

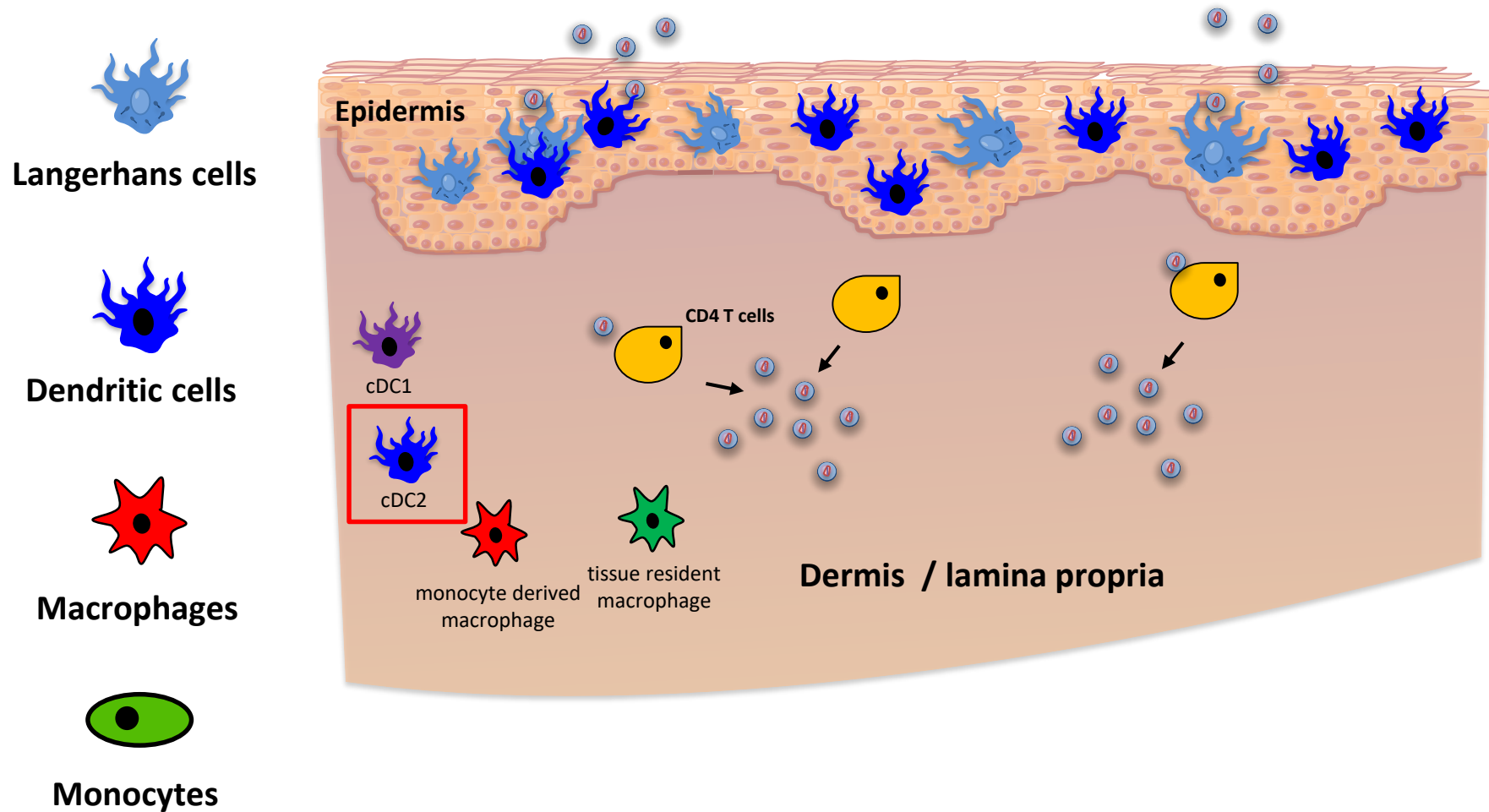
Identification of HIV Transmitting CD11c⁺ Human Epidermal Dendritic Cells

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Mononuclear Phagocytes and Sexual Transmission of HIV



Turville et al., *Blood* 2004
Harman et al., *J. Immunol* 2006
Harman et al., *Blood* 2009
Mercier et al., *PLoS Pathog* 2013
Nasr et al., *J. Immunol* 2015

Paterson et al., *Am J Pathol* 2002
Hladik et al., *Immunity* 2007
Ganor et al., *Mucosal Immunol* 2010

Phenotypic and functional consequences of different isolation protocols on skin mononuclear phagocytes

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RECEIVED NOVEMBER 28, 2016; REVISED FEBRUARY 6, 2017; ACCEPTED FEBRUARY 14, 2017. DOI: 10.1189/jlb.4A1116-496R



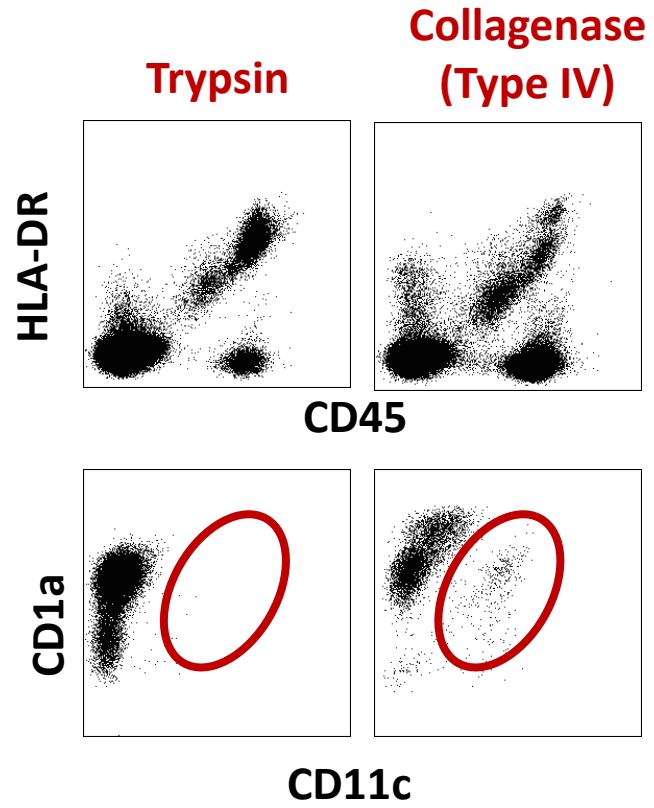
Enzymatic Cleavage of Surface Markers

 minimal cleavage

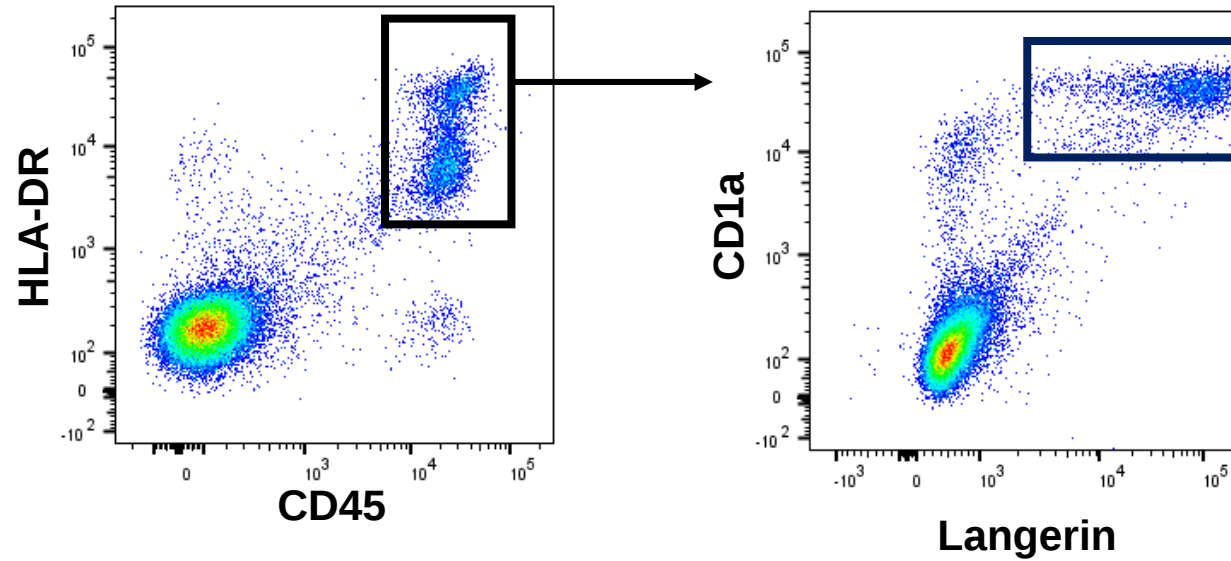
 partial cleavage

 complete cleavage

		Trypsin 	Collagenase <i>Blend F</i>	Collagenase  <i>Type IV</i>
Langerhans cells markers	HLA-DR			
	CD45			
	CD1a			
	Langerin			
Dermal MNP markers	CD1c			
	CD11c			
	CD14			
	CD141			
HIV entry receptors	CD4			
	CCR5			
	CXCR4			

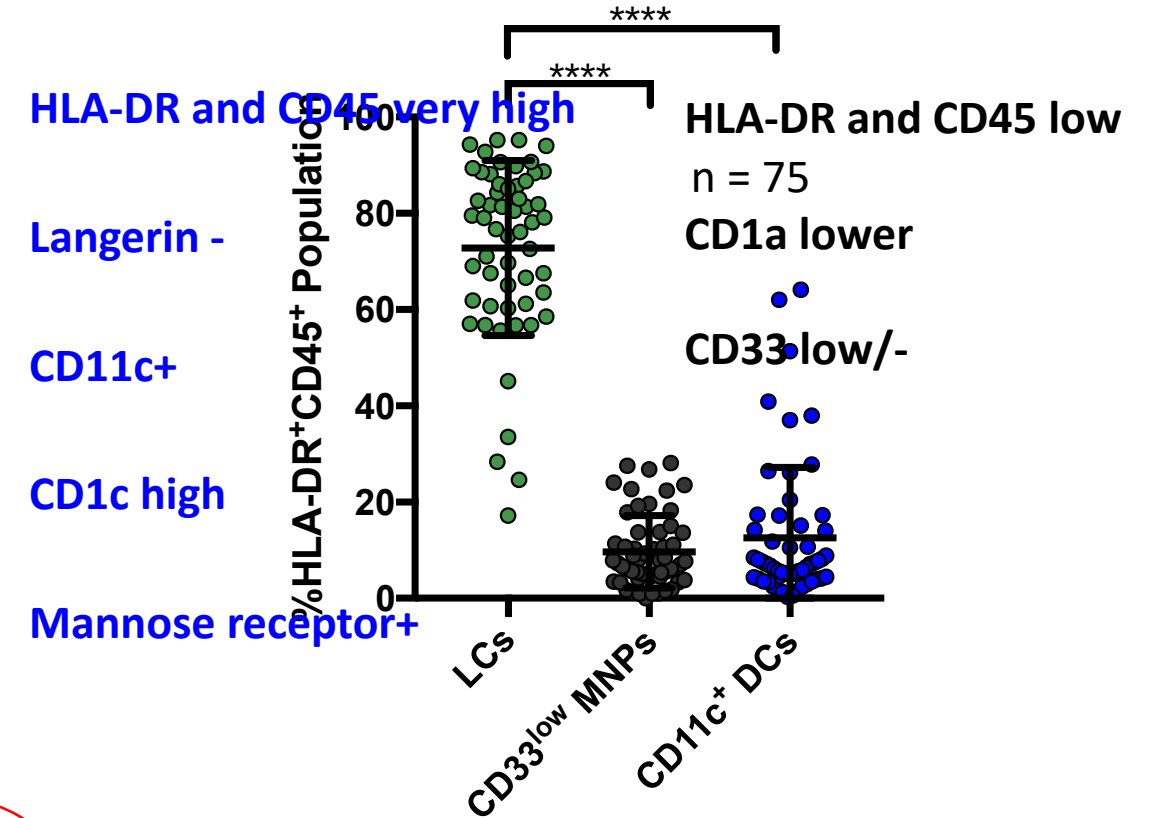
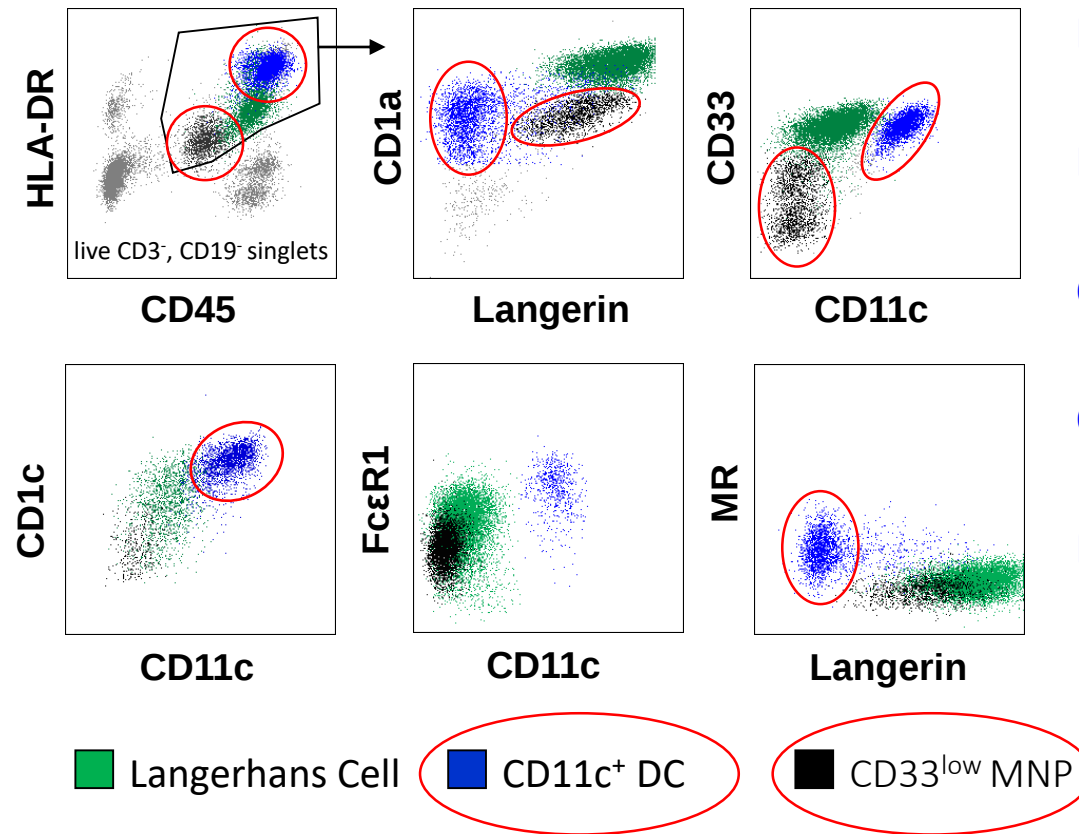


Identification of Langerhans Cells Using Trypsin



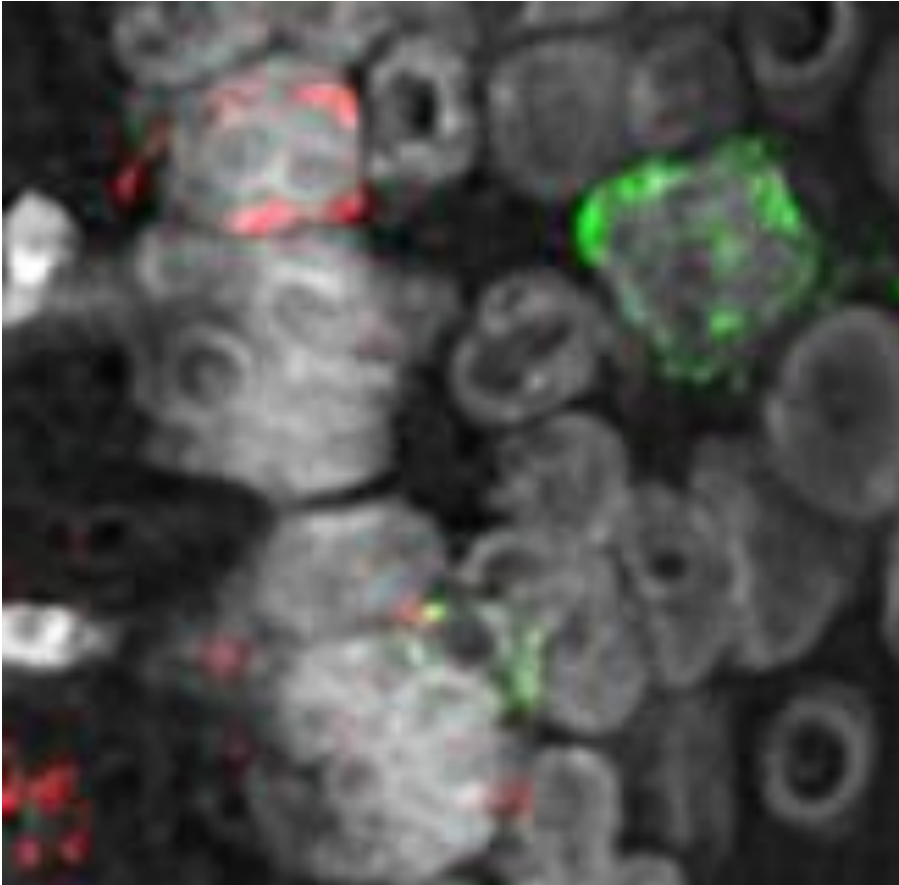
Identification of Novel Epidermal Mononuclear Phagocytes

Collagenase Digestion



Visualisation of Epidermal CD11c⁺ Dendritic Cells *in situ*

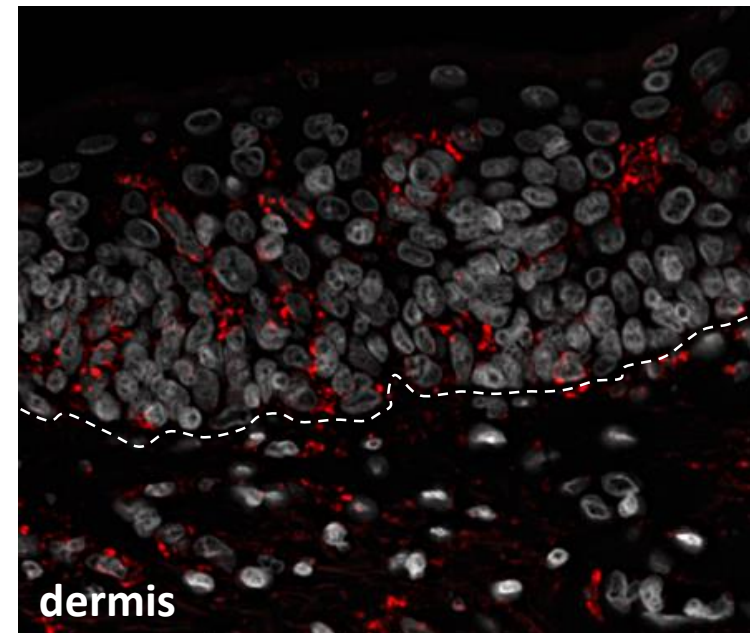
DAPI Langerin CD11c DIC



Epidermal CD11c⁺ DC

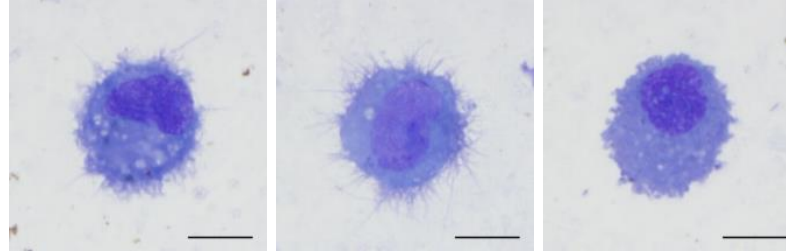


Epidermal Langerhans cell

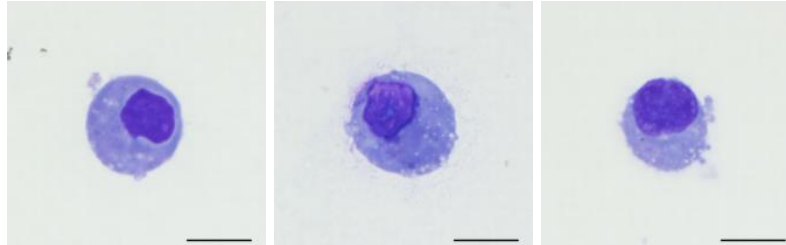


Epidermal Mononuclear Phagocyte Morphology

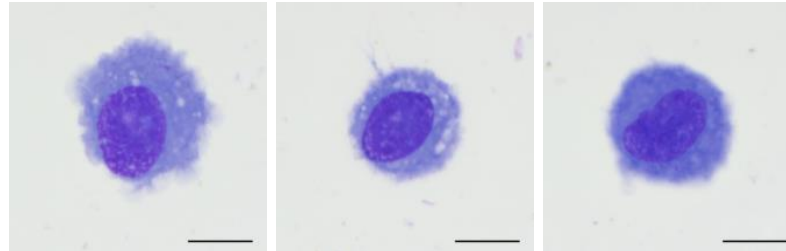
Langerhans Cell



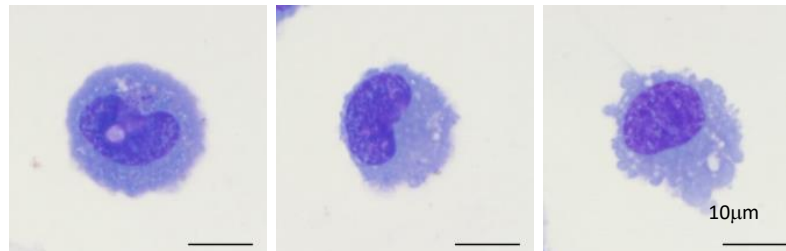
CD33^{low}MNP



Epidermal
CD11c⁺ DC

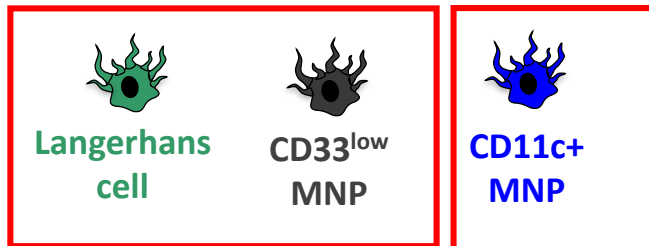


Dermal
CD11c⁺ cDC2

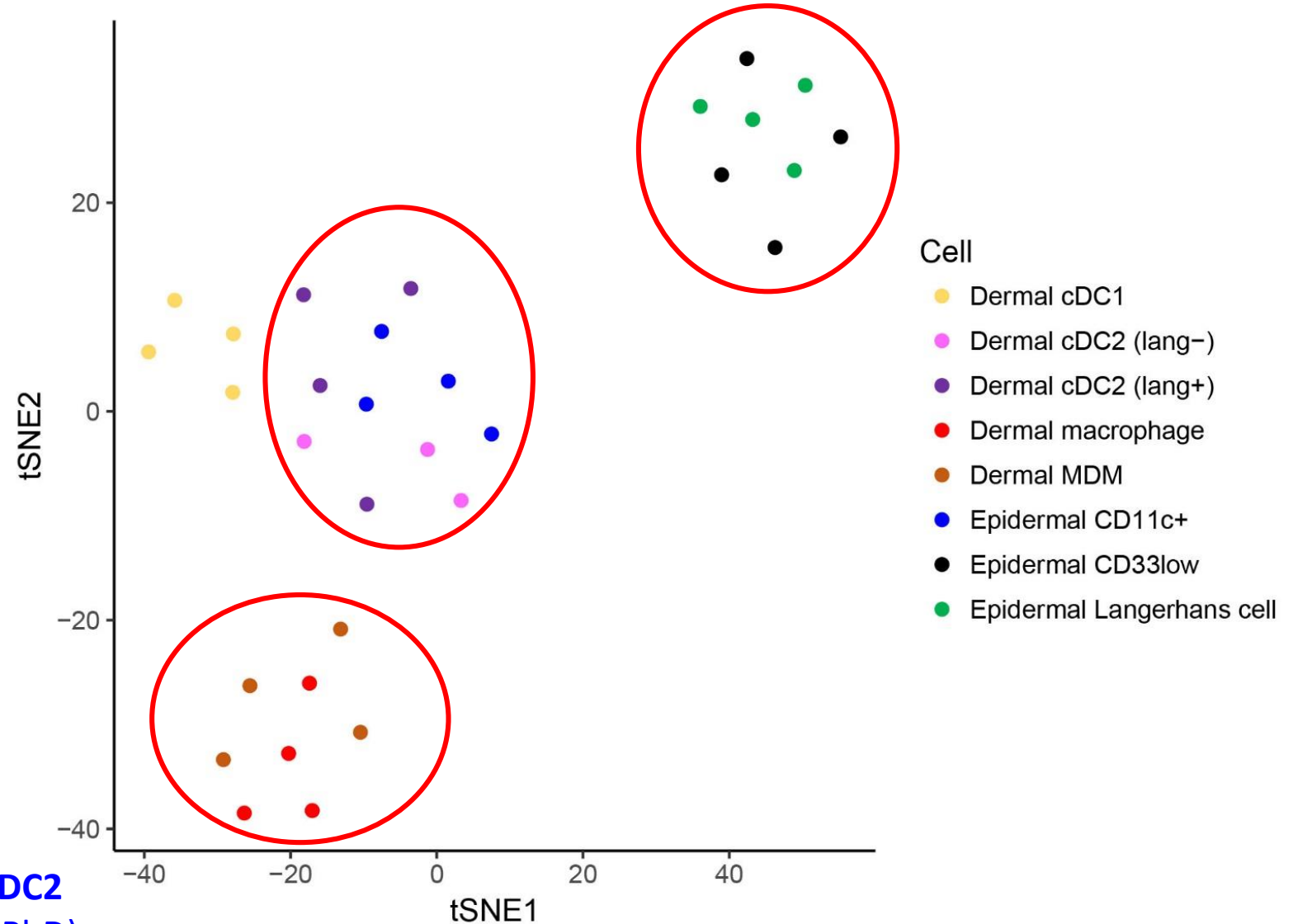
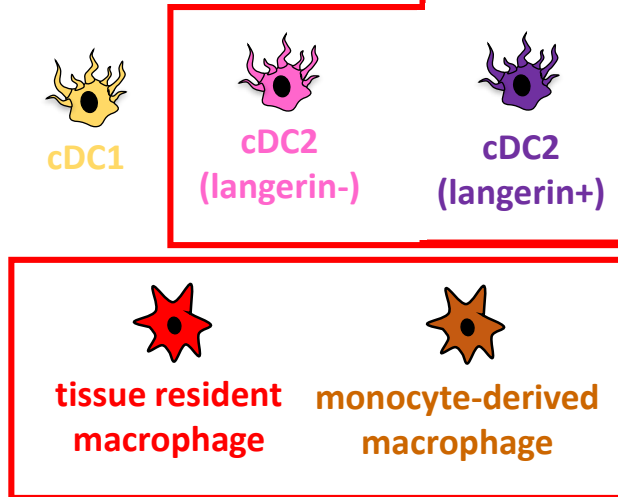


Transcriptional Profiling of Epidermal Mononuclear Phagocytes

Epidermis



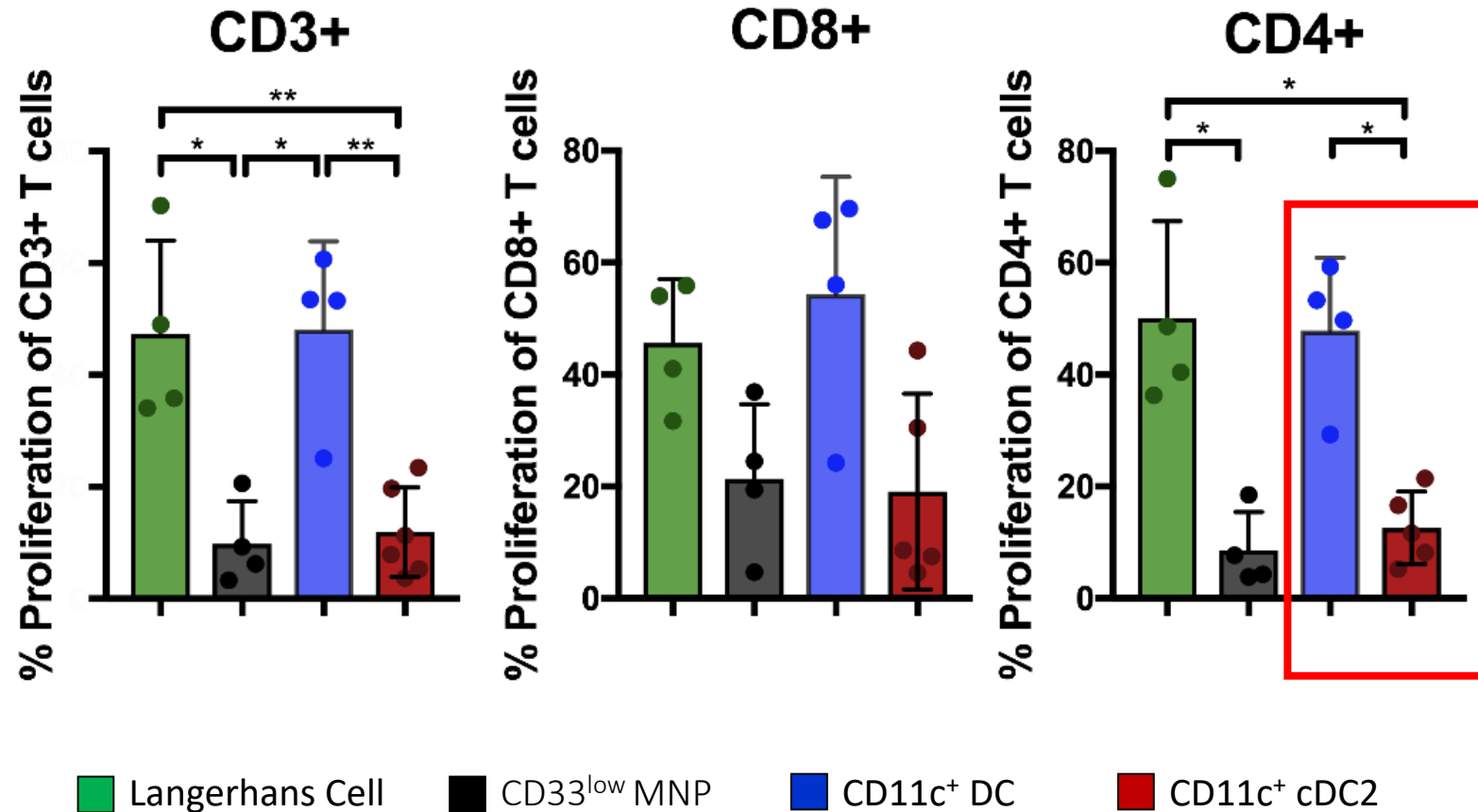
Dermis



Epidermal CD11c DCs are probably dermal cDC2 that migrate into the epidermis (Hafsa Rana PhD)

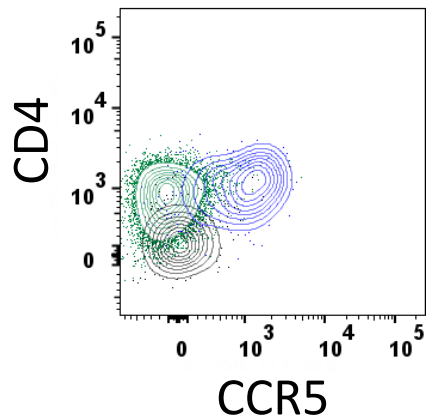
Epidermal CD11c⁺ Dendritic Cells are more Potent APCs than their Dermal CD11c⁺ cDC2 Counterparts

T cell activation assay

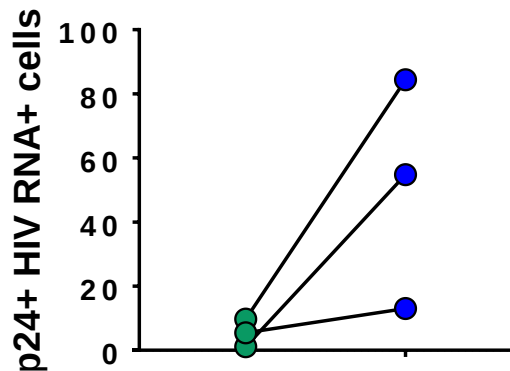


Epidermal CD11c⁺ Dendritic Cells are Preferential Targets for HIV

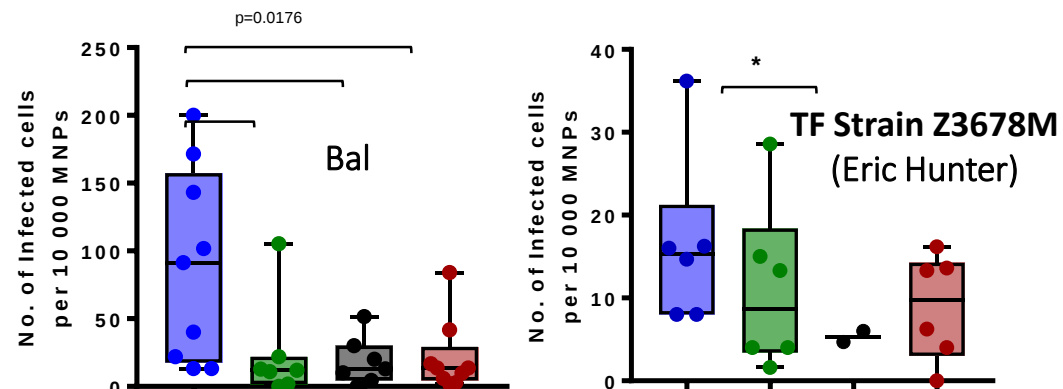
HIV entry receptor expression



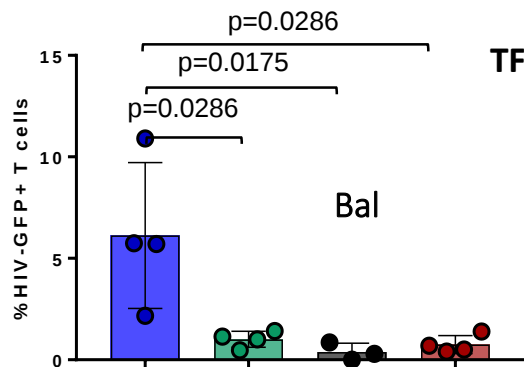
Uptake (2 hours) not CCR5 mediated



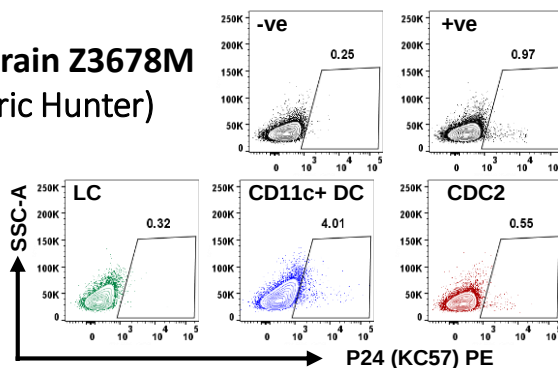
Infection (96 hours)



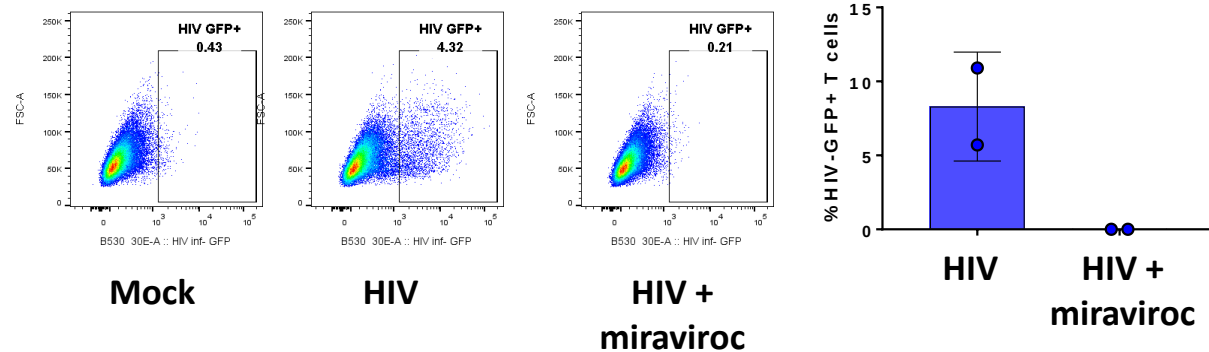
MNP – CD4 T Cell Transfer Assay



TF Strain Z3678M (Eric Hunter)



CCR5 Blocking Assay



Access to Human Anogenital Tissue

extensive collaborations with surgeons at Westmead for access to all human anogenital tissue types

female genital tract

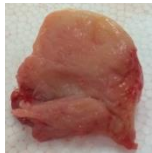
Labia



Vagina



Cervix



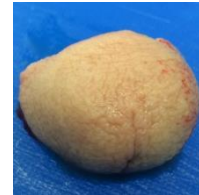
male genital tract

Foreskin



inner outer

Glans



Urethra



anorectal tract

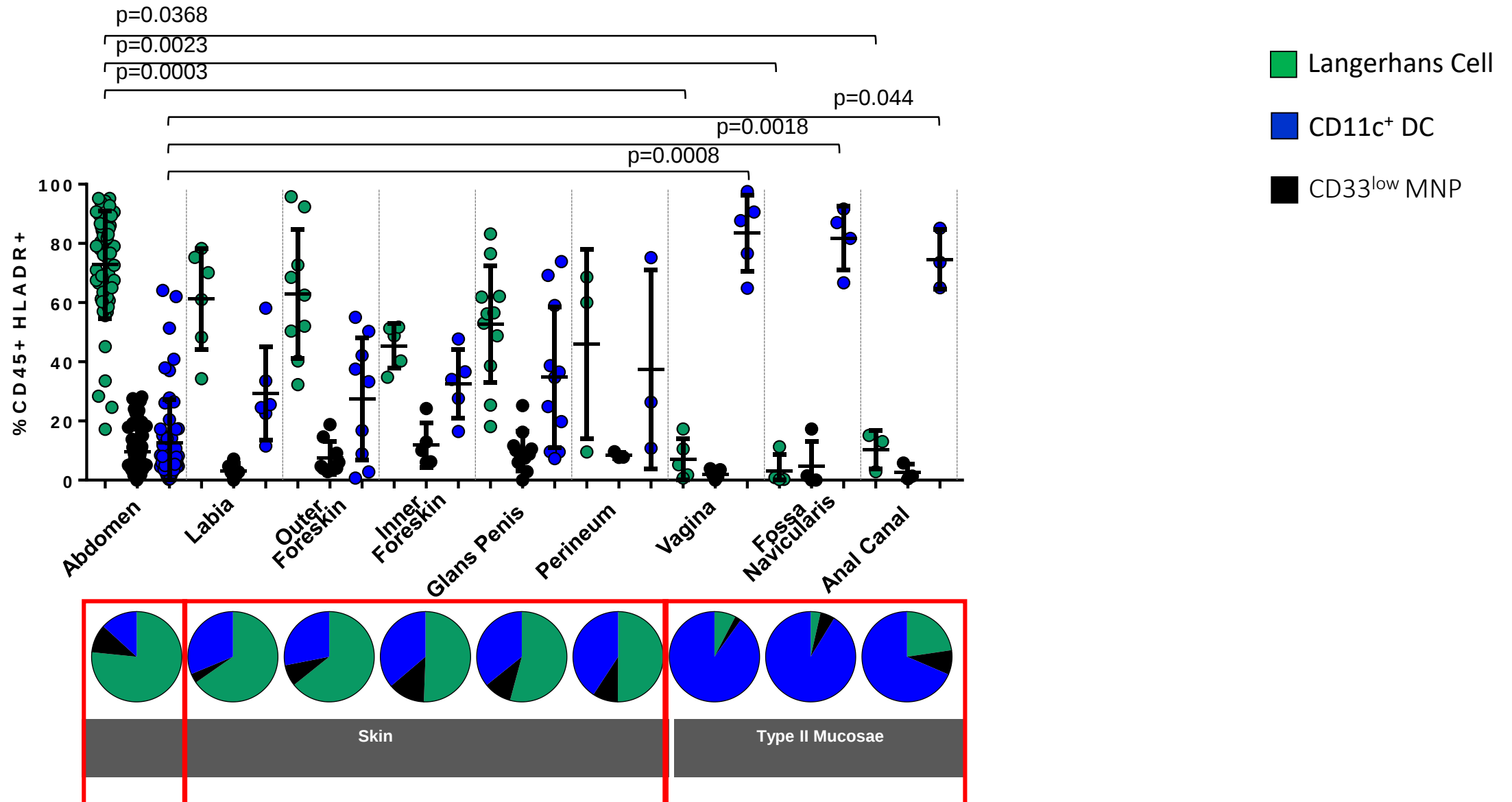
Perineum

Anal Canal

Rectum

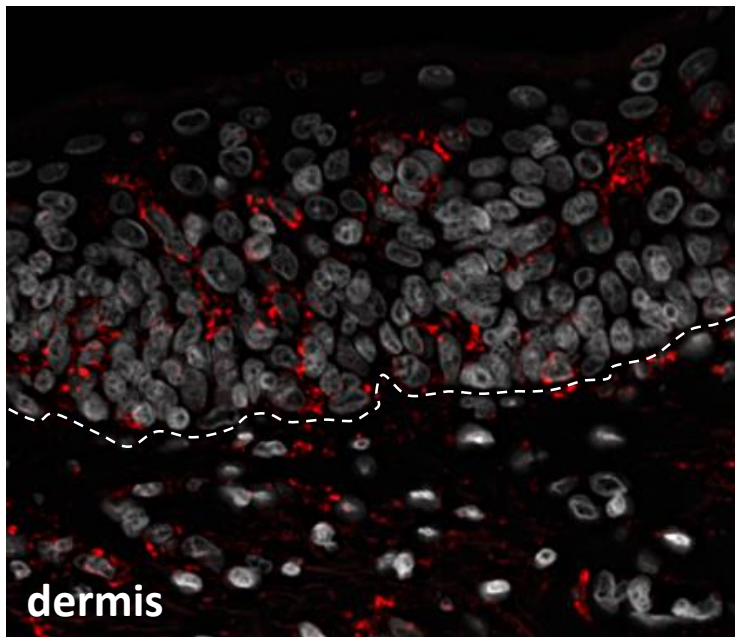


Epidermal CD11c⁺ Dendritic Cells are Enriched in Anogenital Tissues (*ex vivo* Flow Cytometry)

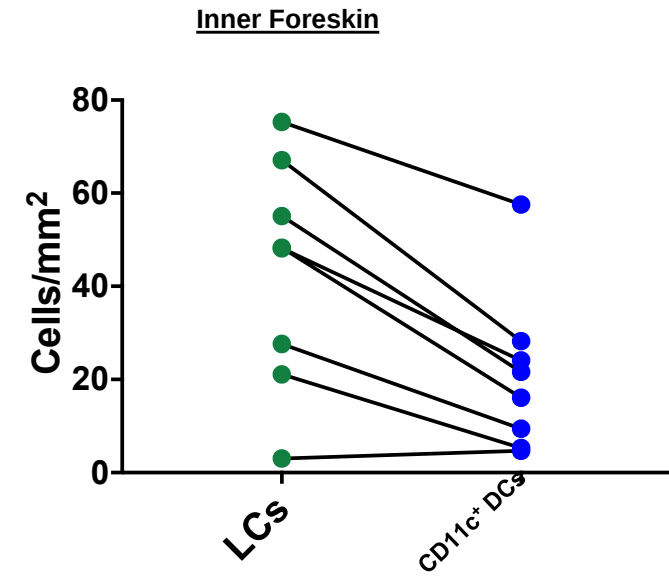


Epidermal CD11c⁺ Dendritic Cells are Enriched in Anogenital Tissues (*in situ* fluorescence microscopy)

Inner foreskin

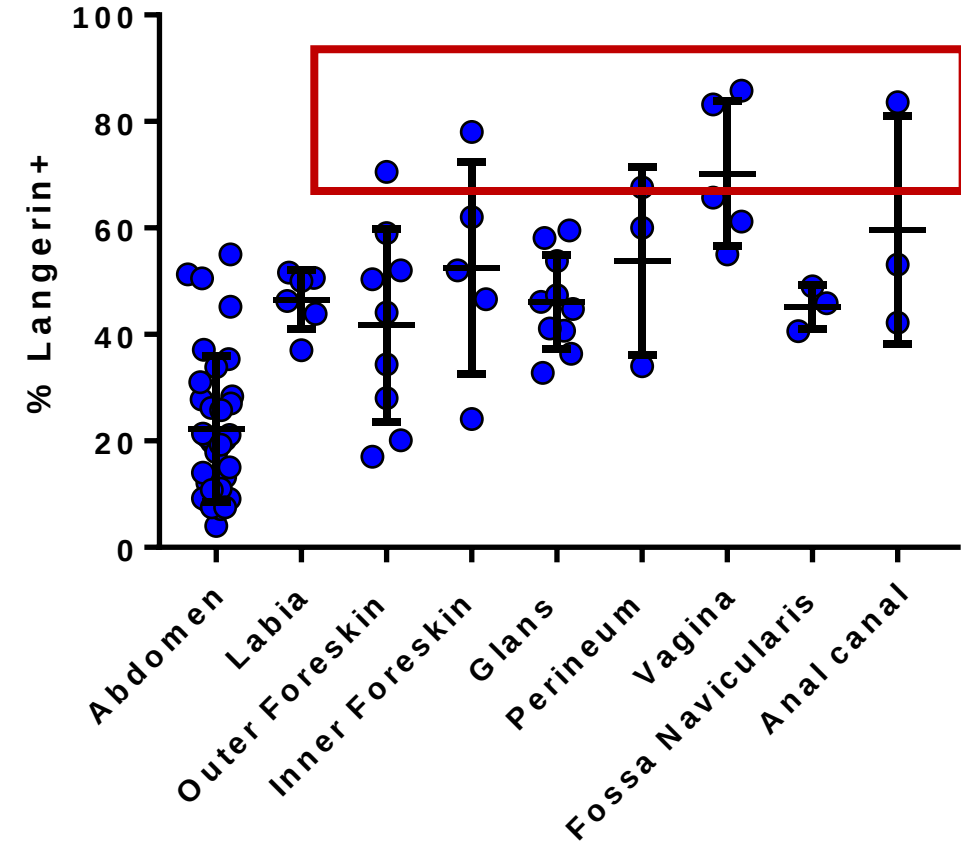
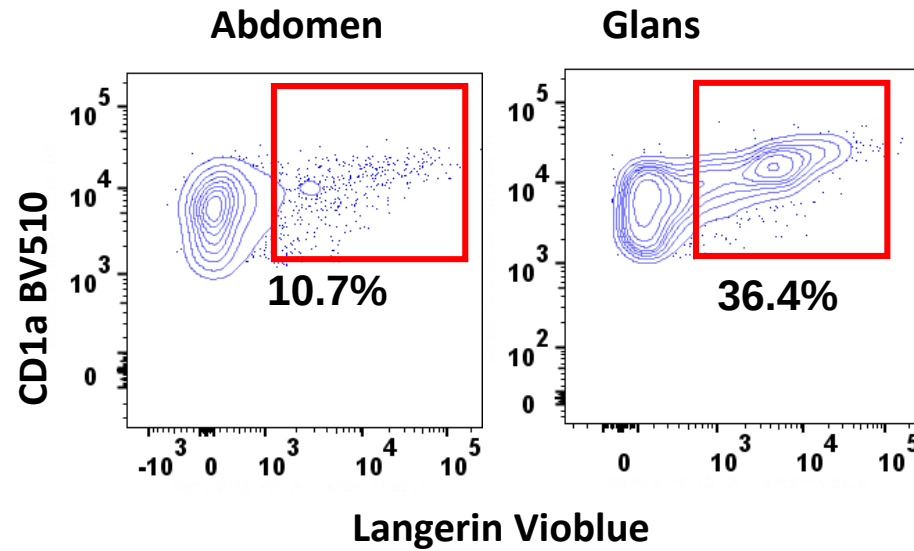


DAPI **CD11c**



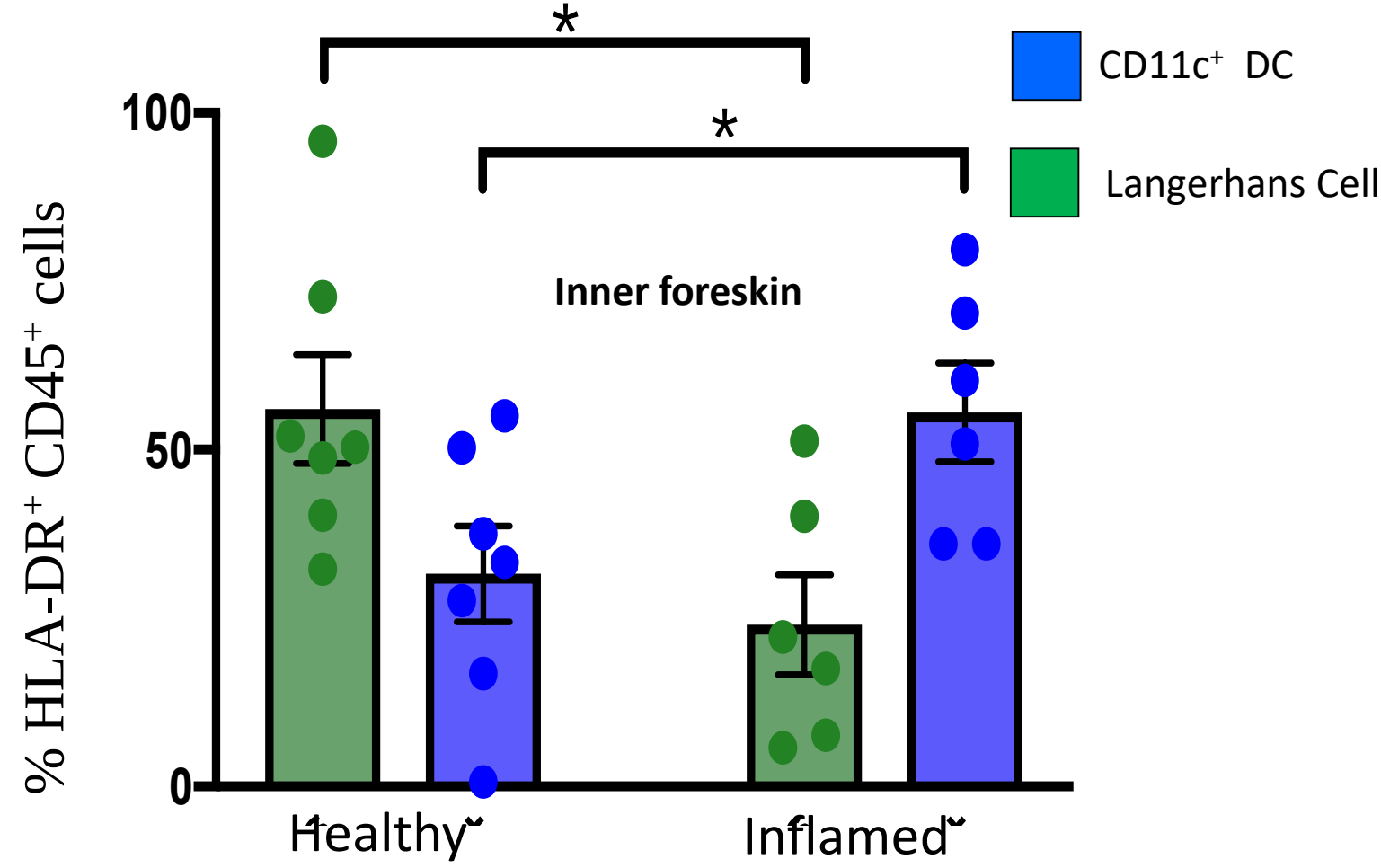
Anogenital Epidermal CD11c⁺ Dendritic Cells Express Langerin

Live, HLA-DR⁺, CD45⁺ singlets , CD11c⁺ DCs

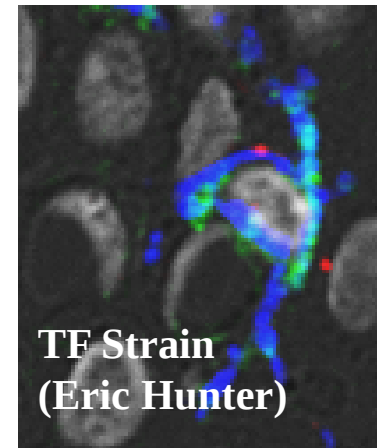
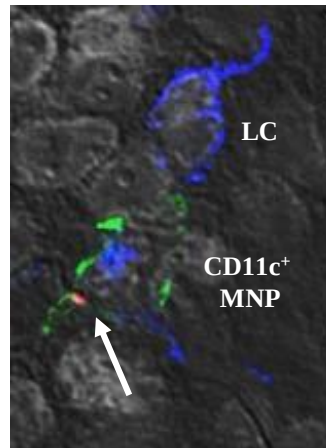
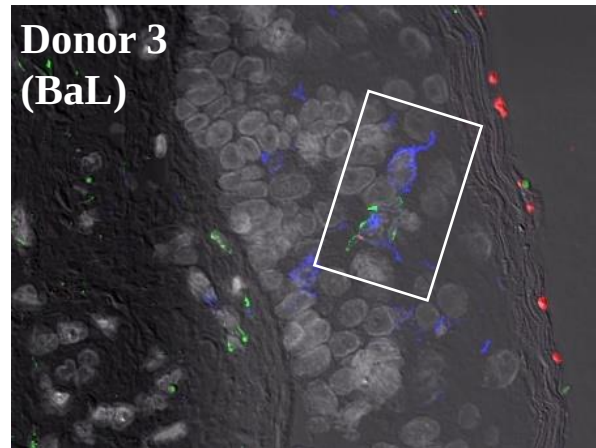
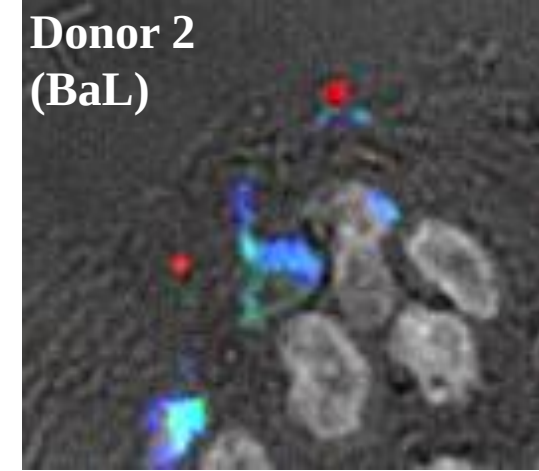
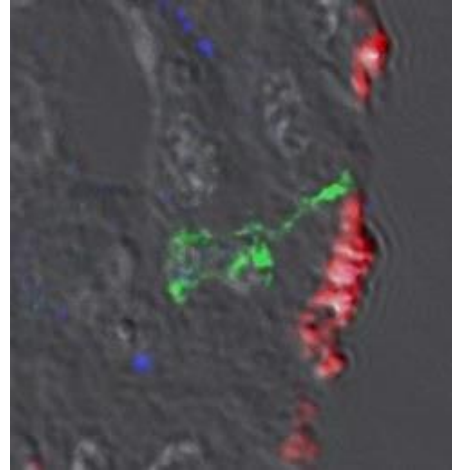
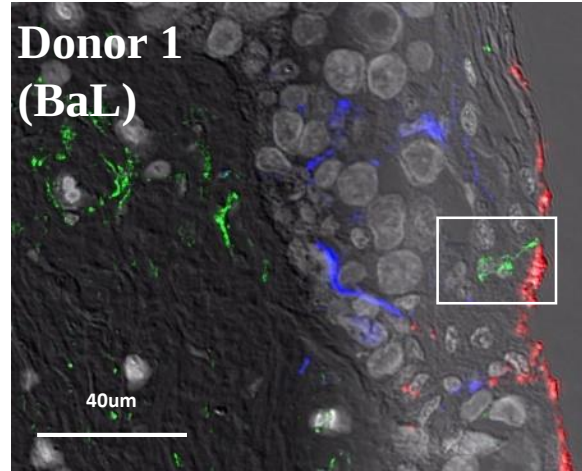


Anogenital CD11c⁺ DCs cannot be discerned from LCs unless CD11c staining is included !!!

CD11c⁺ Dendritic Cells are Enriched in Inflamed Tissue

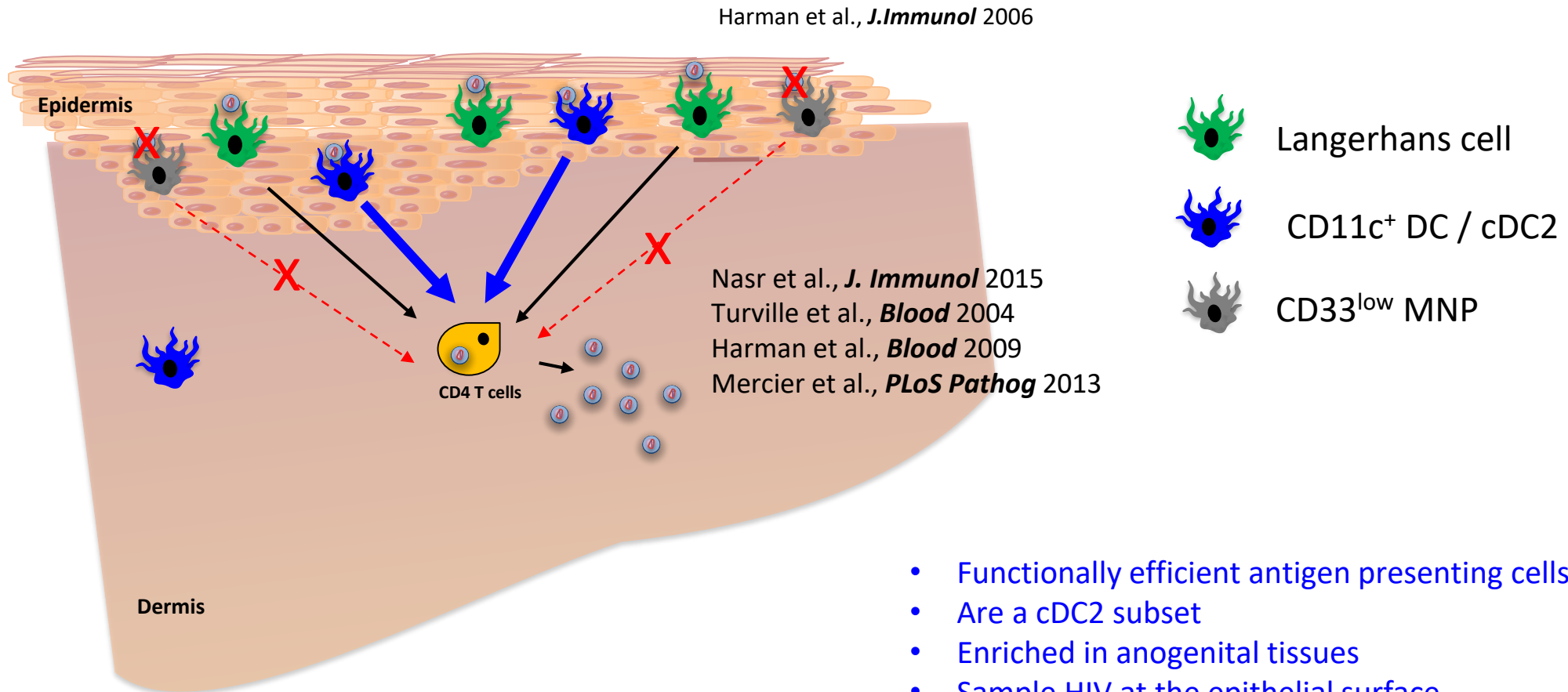


Epidermal CD11c⁺ Dendritic Cells Capture HIV *in situ*



DAPI CD11c HIV RNA Langerin DIC

Overall Summary



- Functionally inefficient antigen presenting cells
- A Langerhans cell precursor?
- Do not bind HIV or transfer it CD4 T cells

- Functionally efficient antigen presenting cells
- Are a cDC2 subset
- Enriched in anogenital tissues
- Sample HIV at the epithelial surface
- Express higher HIV entry receptor levels (CCR5)
- Support higher level of HIV infection
- Most efficient at transfer HIV to CD4 T cells

Acknowledgements



Martijn Gosselink **Jake Rhodes** Toby Plasto **Heeva Baharlou**
Chloe Doyle **Kirstie Bertram** Andrew Harman **Hafsa Rana**

Plastic Surgeons

Tim Papadopoulos
Jake Lim
Laith Barnouti
Mark Kohout
Steve Merten
Gavin Sandercoe
Peter Laniewski

Colorectal Surgeons

Grahame Ctercteko
Martijn Gosselink
Kenneth Loon
Nimala Pathma-Nathan
Mark Gladman
Alan Meagher

Urologists

Ralph Cohen
Andrew Brooks
Michale Wines



Tony Cunningham



Eric Hunter



Jacob Estes



Muzlifah Haniffa



Rachel Botting