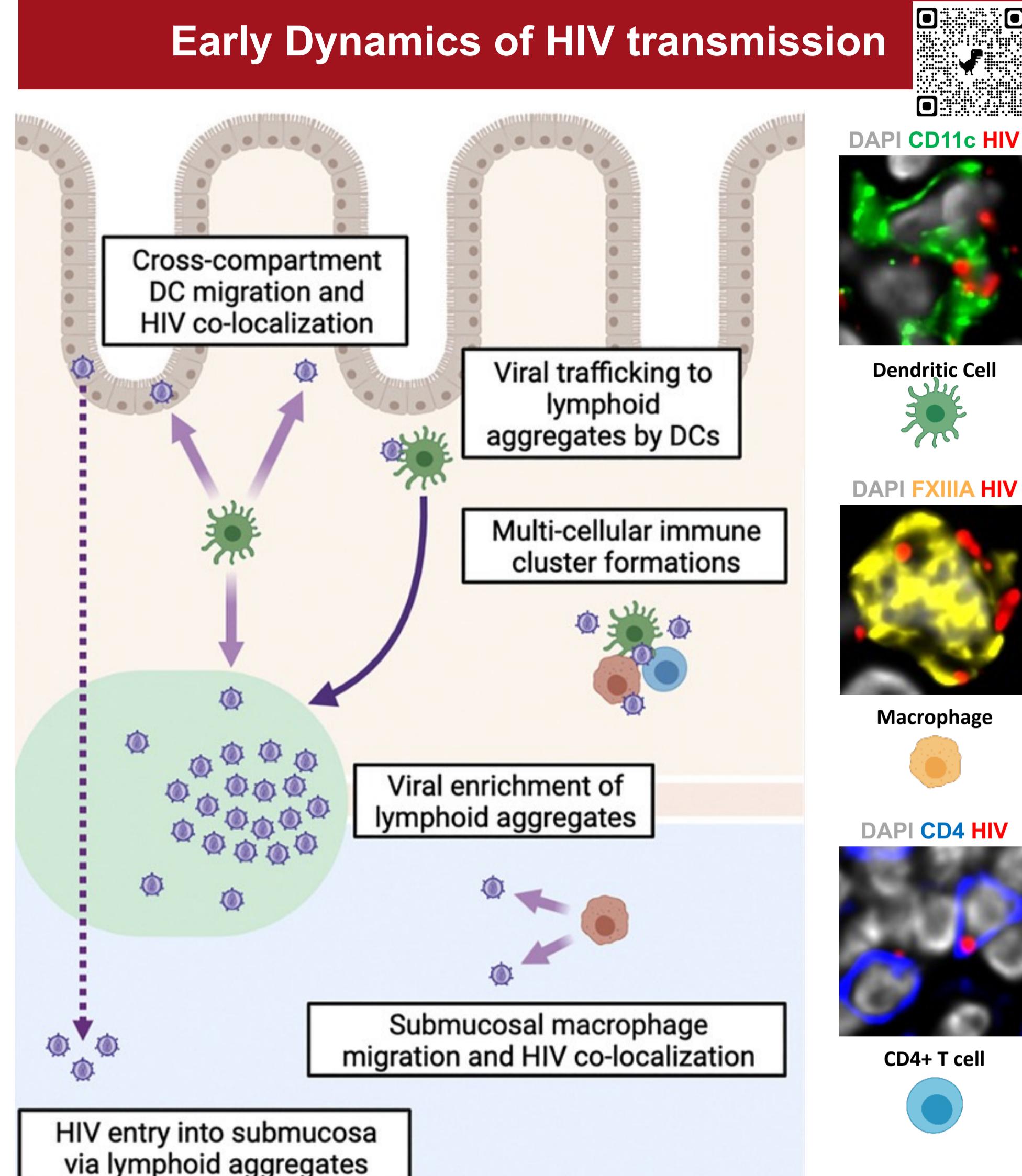


An in situ quantitative map of initial immune cell dynamics during human colorectal HIV transmission

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Early Dynamics of HIV transmission

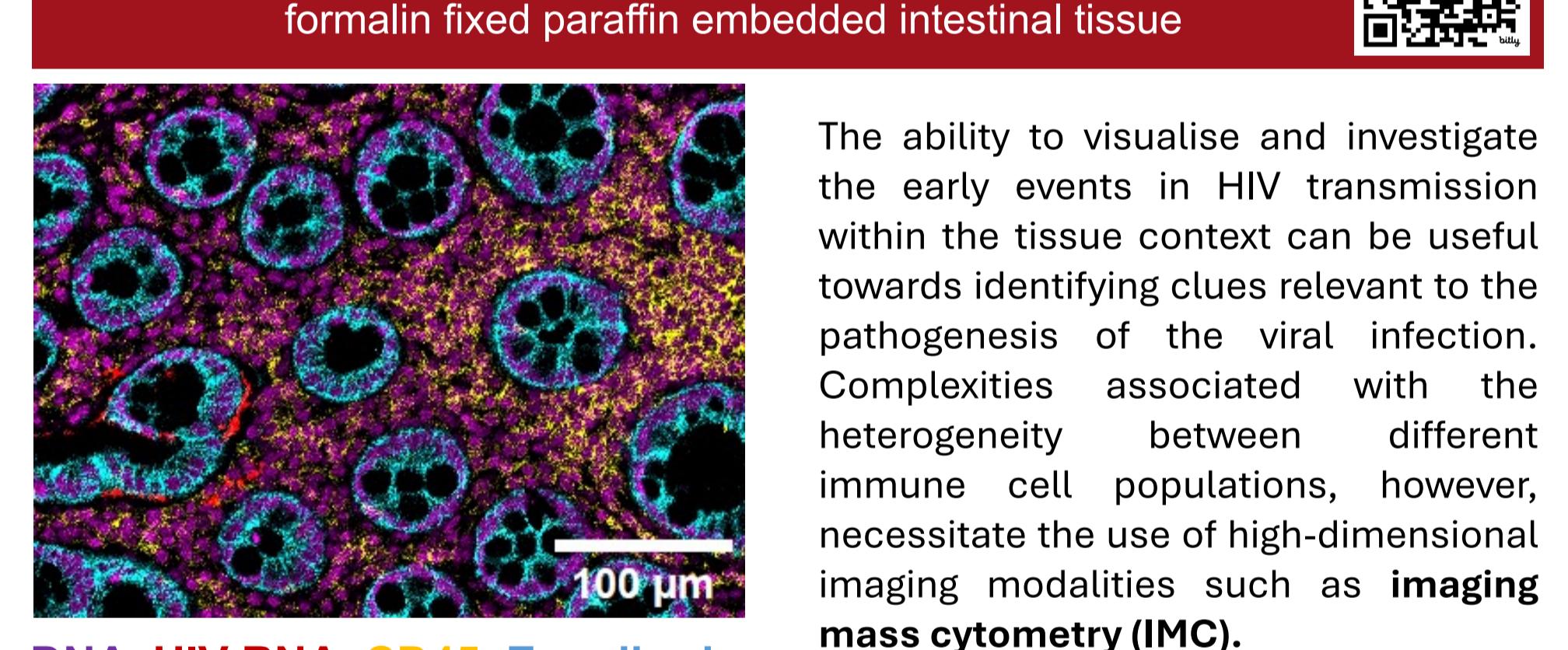


The degree to which HIV can establish infection and persist in a host system is highly dependent on its initial interactions with the immune system. However, our understanding of early events in HIV transmission remains elusive.

By combining RNAscope, cyclic immunofluorescence, and image analysis tools, we have previously quantified HIV transmission signatures in intact human colorectal explants within 2h of topical exposure (PMID: 36130503). We found that HIV is enriched in mucosal DCs, and that the HIV+ DCs accumulate near and within **lymphoid aggregates (LA)**, which act as early sanctuaries of high viral titres while facilitating HIV passage to the submucosa.

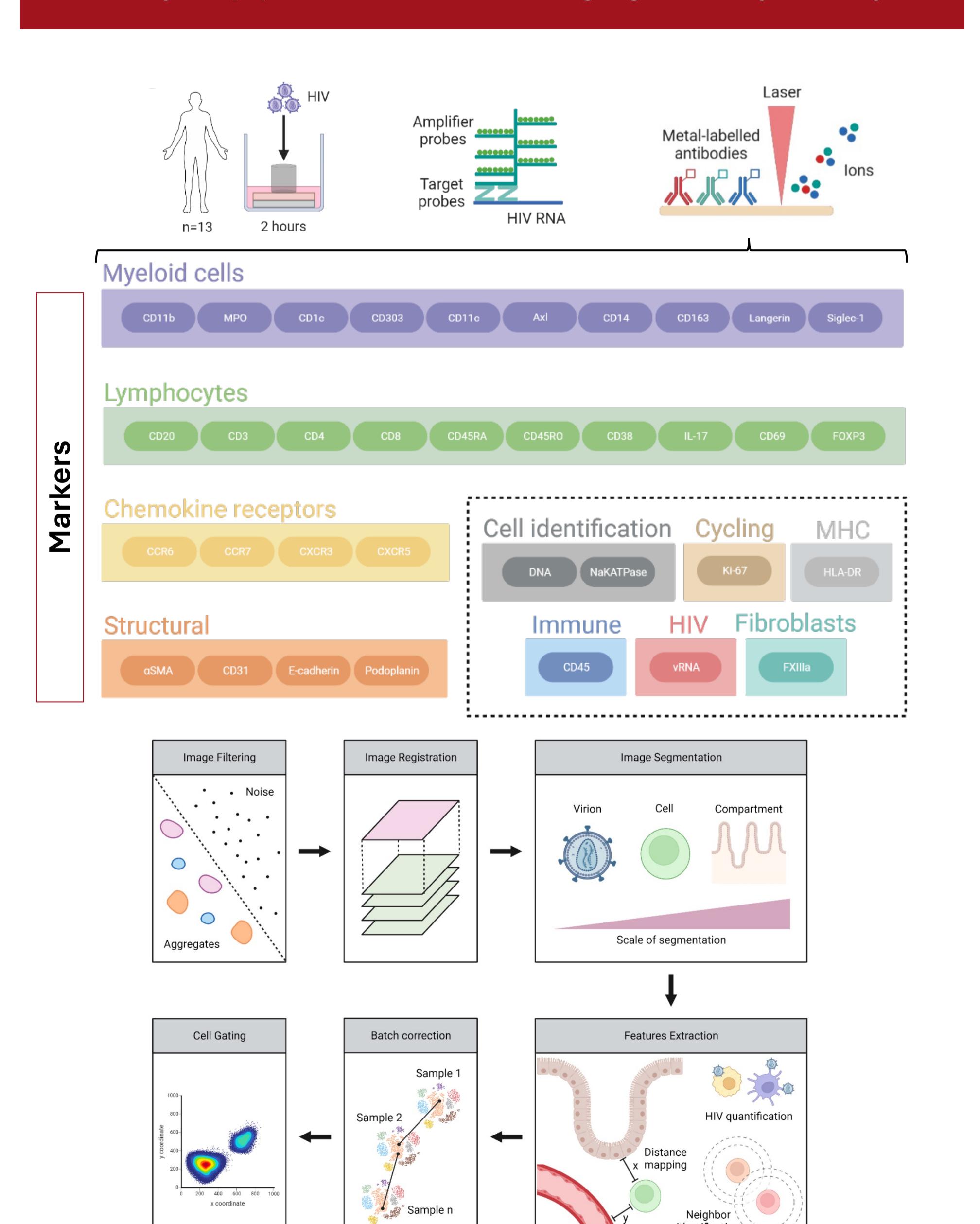
However, the human colorectal immune landscape is heterogeneous and complex. There is a significant need for higher parameter interrogations of the specific immune populations involved in the events summarised above. Here we present our 35-parameter imaging mass cytometry (IMC) panel and the insights it provides on the role of the lymphoid aggregate in HIV transmission.

OMIP-103: A 35-marker imaging mass cytometry panel for the co-detection of HIV and immune cell populations in human formalin fixed paraffin embedded intestinal tissue

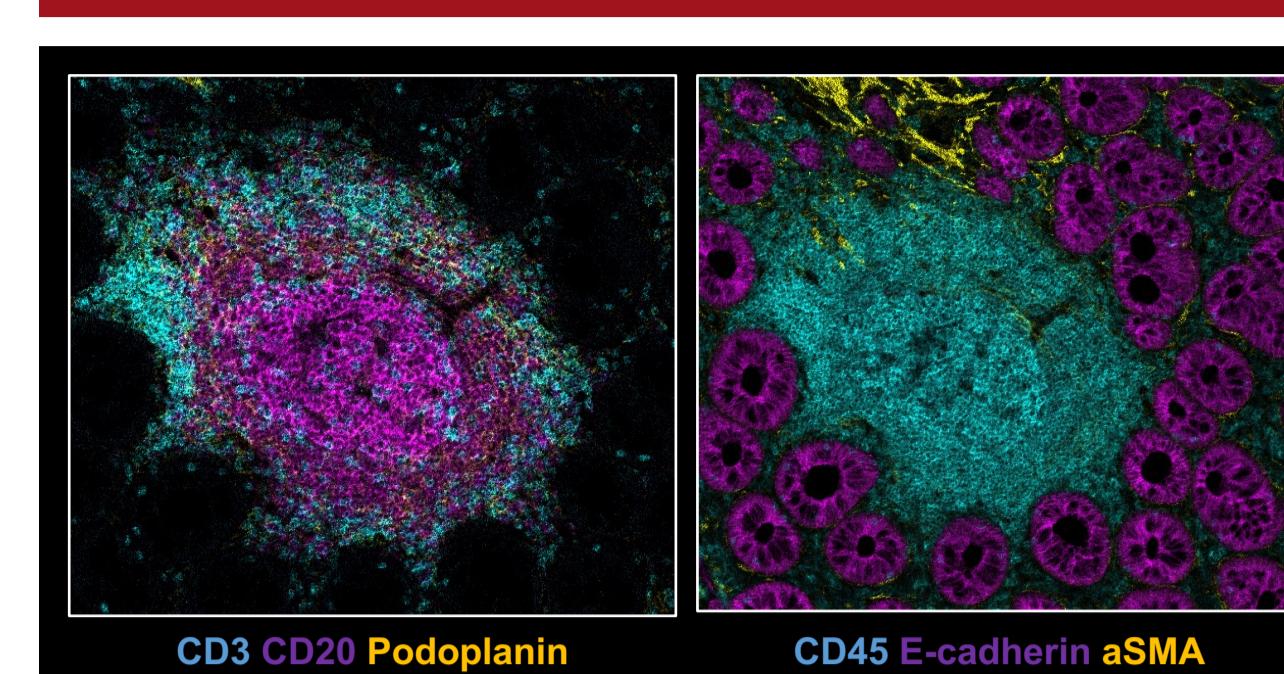


The ability to visualise and investigate the early events in HIV transmission within the tissue context can be useful towards identifying clues relevant to the pathogenesis of the viral infection. Complexities associated with the heterogeneity between different immune cell populations, however, necessitate the use of high-dimensional imaging modalities such as **imaging mass cytometry (IMC)**.

Analysis pipeline of 35-color imaging mass cytometry



Lymphoid aggregates



Lymphoid aggregates (or lymphoid follicles) are found in peripheral tissues, including uninflamed colon. They are a dense collection of semi-organized immune cells, including CD20+ (B cells) and CD3+ (T cells). Their main immunological function is unknown. However, we have found that they play an important role in early HIV transmission (right panel).

Cyclic-immunofluorescent microscopy (Baharlou et al. 2022)

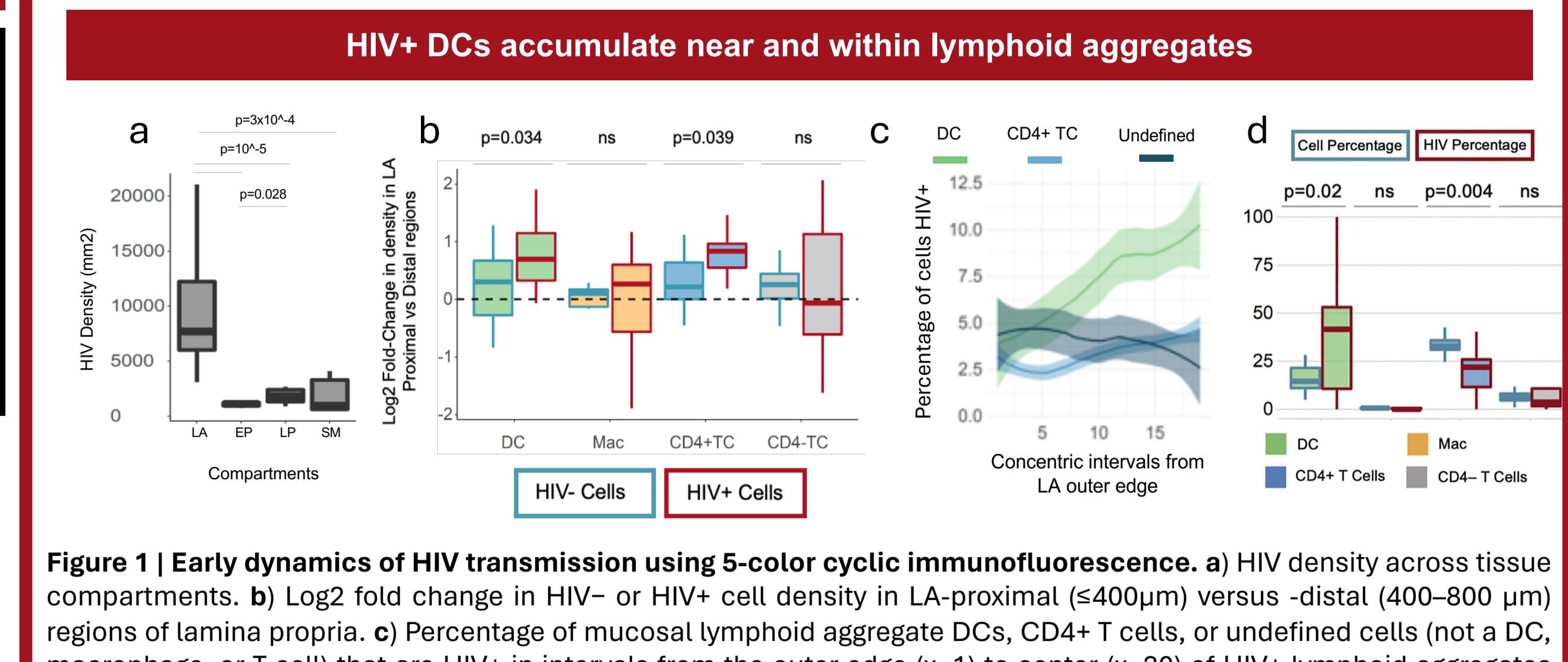
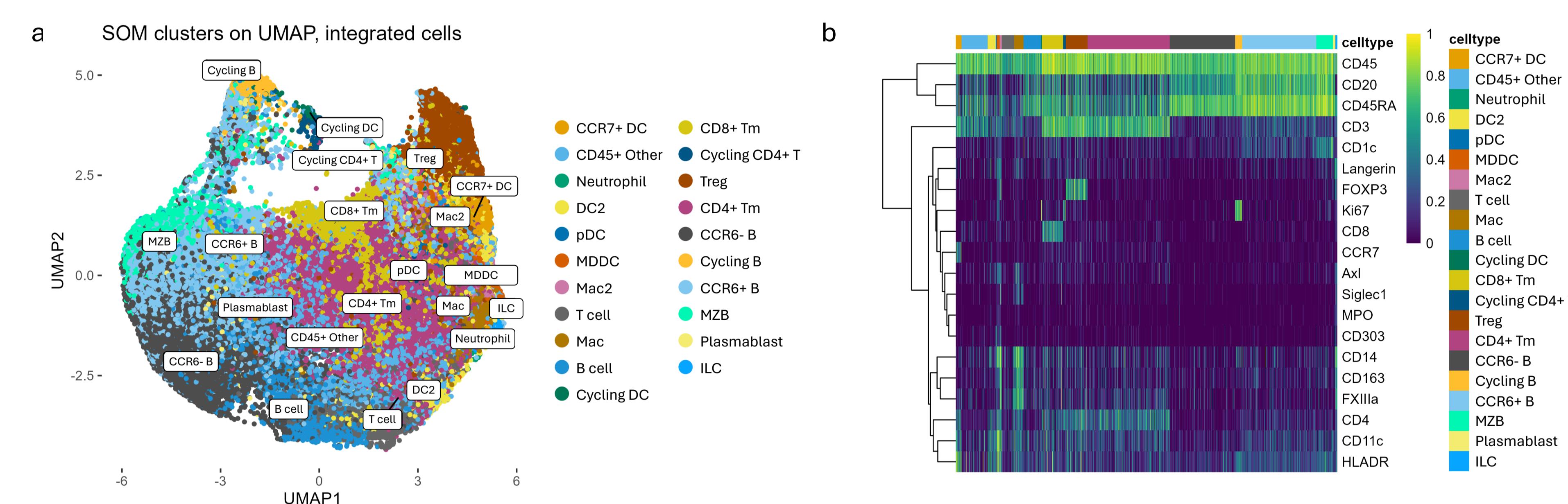


Figure 1 | Early dynamics of HIV transmission using 5-color cyclic immunofluorescence. a) HIV density across tissue compartments. b) Log fold change in HIV- or HIV+ cell density in LA-proximal ($\leq 400\mu\text{m}$) versus -distal (400–800 μm) regions of lamina propria. c) Percentage of mucosal lymphoid aggregate DCs, CD4+ T cells, or undefined cells (not a DC, macrophage, or T cell) that are HIV+ in intervals from the outer edge ($x=1$) to center ($x=20$) of HIV+ lymphoid aggregates (≥ 2 virions). d) Cell type percentage (of all cells) versus percentage of all HIV+ target cells in the LA.

Immune diversity of lymphoid aggregates using higher parameter imaging mass cytometry



We had previously identified an enrichment of HIV in CD11c+ dendritic cells, and not the primary viral target, CD4+ T cells, in the lamina propria and lymphoid aggregates (Figure 1). However, we were limited by the number of parameters capable in high resolution imaging.

We have now optimised and applied a 35-colour imaging mass cytometry panel to colon tissue, also with HIV detection via RNAscope (Figure 2). After improving on current analysis pipelines, we were able to segment the LA tissue compartments and interrogate and characterise the CD45+ immune cells with increased granularity.

We found an overall predominance of **T cells** (particularly CD4+) and **B cells**. We were able to identify sub-populations, including Tregs, plasmablasts, marginal zone (MZB), and CCR6⁺ B cells. Myeloid populations were also present, including macrophages, migratory DC (CCR7+), DC2, monocyte-derived DC (MDDC), with small frequencies of neutrophils and pDCs.

We found that there was a specific enrichment of **B cells** and **monocyte-derived DCs**. Again, we found that the CD4+ T cells are **not** the initial primary target cell in early transmission events.

Figure 2 | Early HIV penetration of lymphoid aggregates and cell type enrichment using 35-colour imaging mass cytometry. a) UMAP of clustered cells from the lymphoid aggregate compartment. b) Heatmap of the expression of key defining markers from the panel across the different cell phenotypes. c) Cell type percentage (of all cells) versus percentage of all virions across target cells in lymphoid aggregates.

Conclusions

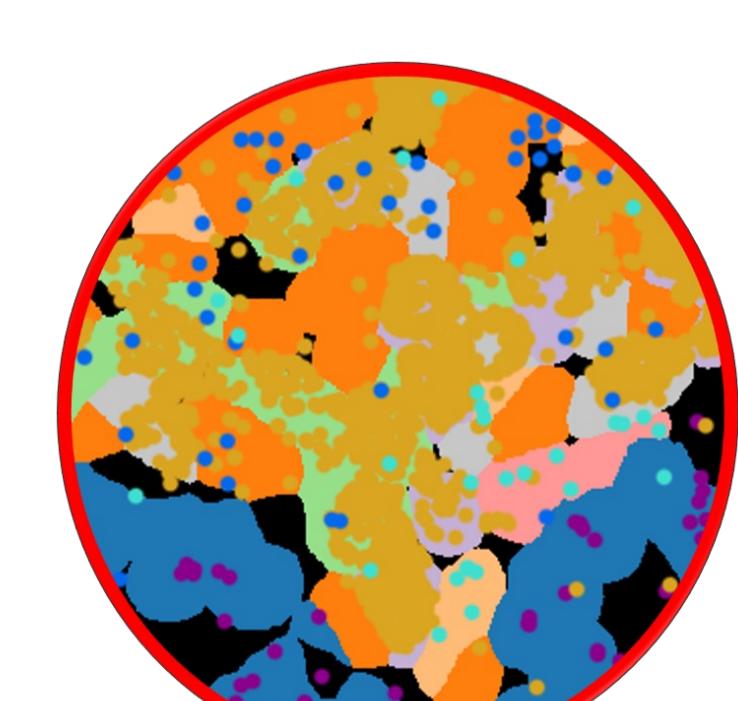
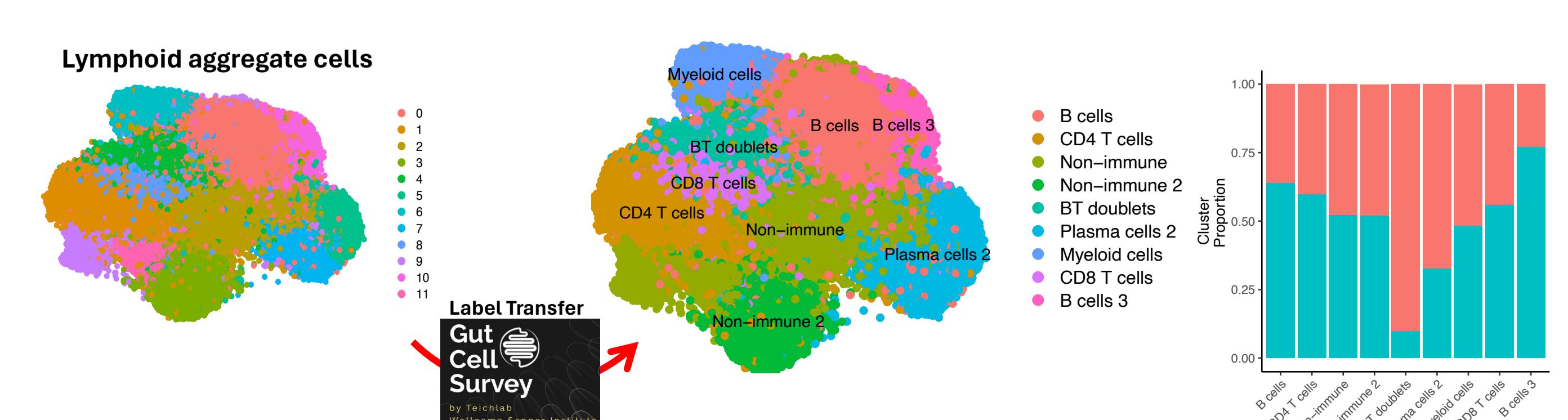
Our understanding of the spatial biology of HIV entry and its dynamic attributes which facilitate transmission is incomplete, largely in part due to the limitations of human tissue models and traditional imaging techniques. However, we have developed a physiologically relevant human *in situ* model in concurrence with the application of high parameter imaging modalities. Thus, we provide an expansive definition of the repertoire of cells that can interact with HIV *in situ*.

Future Directions

We have achieved finer granularity by increasing imaging parameters, allowing for a more comprehensive protein atlas of the human colon. Additionally, it has provided a more accurate insight into the immune cells involved with the early HIV interactions.

However, not all ideal markers were compatible with the IMC protocol (such as CD123), nor was it possible to accommodate enough markers to delineate all immune cell subsets and functionalities. Therefore, we sought the single-cell resolution transcriptomic modality, CosMX. Using the same *in situ* infection assay, we will be able to interrogate transcriptional changes that occur in tissue immune cells during early exposure to HIV.

Preliminary data below demonstrates the lymphoid aggregate heterogeneity via single cell spatial transcriptomics.



CosMX

980 genes + HIV RNA