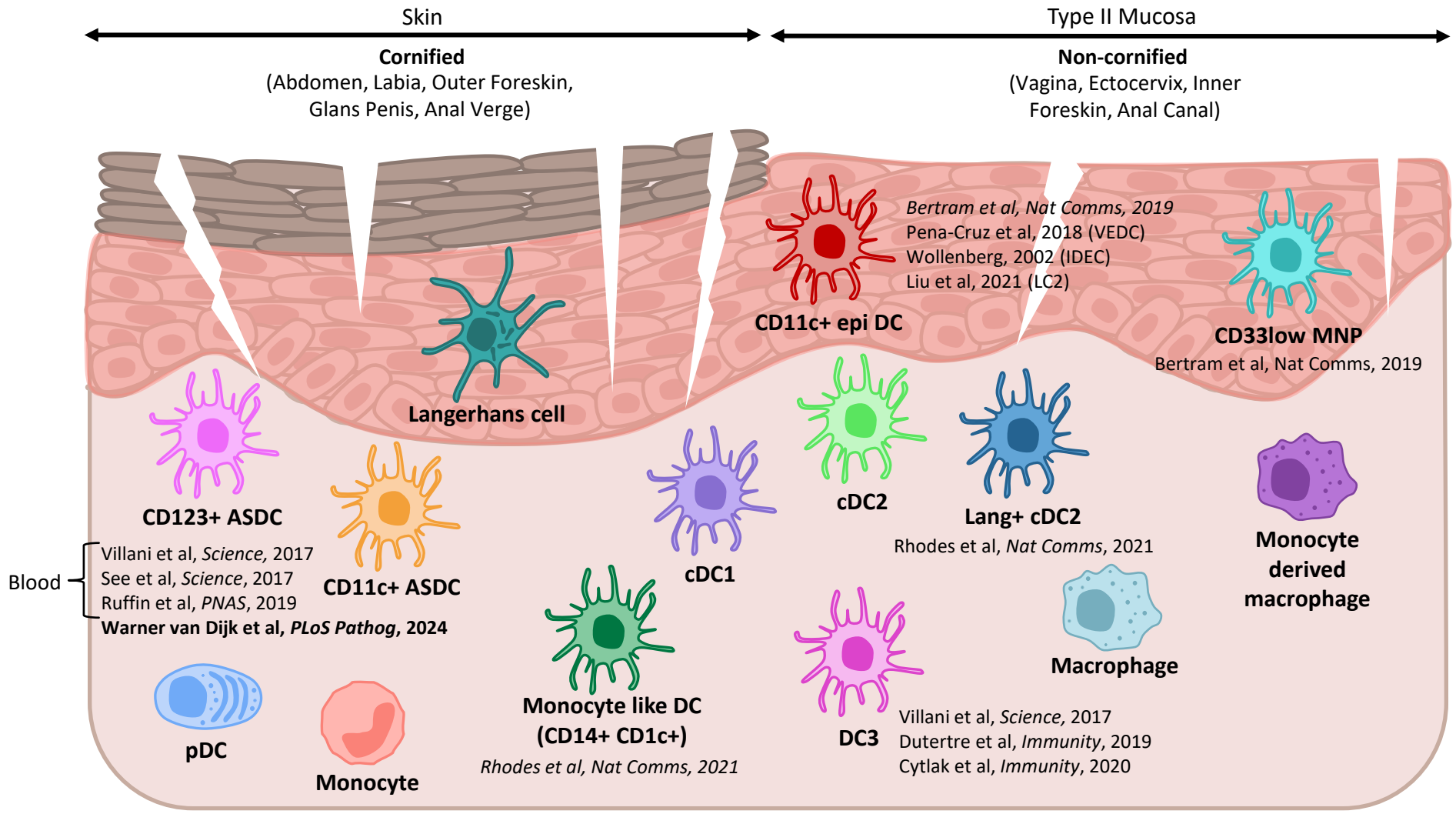
MNPs and HIV transmission in tissue



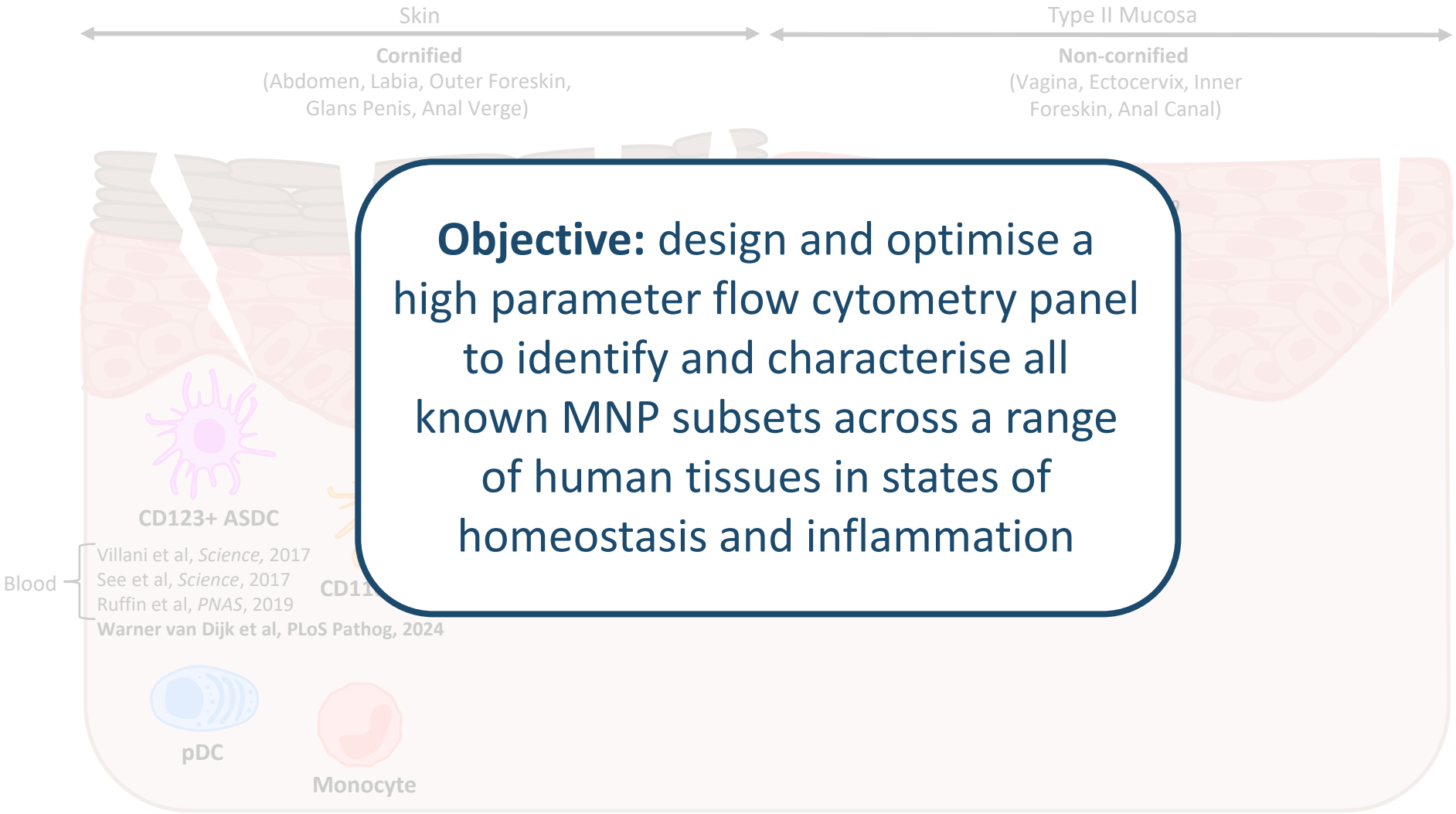
MNP subsets in human tissue



Inflammatory MNP subsets in human tissue

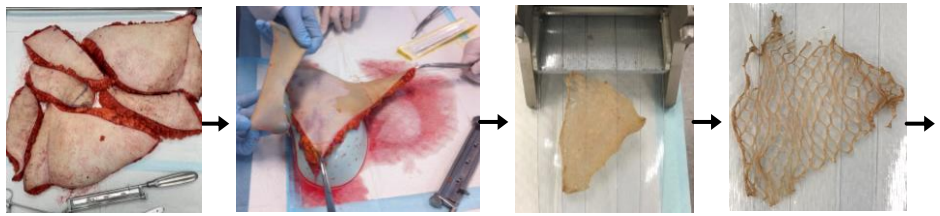


Inflammatory MNP subsets in human tissue



Objective: design and optimise a high parameter flow cytometry panel to identify and characterise all known MNP subsets across a range of human tissues in states of homeostasis and inflammation

Tissue processing methods



Abdominal skin

Skin grafted to 1mm thickness

Skin graft passed through mesher

Meshed skin

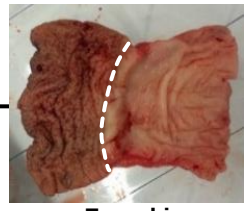
Dispase digestion 4°C (overnight)

Epidermis peeled from dermis

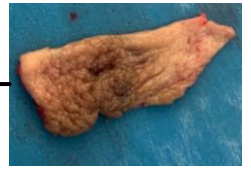
Dermis cut into 5 x 5 mm pieces

Collagenase Type IV digestion (120min)

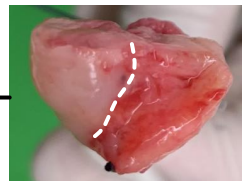
Antibody staining for MNP phenotyping



Foreskin



Labia



Cervix

Thinned and scalpel cuts

Vagina

Genital skin and mucosa

Long overnight dispase digestion and rapid collagenase essential for isolating cells in immature tissue resident state.

Botting et al, *J Leukoc Biol*, 2017
Doyle et al, *Front Immunol*, 2021

A ~~32~~ ~~30~~ ~~29~~ 26-parameter flow cytometry panel to characterise MNPs in human tissue

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MNP defining markers
HLA-DR
CD103
CD4
MR (CD206)
Siglec-1 (CD169)
DC-SIGN (CD209)
XCR1
CD1a
Langerin (CD207)
CD11c
CD11b

MNP defining markers
CD5
CD1c
CD163
CD88
Calprotectin
CD16
CD14

Inflammatory MNP markers
CD123
Siglec-6
Axl

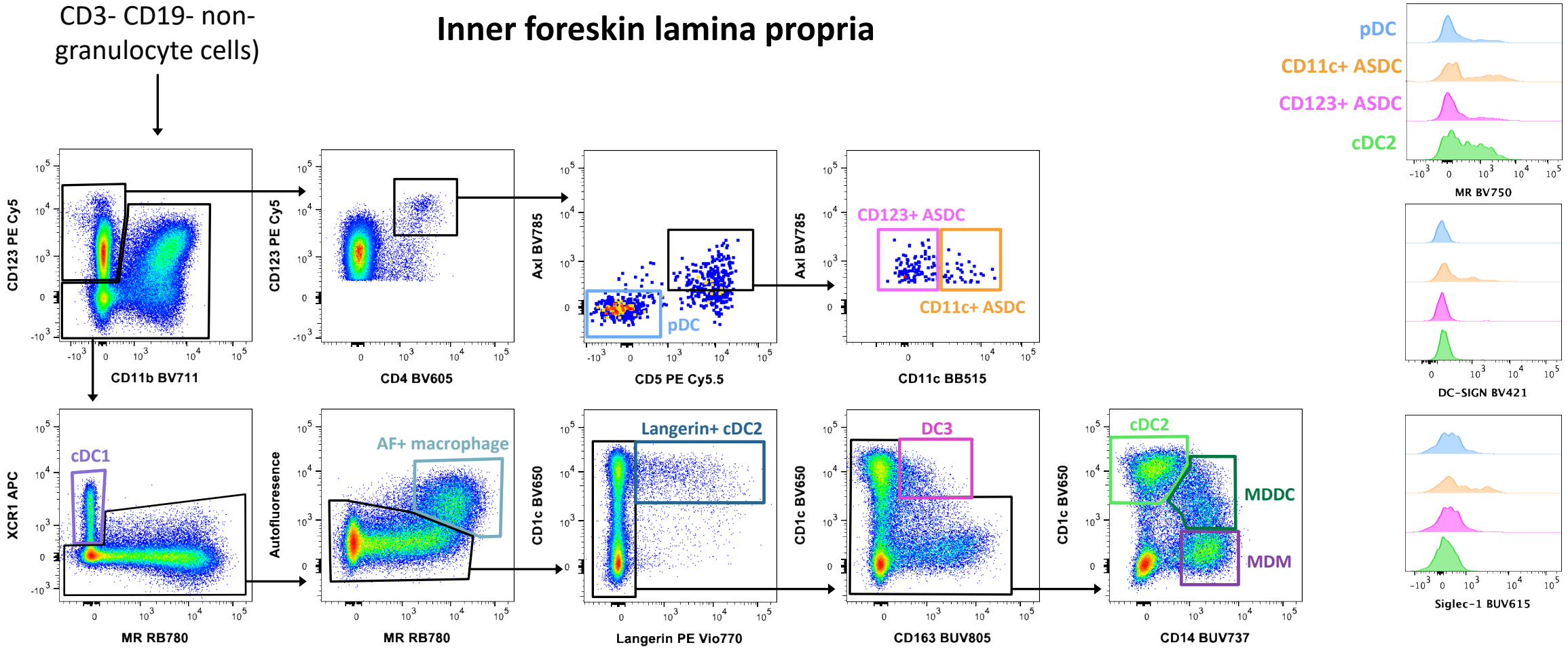
Exclusion/lymphocyte markers
Live/Dead
CD3
CD19

Migratory/cycling
CCR7
Ki67

Planned inclusion of P24 for HIV binding

Gating strategy to identify MNPs in mucosal tissue

MNPs
(Live, single, HLA-DR+ CD3- CD19- non-granulocyte cells)



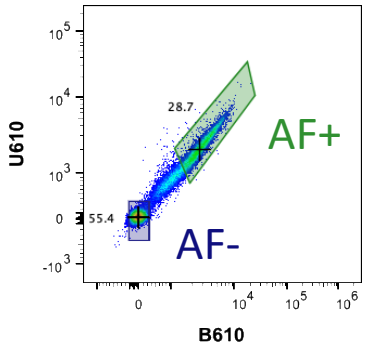
Autofluorescent compensation correction

Sample

Full panel stain

Unstained

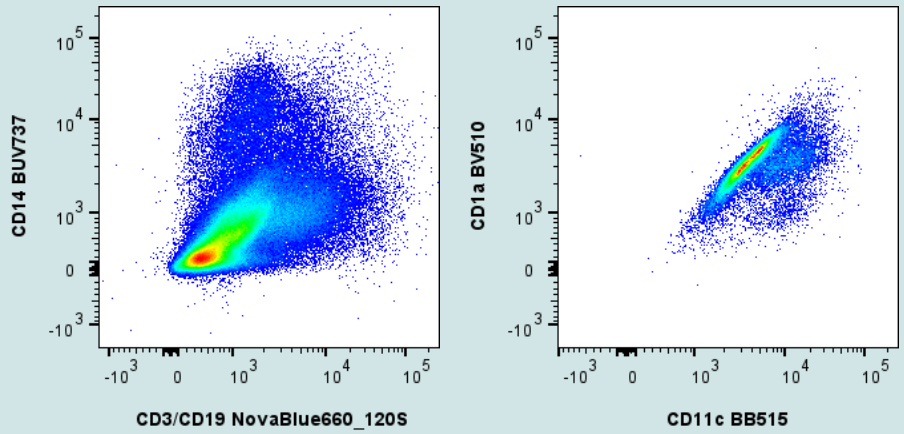
Acquire on BD FACSymphony



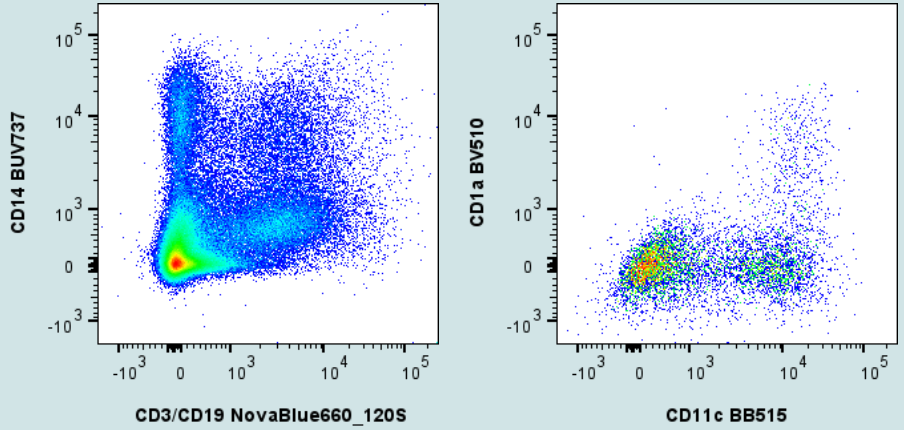
Manually compensate in FlowJo using unstained

Unstained sample must be donor matched

Acquisition compensation

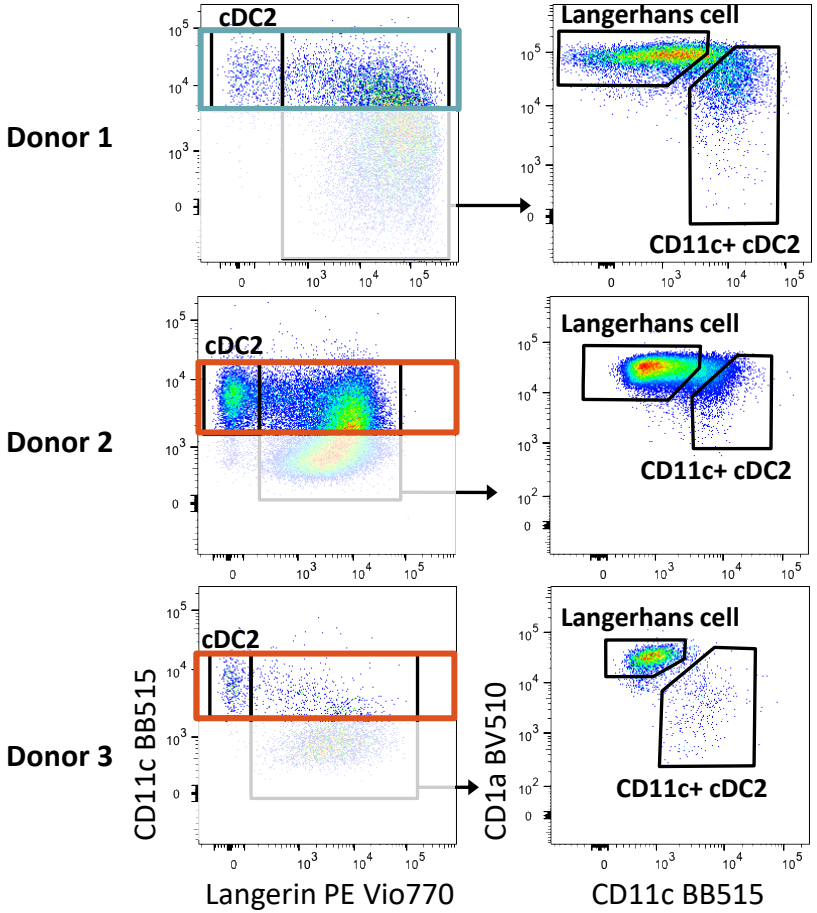


Autofluorescent corrected compensation



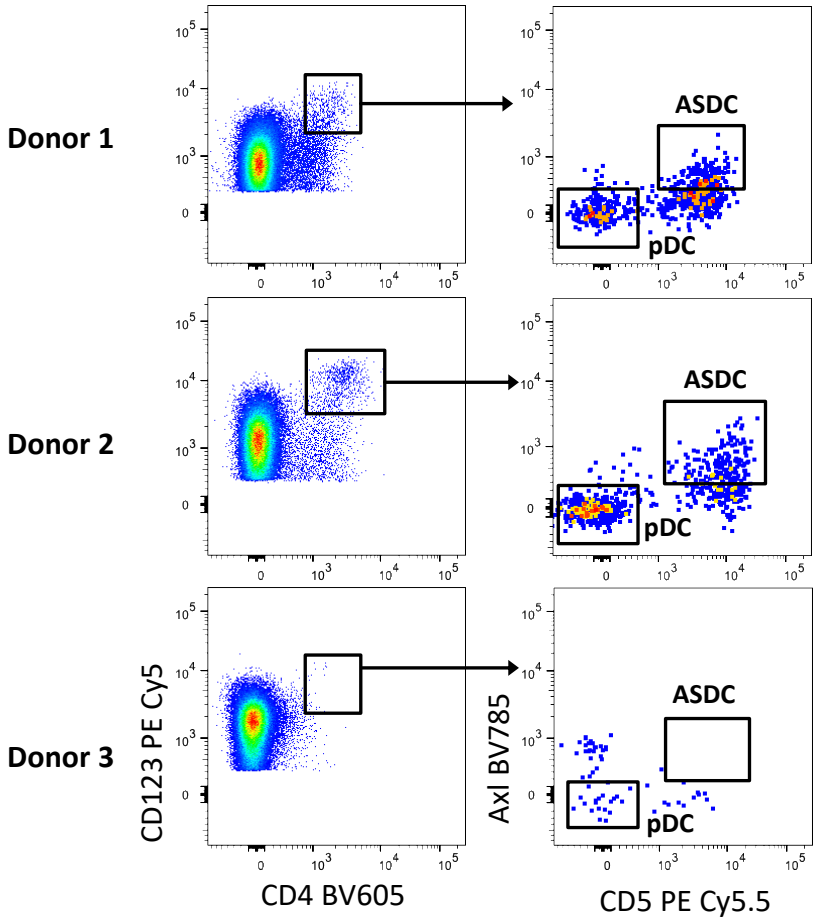
Donor variability

Innate variability



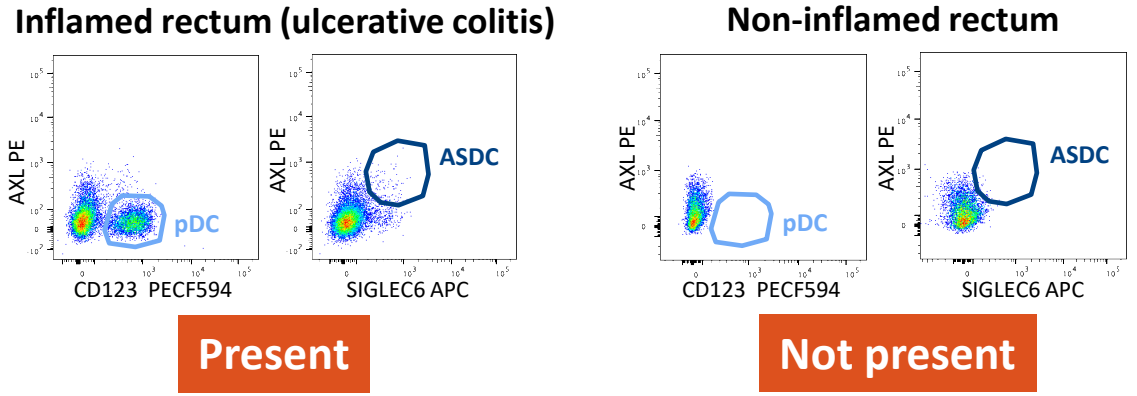
Inner foreskin epidermis

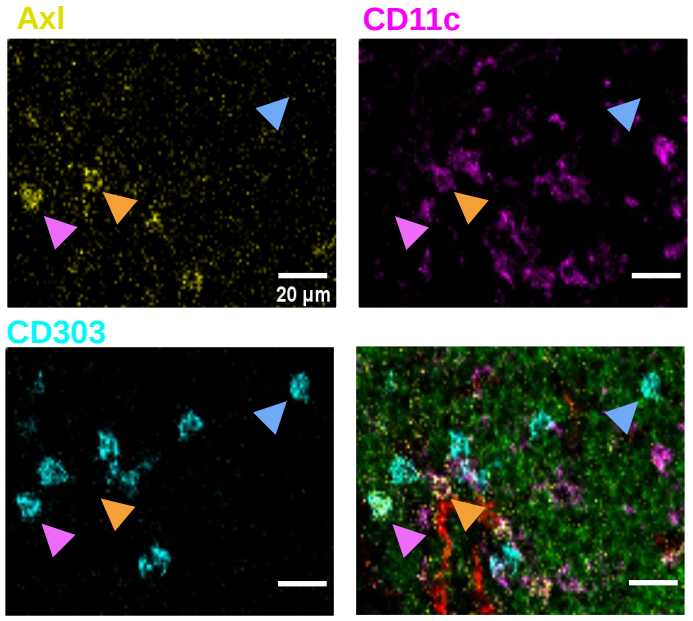
Inflammatory status



Inner foreskin lamina propria

Identification of ASDCs in human mucosal tissue





Further confirmed ASDCs in human tissue in different inflammatory disease settings:

- Imaging mass cytometry (Diverticulitis)
- scRNAseq (psoriasis, colon cancer) (data not shown).

ASDCs move into mucosal tissues in inflammatory environments (like pDCs).

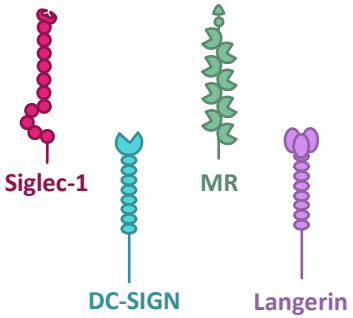
Adapted from Warner van Dijk et al, *PLOS Pathogens,* 2024

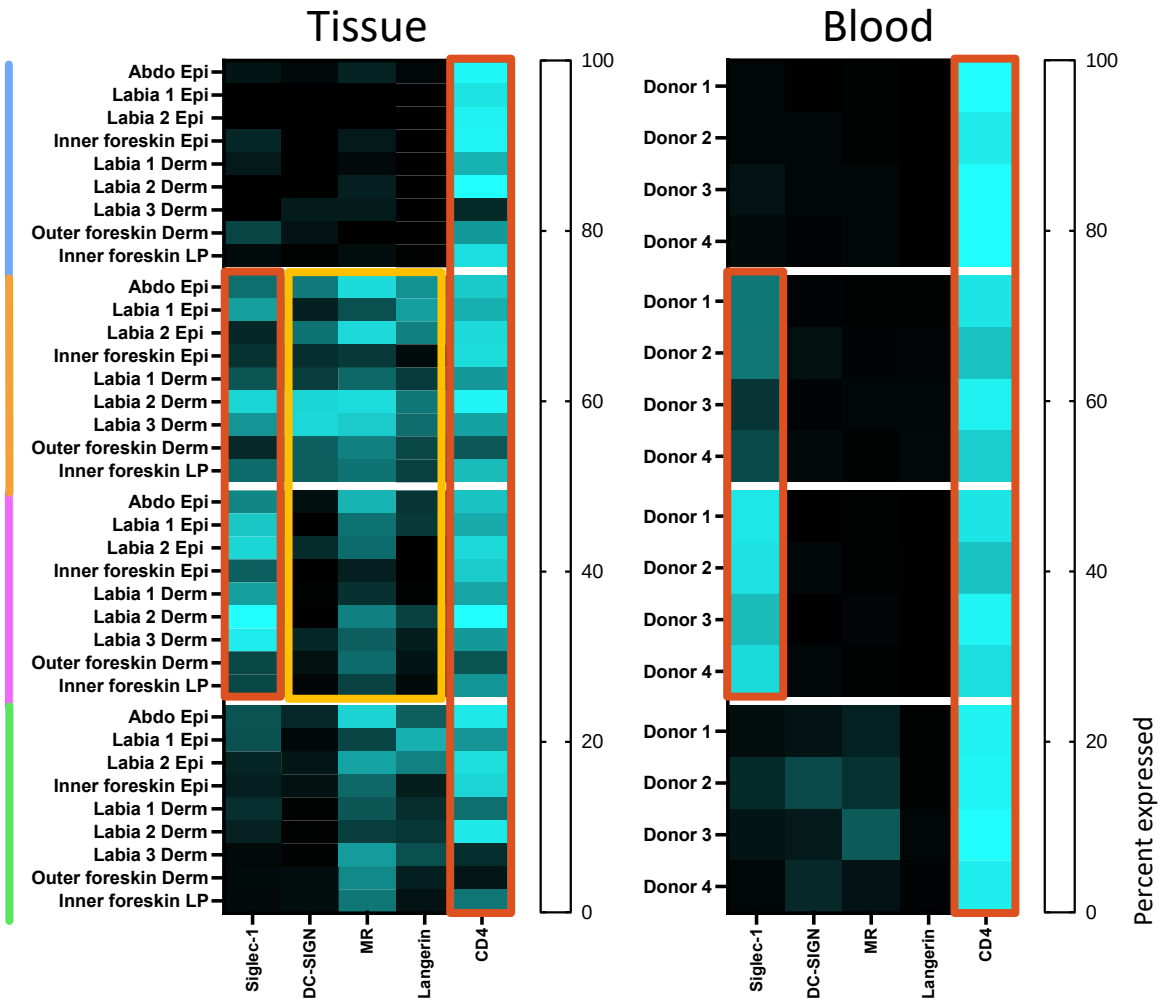
HIV binding and entry receptor expression on ASDCs in tissue

HIV entry receptor (second phase transfer)

CD4

Lectin receptors (first phase transfer)



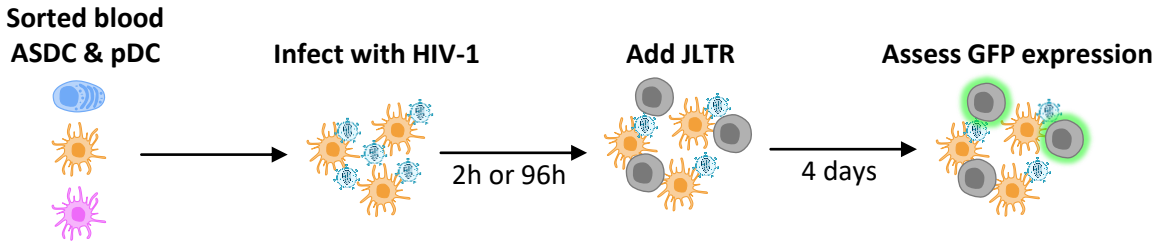


Both blood and tissue ASDCs express high levels of CD4 and Siglec-1.

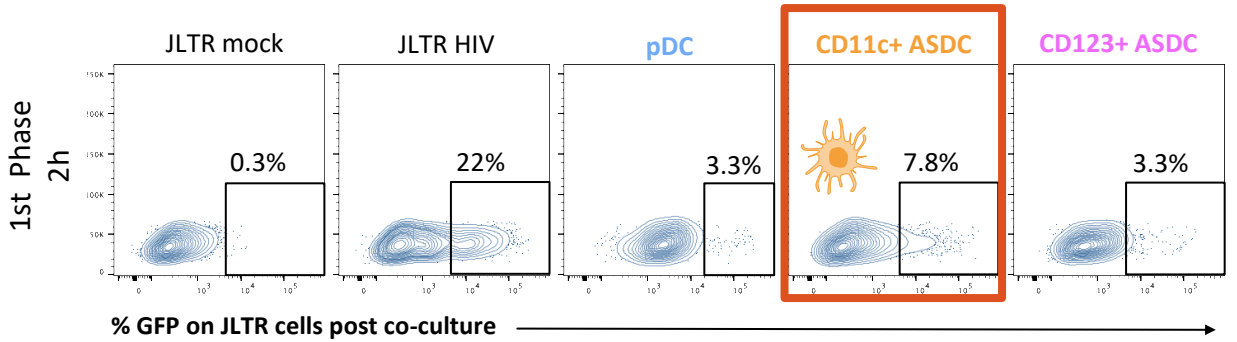
Tissue ASDCs express high levels of DC-SIGN, MR and Langerin.

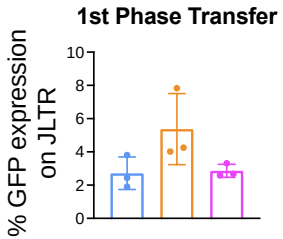
Adapted from Warner van Dijk et al, *PLOS Pathogens,* 2024

HIV Infection of blood ASDCs compared to pDCs

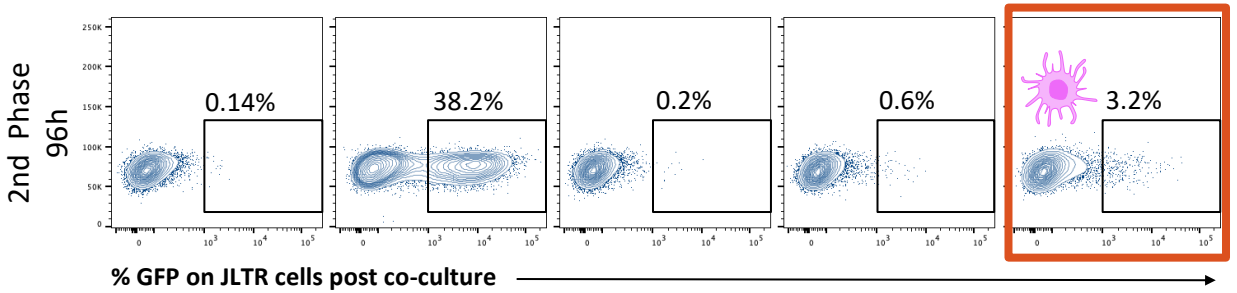


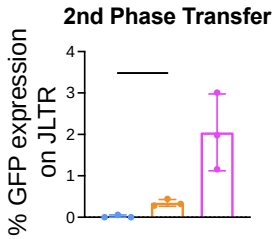
Infection independent





Productive infection (de novo synthesis)





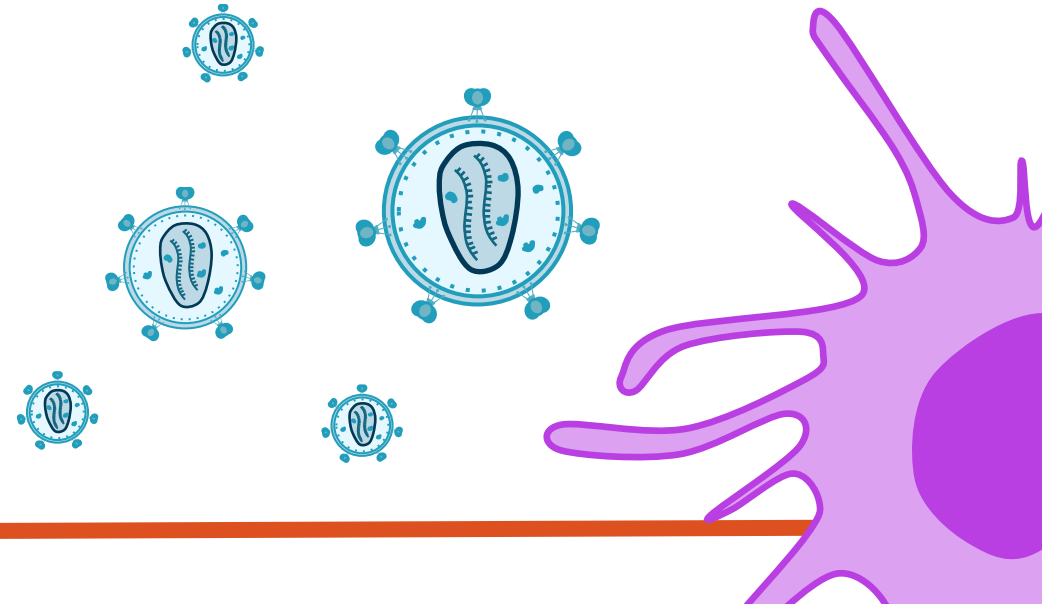
Also shown by Ruffin et al, *PNAS*, 2019

Adapted from Warner van Dijk et al, *PLOS Pathogens,* 2024

Concluding remarks

- Undeniable body of evidence that anogenital inflammation is a causative factor in HIV transmission, particularly in sub-Saharan Africa. Yet **key inflammatory HIV target cells have not been identified**.
- Developed a 26-parameter flow cytometry panel able to identify and characterise all known MNP subsets across a range of human tissues in states of homeostasis and inflammation.
- Of importance, we've identified **ASDCs**, a specifically inflammatory population, to be **present in human anogenital tissue (key HIV transmission site)** and **capable of mediating HIV transmission to CD4 T cells**.

Significance: ASDCs are a new HIV transmitting cell present in inflamed anogenital tissues and may have important implications in improved PrEP design.



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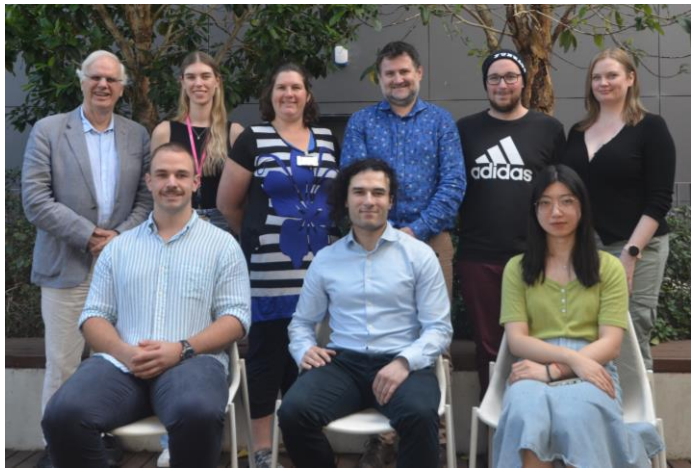

HIV & mucosal immunology group

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